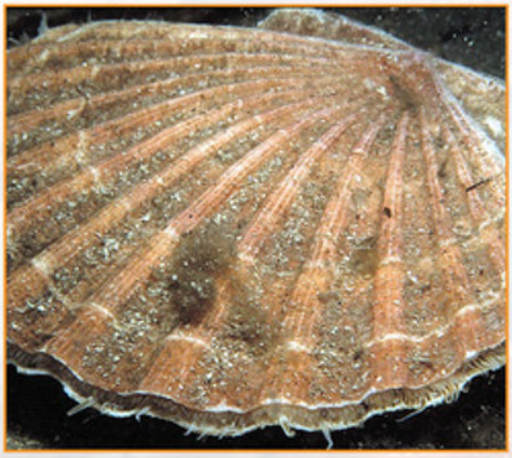
Second Edition

Marine Bivalve Molluscs



Elizabeth Gosling

WILEY Blackwell

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Preface

The phylum Mollusca is one of the largest, most diverse and important groups in the animal kingdom. There are at least 50 000 described species, and about 30 000 of these are found in the sea. The class Bivalvia is one of the eight classes of molluscs and comprises animals enclosed in two shell valves. Examples are mussels, oysters, scallops and clams. Although this class contains a relatively small number of species, about 10 000, it elicits substantial interest chiefly because these species are widely consumed by people. In 2011, production of marine bivalves from fisheries and aquaculture was almost 14 million metric tonnes worldwide with more than 90% of this figure coming from aquaculture.

Some years ago while teaching a course on bivalve biology to aquaculture students, I realized that although the students could access much relevant information from several texts, a single book covering all aspects of the biology, ecology and culture of bivalve molluscs did not exist. Thus the idea to write such a book was conceived. What started off as a small undertaking very quickly snowballed into a substantial task, which took several years to complete, primarily due to my having to read and digest the wealth of published information. The first edition of *Bivalve Molluscs*, published in 2003, was very well received by reviewers, did well in terms of sales, and in 2011 Wiley-Blackwell invited me to prepare a second edition.

In the intervening years, there have been many significant advances, along with the emergence of new areas of research, one reason being the range of new techniques being applied in bivalve biology and ecology. For example, DNA genetic markers play an increasingly important role in disease diagnosis (Chapter 11), identification and tagging of larvae (Chapters 5 and 10), in detecting different strains of pathogenic bacteria (Chapter 12), and in the completely new area of bivalve genomics (Chapter 10). Also, isotope ratios and fatty acid signatures are being used to identify dietary food sources in bivalves (Chapter 4), while measurements of stable oxygen isotopes in fossil and contemporary bivalve shells are allowing reconstruction of past sea surface temperatures. Trace elemental fingerprinting, based on naturally occurring elements in bivalve shells, is being used to assess larval origins and trajectories, while fluorescence *in situ* hybridization (FISH) combined with cell sorting can identify and rapidly count and sort larvae of several bivalve species in diverse plankton samples (Chapter 5). Immunosensors – devices that use specific biochemical reactions mediated by antibodies to detect chemical compounds – may soon replace liquid chromatography, which has long been the official method for detection and quantification of algal toxins in shellfish (Chapter 12).

Another significant development has been the increasing use of sophisticated mathematical models to describe and predict growth and bioenergetics of individual animals (Chapters 4 and 6), to track larval movement and recruitment (Chapter 5) and to predict geographic range of species in global warming scenarios (Chapter 3). Models are also being employed to simulate disease and forecast outbreaks (Chapters 11 and 12), to describe carrying capacity and environmental effects in aquaculture areas (Chapter 9). Also, several model-based approaches are in use to assess fisheries stocks (Chapter 8).

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Another area that has changed is the management of bivalve fisheries and aquaculture systems, where the emphasis is now more on the use of legislation and control measures, particularly in Europe, North America, Australia and New Zealand (Chapters 8 and 9). This also pertains to the control of diseases in oysters and clams (Chapter 11) and to hygiene standards for shellfish and shellfish waters (Chapter 12).

The focus of the book, like the first edition, is on marine bivalves of commercial importance, and while the book is written primarily for undergraduate students, it will be of value to graduate students, investigators engaged in bivalve research, and fishery management and aquaculture personnel. This second edition has been extensively revised and updated; it now has more than 2500 references, of which more than 70% is new, and 250 figures and tables, of which 50% is new. Chapter 1 covers the phylogeny and evolution of Bivalvia – one of the hottest topics in bivalve biology in the past 10 years. Chapter 2 provides a detailed description of external and internal anatomy, while Chapter 3 describes global and local distribution patterns, and the physical and biological factors influencing distribution and abundance, with a new section on climate change and the actual and potential impacts of global warming, ocean acidification and hypoxia on bivalves. Factors influencing filter feeding, together with dietary components and assimilation efficiency are covered in Chapter 4; new material is presented on the influence of bivalves in marine ecosystems. Chapter 5 deals with reproduction, larval development and settlement, and has new sections on sperm–egg interaction, larval dispersal, connectivity, identification and abundance estimates. Chapter 6 covers methods of measuring growth and the various factors influencing growth; new sections deal with the use of the bivalve shell as a marine archive and endogenous modulators of growth. The processes of circulation, respiration, excretion and osmoregulation are described in Chapter 7. The fishery assessment and management methods that are used in commercial fisheries of mussels, oysters, scallops and clams are covered in Chapter 8, with new material on legislation and enforcement measures. The fundamentals of bivalve aquaculture are dealt with in Chapter 9, focusing on a number of key species for detailed treatment. New material deals with larval nutrition, spawning, cryopreservation, and the effects of bivalve culture on the environment, with a new, large section on facilitating sustainable aquaculture development. Chapter 10 describes the application of genetic methods, with new sections dealing with global breeding programmes and the relatively new area of bivalve genomics. Chapter 11 deals with diseases and parasites, with a large amount of new information on diagnostic methods and the diverse defense mechanisms utilized by bivalves. Finally, Chapter 12 deals with the role of bivalves in disease transmission to humans, with new sections on production and processing controls, regulation of monitoring and quality control, including the HACCP system.

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1 Phylogeny and evolution of bivalve molluscs

The phylum Mollusca is one of the largest, most diverse and important groups in the animal kingdom, with at least 50 000 described species and probably as many as 200 000 living species, most of which are marine. The phylum has a remarkable fossil record going back to the Early Cambrian some 540 million years ago (Ponder & Lindberg 2008). Molluscs are soft-bodied animals but most have a hard protective shell. Inside the shell is a heavy fold of tissue called the mantle. The mantle encloses the internal organs of the animal. Another feature of the phylum is a large muscular foot that is generally used for locomotion.

Although most molluscs share this basic body plan the group is characterised by a great diversity of form and habit. As Morton (1967) aptly puts it:

> *Molluscs range from limpets clinging to rocks, to snails which crawl or dig or swim, to bivalves which anchor or burrow or bore, to cephalopods which torpedo through the water or lurk watchfully on the bottom. They penetrate all habitats: the abysses of the sea, coral reefs, mudflats, deserts, and forests, rivers, lakes and under ground. They may become hidden as parasites in the interior of other animals. They feed on every possible food and vary in size from giant squids and clams to little snails a millimetre long.*

Phylogeny of the Mollusca

Eight classes of molluscs are recognized (Figure 1.1), mostly based on cladistic[1] (phylogenetic) analysis of morphological characters in extant and fossil taxa (Haszprunar *et al.* 2008). Aplacophora contains two classes: Solenogastres (~250 species) and Caudofoveata (~150 species). These are worm-shaped, deep-water animals lacking a shell but covered by a cuticle and aragonite spicules. Polyplacophora (~100 species), often referred to as chitons, inhabit hard substrates on rocky shores, and are characterized by eight dorsal shell plates. Aplacophora and Polyplacophora are grouped in Aculifera, which is regarded as monophyletic, that is all taxa in this group share a common ancestor (Sigwart & Sutton 2007). There are only 30 or so species in the class Monoplacophora (not shown in Figure 1.1)

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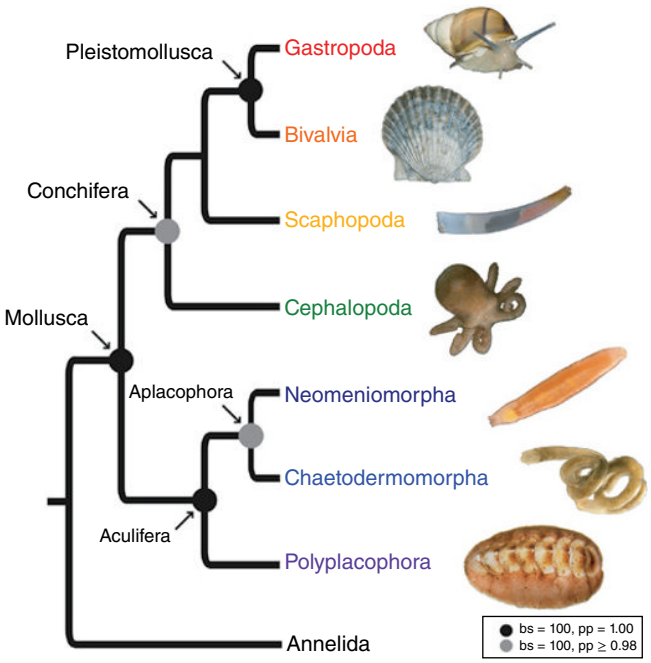


Figure 1.1 Molluscan phylogenetic tree based on transcriptome and genome data from all major lineages, except the Monoplacophora. Black circles represent nodes with bootstrap support (bs) = 100 and posterior probabilities (pp) = 1.00. Grey circles represent nodes with bs = 100 and pp ≥ 0.98. Bootstrapping is used to assess the stability of taxon groupings in a phylogenetic tree; posterior probability measures the likelihood that an event will occur given that a related event has already occurred. Photos of the major lineages are not to scale. Neomeniomorpha = Solenogastres; Chaetodermomorpha = Caudofoveata.
From Kocot *et al.* (2011). Reproduced with permission of Nature Publishing Group.

and all live in deep waters, and are small and limpet-like with a single cap-like shell. Scaphopoda (~600 species), commonly known as tusk shells because of their conical and slightly curved shell, live in marine mud and sediments. The class Gastropoda is the largest (>100 000 species) and most diverse, containing spirally coiled snails, flat-shelled limpets, shell-less sea slugs and terrestrial snails and slugs. The class Bivalvia with about 9200 species (Huber 2010) includes laterally compressed animals enclosed in two shell valves, such as mussels, oysters, scallops and clams. Octopus, squid and cuttlefish are in the class Cephalopoda. There are about 1000 species in this class and they represent the largest, most organised and specialised of all the molluscs. These four shelled classes are grouped as Conchifera, which is regarded as a monophyletic group. The Monoplacophora are generally accepted as the earliest extant offshoot of the Conchifera.

The hypothetical ancestral mollusc (HAM) is believed to have been either an advanced flatworm or a reduced annelid. It is envisioned as a small (1–3 mm) shelled animal that lived in shallow, pre-Cambrian seas, and crept over the substrate on a large foot, scraping algae off the rocks with its specialised mouthparts. At the posterior of the animal was a pair of ciliated filamentous ctenidia (gills), which functioned solely as respiratory organs (see Haszprunar *et al.* (2008) and references therein). Whether such a creature really existed is a moot point. Lindberg and Ghiselin (2003) regard it as 'a pest preserved in a textbook refugium' and made a strong case for its 'extinction', on the basis that it has hindered rather than helped evolutionary biologists and palaeontologists in solving problems.

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Until relatively recently morphological data were the only source used to deduce phylogenetic relationships within the Mollusca. In the 1980s the application of molecular methods was seen as a potentially important advance towards elucidating relationships of this major taxon. Most analyses have focused on single nuclear genes, for example small 18S ribosomal subunit (SSU) and large 28S ribosomal subunit (LSU). However, fundamental questions in mollusc evolution remain largely unanswered by the morphological and molecular data, which often give non-congruent results. Examples of such questions are whether the worm-like Aplacophora diverged before the Conchifera or lost their shells secondarily; whether the Polyplacpophora is a sister group to Conchifera, or Monoplacophora; and what are the interrelationships of conchiferan groups (Smith *et al.* 2011). Recently, researchers have adopted a multigene approach in an attempt to answer these questions. This approach uses sequences derived from genome and transcriptome data (Chapter 10) that allow numerous orthologous[2] protein-coding genes to be identified and employed in phylogeny reconstruction. Kocot *et al.* (2011) identified more than 300 orthologous genes, which they used in a phylogenetic study on 42 taxa from all major lineages within the Mollusca, except the Monoplacophera (Figure 1.1). Their results strongly supported two major clades[3]: Aculifera, which included a monophyletic Aplacophora sister to Polyplacophora, and Conchifera, which supported a sister–taxon relationship between Gastropoda and Bivalvia. They proposed the name Pleistomollusca for this grouping, which contains greater than 95% of all molluscan species (Figure 1.1). Scaphopoda was sister to Pleistomollusca, albeit with only moderate support, and Cephalopoda was found to be the sister taxon of all other Conchifera. Smith *et al.* (2011), using a similar approach but this time including Monoplacophra, also found strong support for the same two major clades, but within the Conchifera, Monoplacophora was not the sister group to all other Conchifera, as has been suggested by most authors, but was instead the sister group to Cephalopoda. They also found support for a clade comprising Bivalvia, Scaphopoda and Gastropoda, with the last two as sister groups. Just to highlight the complexity of deducing molluscan phylogeny, Vinther *et al.* (2012), using seven nuclear genes, found support for Cephalopoda as a sister group to Aculifera.

Because this book is concerned with bivalve molluscs, attention will now be fully focused on the class Bivalvia.

Phylogeny and evolution of Bivalvia

Classification and phylogeny

Bivalves are the second largest class within the Mollusca. Over evolutionary time they have become flattened side to side. Two mantle lobes cover the body organs and secrete the two shell valves that are hinged dorsally (Figure 1.2 and Chapter 2). Extant bivalves are an important component of marine and freshwater ecosystems, with more than 80% of species living in ocean habitats, and exhibiting varied ecologies. Sessile epifaunal bivalves, such as oysters and mussels, attach themselves to hard surfaces using cement or byssal threads, while infaunal burrowers bury themselves to different depths in sand or sediment on the seafloor or in riverbeds. Other sessile forms bore into hard sediments, coral or wood. Some species such as scallops are free-living and can move through the water by clapping the two shell valves together, or can dig into the sediment using their muscular foot. Although some bivalves are deposit feeders, the majority use greatly enlarged gill surfaces to filter food particles from the surrounding water (Chapter 4). Some species obtain all or part of their food through symbiosis with bacteria or zooxanthellae. Because bivalves are rich in

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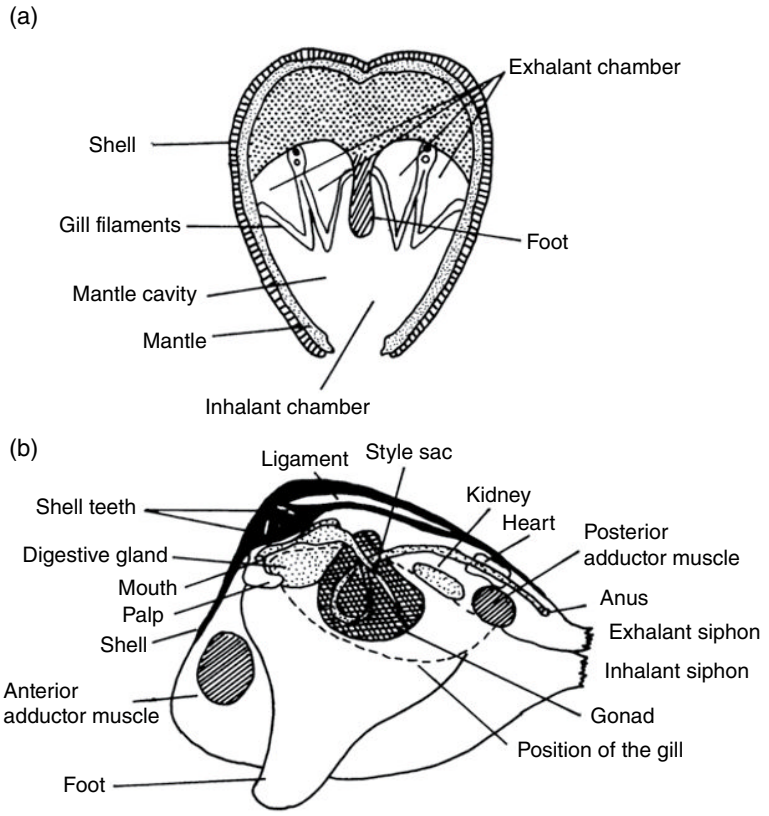


Figure 1.2 (a) Transverse section through a bivalve illustrating lateral compression and the position of the mantle, foot and gills. (b) Longitudinal section showing the major organs; gill omitted for clarity. Adapted from Barnes *et al.* (1993).
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protein they form the basis of valuable fisheries and aquaculture industries worldwide (Chapters 8 and 9). However, because of their mode of feeding they pump large volumes of water and thus have the potential to accumulate contaminants, bacteria, viruses and toxins, frequently posing significant public health risks (Chapter 12).

Despite the fact that bivalves, because of their strong shells, provide one of the most complete fossil record of any animal group, their systematics,[4] until recent times, lagged behind that of other animal groups. However, this situation has changed with researchers becoming increasingly involved in large-scale phylogenetic analyses using combinations of morphological, palaeontological and molecular data sources (Bieler & Mikkelsen 2006). Historically, there was a heavy reliance on single-character systems, for example shell hinge teeth, shell ligament, gill structure, gill ciliation, stomach morphology, mantle edge fusion and shell microstructure. This changed in the 1970s with the development of numerical systematics based on simultaneous analysis of multiple character systems. From the early 1990s gene sequence data became available and over the past two decades this data source has made a significant contribution in systematic studies, encompassing all Bivalvia as well as major groups within the class (Giribet 2008 and references therein; Plazzi & Passamonti 2010; Plazzi *et al.* 2011; Tsubaki *et al.* 2011; Sharma *et al.* 2012; Yuan *et al.* 2012).

Traditionally, bivalves were divided into five extant subclasses (Protobranchia, Pteriomorpha, Palaeoheterodonta, Heterodonta and Anomalodesmata), established mainly on the basis of shell structure and anatomy. However, a new classification was recently

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Table 1.1 Classification of Bivalvia after Bieler *et al.* (2010).

Class Bivalvia	
Subclass Protobranchia	
Order	Nuculida
	Superfamily Nuculoidea
Order	Solemyida
	Superfamily Manzanelloidea
	Superfamily Solemyoidea
Order	Nuculanida
	Superfamily Nuculanoidea
Subclass Autobranchia	
Superorder Pteriomorphia	
Order	Arcida
	Superfamily Arcoidea
	Superfamily Limopsoidea
Order	Limida
	Superfamily Limoidea
Order	Mytilida
	Superfamily Mytiloidea
Order	Ostreida
	Superfamily Ostreoidea
Order	Pectinida
	Superfamily Anomioidea
	Superfamily Dimyoidea
	Superfamily Pectinoidea
	Superfamily Plicatuloidea
Order	Pteriida
	Superfamily Pinnoidea
	Superfamily Pterioidea
Superorder Heteroconchia	
Clade Palaeoheterodonta	
Order	Trigoniida
	Superfamily Trigonioidea
Order	Unionida
	Superfamily Etherioidea
	Superfamily Hyrioidea
	Superfamily Unionoidea
Clade Heterodonta	
Order	Lucinida
	Superfamily Lucinoidea
	Superfamily Thyasiroidea
Order	Carditida
	Superfamily Carditoidea
	Superfamily Crassatelloidea
Order	Venerida
	Superfamily Arcticoidea
	Superfamily Cardioidea
	Superfamily Chamoidea
	Superfamily Cyamioidea
	Superfamily Cyrenoidea
	Superfamily Cyrenoidoidea
	Superfamily Dreissenoidea
	Superfamily Gaimardiodea
	Superfamily Galeommatoidea
	Superfamily Glossoidea
	Superfamily Mactroidea

(*Continued*)

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Table 1.1 (Continued)

Class Bivalvia	
	Superfamily Sphaerioidea
	Superfamily Tellinoidea
	Superfamily Ungulinoidea
	Superfamily Veneroidea
Order	Myida
	Superfamily Myoidea
	Superfamily Pholadoidea
Order	Uncertain
	Superfamily Hiatelloidea
	Superfamily Solenoidea
Order	Uncertain
	Superfamily Gastrochaenoidea
Order	Pholadomyida (=Anomalodesmata)
	Superfamily Ceratomyoidea
	Superfamily Clavagelloidea
	Superfamily Cuspidarioidea
	Superfamily Myochamoidea
	Superfamily Pandoroidea
	Superfamily Pholadomyoidea
	Superfamily Poromyoidea
	Superfamily Thracioidea
	Superfamily Verticordioidea

Adapted from Bieler *et al.* (2010). © BioOne.
Only extant taxa have been included.

proposed and adopted by the World Register of Marine Species (WoRMS; Bieler *et al.* 2010). The authors used a variety of phylogenetic information including molecular analysis, anatomical analysis, shell morphology and microstructure, as well as biogeographic, paleobiogeographic and geological information. This new classification of Bivalvia has been used for the following section.

Protobranchia are primitive, marine, infaunal bivalves that use their large labial palps in deposit feeding, the ctenidia being used solely for respiration, in contrast to other subclasses within Bivalvia. Protobranchs belong to the orders Solemyida, Nuculida and Nuculanida (Table 1.1). There is general agreement that Protobranchia is the first emerging lineage of Bivalvia and although most morphological studies have shown the subclass to be monophyletic, this has been questioned in more recent studies using molecular data (Giribet 2008 and references therein; Sharma *et al.* 2013). The subclass Autobranchia is divided into the superorders Pteriomorphia and Hereroconchia and includes all bivalves that have their ctenidia modified for filter feeding. Pteriomorphia contain entirely marine, mostly byssate and infaunal forms, such as many familiar and commercially important bivalves, for example mussels, oysters, arks and scallops. There are 11 superfamilies in this group (Bieler *et al.* 2010). The Heteroconchia comprise the clades Paleoheterodonta and Heterodonta (Table 1.1). Palaeoheterodonta contains two very distinct orders: the marine Trigoniida, remnants of a once diverse group, and the diverse freshwater Unionida (freshwater mussels and pearl mussels). This clade is regarded as basal to the remaining Autobranchia (Plazzi *et al.* 2011). Heterodonta, which now includes Anomalodesmata, is the largest, most widely distributed and most diverse of all bivalve clades, and includes the majority of familiar burrowing bivalves (cockles and clams), some of which live in freshwater, notably the invasive zebra mussel. Bieler *et al.* (2010) list 33 superfamilies in this clade (but see Giribet 2008).

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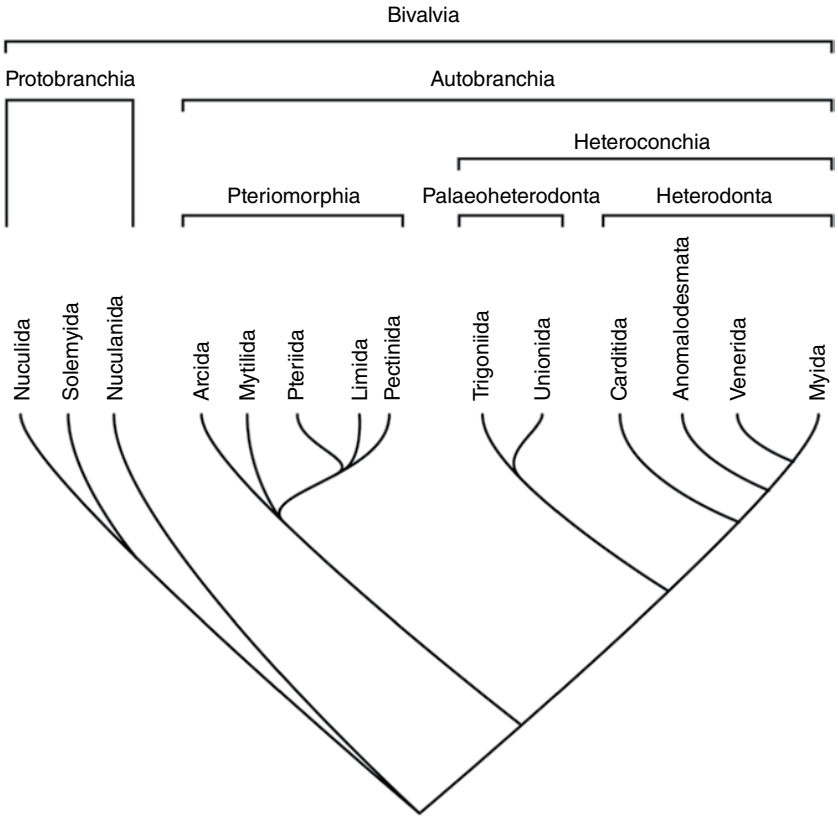


Figure 1.3 Phylogenetic diagram showing hypothesized relationships between the major clades recognized for the living members of the class Bivalvia. Note that the order Ostreida in the superorder Pteriomorphia and the order Lucinida in the clade Heterodonta (as per Table 1.1) are not included in the figure.
From Bieler and Mikkelsen (2006). Reproduced with permission of John Wiley & Sons.

Results from the combined analysis of morphological and molecular data sets support the monophyly of Autobranchia, Pteriomorphia, Heteroconchia, Palaeoheterodonta and Heterodonta (see Giribet 2008 for details). Those wishing to further explore relationships within and between different bivalve groups should consult Johnson and Haggart (1998); Harper *et al.* (2000); Bieler (2006); Taylor *et al.* (2007) and Ponder and Lindberg (2008). Figure 1.3 illustrates the hypothesized phylogenetic relationships between some of the major clades recognized for extant members of the class Bivalvia.

Evolution and adaptive radiation in Bivalvia

It is generally agreed that the early bivalves were shallow burrowers in soft substrates. They belonged to the Protobranchia and are represented today by fossil forms that date back to the Cambrian era of the Paleozoic period (500 mya), and also by some living forms such as the little nut shells, genus *Nucula*. These lie just barely covered in muddy sand, with the anterior end directed downward and the posterior end directed towards the soil–water interface. *Nucula* is a typical isomyarian bivalve, that is anterior and posterior adductor muscles are

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about the same size. Unlike most other bivalves the flow of water into the animal is from anterior and posterior directions (see Figure 1.4a and b). *Nucula* feeds on surface deposits by means of palps, long fleshy extensions of the mouth. Therefore, the ctenidia are primarily respiratory organs. The development of labial palps was perhaps a necessary stage in the evolution of filter feeding, making it possible for the mouth to be lifted off the substrate. There is no doubt that study of this group makes it easier to understand the transition from the primitive mollusc to more modern bivalves.

One of the most important developments in the evolution of modern bivalves was moving the site of water intake to the posterior of the animal (Figure 1.4b and d). This made it possible for bivalves to penetrate sand or mud 'head first' with the posterior end in free communication with the water above. Extensions of the mantle to form siphons at the posterior enabled the animals to live deeper and deeper under the surface. As bivalves evolved, plankton in the incoming current was increasingly adopted as a source of food, the ctenidia replacing the palp processes as the feeding organs. The chief modification of the ctenidia for filtering was the lengthening and folding of individual gill filaments.

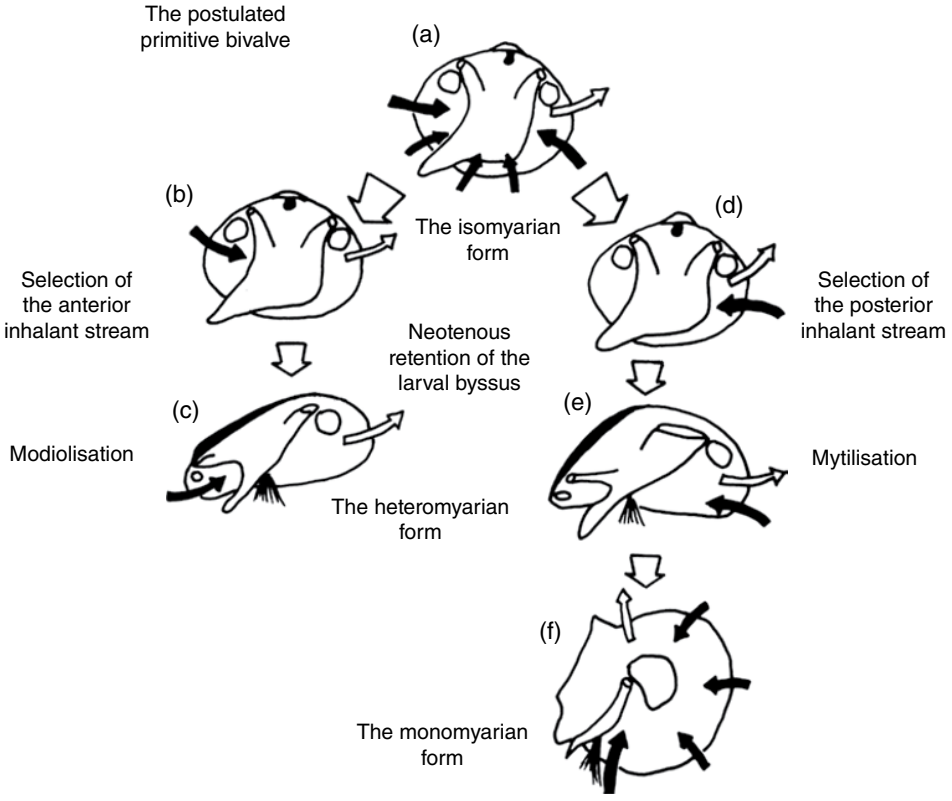


Figure 1.4 The evolution of the heteromyarian form, and ultimately monomyarian form, from an isomyarian ancestor. (a) Postulated primitive isomyarian bivalve such as *Nucula* or *Glycymeris* with water capable of entering the mantle from anterior and posterior directions. (b) Selection of the anterior inhalant stream by representatives of such groups as the Lucinoidea (shallow burrowers in tropical mud) can only result in the process of heteromyarianisation leading to (c) a modioliform shell found in ark shells, *Arca*. (d) Selection of the posterior inhalant stream can result in full expression of the heteromyarian form (e), for example in the mussel, *Mytilus*, and ultimately, the monomyarian form (f), in oyster and scallop species.
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In addition, many extra filaments were added so that they extended as far forward as the labial palps. Both of these modifications greatly increased the surface area of the ctenidia. It is believed that the triangular-shaped filaments of the primitive bivalve gill progressively changed over evolutionary time to the W-shaped filaments of the modern bivalve gill (see Cannuel *et al.* 2009). A notch at the bottom of each side of the W lines up with similar notches on adjacent filaments to form a food groove that extends the length of the underside of the ctenidia. Yonge (1941) suggested that since the food groove was necessary for nutrition these notches probably preceded folding of the gill filaments. Changes in both ciliation and water circulation followed. The exploitation of filter feeding led to the first increase in bivalve diversity and body plan disparity so that by the Ordovician period of the Paleozoic era (~450 mya) all extant higher lineages and feeding types were present and had colonised a wide variety of habitats that had hitherto been inaccessible to their protobranch ancestors (Giribet 2008 and references therein).

An important factor in this diversification was the development of a larval byssal apparatus in the basal Autobranchia, which was absent in Protobranchia, and which was later retained in some adult forms (Morton 1996). Byssus threads fixed one valve to the substrate, thus providing attachment. Consequently, individuals could exploit a variety of hard substrates, allowing them to adopt an epibenthic lifestyle in new adaptive niches (Giribet 2008). The byssal apparatus is seen as a persistent post-larval structure that evolved for temporary attachment of the animal to the substrate during the vulnerable stage of metamorphosis. In most species of oysters, clams and scallops the byssal apparatus is subsequently lost. However, in mussels it persists into adult life (Figure 1.4c and e), allowing them to anchor themselves and live in more wave-exposed habitats (Morton 1996). In byssally attached forms there has been a tendency for the anterior (head) end of the animal to become smaller with a corresponding enlargement of the posterior end. Accompanying this change there has also been a reduction of the anterior adductor muscle and an increase in the size of the posterior adductor muscle. The evolution of this heteromyarian form led to the development of a pronounced triangular shape (Figure 1.4c). This is very marked in mussels in the order Mytilidae (see Figure 1.4e) and is believed to be an adaptation to living in clusters, expansion of the posterior shell allowing free access, posteriorly, to the water above (Morton 1992). The heteromyarian condition has been seen as a stepping-stone towards the monomyarian form and the adoption of a horizontal posture (Figure 1.4f). Monomyarian bivalves, such as scallops and oysters, have largely circular shells, all trace of the anterior adductor muscle is lost, and the body has been reorganised around the enlarged and more or less centrally placed posterior muscle. Water enters around two-thirds or more of the rounded margins of the shell. Shell attachment has led to varying degrees of inequality in the size of the two shell valves. In scallops the shell valves are circular but both can be concave and similar, or the left (uppermost) valve may be flat. Like oysters they also lie in a horizontal position on the substrate. However, scallops far from being fixed are active, swimming bivalves. In early life they use byssus threads for attachment to algae, but before they attain a size of 15 mm the majority of species have detached themselves to take up a free-living existence on the seabed.

Cementation also evolved during the Paleozoic era. This adaptation arose independently in several marine pteriomorphians, heterodonts and anomalodesmatans, as well as in freshwater unionids, peaking in the Late Triassic and Jurassic periods of the Mesozoic era (150–220 mya) as a possible response to the appearance of many predatory groups (Vermeij 1977; Harper 1991). During the Triassic another important development occurred when an ancestral unionid (Paleoheterodonta) colonised freshwater environments. Giribet (2008) suggests that this move might have been triggered by evolution of a novel mode of development using microscopic glochidia larvae with fish as intermediate hosts.

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Burrowing into the substrate is the habit most extensively exploited by bivalves. Contact is maintained with the surface by way of siphons that extend from the posterior end of the animal. During the Cenozoic era (up to 60 mya) soft, nutrient-rich sediments on continental margins allowed diversification of shallow burrowing, globular, strongly ribbed forms and deep burrowers with smooth, blade-like shells (Giribet 2008). Cockles (e.g. *Cardium* spp.) are shallow burrowers, while many clam species, for example razor clams (*Siliqua*, *Ensis*), burrow as deep as 60 cm. The geoducks (*Panopea*) on the West Coast of the United States are among the deepest burrowers, digging down to a depth of over a metre, aided by a streamlined shell for fast burrowing and fusion of the mantle edges (apart from a small gape for the large muscular foot) to prevent entry of sediment into the mantle cavity. Geoduck siphons are so large that they can no longer be retracted into the shell. Many bivalves that burrow deeply (>30 cm) live in permanent burrows, moving deeper as they grow larger. This lifestyle is brought to an extreme by bivalves that bore into hard substrates such as shell, coral, wood and rock and are permanently locked in their burrows and are, therefore, inevitably dependent on outside sources of food. However, in wood-boring bivalves excavated 'sawdust' is the principal food source and phytoplankton is only used to supply the nitrogen and vitamins missing from an all-wood diet.

While the number of species of bivalve molluscs is only about 10% of that documented for gastropods, there is substantial interest in this group chiefly because so many of its members are eaten by humans in large amounts. In the following chapters attention will be focused only on bivalves of commercial importance: mussels, oysters, scallops and clams. Although the general term 'shellfish' will sometimes be used to refer to this group, the author is well aware that for many people the term has a wider meaning and incorporates many other non-bivalve molluscs not dealt with in this book, such as abalone, periwinkles, whelks and even crustaceans such as crabs, prawns and shrimp.

Notes

1 A system of classification based on the phylogenetic relationships and evolutionary history of groups of organisms, rather than on purely shared features.
2 Orthologs are genes whose encoded proteins fulfill similar roles in different species.
3 A clade is a monophyletic group.
4 The classification of organisms and the evolutionary relationships among them.

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2 Morphology of bivalves

Introduction

In this chapter the approach is to use much studied, representative species from each group of mussels, oysters, scallops and clams rather than to attempt an exhaustive description of the morphology of all bivalve species. So the following species have been chosen: for mussels the genus *Mytilus*, for oysters *Ostrea* and *Crassostrea*, for scallops *Pecten maximus* and for clams *Mercenaria mercenaria*.

The general morphology and functions of the shell, mantle, foot, gill, alimentary canal, gonad, heart, kidney and nervous tissue are described in the following sections. Additional information on their particular roles in feeding, reproduction, and circulation, excretion and osmoregulation, is presented in Chapters 4, 5 and 7, respectively.

Shell

Bivalves have two shell valves that are hinged dorsally and connected by an elastic ligament. Adductor muscles hold the valves together, and relaxation of the ligament and contraction of these muscles open and close the shell, respectively. A series of interlocking teeth and sockets along the hinge line prevent the valves from sliding against one another. The shell has several functions: it acts as a skeleton for the attachment of muscles, it protects against predators, and in burrowing species it helps to keep mud and sand out of the mantle cavity.

The main component of the shell is calcium carbonate, formed by the deposition of crystals of this salt in an organic matrix, which is composed of the polysaccharide β-chitin, a hydrophobic silk protein and a complex assemblage of hydrophilic proteins, many rich in aspartic acid (Addadi *et al.* 2006). The mineral component represents more than 95% of the shell weight, while 1–5% represents the organic matrix. Three layers make up the shell: (1) a thin outer periostracum of horny conchiolin, a fibrous insoluble protein, often much reduced due to mechanical abrasion, fouling organisms, parasites or disease; (2) a middle prismatic layer of aragonite or calcite, crystalline forms of calcium carbonate and

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(3) an inner nacreous layer composed of tablets of aragonite arranged in layers that are separated by sheets of organic matrix composed of elastic biopolymers of chitin and silk-like proteins; nacre is either of dull texture or iridescent mother-of-pearl, depending on the species.

Techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray and electron diffraction patterns have played an important role in clarifying shell microstructure (Weiner *et al.* 1983; Levi–Kalisman *et al.* 2001; Nudelman *et al.* 2008), while analytical methods such as secondary ion mass spectrometry (SIMS; Shirai *et al.* 2008), electron probe microanalysis (EPMA; Jacob *et al.* 2008) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Jacob *et al.* 2008) have been used to elucidate the chemical composition of the shell in several species. To date, more than 10 morphological types of shell structure are discriminated among bivalves (Kobayashi & Samata 2006).

The construction of the shell begins very early in larval development. An area of ectodermal cells in the dorsal region of the developing embryo secretes the first larval shell. The secretion of a second larval shell by the mantle, rather than the shell gland, follows soon after. After metamorphosis the secretion of the adult shell begins. This is more heavily calcified, has different pigmentation and more conspicuous sculpturing than the larval shell. Growth takes place at the edge of the shell in a small fluid-filled space sealed by the periostracum and the epithelial cells of the mantle. Into this space these cells release all the precursors for mineralization: mineral ions actively extruded from the cytoplasm, and organic components of the shell matrix that are secreted by exocytosis (Marin & Luquet 2005). The outer mantle fold secretes the periostracum and prismatic layers, while the inner nacreous layer is secreted by the general mantle surface (see later). Shell matrix proteins play a key role in the mineralization process (see Marin & Luquet 2004 for a review), and recently several genes that code for nacreous and prismatic layer proteins in bivalves have been identified (Inoue *et al.* 2010; Jackson *et al.* 2010).

The shell grows in circumference by the addition of material from the edge of the mantle, and grows in thickness by deposition from the general mantle surface. Calcium for shell growth is obtained from the diet or taken up from seawater. Carbonate is derived from the CO_2/bicarbonate pool in the animal's tissues. The energy required for shell growth is not an insignificant portion of a bivalve's total energy budget (Hawkins & Bayne 1992).

The colour, shape and markings on the shell vary considerably between the different groups of bivalves. Not surprisingly, therefore, shell characters are consistently used in species identification (Table 2.1).

Mussels

In mussels the two shell valves are similar in size, and are roughly triangular in shape (Figure 2.1a and b). The valves are hinged together at the anterior by means of a ligament. This area of the shell is called the umbo. The interior of the shell is white with a broad border of purple or dark blue. This is called the pallial line and is the part of the shell along which the mantle is attached when empty shells are examined. On the inside of each valve are two muscle scars, the attachment points for the large posterior adductor muscle and the much-reduced anterior adductor muscle. In *Perna* species the anterior adductor scar in absent. Anterior and posterior retractor muscles are also attached to the shell; these control the movement of the foot (see later). The foot in turn secretes a byssus, a bundle of tough threads of tanned protein. These threads emerge through the ventral part of the shell and serve as mooring lines for attachment of the mussel to the substrate, and to other mussels.

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Table 2.1 The major shell characters used in species identification.

Character	Variations
Shell shape	Oval, circular, triangular, elongate, quadrate
Shell valves	Similar (equivalve), or dissimilar (inequivalve)
Colour	Shell exterior: background/surface patterns; shell interior: white, pearly, etc.
Ribs	Number, width, prominence (distinct, flattened)
Sculpturing	Concentric lines, ridges, grooves
Ligament	Shape, position (internal, external), structure
Umbo	Position (anterior, terminal, subterminal)
Adductor scars	Number, size, position
Hinge line	Straight or curved, presence of 'ears' (size, shape)
Hinge teeth	Number, type
Pallial line	With or without a sinus
Pallial sinus	Size
Shell microstructure	Mineral composition and structure of shell layers

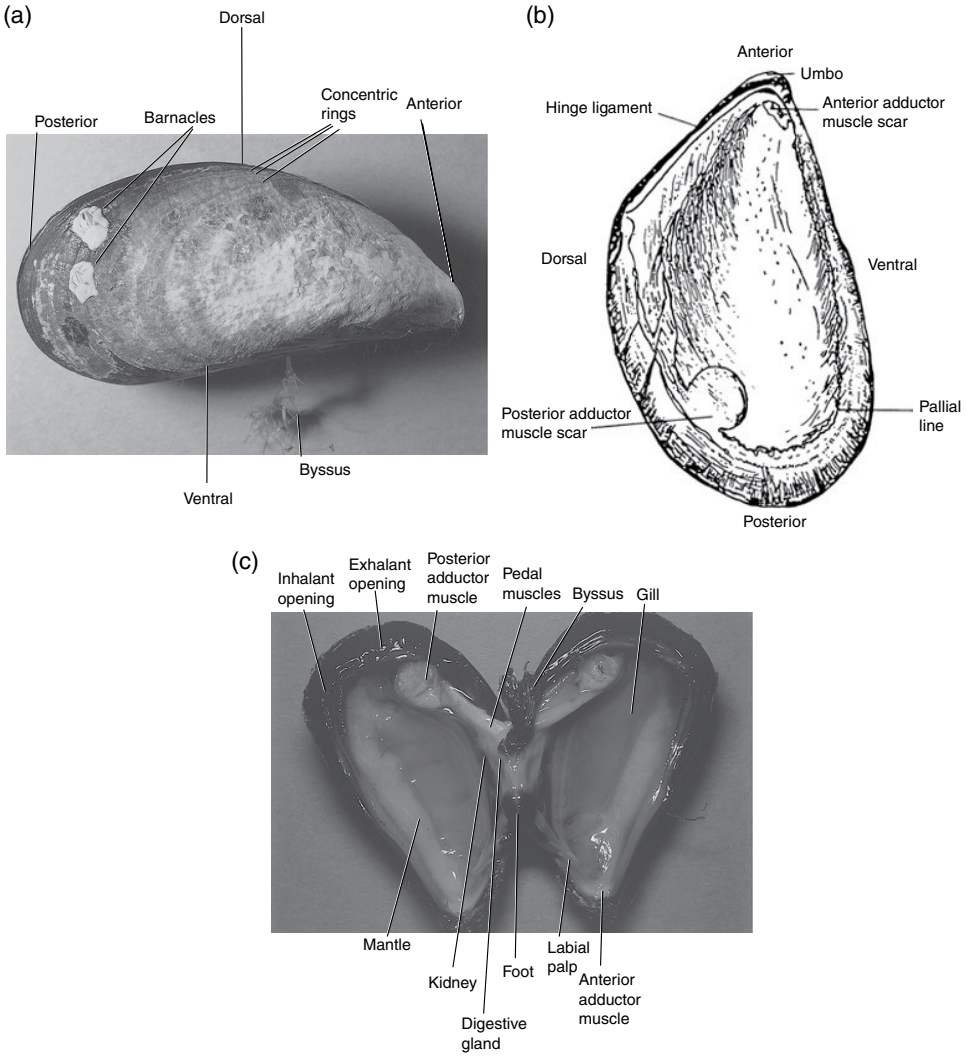


Figure 2.1 The (a) external, (b) internal shell and (c) internal features of the mussel *Mytilus edulis*. Photograph and permission to reproduce by Craig Burton.

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The colour of mussel shells is controlled by several genes (Innes & Haley 1977; Newkirk 1980) and also varies depending on the age and location of the animal (Mitton 1977). In the intertidal zone the blue mussel *Mytilus edulis* has a blue-black and heavy shell, while in the sublittoral region, where mussels are continuously submerged, the shell is thin and brown with dark brown to purple radial markings. In the rock mussel *Perna perna* the shell is red-maroon with irregular patches of light brown and green. Juvenile green mussels, *Perna viridis*, have bright green or blue-green shells, but older individuals tend to have more brown in the shell (Siddall 1980).

The presence of concentric rings on bivalve shells has been extensively used to estimates their age. In many species of scallops and clams these rings have been shown to be annual in origin and therefore can be used as a reliable estimate of age. However, in mussels there are few geographic locations where the rings provide an accurate estimate of age (Lutz 1976). One must therefore resort to shell sectioning for age determination. When longitudinal sections are examined microscopically distinct growth bands in the inner nacreous layer are clearly seen (see Figures 6.8 and 6.9 in Chapter 6). These are formed at a rate of 1 year^{-1} during spring (Lutz 1976). In addition, in the middle prismatic layer there are micro-growth bands, which have a tidal periodicity (Richardson 1989). These bands, together with the annual bands, can thus be used to track individual short-term and long-term variations in growth rates, respectively.

Figure 2.2 illustrates the convention used for the principal shell parameters. Height is the distance from the hinge line to the shell margin. Length is the widest part across the shell at 90° to the height. The width is measured at the thickest part of the two shell valves (Dore 1991).

Under optimal conditions, such as in the sublittoral zone, *M. edulis* and the Mediterranean mussel *Mytilus galloprovincialis* attain a shell length of 100–130 mm, whereas in marginal conditions, for example the high intertidal zone on an exposed shore, mussels may measure as little as 20–30 mm, even after 15–20 years (Seed 1976). This is not, however, a universal pattern. In South Africa the native mussel *P. perna* is largest on more exposed shores,

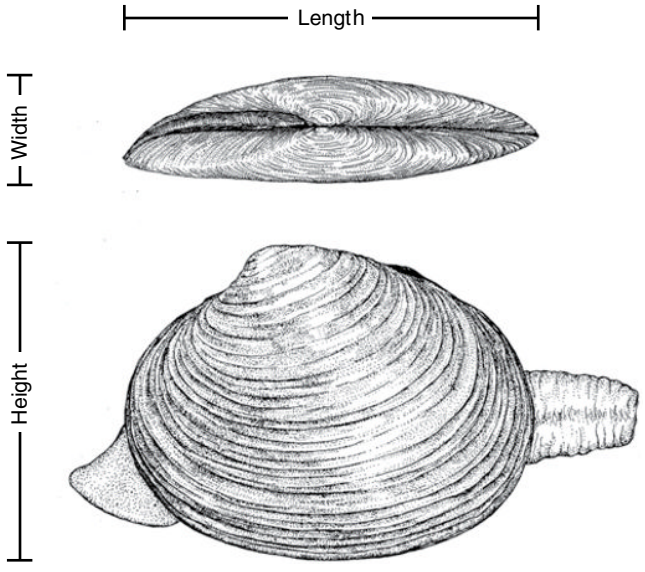


Figure 2.2 The convention used for the main shell measurements in bivalves.
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whereas the invasive mussel *M. galloprovincialis* is largest at intermediate levels of shore exposure (McQuaid *et al.* 2000; Hammond & Griffiths 2004). Shell shape is also very variable in mussel species. The shells of densely packed mussels have higher length-to-height ratios than those from less crowded conditions. This is most extreme in older mussels and ensures that they can more readily exploit posterior feeding currents, as they are effectively elevated above younger mussels in the same clump (Seed & Suchanek 1992). Shell morphology can also be correlated with wave exposure; on the west coast of Canada both juvenile and adult *Mytilus trossulus* at wave-exposed sites show a lower shell height-to-shell width ratio, and a thicker shell than mussels from sheltered locations (Akester & Martel 2000). Shell shape has also been used in taxonomic studies to differentiate both within and between various *Mytilus* taxa (Innes & Bates 1999; Krapivka *et al.* 2007; Gardner & Thompson 2009).

Mussels have been extensively used to assess environmental contamination. Radionucleotides and metals such as uranium, vanadium, silver, cadmium, cobalt, zinc and lead are highly concentrated in the shell (Livingstone & Pipe 1992; Widdows & Donkin 1992; Fisher *et al.* 1996; Boisson *et al.* 1998). Antifouling agents, for example tributyl tin (TBT), cause shell deformities, characterised by the production of cavities within the shell, in both mussels and oysters.

Oysters

In the European flat oyster *Ostrea edulis*, the shell valves are approximately circular and are hinged together on the dorsal side by a horny ligament (Figure 2.3a(i)). The right valve is flat while the left is cupped (Figure 2.3a(ii)). At rest on the sea bottom the flat valve is uppermost and the cupped valve is cemented to the substrate. The American Eastern oyster *Crassostrea virginica* also has dissimilar valves, but the general shell shape is more elongated and the left valve is more deeply cupped than in *Ostrea* (Figure 2.3b(i) and b (ii)). In both oyster species the shell colour is off-white, yellowish or cream but often with purple or brown radial markings in *C. virginica*. The inside of the shell valves is pearly-white and there is a single large adductor scar. The shell in both oyster groups is thick and solid and both valves have distinct concentric sculpturing, with the surface of the cupped valve more raised and frilled in *Crassostrea*. The concentric markings cannot be used to determine the age of oysters, and one must resort to sectioning of the hinge plate for an accurate estimate (Kraeuter *et al.* 2007). In general, *O. edulis* has a maximum shell height of 100 mm, while *C. virginica* grows as large as 350 mm; *Saccostrea* species tend to be smaller, with a maximum shell height of 60 mm. For a very comprehensive account of larval and adult shell structure in *C. virginica* see Carriker (1996).

Scallops

Scallops more than any other group of bivalves have attracted the interest of naturalists and collectors for centuries. 'In appearance no other molluscan shells have so pleasing a design and range of colours as pecten shells' (Cox 1957). In the king scallop *P. maximus*, the left valve is flat and is slightly overlapped by the right one, which is convex (Figure 2.4a). Adults recess in the substrate with the flat valve uppermost. The two valves, which are roughly circular, are held together along the hinge line by a rubbery internal ligament (see Kitagawa & Li 2004 for details). Typically, there are 15–16 ribs radiating from the hinge. These alternate with grooves and give the scallop its distinct comb-like appearance. There are two projecting 'ears' or auricles on either side of the umbo; these vary in size and shape and are used, along with other shell characters, to differentiate one species of scallop from

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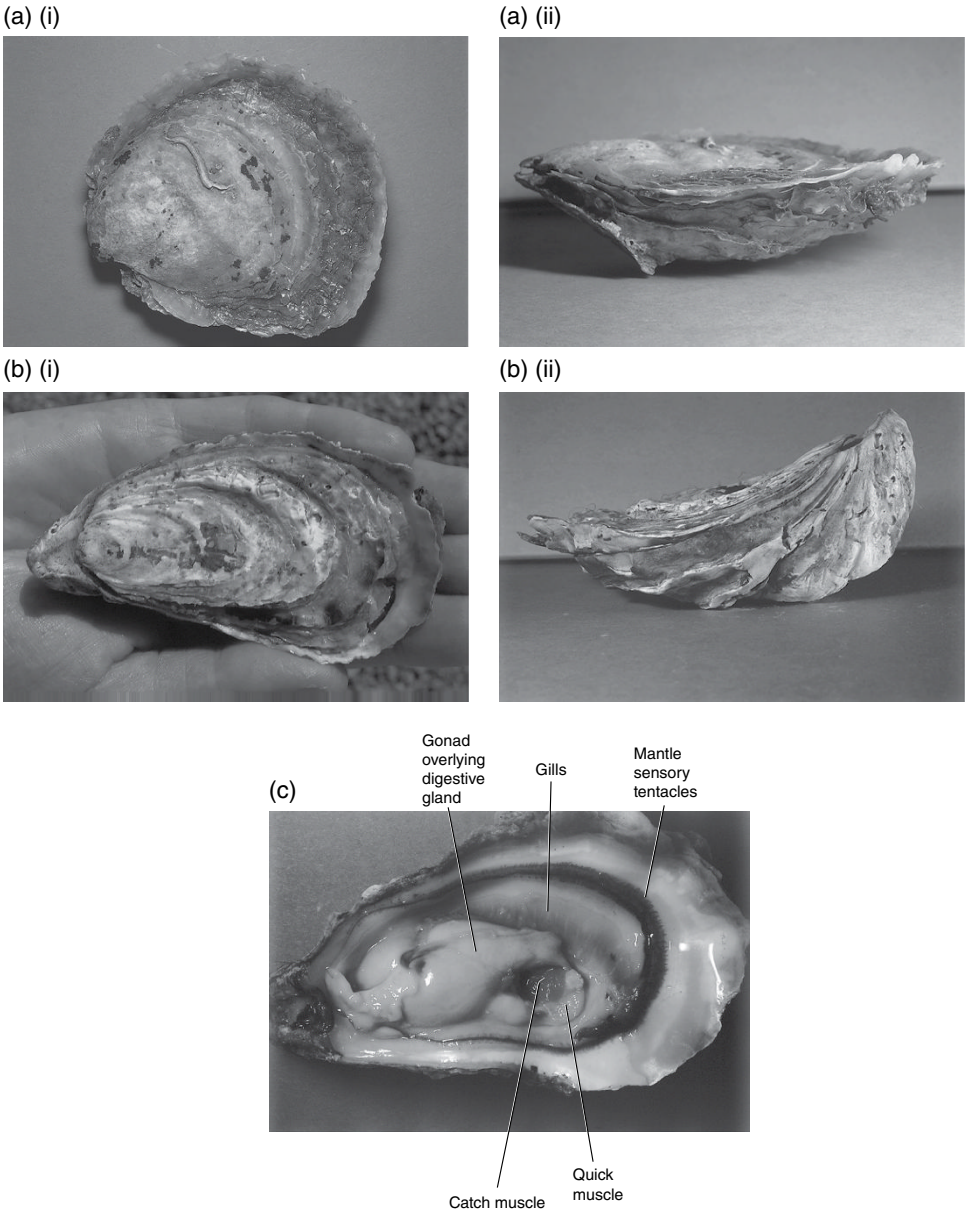


Figure 2.3 External shell of (a) *Ostrea edulis* (i) & (ii) and (b) *Crassostrea gigas* (i) & (ii). (c) Internal features of *C. gigas*.
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the next (see Table 2.1 for shell characters used in species identification). There is a large, centrally placed, adductor muscle, a standard seafood commodity that is widely traded and universally available. Distinct annual rings on the shell make ageing of scallops a relatively easy task, compared to mussels and oysters. Shell size in scallops varies quite a bit depending in the species: *P. maximus* can be up to 150 mm in length, while the sea scallop *Placopecten magellanicus* and the yesso scallop *Placopecten (=Mizuhopecten) yessoensis* can reach a size of 200–230 mm. Other species such as the queen scallop *Aequipecten (Chlamys)*

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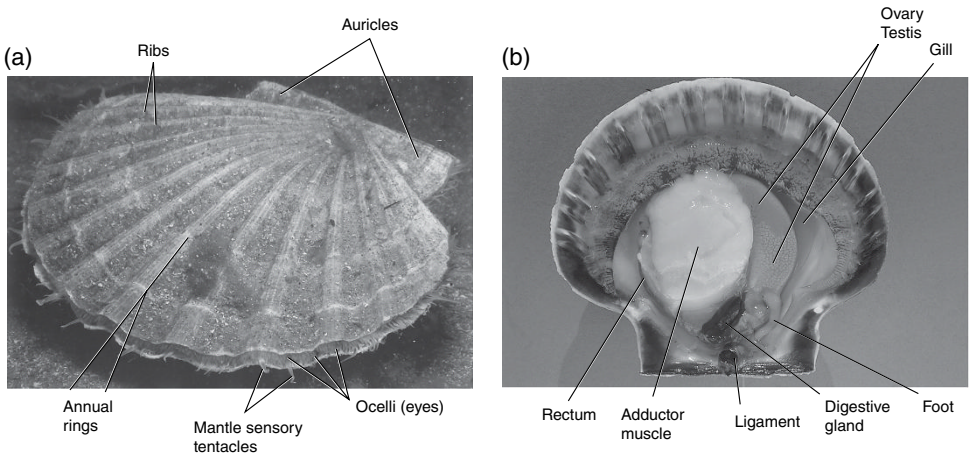


Figure 2.4 (a) External shell and (b) internal anatomy of the scallop, *Pecten maximus*. Photograph and permission to reproduce by Craig Burton.

opercularis, the Icelandic scallop *C. islandica* and the bay scallop *Argopecten irradians* seldom grow larger than 100 mm.

The beautiful colours that are a feature of scallop shells are laid down when the shell is being formed. In *P. maximus* the colours range from off-white to yellow to light brown, often overlaid with bands or spots of darker pigment. In *A. irradians*, background colour of the shell and overlying pigment distribution is coded by one gene locus (Adamkewicz & Castagna 1988), which has recently been mapped (Qin *et al.* 2006). In the Chilean scallop *Argopecten purpuratus*, there are at least three separate loci controlling shell colour (Winkler *et al.* 2001), which aside from their particular phenotypic effects have been associated with differences in growth and survival (Wolff & Garrido 1991).

The scallop shell, as well as the digestive gland, kidney and gills, accumulate trace elements, such as cobalt, manganese and zinc, from their environment, as well as natural and anthropogenic radionuclides (see Metian *et al.* 2011 for references).

Clams

Clams are a very diverse group of bivalves in that there is notable variation in the shape, size, thickness, colour and degree of sculpturing of the shell from one species to the next. The one feature that all clams have in common is that they burrow into the seabed. Consequently, both shell and body (see later) display modifications necessary for this type of existence. The quahog clam *M. mercenaria* has a thick, triangular shell (Figure 2.2). It is grey or brown with a sculpturing of numerous shallow concentric rings that run around the shell, parallel to the hinge. Annual rings are clearly visible on the shell exterior and thus ageing in this species, and indeed in many of the other commercially important clam species, is an easy task. The inside of the shell is glossy white, often with bluish-purple tints. It was this feature that made them valued as currency in earlier times (Dore 1991). There are three conspicuous teeth on each valve and each tooth fits into a corresponding socket on the opposing valve (Figure 2.5). This ensures an intimate fit when the valves are closed. The shell interior is marked by an anterior and posterior adductor muscle scar, a distinct pallial line and a short pallial sinus – the indentation indicating the position of the retracted siphons in the closed shell (see later). The depth of the pallial sinus is a very reliable indicator of the length of the siphons, and thus the burrowing depth of a particular clam species.

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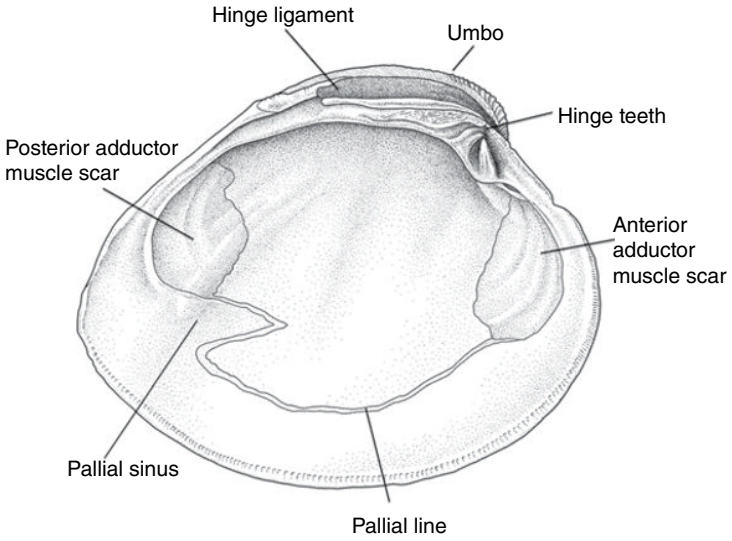


Figure 2.5 Internal features of the left shell valve of the hard shell clam *Mercenaria mercenaria*. Figure and permission from R. Bieler & P. Mikkelsen, Partnerships for Enhancing Expertise in Taxonomy (PEET-Bivalves project), Field Museum of Natural History, Chicago, Illinois, United States. Drawing by L. Kanellos, project illustrator.

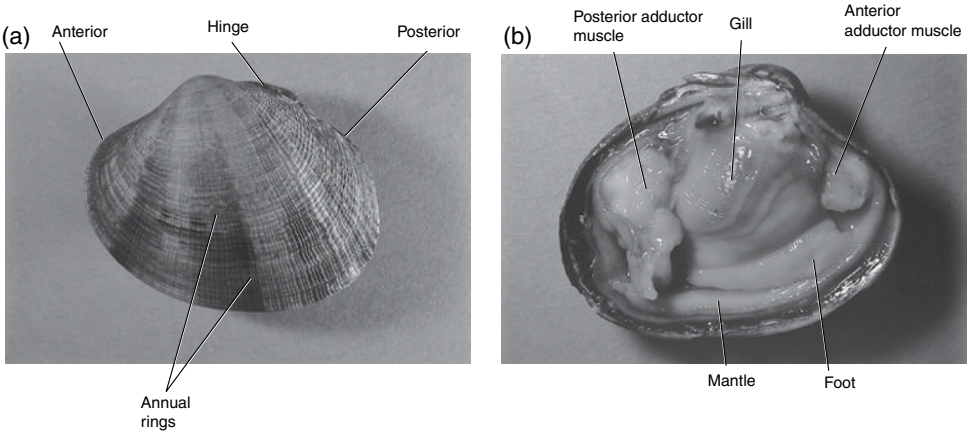


Figure 2.6 (a) Shell and (b) internal anatomy of the clam *Ruditapes philippinarum*. Photograph and permission to reproduce by Craig Burton.

M. mercenaria and the softshell clam *Mya arenaria* can reach a shell length of 150 mm, while the surf clam *Spisula solidissima* grows as large as 220 mm. The palourde *Ruditapes decussata* and the Manila clam *Ruditapes philippinarum* (Figure 2.6a) are much smaller, with a maximum shell length of about 75 mm. The European cockle *Cardium edule* is even smaller with a maximum shell length of 65 mm. Cockles and arkshells are close relatives of clams and for most practical purposes can be considered as clams (Dore 1991). In 2007 a specimen of the ocean quahog *Arctica islandica* was dredged from the seabed off Iceland by researchers from Bangor University, United Kingdom. By counting the rings on its 850 mm shell they estimated that the clam was 405 years old, the world's oldest living creature (*The Sunday Times* 2007).

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In bivalves where the two shell valves are the same shape, such as mussels and clams, the valves are drawn together by anterior and posterior adductor muscles. When these are relaxed the shell is opened by the elasticity of the ligament. Contraction of the adductor muscles closes the shell. When a bivalve dies these muscles can no longer contract and the ligament forces the shell open. A dead bivalve has always a gaping shell.

In bivalves with dissimilar valves, for example oysters and scallops, there is a single centrally placed adductor muscle. This performs the same function as the two adductors in mussels and clams. The muscle is divided into two visually distinct parts: the catch, made up of smooth, opaque muscle fibres is responsible for sustained closure of the valves with little energy expenditure, and the quick, composed of striated, translucent muscle fibres, is concerned with fast repetitive movements such as swimming. The catch and quick portions can be clearly seen in Figure 2.3c. In mussels and clams these two types of fibres are intermingled so that the adductor muscles appear homogeneous.

In a 'swimming' scallop repeated rapid closure of the widely open valves by contraction of the quick muscle, and backward ejection of water on either side of the hinge, causes the scallop to move forward in a series of jerks (Figure 2.7a; reviewed in Brand 2006). By suitably adjusting the edges of the mantle to direct a stream of water downwards the scallop can move both forwards and upwards in an erratic movement, looking like it is taking a series of bites out of the water. In some situations water is jetted through only one side of the shell causing the animal to rotate (Figure 2.7b). An approaching predator such as a starfish can provoke a different type of movement; forceful expulsion of water from the margins of the shell drives the animal backward in what looks like a very definite 'escape' movement (Figure 2.7c). Using a slight variation of jet propulsion the scallop can execute a somersault if it finds itself in an overturned position (Figure 2.7d). By adjusting the mantle margins it rights itself by suddenly expelling water downwards all around the free margins of the shell (Cox 1957). While the majority of investigations on swimming behaviour have been laboratory-based, observational and experimental studies in the wild have proved

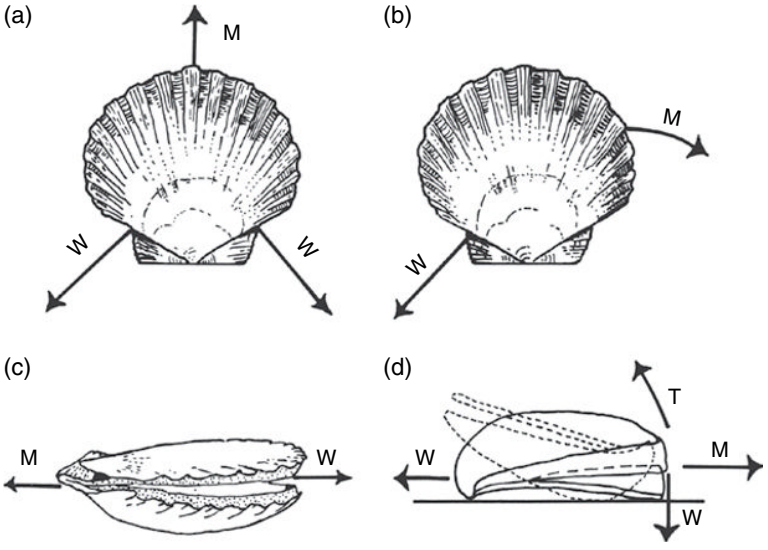


Figure 2.7 Swimming and escape movements in scallops. Arrows indicate direction of movement (M), direction of jet stream (W) and turning (T). (a) Swimming movement, (b) twisting movement, (c) escape movement and (d) somersaulting movement.
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particularly important in assessing scallop reaction to environmental conditions, fishing gear and contact with predators. In general, most species utilise rapid movements over very short distances (<5 m), a strategy well suited for escape reactions but not for efficient long-distance movement (Brand 2006).

Scallops in the process of burying themselves on the seabed (recessing) also utilise similar mechanisms. A series of powerful contractions of the adductor muscle eject water from the mantle cavity in the region of the posterior auricle (one of the shell projections at the hinge line). The water jets are directed downwards by muscular control of the velum (inner mantle fold); this lifts the shell at an angle to the seabed and subsequent water jets blow a hollow in the sediment. Once the hollow is of sufficient depth an extra powerful contraction lifts the scallop and it lands precisely in the recess. Sediment settles on the shell and after a few days this may be sufficiently thick to make detection difficult. Surprisingly, the main benefit is feeding-related as recessing lowers the inhalant water current to the level of the sediment surface and detritus can be more easily drawn into the mantle cavity (see Chapter 4).

When bivalves are submerged and are feeding, the shell valves are always open. In burrowing bivalves the siphons are extended above the substrate through a gap in the shell. Closure of the shell occurs when the animal is out of the water, or when predators or adverse environmental conditions, for example sudden salinity changes or high levels of toxicants, threaten it.

Figures 2.1c, 2.3c, 2.4b and 2.6b, which accompany the shell photos of mussels, oysters, scallops and clams, show the various organ systems described in the following sections of this chapter.

Mantle

In bivalves the mantle consists of two lobes of tissue that completely enclose the animal within the shell. Between the mantle and the internal organs is a capacious mantle cavity. With the exception of mussels (see later) the mantle is thin and transparent but the edges of the mantle and siphons are usually darkly pigmented, which probably gives protection from the harmful effects of solar radiation (Seed 1971). The mantle consists of connective tissue with haemolymph ('blood') vessels, nerves and muscles that are particularly well developed near the mantle margins. Cilia on the inner surface of the mantle play an important role in directing particles onto the gills and in deflecting heavier material along rejection tracts towards the inhalant opening, the entry point on the mantle for incoming water. Periodically, the rejected material is discharged by sudden and forceful closure of the shell valves; this is sufficient to blow the rejected material out of the mantle cavity through the inhalant opening.

In mussels the mantle contains most of the gonad (Figure 2.1c). Gametes proliferate within the mantle and are carried along ciliated channels to paired gonoducts that discharge into the mantle cavity (see later). After mussels have released their gametes the mantle is thin and transparent. The mantle is not only the site of gametogenesis but is also the main site for the storage of nutrient reserves, especially glycogen. In *M. edulis* reserves are laid down in summer and are utilised in autumn and winter in the formation of gametes. For a full discussion of energy metabolism in the mantle and other tissues see de Zwann and Mathieu (1992).

Pearl formation

Sometimes a foreign object like organic material or a parasitic larva lodges between the mantle and the shell. In self-defence the bivalve encapsulates the object with layers of nacreous shell and so a pearl is formed. Although all bivalves are capable of forming pearls

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it is only those with an inner mother-of-pearl layer that can produce pearls of commercial importance. The best quality natural pearls are produced by pearl oysters, *Pinctada* spp., that live throughout the tropical Indo-Pacific. Nowadays, most oyster pearls are produced artificially by inserting a small piece of freshwater mussel shell surrounded in mantle tissue from a donor oyster, into the gonad at the base of the foot. The implanted tissue develops and covers the nucleus with layers of nacre. Pearls may take 1–3 years to form in this way but the industry is a lucrative one; global production of marine cultured pearls in 2004 had an estimated value of about $475 million (Southgate 2007). Mussels (*Mytilus* sp.) produce pearls but this is in response to infection by the larva of a small parasitic flatworm. In fact, these pose a commercial problem. Unfortunately, the high incidence of pearls in bottom-grown mussels on the Atlantic coast of North America, Denmark, England and France is an impediment to marketing these mussels. The problem can be eliminated if mussels are grown on ropes and brought to market before the pearls reach a detectable size (Lutz 1980).

Mantle margins

The mantle margins are thrown into three folds: the outer one, next to the shell, is concerned with shell secretion (see earlier); the middle one has a sensory function (see later) and the inner one is muscular and controls water flow in the mantle cavity. A minute space that contains pallial fluid separates the mantle from the shell, except in the regions of muscle attachment. The calcareous and organic materials for shell secretion are deposited into this space.

The mantle is attached to the shell by muscle fibres in the inner fold; the line of attachment, the pallial line, runs in a semicircle a short distance from the edge of the shell (Figure 2.5). In most bivalves the mantle margins are fused between the inhalant and exhalant openings. In mussels the exhalant opening is small, smooth and conical, and the inhalant aperture is wider and fringed by sensory papillae (Figure 2.8). In oysters the inhalant opening is very large, mirroring the gills, and the exhalant opening is again small. There is no mantle fusion in scallops so water enters around the entire mantle edge and exits on either side of the hinge line.



Figure 2.8 Exhalant (white and smooth) and inhalant (fringed with tentacles) openings in the mantle of the mussel *Mytilus edulis*.
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Additional modification of the mantle edge occurs in deep-burrowing forms such as clams. To minimise fouling of the mantle cavity there is complete fusion of the mantle edges except for three openings: the inhalant and exhalant apertures at the posterior end, and an opening at the anterior end through which the foot protrudes. In addition, the margins of the exhalant and inhalant openings are elongated to form siphons that extend out of the substrate for feeding, but which can be retracted into the shell. Siphon extension is mediated by haemolymph pressure or by water pressure in the mantle cavity when the shell is closed. Muscles in the inner mantle fold control siphon retraction. Some siphonate forms, for example the softshell clam *M. arenaria*, are not capable of totally retracting the siphons. Consequently, their siphons have a protective covering of periostracum to protect against predation. Predators such as sea otters, fish, birds and crabs crop extended siphons of buried clams, potentially causing them to reduce their burial depth, thus making them more susceptible to excavating predators (Meyer & Byers 2005; Cledón & Nuñez 2010). Whitlow (2010), however, has recently shown that *M. arenaria* exhibits a phenotypic response to crab predator cues by growing longer siphons, thus enabling greater burrowing depths and, consequently, increased survival rates. A novel strategy to avoid predation is used by the butter clam *Saxidomus giganteus*. This species sequesters diet-derived paralytic algal toxins, highly potent neurotoxins, in its siphons as an innate, rather than an acquired, defence mechanism against siphon-nipping predators (Kvitek 1991; Kvitek & Beitler 1991).

As mentioned already, the outer mantle fold secretes the shell. The middle fold is primarily sensory, having assumed this role in the evolution of the bivalve form from the ancestral mollusc – a change that involved the loss of the head and associated sense organs. The middle fold is frequently drawn out into short tentacles that contain tactile and chemoreceptor cells. Both of these cell types play an important role in predator detection and avoidance. Ocelli, which are sensitive to sudden changes in light intensity, may also be present on the middle fold. These 'eyes' can be simple invaginations lined with pigment cells and filled with a mucoid substance or 'lens'. However, in scallops the eyes, numbering 50–100 depending on the species, are unquestionably better developed than in other bivalves. Each eye consists of a cornea, a lens and a retina and produces a low contrast image (Colicchia *et al.* 2009). While sensory receptors are found at the edge of the open mantle in sessile or surface-dwelling bivalves, in burrowing forms they are concentrated at the tips of the siphons. Further information on the nervous system in bivalves is given later.

The inner mantle fold, or velum, is the largest of the three mantle folds and is particularly conspicuous in scallops. Small sensory tentacles or papillae (Figure 2.9) usually fringe the fold and there is a large muscular component, especially on the inhalant opening. The velum plays an important role in controlling the flow of water into and out of the mantle cavity. It also plays a very important role in the so-called escape response and swimming movement of scallops described earlier.

Many algae are seen as symbionts of marine bivalves. For example, in the giant tropical clam *Tridacna gigas*, the edges of the mantle are packed with symbiotic zooxanthellae. When clams gape the mantle is extruded and the zooxanthellae are exposed to sunlight, thus allowing photosynthesis to take place. The clams utilise the photosynthetic products as nutrients, rather than the zooxanthellae themselves (Dame 2012 and references therein). This explains why these clams grow as large as 100 cm in length in impoverished coral reef waters. Not all algae found in bivalves are so benign. *Zoochlorella* spp. produce lesions of the mantle, tentacles and eyes in the scallops *A. irradians* and *P. magellanicus*, and *Coccomyxa parasitica* infect the mantle of *P. magellanicus*, causing gross deformities of the shell valves (see McGladdery *et al.* 2006 for references). A full account of diseases, parasites and pests of bivalves is presented in Chapter 11.

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Figure 2.9 The scallop *Pecten maximus* resting on the seabed with sensory tentacles and muscular velum of the mantle clearly visible.
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Gills

Filter feeding is believed to have evolved in some group of early protobranch molluscs, giving rise to the lamellibranchs, the dominant class of modern bivalves. Lamellibranchs feed by using the incoming current as a source of food, the gills having replaced the palps as the feeding organs. One important development in the evolution of filter feeding was movement of the site of water intake to the posterior of the animal (see Chapter 1).

Structure

The lamellibranch gills, or ctenidia, are two large, curtain-like structures that are suspended from the ctenidial axis that is fused along the dorsal margin of the mantle (Figures 1.2a in Chapter 1 and Figure 2.10a). Within the ctenidial axis are the branchial nerves and afferent and efferent branchial haemolymph vessels. Generally, the gills follow the curvature of the shell margin with the maximum possible surface exposed to the inhalant water flow (Figures 2.3c and 2.4b). Each gill is made up of numerous W-shaped (or double V) filaments, and an internal skeletal rod rich in collagen strengthens each filament. Each V is known as a demibranch and each arm is called a lamella, giving an inner descending and outer ascending lamella (Figure 2.10b(i)). In the space between the descending and ascending lamellae is the exhalant chamber, connected to the exhalant area of the mantle edge; the space ventral to the filaments is the inhalant chamber connected to the inhalant area of the mantle edge (Figure 2.10a).

In more primitive lamellibranchs neighbouring gill filaments are attached to one another simply through interlocking clumps of cilia (Figure 2.10b(i)). This rather delicate gill type is termed fillibranch, and is seen in mussels and scallops. In more advanced bivalves neighbouring filaments are joined to each other at regular intervals by tissue connections (interfilament junctions), leaving narrow openings or ostia between them (Figure 2.10c(i)). This gill type, termed eulamellibranch, is a solid structure and is found in the majority of

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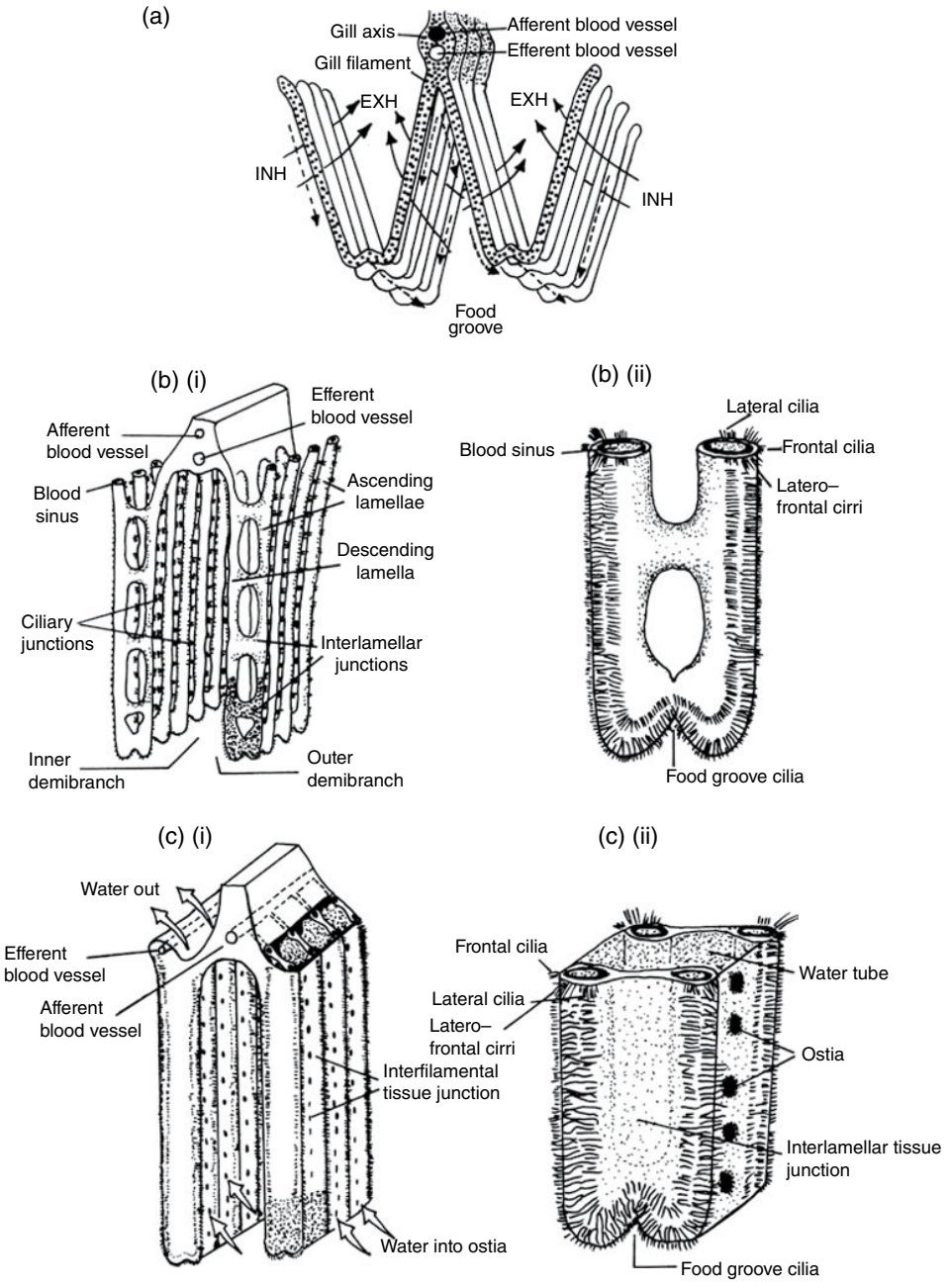


Figure 2.10 (a) Section of a lamellibranch gill showing the ctenidial axis and four W-shaped filaments. For greater clarity the descending and ascending lamellae of each demibranch have been separated. Solid arrows indicate direction of water flow through the filaments from inhalant (INH) to exhalant (EXH) chambers and broken arrows indicate path of particle transport to the food grooves. (b) (i) section of a fillibranch gill in the mussel *Mytilus edulis*. Adjacent filaments are joined together by ciliary junctions. (b) (ii) Transverse section through one fillibranch gill filament (shaded in (b) (i)) showing pattern of ciliation. (c) (i) Section of a eulamellibranch gill in the clam *Mercenaria mercenaria*. Adjacent filaments are joined by tissue connections, called interfilamental junctions. Water enters the gill through perforations (ostia). (c) (ii) Transverse section through a demibranch (shaded in (c) (i)) showing pattern of ciliation. (a) From Barnes *et al.* (1993). Reproduced with permission of Blackwell Scientific Publications; (b) and (c) from Pechenik (1991). Reproduced with permission of the McGraw-Hill Companies.

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bivalves. In oysters interfilament junctions are less extensive than in most eulamellibranch species, so the gills are often referred to as pseudoeulamellibranch.

In some bivalves, for example cockles, razor clams, oysters and scallops, the surface area of the gills has been greatly increased by folding or plica. This is believed to be an adaptation to living on coarse substrate; the plicate surface allows coarse filtration of large particles, while cilia on the filaments deal with finer filtration. Filaments are often structurally differentiated into principal and ordinary filaments, in which case the gill is termed heterorhabdic (Chapter 4, Figure 4.9). The principal filaments are located in the troughs of plicae, separated from each other by a variable number (10–20) of ordinary filaments. A gill with undifferentiated filaments is called homorhabdic. The filibranch homorhabdic gill in adult mussels is regarded as the ancestral condition from which the other gill types evolved (Beninger & Dufour 2000).

Functions

Cilia on the gill filaments have specific arrangements and functions (Figure 2.10b(ii) and c(ii)). Lateral cilia are set along the sides of the filaments in fillibranch gills and in the ostia of eulamellibranch gills. These cilia are responsible for drawing water into the mantle cavity and passing it through the gill filaments or through the ostia, and then upwards to the exhalant chamber and onwards to the exhalant opening. Lying between the lateral and frontal cilia (see later) are the large feather-like latero-frontal cilia that are unique to bivalves. When the incoming current hits the gill surface these cilia flick particles from the water and convey them to the frontal cilia. The frontal cilia, which are abundantly distributed on the free outer surface of the gill facing the incoming current, convey particles aggregated in mucus downwards towards the ciliated food grooves on the ventral side of each lamella. In some bivalves there are actually two sets of frontal cilia: large coarse ones that carry larger particles for eventual rejection as pseudofaeces, and rows of small fine cilia that convey small particles towards the labial palps and mouth. The movement of cilia is under nervous control. Each gill axis is supplied with a branchial nerve from a visceral ganglion (Figure 2.16), which subdivides to innervate individual groups of filaments. A more detailed description of the structure and function of the gills in filtration and feeding is presented in Chapter 4.

In bivalves the gills have a respiratory as well as a feeding role. Their large surface area and rich haemolymph supply make them well suited for gas exchange. Deoxygenated haemolymph is carried from the kidneys to the gills by way of the afferent gill vein. Each filament receives a small branch of this vein. The filaments are essentially hollow tubes within which haemolymph circulates. Gas exchange takes place across the thin walls of the filaments. The oxygenated haemolymph from each filament is collected into the efferent gill vein that goes to the kidney and into the heart (Figure 2.15). It is probable that gas exchange also occurs over the general mantle surface.

Because of their role in ingestion and respiration the gills are among the main target organs in the bioaccumulation of pesticides, soluble heavy metals and hydrocarbons. Complex mixtures of heavy metals and polycyclic aromatic hydrocarbons (PAHs) cause morphological changes in gill epithelium and an increase in haemocyte number, resulting in an increased turnover rate in gill epithelial cells. These are possible mechanisms to compensate for cell injury and prevent entry of pollutants from gill filaments into the entire organism (David *et al.* 2008). Metallothioneins (MTs) are metal-binding, heat-stable and low–molecular weight proteins that play an important role in detoxifying trace metals, and are widely distributed in tissues such as gill and digestive gland. MT levels in bivalves are now used as a biomarker of heavy metal contamination in coastal ecosystems (reviewed by Amiard *et al.* 2006).

The gills perform an additional function in hydrothermal vent mussels, which depend almost entirely on endosymbiont chemosynthetic bacteria in the gill filaments as an energy

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source. The bacteria use the energy obtained from the oxidation of reduced sulphur compounds and methane from hydrothermal fluid for the fixation of the CO_2 required for primary production (Yamanaka *et al.* 2003). Recently, Petersen *et al.* (2011) reported that bacteria also use hydrogen, a natural example of a living hydrogen-powered 'fuel cell'. Such a finding may have wider implications than purely academic ones, as hydrogen-powered fuel cells are regarded today as one of the most promising clean-energy alternatives (ScienceDaily 2011).

The foot

The foot first appears when bivalve larvae are about 200 µm in length, and it becomes functional in crawling and attachment at approximately 260 µm shell length (Bayne 1971). This is the pediveliger stage of development, which immediately precedes settlement and metamorphosis (see Chapter 5). The foot is proportionately very large and sock-shaped, and is made up of layers of circular and longitudinal muscles surrounding a capacious haemolymph space. The ventral surface or 'sole' of the foot is covered in cilia and in the mussel *M. edulis* there are as many as nine different kinds of gland, each of which plays it own specific role in crawling and attachment (Lane & Nott 1975). A byssal duct opens at the 'heel' of the foot and a byssal groove extends forward along the 'sole' from this opening (Hodgson & Burke 1988).

At the onset of settlement, when the pediveliger larva is between 250 and 300 µm shell length, it slowly descends from the plankton to the seabed. Then follows a period of swimming and crawling behaviour before attachment and metamorphosis occurs.

Attachment in mussels

While swimming, the foot is fully extended, and periodically the velum (larval swimming organ) is withdrawn, and the larva sinks to the bottom and begins to crawl. If the substrate is unsuitable, that is one that does not stimulate the secretion of byssus, the foot is withdrawn and the larva once again swims off (Lutz & Kennish 1992). This cycle can be repeated many times over a period of a few days. In *Mytilus*, when a suitable substrate is found the larva continues to crawl for some time, gradually ceases movement, protrudes the foot and quickly secretes a single byssal thread. In the newly attached mussel larva this thread can be repeatedly broken and reformed before final settlement takes place. As the mussel grows in length an increasing number of attachment threads are secreted, thus tethering the animal to the substrate. To resist dislodgement mussels cluster their threads in the direction of applied forces (e.g. facing ebb and flow of the tide).

There are four distinct regions in the mussel byssus: root, stem, thread and plaque (Figure 2.11). The root is embedded in the muscular tissue at the base of the foot; the stem is divided into sections, each with a thread attached; each thread ends in a plaque, where attachment to the substrate takes place. Byssal threads are approximately 0.1 cm in diameter and 2–4 cm in length. Mussels secrete one thread at a time and the process takes 3–10 min per thread (Waite 1992). Each thread has a flexible, collagenous inner core covered by a tough, durable cuticle of polyphenolic protein (details in Sagert & Waite 2009). The mechanical properties of threads vary depending on the species. For example, in *Mytilus californianus* distal threads are 2–3 times stiffer and 30% more extensible than those in either *M. trossulus* or *M. galloprovincialis*, which may contribute to the strong attachment strength of *M. californianus* and its ability to dominate wave-exposed shores (Bell & Gosline 1996; Bell & Gosline 1997); see Carrington 2002 and Babarro *et al.* 2008 for details on ecomechanics of mussel attachment).

Marine mussels use a natural adhesive in the plaques to attach themselves to a wide variety of substrates in an aqueous environment, and to date there are no synthetic glues that

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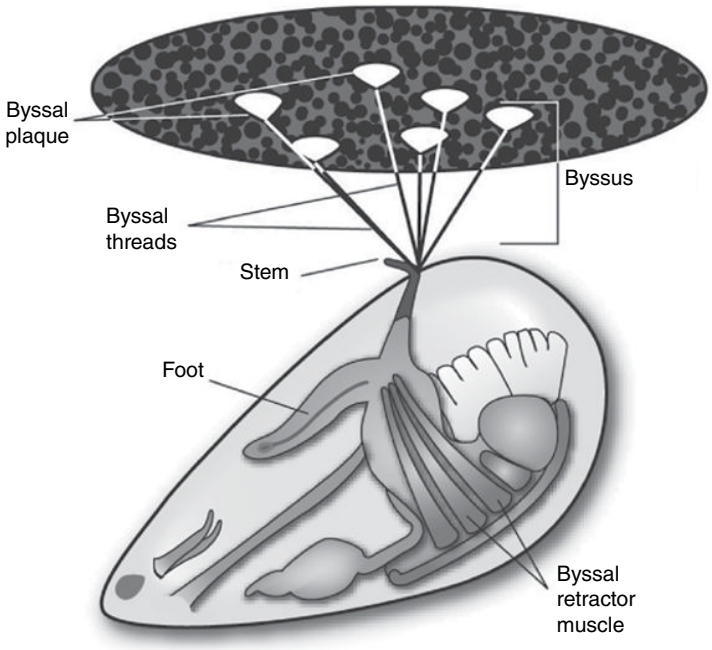


Figure 2.11 Anatomy of the byssus in the mussel *Mytilus edulis*.
From Silverman and Roberto (2007). Reprinted from *Marine Biotechnology* (Springer Open Access).

can perform this function. It is not surprising, therefore, that over the past decade mussel adhesive proteins are attractive targets for biomimetic technology, which entails using designs from nature to solve problems in engineering, materials science, medicine and other fields. So far, more than a dozen adhesive proteins have been identified and characterized, and recombinant DNA technology has been used to obtain these in large amounts for conventional adhesion tests and practical applications (Silverman & Roberto 2007; Cha *et al.* 2008; Roberto & Silverman 2011). Alternatively, the exceptional adhesive properties exhibited by the native proteins can be captured in synthetic polymer systems (see Lee *et al.* 2011 for a review). These have potential use as coatings for a wide range of organic and inorganic materials (Lee *et al.* 2007), for medical adhesion and sealing, for example fetal membrane rupture (Haller *et al.* 2011) and as antifouling coatings (Statz *et al.* 2006).

Attachment threads are not to be confused with the long drifting threads secreted by young post-larval mussels and many other bivalves that prolong the planktonic phase (see Chapter 5). Although similar in diameter and structure to attachment threads, they differ in that they are long, single filament structures that are secreted by special glands that atrophy and disappear soon after settlement (Lane *et al.* 1985).

Attachment in other species

Oyster larvae use cement for attachment, squeezing this out from the byssal gland and then applying the left shell valve to the cement. The adhesive is an organic–inorganic hybrid of cross-linked phosphorylated protein with $CaCO_3$ (Burkett *et al.* 2010). Once settled oysters are not capable of detaching and reattaching (see Chapter 5). The attached larva undergoes metamorphosis, a series of morphological changes that herald the transition from a pelagic to a sessile existence. In mussels, scallops and clams the larval foot is retained in adult life. However, in oysters such as *Ostrea* and *Crassostrea* spp., which attach by cement, there is

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complete loss of the foot at metamorphosis. In scallops, such as *P. maximus* and *A. opercularis*, the byssus serves only for temporary attachment, and the animals break away on reaching a certain size. Consequently, the foot is a very small degenerate structure in adults of these species. Some scallops, however, for example *Mimachlamys* (formerly *Chlamys*) *varia*, spend their whole life attached by byssus, as do all mussels. The foot is therefore a prominent feature in such species. In young mussels the foot also has a role in locomotion and removal of debris from the surface of the shell.

Digging

Clams initially use a byssus for attachment to particles of sediment but they soon abandon this and use their large foot to burrow into the substrate. The burrowing movement is brought about by a combination of haemolymph pressure and muscle action in the foot. When a bivalve is placed on a suitable substrate it proceeds to burrow into it in a series of steps or 'digging cycles', which continue until the animal is beneath the surface. The activity from initiation to completion of burrowing is called the digging period. Digging cycles are repeated many times during a digging period.

A digging cycle consists of a series of coordinated activities, similar in all burrowing bivalves investigated to date; Figure 2.12a illustrates the various shell and pedal muscles involved in a digging cycle:

- The shell adductor muscles relax and the two valves open a little and press against the substratum because of the elastic nature of the ligament. The projecting foot probes and pushes into the surrounding sand. Rhythmically repeated contraction and relaxation of the pedal protractor and transverse muscles initiate protraction of the foot (Figure 2.12b(i)).
- As the foot protrudes the shell valves begin to close by contraction of the adductor muscles (Figure 2.12b(ii)). Some water is expelled from the mantle cavity, which helps to loosen the sand and facilitates the movement of the foot. The remaining water in the mantle cavity and the haemolymph act as a hydrostatic skeleton. Closure of the shell valves serves to increase the pressure of these two fluids. Haemolymph from the body is forced down into the foot, causing the foot to swell and anchor into the substrate.
- Once the foot is anchored pedal retractor muscles contract, pulling the shell downwards into the loosened sand (Figure 2.12b(iii)). These muscles insert on the shell in the region of the adductor muscles. Contraction of the anterior retractor occurs before that of the posterior retractor muscle. This has the effect of rocking the shell, which in turn facilitates its movement through the sand. Ridges or projections on the valves often aid anchorage, especially during the rocking movements.
- The shell adductors relax, the valves open and pedal dilation and anchorage is lost.
- There is now a static period during which the foot repeatedly probes the surrounding substrate.

As the animal penetrates further into the substrate the digging period shortens. This is probably not due to fatigue as animals will repeatedly burrow when they are removed from the substrate after completing a digging period (Trueman 1968). To ascend towards the surface they back out, pushing against the anchored end of the foot, although some actually turn around and burrow upwards.

Burrowers such as the razor shells *Ensis* or *Solen* have long, thin shells and a large foot that takes up half of the mantle cavity. Such clams can burrow as fast as one can dig for them. On the other hand, *M. mercenaria* and *M. arenaria* with their smaller foot and ovoid shells are not well suited for fast burrowing. In general, smaller individuals burrow faster than larger individuals of the same species (Tallqvist 2001). Sediment grain size is also an

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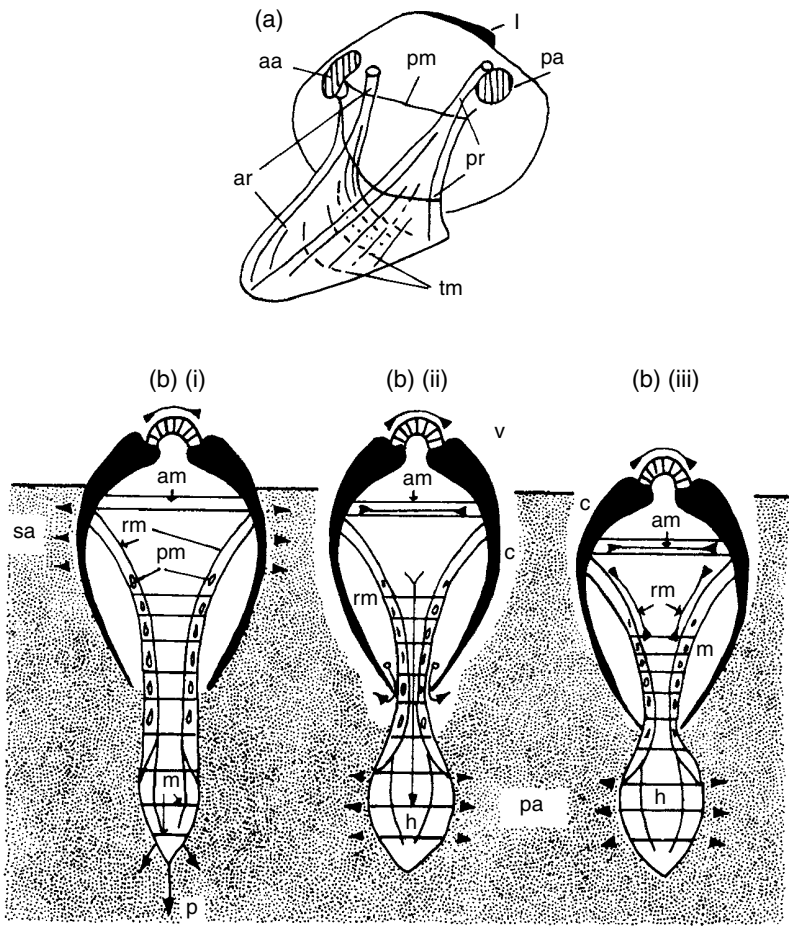


Figure 2.12 (a) The principal muscles used in burrowing. aa, anterior adductor; ar, anterior retractor; l, ligament; pa, posterior adductor; pm, protractors, circular muscles around the upper part of the foot; pr, posterior retractor; tm, transverse pedal muscles. (b) Successive stages in the burrowing of a generalized bivalve showing shell (sa) and pedal (pa) anchorages (arrowheads). (i) Valves press against the substrate by means of the opening thrust of the ligament and the foot extends by probing (p). (ii) Contraction of the adductor muscles (am) ejects water from the mantle cavity (m), thus loosening the substrate (c) around the valves (v); high pressure simultaneously produced in the haemocoele (h) gives rise to pedal dilation. (iii) Contraction of retractor muscles (rm) pulls the shell down into the loosened substrate. pm, pedal protractor muscles; tm, transverse pedal muscles; ⟷ tension in ligament, adductor or retractor muscles. Adapted from Trueman (1968). Reproduced with permission of the Zoological Society of London.

important factor in burrowing performance. For example, in the clam species *Donax serra* and *Donax sordidus*, Nel *et al.* (2001) found that fastest burrowing times were measured in fine and medium sediments (125–500 μm grain size) but increased towards the very fine (90–125 μm) and coarse extremes (500–2000 μm). Other factors such as low temperature (Donn & Els 1990), hypoxia and surface algal mats (Tallqvist 2001) all retard burial time.

As seen previously, burrowing is a complex behaviour that consists of a sequence of instinctive reflexes, and is therefore an extremely sensitive indicator of sediment toxicity (Pynnönen 1996). Since the late 1980s burrowing speed in clams has been routinely used as a bioassay for contaminated sediments in estuarine and coastal environments (Phelps 1989; Roper *et al.* 1995; Byrne & O'Halloran 1999; Shin *et al.* 2002; Bonnard *et al.* 2009; Boldina-Cosqueric *et al.* 2010).

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Labial palps and alimentary canal

Each gill terminates within a pair of triangular palps that are situated on either side of the mouth. The inner surface of each palp faces the gill, and is folded into numerous ridges and grooves that carry a complicated series of ciliary tracts. The outer surfaces of the palps are smooth and between the inner and outer surfaces there is muscular-connective tissue (Figure 4.11 in Chapter 4).

The main function of the labial palps is to continually remove material from the food tracts on the gills in order to prevent gill saturation. In dense suspensions sorting and rejection tracts on the palps channel most of the filtered material away from the mouth and deposit it as pseudofaeces, so that the animal can continue to filter and ingest at an optimum rate. The pseudofaeces are carried along rejectory tracts on the mantle to the inhalant opening. Periodically, the pseudofaeces are forcefully ejected through this opening. When the ingestive capacity is not exceeded particles from the gill move along acceptance tracts on the labial palps towards the mouth (see Chapter 4).

Stomach and style

The mouth is ciliated and leads into a narrow ciliated oesophagus. Ciliary movement helps to propel material towards the stomach. Indeed, this method of moving material is found throughout the length of the alimentary canal, primarily because it lacks a muscular wall. The stomach is large and oval-shaped and lies completely embedded in the digestive gland, which opens into it via several ducts (Figure 2.13; see Purchon 1957 for a detailed description of the bivalve stomach). A semi-transparent gelatinous rod, the crystalline style, originates in a style sac at the posterior end of the stomach and projects forward and dorsally across the cavity of the stomach to rest against the gastric shield, a thickened area of the stomach wall. The projecting anterior end of the style is rotated against the gastric shield by the style sac cilia, and in the process the style end is abraded and dissolved,

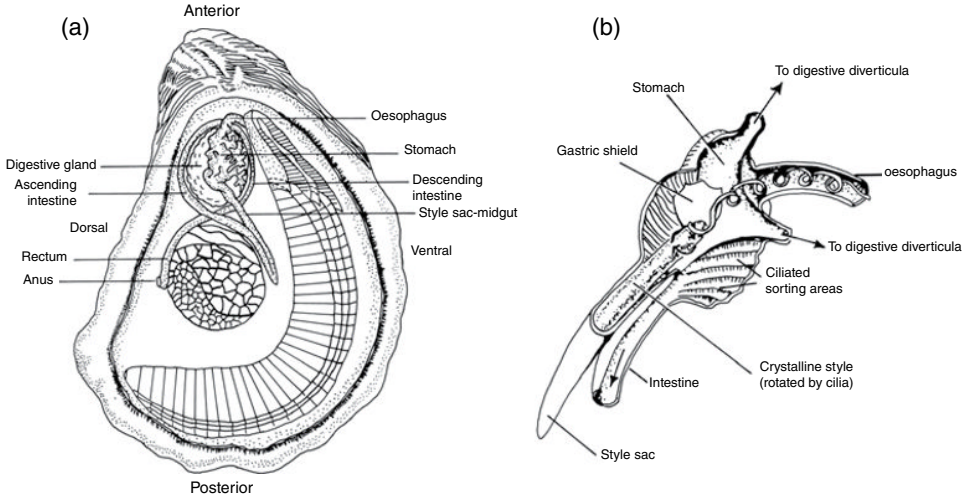


Figure 2.13 (a) The digestive system of the oyster *Crassostrea virginica*. (b) Bivalve stomach showing rotation of crystalline style and winding of food string. Rejectory groove on floor of stomach not shown. (a) Adapted from Langdon and Newell (1996), after Galtsoff (1964). Reprinted with permission from Maryland Sea Grant; (b) From Pechenik (1991). Reproduced with permission of the McGraw-Hill Companies.

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releasing carbohydrate-splitting enzymes in the process. This loss is made good by continual additions by the style sac to the base of the style. This remarkable structure is possibly the only example of a rotating part in an animal. The length of the style is correlated with shell length; the length is generally 50–60% of shell length (Alyakrinskaya 2001). Style length changes with the season, with maximum length in spring when food intake is high; the length also exhibits a tidal cycle with maximum length when the animal is submerged and feeding, although the change was only approximately 5% in *M. balthica* (Hummel *et al.* 1988), but as large as 25% in *M. edulis* (Alyakrinskaya 2001).

The style has additional functions in the digestive process (Figure 2.13b). The low pH of the stomach facilitates the dislodgement of particles from the mucus string. These particles are then mixed with the other contents of the stomach, including the liberated enzymes from the style. The rotation of the style helps the mixing process to take place. While all the mixing and extracellular digestion is taking place, the stomach contents come under the influence of ciliary tracts that cover all areas of the stomach except those occupied by the gastric shield. These ciliated tracts have fine ridges and grooves and act as sorting areas in much the same way as the labial palps. Finer particles and digested matter are kept in suspension by cilia at the crests of the ridges, and this material is continually swept towards the digestive gland duct openings. Larger particles segregate out and are channelled into the intestine along a deep rejectory groove on the floor of the stomach.

Digestion

The digestive gland, which is brown or black and consists of blind-ending tubules that connect to the stomach by several ciliated ducts, is the major site of intracellular digestion. Within these ducts there is a continuous two-way flow: materials enter the gland for intracellular digestion and absorption and wastes leave *en route* to the stomach and intestine (Figure 2.14). The tubules are composed of two cell types: digestive cells and basophil (secretory) cells (Weinstein 1995; and Figure 4.20 in Chapter 4). Digestive cells take up material by pinocytosis (uptake of extracellular fluid into a cell) and digest it within vacuoles. The end products of digestion are released directly into the system (Mathers 1972),

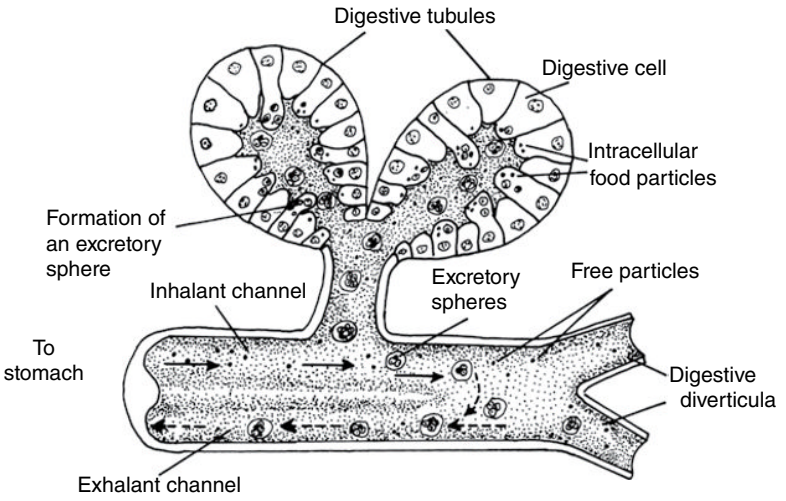


Figure 2.14 A section of the digestive gland showing absorption and intracellular digestion of material coming from the stomach (solid arrows) and outward movement of wastes (broken arrows). Adapted from Owen (1955). Reproduced with permission from *Journal of Cell Science*.

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and the waste is retained in membrane-bound residual bodies that are later released into the lumen of the tubules. The digestive cells eventually rupture and are replaced by new cells. The released waste enclosed in excretory spheres is swept towards the stomach and ultimately into the intestine. This waste may also contain digestive enzymes, which could be utilised by the stomach for extracellular digestion (Mathers 1972). The secretory cells carry out extensive protein synthesis and probably secrete digestive enzymes (Beninger & Le Pennec 2006). The cellular structure and function of the digestive gland has been reviewed for oysters by Langdon and Newell (1996) and for scallops by Beninger and Le Pennec (2006).

The stomach, digestive gland and, sometimes, the intestine produce a wide variety of digestive enzymes (details in Chapter 4). Carbohydrate-splitting enzymes, for example amylases, have been found in high concentration in the style, while esterases, cellulases and chininases have been reported from the stomach. Fat-digesting enzymes, for example esterases and acid and alkaline phosphatases, have been reported from the stomach, digestive gland and intestine of the oysters *O. edulis* and *Crassostrea angulata* (Mathers 1973; Langdon & Newell 1996 and references therein). Proteolytic activity is characteristically low in bivalves but endopeptidases such as chymotrypsin and cathepsin have been reported from the digestive gland, and trypsin activity in the stomach and intestine of some bivalves (Reid 1968).

The digestive gland also plays an important role in the storage of metabolic reserves, which are used as an energy source during the process of gametogenesis (see Chapter 5) and during periods of physiological stress (Bayne *et al.* 1976).

Rejected particles from the stomach as well as waste material from the digestive gland pass into the long coiled intestine (Figure 2.13a). The intestinal cells secrete a range of enzymes: esterases, alkaline and acid phophatases, chitinase and leucineaminopeptidase (Le Pennec *et al.* 1991). Waste is formed into faecal pellets that are voided through the anus and are swept away through the exhalant opening.

Pollutants

Digestive gland cells are the major environmental interface for the uptake of contaminants, especially those associated with natural particulates that are filtered from seawater (Moore & Allen 2002). A range of biomarkers, measured at the molecular or cellular level, have been identified that serve as 'early warning' tools in environmental quality assessment surveys (reviewed by Cajaraville *et al.* 2000). The most used biomarkers in digestive gland tissue are metallothionein induction, lysosomal alterations and peroxisome proliferation. Metallothioneins bind to heavy metals and are also likely to be involved in detoxification processes. Their synthesis in response to specific toxic metals has been well documented for marine bivalves (reviewed by Amiard *et al.* 2006). Lysosomes are ubiquitous cytoplasmic organelles that contain hydrolytic enzymes that degrade macromolecules to low–molecular weight products. They accumulate many toxic metals and organic chemical contaminants that provoke loss of membrane integrity, increase in lysosomal size and accumulation of lipid and the pigment lipofuscin. These responses have been used routinely to test for the effects of toxic contaminants in both experimental investigations and environmental impact assessments (Moore *et al.* 2004). Peroxisomes are cytoplasmic organelles involved in the metabolism of lipids and reactive oxygen free radicals, and peroxisome proliferation is used to assess levels of organic contaminants, for example PAHs and polychlorinated biphenyls (PCBs) in coastal ecosystems (Cajaraville & Ortiz-Zarragoitia 2006).

The formation of granulocytomas (inflammatory lesions) in response to pollutants has also been reported in digestive gland tissue (Garmendia *et al.* 2011).

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Gonads

The reproductive system in bivalves is exceedingly simple. The gonads are paired but are usually so close together that the pair is difficult to detect. Each gonad is little more than a system of branching tubules, and gametes are budded off the epithelial lining of these tubules. The tubules unite to form ducts that lead into larger ducts and eventually terminate in a short gonoduct. In primitive bivalves, for example the nut shell *Nucula*, the gonoducts open into the kidneys, and eggs and sperm exit through the kidney opening (nephridiopore) into the mantle cavity. In most bivalves the gonoducts open through independent pores into the mantle cavity, close to the nephridiopore. In the majority of bivalves, fertilization is external and the gametes are shed through the exhalant opening. However, a number of bivalves brood their young (*Adacnarca*, *Gaimardia*, *Lasaea* and *Mysella* spp.), a successful strategy in regions where there are constraints on the availability of energy to adults and/or larvae such as the deep sea and polar oceans (see Higgs *et al.* 2009).

Mussels are dioecious, that is the sexes are separate, and the gonad develops within the mantle tissue (Figure 2.1c). In ripe mussels (*M. edulis*) the mantle containing the gametes is typically orange in females and creamy-white in males. In oysters the gonad covers the outer surface of the digestive gland (Figure 2.3c). When *O. edulis* is in a ripe condition the gonad forms a layer 2–3 mm thick and its creamy colour obscures the brown colour of the digestive gland. In ripe *Crassostrea gigas* the gonad is often 6–8 mm thick and may comprise a third of the total body weight, exclusive of the shell (Walne 1974). In clams the gonad is situated at the base of the foot. In oysters and clams the sexes are separate but they do have the ability to change sex (see Chapter 5). Most scallop species are hermaphrodites. In *P. maximus* the mature gonad is divided into a dorsal testis, which is white in colour, and a ventral ovary, which is orange-red, and both of these curve around the large central adductor muscle (Figure 2.4b). The sperms are shed before the eggs, thus minimising the chances of self-fertilisation. In dioecious scallop species such as *M. varia* and *P. magellanicus*, the female gonad is pink while the male gonad is white.

Heart and haemolymph vessels

The heart lies in the mid-dorsal region of the body, close to the hinge line of the shell. It lies in a space called the pericardium, which surrounds the heart dorsally and a portion of the intestine ventrally. The heart consists of a single, muscular ventricle and two thin-walled auricles. Haemolymph flows from the auricles into the ventricle, which contracts to drive the haemolymph into a single vessel, the anterior aorta. A posterior aorta is also present in pseudo- and eulamellibranch bivalves. The aorta divides into many arteries, the most important of these being the pallial arteries from the posterior aorta that supply the mantle, and the visceral arteries (gastrointestinal, hepatic and terminal) from the anterior aorta that supply the stomach, intestine, shell muscles and foot with haemolymph (Figure 2.15). The arteries break up into a network of vessels in all tissues and these then join to form veins, which empty into three extensive spaces: the pallial, pedal and median ventral sinuses. The circulatory system is therefore an open system with haemolymph in the sinuses bathing the tissues directly. From the sinuses haemolymph is carried to the kidneys for purification.

In *Mytilus* some of the haemolymph from the kidney network enters the gills, discharging into the afferent gill vein, which gives off a branch to each gill filament, descending on one side and ascending on the other. The ascending vessels join to form an efferent gill vein that passes back to the kidneys. The haemolymph from the kidneys returns to the auricles of the

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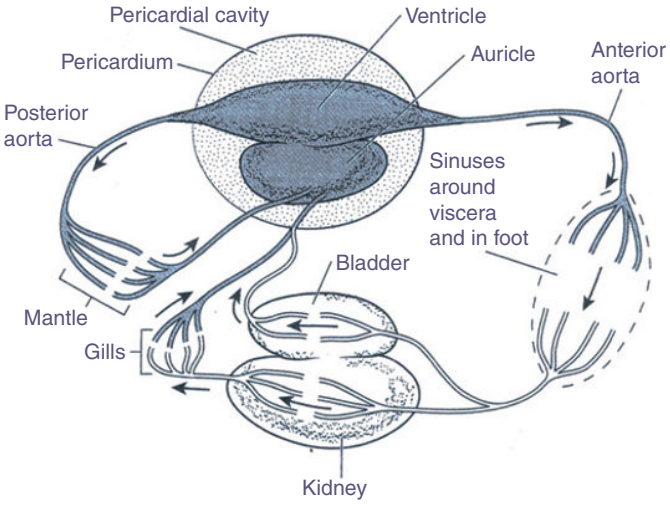


Figure 2.15 Circulatory system of a typical bivalve. The shaded areas indicate the route of oxygenated haemolymph.
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heart. In other bivalves, for example *Pecten* spp., haemolymph from the gills does not return to the kidney but flows directly from the gills to the heart (Figure 2.15). In all bivalves there are well-developed circulatory pathways through the mantle, which therefore serves as an additional site of oxygenation.

Haemolymph plays a number of important roles in bivalve physiology. These include gas exchange, osmoregulation (Chapter 7), nutrient distribution, waste elimination and internal defence (Chapter 11). Because haemolymph constitutes 40–60% of the fresh tissue weight it also serves as a fluid skeleton, giving temporary rigidity to such organs as the labial palps, foot (see earlier), siphons and mantle edges. The haemolymph contains cells called haemocytes that float in a colourless plasma. Most bivalves lack circulating respiratory pigments, probably because their sedentary lifestyle and large exposed surfaces for oxygen uptake preclude the need for such pigments. However, haemocyanin, the typical molluscan respiratory pigment, is found in some protobranch bivalves, while haemoglobin has been reported in several bivalve families (references in Giribet 2008). Haemocytes are not confined to the haemolymph system but move freely out of the sinuses into surrounding connective tissue, the mantle cavity and gut lumen. Therefore, it is not surprising that these cells play an important role in physiological processes such as nutrient digestion and transport, excretion, tissue repair and internal defence (see Chapter 7 for details on haemocyte functions).

Excretory organs

There are two types of excretory organs in bivalves, the pericardial glands and the paired kidneys. In *Mytilus* the reddish-brown U-shaped kidneys lie ventral to the pericardial cavity surrounding the heart, and dorsal to the gill axis, and in fact extend the complete length of the gill axis from the labial palps to the posterior adductor muscle (Figure 2.1c). In scallops the paired kidneys are attached to the anterior margin of the central adductor muscle, partially hidden by the gonad. One arm of each kidney is glandular and opens into the pericardium, and the other end is a thin-walled bladder that opens through a nephridiopore into the mantle cavity.

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The brown-coloured pericardial glands, sometimes referred to as Keber's organs, develop from the epithelial lining of the pericardium and come to lie over the auricular walls of the heart. Waste accumulates in certain cells of the pericardial glands and this is periodically discharged into the pericardial cavity and from there it is eliminated via the kidneys. Other cells of the pericardial glands are involved in filtering the haemolymph, the first stage of urine formation. The filtrate then flows to the glandular part of the kidney where the processes of secretion and reabsorption of ions occur. The end result is urine that has a high concentration of ammonia and smaller amounts of amino acids and creatine (Table 7.5 in Chapter 7). Most aquatic invertebrates excrete ammonia as the end product of protein metabolism. Ammonia is highly toxic but its small molecular size and high solubility in water ensure that it very rapidly diffuses away from the animal.

While the kidneys and pericardial glands are the major excretory organs, excretory products are probably also lost across the general body surface and particularly across the gills (see Chapter 7 for details on excretion and osmoregulation).

The kidney plays a very important role in the storage and elimination of radionuclides and heavy metals such as silver, cobalt, mercury, manganese, lead and zinc (Metian *et al.* 2011 and references therein). In scallops Metian *et al.* (2009) have shown that several of these metals are sequestered in renal concretions, mostly of calcium carbonate, before being eliminated in the urine.

Nerves and sensory receptors

The nervous system of bivalves is fundamentally simple. It is bilaterally symmetrical and consists of three pairs of ganglia and several pairs of nerves (Figure 2.16). The cerebral ganglia are joined by a short commissure dorsal to the oesophagus. From each cerebral ganglion two pairs of nerve cords extend to the posterior of the animal. One pair extends directly back to the visceral ganglion, which is located on the surface of the posterior adductor muscle. The second pair extends posteriorly and ventrally to the pedal ganglia in the foot. The cerebral ganglia innervate the palps, anterior adductor muscle and part of the mantle, as well as the statocysts and osphradia (see later). The pedal ganglia control the foot. The visceral ganglia control a large area: gills, heart, pericardium, kidney, digestive tract, gonad, posterior adductor muscle, part or all of the mantle, siphons and pallial sense organs. In scallops the paired visceral ganglia are completely fused, thus forming an elementary 'brain' with distinct optic lobes that innervate the eyes on the edge of the mantle.

The ganglia also have a major neurosecretory role in bivalves. Several different types of neurosecretory cells have been identified in mussels and most of these are located in the cerebral ganglia (Illanes–Bucher 1979). These cells produce peptides that are released into the circulatory system. At least four different neuropeptides have been identified that mediate reproductive-related events in bivalves (Morishita *et al.* 2010). One of these, gonadotropin-releasing hormone (GnRH), is a neurohormone that is also important in the control of reproduction in vertebrates. Vertebrate steroid hormones, for example estradiol-17β, progesterone and testosterone, have also been identified in bivalves, but evidence of their endogenous origin and mechanism of action is still lacking (Lafont & Mathieu 2007). Insulin-related peptides have also been identified in neurosecretory cells of several bivalve species, and have been shown to be involved in growth regulation by stimulating protein synthesis in mantle edge cells involved in shell and soft tissue growth in *C. gigas* (Gricourt *et al.* 2003) and, more recently, in early developmental stages in this species (Gricourt *et al.* 2006). More details on the role of neurohormones in gametogenesis, and growth are provided in Chapters 5 and 6, respectively.

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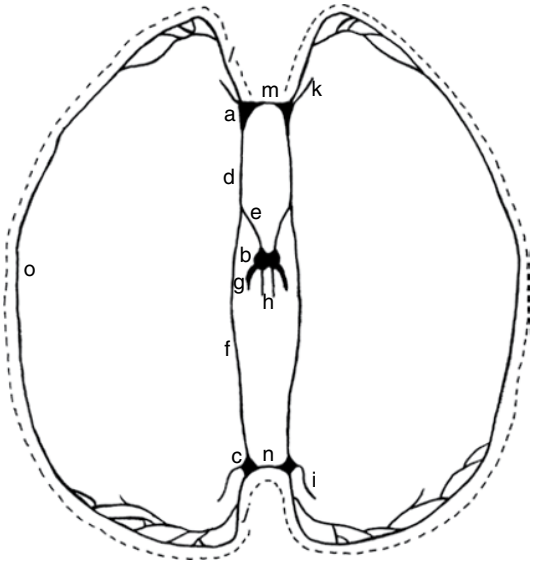


Figure 2.16 Schematic representation of the nervous system in the mussel *Mytilus edulis*. Dashed line indicates the outline of the two shell valves. a, cerebral ganglia; b, pedal ganglia; c, visceral ganglia; d, cerebrovisceral pedal connective; e, cerebropedal connective; f, cerebrovisceral connective; g, pedal nerve; h, byssal retractor nerve; i, branchial nerve; j, posterior pallial nerve; k, anterior pallial nerve; l, buccal nerve; m, cerebral commissure; n, visceral commissure; o, circumpallial nerve.
From de Zwann and Mathieu (1992). Reproduced with permission of Elsevier.

During the evolution of bivalves, with loss of a distinct head, most of the sense organs withdrew from the anterior end and have came to lie at the edge of the mantle. In burrowing forms they are concentrated at the tips of the siphons. Most sensory receptors are located on the middle fold of the mantle. This fold is thick and bears a large number of pallial tentacles, their length and number varying with the species. The tentacles are covered in epithelial sensory cells that are sensitive to touch. A slight tactile stimulus elicits local contraction of the mantle or siphon musculature. This is a reflex action and is not under the control of the central nervous system. A strong stimulus produces a coordinated retraction of the whole animal into its shell. This more general and clearly adaptive type of contraction is under the control of the visceral ganglion. Chemoreceptor cells are probably also present on the tentacles. In scallops, for example, starfish extracts elicit tentacle withdrawal, contraction of the velum and even the swimming response at higher concentrations (Wilkens 2006).

Ocelli, which can detect sudden changes in light intensity, may also be present on the middle fold of the mantle or siphons. These may take the form of invaginated eyecups lined with pigmented sensory cells and filled with a mucoid substance that acts as a 'lens', or they may be very well-developed structures as in scallops. As mentioned earlier, scallop eyes produce a low-contrast image. Recent research shows that swimming scallops tend to have better vision than sessile scallops, and this suggests that mobile scallops may visually detect preferred habitats (Speiser & Johnsen 2008a). Moreover, during shell gaping, when eyes on the mantle edges are exposed, scallops may visually detect the size and speed of moving particles and use this information to help identify favourable feeding conditions (Speiser & Johnsen 2008b). For more information on the visual physiology of scallops see Speiser *et al.* (2011).

Sensory receptors called osphradia are well known in gastropods but in bivalves they are difficult to detect, either because of their small size or because they are in fact absent in

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some species. These receptors, usually paired, consist of a band of sensory and secretory cells located in the gill axis and enervated by the branchial nerve. There has been some debate as to the role of these structures in bivalves (reviewed by Haszprunar 1987), but the general consensus is that they have a dual function: reception of chemical spawning cues by the sensory cells, and synchronization of gamete emission by the secretory cells through the release of serotonin, a powerful stimulant for spawning in bivalves (Beninger *et al.* 1995).

A pair of statoreceptors lies in the foot near the pedal ganglia and is innervated by the cerebral ganglia. There are two types, statocysts that are closed vesicles containing either single or small multiple endogenous concretions, or statocrypts that communicate with the exterior and contain many exogenous particles, for example sponge spicules cemented by mucous. The cavity of the statoreceptor is fluid-filled and lined with hair cells, so called because of their sensory apical cilia. The solid concretion(s) interact with the cilia and convey information to the organism on its orientation in space. Statoreceptors could also be important in the control of the swimming reflex in scallops (Beninger & Le Pennec 2006).

Finally, another sensory receptor, the abdominal sense organ (ASO), first described in scallops, but now reported from at least 19 bivalve families (Haszprunar 1983), is a small piece of tissue situated on the adductor muscle near the anus. ASO epithelium contains ciliated sensory cells and mucocytes, the former making up 90% of the total cell number. While some believe that the ASO functions in the regulation of water flow within the pallial cavity (Beninger & Le Pennec 2006), others have suggested that it may be involved in the perception of water-borne vibrations – similar in some respects to the acoustic lateral line system in fish – and Zhadan (2005) has indeed shown that the scallops *Mizuhopecten yessoensis* and *Chlamys swifti* are sensitive to ultrasonic vibrations in the range 30–1000 Hz. This type of sensory input could give warning of the approach of a predator.

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3 Ecology of bivalves

Introduction

Ecology is the study of interactions between organisms and their environment. Environment embodies everything outside the organism that impinges on it, for example physical factors such as temperature, salinity and light, and biological factors such as predators, competitors and parasites. An organism's response to these factors influences its distribution and abundance both on a local and regional scale.

In this chapter the effects of temperature and salinity, probably the two most important physical factors governing the distribution of marine organisms, are described in detail. Their effects on other aspects of bivalve biology, such as feeding, reproduction, growth, and respiration and osmotic regulation, are covered in Chapters 4, 5, 6 and 7, respectively. Other limiting factors such as aerial exposure, oxygen concentration, currents and substrate type are briefly reviewed. In addition, effects of biological factors such as predation and competition on bivalve distribution and abundance are considered. Pathogens and parasites, which also influence the ecology of bivalve populations, are dealt with in Chapter 11. Finally, the potential and observed impacts of climate change on marine ecosystems are also discussed.

Before describing the major factors that influence bivalve distribution patterns, some information on the global and local ranges of representative species is presented in the following section.

Global and local distribution patterns

The World Register of Marine Species (WoRMS; http://www.marinespecies.org) is a database that provides an authoritative and comprehensive list of names of marine organisms. The content of the registry is edited and maintained on an ongoing basis by scientific specialists on each group of organisms. The register also provides information on the global distribution of individual species in specific geounits, for example Baltic Sea, Adriatic Sea

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and Gulf of Mexico in the case of the clam *Mercenaria mercenaria*; records of published source occurrences are also included. The register is linked to numerous web sites, including the *Encyclopedia of Life* (http://eol.org), which provides distribution maps plus records for individual species.

In relation to bivalve global distribution some points are worth noting:

1. Some parts of the world, for example Asia, Africa and South America, are underrepresented simply because there are few if any comprehensive accounts of species distributions from these regions.
2. Several species, in particular oysters and clams, have become established, either through planned or accidental introductions, in regions outside of what would be considered their native range.
3. Until relatively recent times distributions have been mapped solely on the basis of external features of the shell. With the advent of molecular markers some distributions have had to be revised, either by extending or reducing the previously reported geographic range (see later). Sometimes species distributions are based solely on molecular markers. This highlights the fluidity of current species distributions and boundaries.

In the following sections the geographic distribution and habitats of representative, and in some cases commercially important, genera from each of the four bivalve groups will be considered.

Mussels

Mussels in the genus *Mytilus* are a dominant component of rocky shore communities in cooler waters of the northern and southern hemispheres. The blue mussel *Mytilus edulis* has a wide distribution in the northern hemisphere, occurring in European waters from Spitsbergen (74–81°N; Berge *et al.* 2005) to western France, and on the Atlantic coast of North America from the Canadian Maritimes southward to North Carolina (Gosling 2003 and references therein). It has also been reported in Iceland (Varvio *et al.* 1988) and Greenland (Wanamaker *et al.* 2007). *Mytilus galloprovincialis* occurs in the Mediterranean Sea, the British Isles, continental Europe, North Africa and parts of the northern Pacific (Westfall *et al.* 2010 and references therein). Some of the present distribution is due to accidental introductions into Japan, Hong Kong, southern California and South Africa (references in Gosling 1992). Other *Mytilus* species have a more restricted range. For example, the Californian mussel *Mytilus calfornianus* is confined to the Pacific coast of North America, while *Mytilus trossulus* is restricted to cool water regions of the northern hemisphere. The latter is an example of a species whose distribution has been mapped solely using molecular markers. In the northern hemisphere, where the ranges of *M. edulis*, *M. trossulus* and *M. galloprovincialis* overlap, variable amounts of hybridisation occur between species pairs. In the southern hemisphere the distribution of *M. edulis and M. galloprovincialis* is much less clear. Results from molecular analyses indicate that *Mytilus* populations in Argentina and Chile are similar (but not identical) to northern hemisphere *M. edulis*, while those in Australia, Tasmania and New Zealand are similar (but not identical) to northern hemisphere *M. galloprovincialis* (Gérard *et al.* 2008; but see Borsa *et al.* 2012). It has been suggested that mussels in the southern hemisphere are derived from two ancient migration events from the northern hemisphere (Hilbish *et al.* 2000). Interestingly, Westfall *et al.* (2010) recently identified a southern hemisphere *M. galloprovincialis* lineage that is native to New Zealand.

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Species in the genus *Perna* have a subtropical to tropical distribution in the southern hemisphere. The green mussel *Perna viridis* has the most widespread distribution, extending from India eastwards to south-east Asia, the China coast, Indonesia, Philippines and Samoa. The species is a recent invader to the Caribbean Sea, including the subtropical south-eastern United States region (Baker *et al.* 2007). The brown mussel *Perna perna* is found on the east, west and south coasts of Africa, and the coast of South America, extending into the Caribbean Sea. It has invaded the western Gulf of Mexico, including Texas and northern Mexico (Hicks & McMahon 2002). The green-lipped or New Zealand mussel *Perna canaliculus* is restricted to New Zealand where it was cultured and marketed as the Greenshell® mussel. The ribbed mussel *Geukensia demissa* occurs along the Atlantic coast of North America from the Gulf of Maine to Florida and the Gulf of Mexico (Franz 2001).

On a local scale mussels (*Mytilus*) dominate the intertidal to subtidal regions of rocky shores. *M. edulis* has the widest distribution pattern in the genus, extending from high intertidal to subtidal regions, from estuarine to fully marine conditions and from sheltered to extremely wave-exposed shores. At exposed sites the species prefers gently sloping, slow-draining platforms to steep rock faces. On such shores dense beds of small mussels, often several layers thick, blanket the rocks (Figure 3.1). On sheltered shores and in estuaries fewer but larger mussels are found (Figure 3.2). Subtidal populations on pier pilings, oil



Figure 3.1 Mussels (*Mytilus*) on an exposed rocky shore on Achill Island, West of Ireland. Individuals only attain a maximum shell length of 2.5 cm, and that after many years. Plastic tag in the foreground is 25 cm long.
Photograph by Elizabeth Gosling.

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Figure 3.2 A cluster of mussels (*Mytilus*) on a sheltered shore at Lough Hyne, Cork, Ireland. Mussels in this habitat can reach shell lengths of 50–60 mm or greater.
Photograph by Elizabeth Gosling.

platforms and culture ropes enjoy a virtually predator-free environment and this, combined with continuous submersion, allows *M. edulis* to reach a large size in a relatively short period of time, for example 50 mm shell length in 6–8 months (Page & Hubbard 1987). *Perna* spp. are found both in brackish estuaries and in open, but not very exposed, waters in the lower intertidal to subtidal regions of the shore. Some mussel species prefer salt marshes (*Guekensia demissa*) or quiet bays (*P. viridis*), while others (*Bathymodiolus* spp.) have colonized abyssal depths near hydrothermal vents.

Mussels settle on a wide variety of substrates, for example rock, stones, pebbles, shell, cement and wood, once the substrate is firm enough to provide a secure anchorage. In the case of *Perna*, mangrove mudflats represent a major habitat for tropical species. Early *M. edulis* spat either attach to filamentous algae, from which they eventually migrate onto adult mussel beds, or else they settle directly onto adult beds (Chapter 5). On rocky shores mussel beds provide a habitat for a few but abundant macroinvertebrate species (Seed 1996). Upper distribution limits for mussels are usually governed by physical factors, primarily temperature, while predators are mainly responsible for setting lower limits (Seed & Suchanek 1992).

Oysters

In comparison to mussels there is a lot more information on the global distribution of oyster species, presumably because of their greater economic importance. Several of them have broad geographic ranges. For example, the Eastern oyster *Crassostrea virginica* extends from the Gulf of St. Lawrence in Canada (48°N) to the Gulf of Mexico. Similarly, the range of the Indian Rock oyster *Saccostrea cucullata* extends from the tropical coast of West Africa (10°W) eastward across the Indian Ocean as far as the Philippines (130°E). Other species with broad ranges are the Columbian oyster *Crassostrea columbiensis*, the European flat oyster *Ostrea edulis* and the black-bordered oyster *Saccostrea echinata*. The Pacific oyster *Crassostrea gigas* is native to the Indo-West Pacific region, but because of successful

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introductions mainly for aquaculture purposes, on the Pacific coast of North America, the Pacific and Atlantic coasts of South America, western and north-western Europe, northern Africa and Australia, it now has a relatively widespread global distribution (Miossec *et al.* 2009 and references therein). As mentioned already, distributions may need to be revised in the light of new evidence. For example, the Chilean flat oyster *Ostrea puelchana*, which has a circum-global distribution between latitudes 35° and 50°S, is probably not a single species. Results from DNA molecular phylogenetic analyses suggest that in Argentina the species is indeed *O. puelchana*, but that in Chile and New Zealand it is *Ostrea chiliensis*, in South Africa, *Ostrea algoensis* and in Australia, *Ostrea angasi* (Jozefowicz & Ó Foighil 1998; Ó Foighil *et al.* 1999). There is genetic evidence that the species on the coasts of Brazil and Argentina, once believed to be *C. virginica*, is the mangrove oyster *Crassostrea rhizophorae* (Hedgecock & Okazaki 1984). There is also evidence that a second species, *Crassostrea brasiliana*, occurs sympatrically with *Crassostrea rhizophorae* (Ignacio *et al.* 2000; see also Lazoski *et al.* 2011). The oyster *Ostrea lurida*, believed to be the only native oyster on the west coast of North America, is instead two distinct species, with *O. lurida* inhabiting waters from Baja, California, northward to Alaska and *Ostrea conchaphila*, from central Mexico southward to Panama (Polson *et al.* 2009). It is clearly important in mapping the geographic distribution of a particular species that we determine whether it is genetically homogeneous or a mixture of two or more species (Chapter 10).

Oysters are commonly found in the low intertidal to subtidal regions of shallow, sheltered estuaries (Table 3.1). Compared to mussels they settle on a more restricted range of

Table 3.1 Habitat type of 12 commercially important oyster species.

Species	Common name(s)	Habitat
Crassostrea virginica	Eastern oyster	Forms reefs in intertidal and subtidal regions of estuaries and coastal areas of reduced salinity
Crassostrea gigas	Pacific oyster, Japanese oyster	Attach to hard or rocky surfaces in shallow or sheltered salinity (23–28 psu) waters up to 40 m deep
Crassostrea gasar	West African, mangrove oyster	Attached to roots and branches of mangroves in intertidal zones, and on muddy bottom of brackish regions of estuaries
Crassostrea columbiensis	Columbian oyster	Adheres to rocks or mangrove roots or other solid substrata in the mid-intertidal zone
Crassostrea rhizophorae	Mangrove oyster, gureri	In the intertidal area attached to roots and branches of mangrove trees in high salinity seawater
Ostrea edulis	European flat oyster	On substrates of mud, rocks or muddy gravel in shallow, sheltered estuarine waters
Ostrea puelchana	Chilean flat oyster, Patagonian oyster, puelche	Forms extensive banks from low tide to 15 m in estuarine conditions
Ostrea conchaphila	Native Pacific oyster, Olympia oyster	Attached to hard substrate or loose on soft substrate singly or in small groups in estuaries and salt water lagoons
Saccostrea glomerata	Sydney rock oyster	Attached to rock and shell in intertidal zone of estuaries, but also occurs subtidally
Saccostrea cucullata	Indian Rock oyster, Bombay oyster, curly oyster, Red Sea oyster	Intertidal and shallow subtidal rocky or firm substrata at depths of 1–15 m
Saccostrea echinata	Black-bordered, black-edged, black-lipped oyster	Attached to intertidal and shallow subtidal rocks and other hard surfaces
Saccostrea palmula	Palmate oyster	Attached to mangrove roots or rocks in intertidal and shallow subtidal areas of estuaries and mangrove forests

From Carriker and Gaffney (1996); Gillespie (1999); Jackson (2003); Romero *et al.* (2013).

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substrates, mainly rock and shell, and roots and branches in the case of mangrove species. Oyster reefs and beds provide numerous microhabitats for both motile and sessile species.

Clams

The softshell clam *Mya arenaria* occurs in many temperate and subarctic areas along the north-east and north-west coast of the Atlantic Ocean and the north-east coast of the Pacific Ocean. This wide geographic range has resulted from intentional and unintentional introductions, as well as range expansions over the past 700 years (Powers *et al.* 2006). The hard clam *Mercenaria mercenaria* is distributed on the Atlantic coast of North America from the Gulf of St. Lawrence to Florida, and is particularly abundant from Maine to Virginia. In the southern part of its range it is sympatric with *Mercenaria campechiensis*, which extends into the Gulf of Mexico. *M. mercenaria* has been successfully introduced into California, but with limited success into western Europe. In contrast, the Manila clam *Ruditapes philippinarum*, indigenous to the western Pacific, was introduced first onto the West coast of North America and from there into western Europe. In Europe it has proved to be a hardy, fast-growing but generally non-reproducing species with substantial potential for commercial production (Chapter 9). There are two important fisheries species: the surf clam *Spisula solidissima* occurs off the east coast of the United States from Nova Scotia to South Carolina, while the quahog clam *Arctica islandica*, whose range overlaps with the surf clam in North America, is found from Iceland southwards to the Bay of Biscay, France. The blood cockle *Anadara granosa*, an important fisheries species in China, is widespread throughout the Indo-West Pacific, from east Africa to Polynesia, north to Japan and south to North and East Australia. The Pacific geoduck *Panopea generosa* occurs on the west coast of North America and is extremely abundant in the inland waters of Puget Sound, British Columbia and Alaska, where subtidal populations support important fisheries (Brown & Thuesen 2011). Giant clams (*Tridacna* spp.) are found throughout the tropical Indo-Pacific, generally inhabiting the shallow water of coral reefs. Other clams that also have a broad geographic range in tropical waters are species of *Arca*, *Mactra*, *Meretrix* and *Paphia*.

Of the four bivalve groups, clams occupy the broadest range of habitats (Table 3.2). They are found from open coasts to sheltered, saline and estuarine locations. They extend from the upper intertidal to subtidal regions of shores, in some cases to depths of 200 m. They settle on a variety of substrates, for example mud, sand or gravel, or combinations of these, and bury themselves at depths ranging from 5 to 100 cm, depending on the species. Giant clams, *Tridacna* spp., do not burrow but byssally attach to coral reef early in life.

Scallops

Scallops are found in all waters of the northern and southern hemispheres and show a more extensive global distribution than any of the groups mentioned so far. The Iceland scallop *Chlamys islandica* is the northernmost species of scallop, extending into Arctic regions, as far as 75°N. It occurs from Alaska to Puget Sound on the Pacific coast of North America, from the Arctic seas to southern Massachusetts, from Spitsbergen south to the Lofoten Islands, Norway, and from Greenland and Iceland east to the Barents and White seas (Strand & Parsons 2006). The great scallop *Pecten maximus* and the queen scallop *Aequipecten opercularis* have similar geographic ranges along east coasts of the North Atlantic from northern Norway to West Africa, although *A. opercularis* extends further into the Meditteranean and Adriatic seas (Brand 2006). The sea scallop *Placopecten magellanicus* is confined to the North-west Atlantic with a geographical range from the north shore of the

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Table 3.2 Habitat type of commercially important clam species.

Species	Common name(s)	Habitat
Mercenaria mercenaria	Hard clam	Estuarine intertidal to shallow subtidal; sand, sand-mud, sand-shell; 5–10 cm
Mya arenaria	Softshell clam	Estuarine, upper intertidal to shallow subtidal to ~200 m depth; firm mud/sand; 15–25 cm
Spisula solidissima	Surfclam	Open ocean to depths of ~50 m; coarse or fine sand; 5–15 cm
Cerastoderma edule	Edible cockle	Estuarine to fully marine; mid-tide to low-water; sand, soft mud, gravel; <5 cm
Ruditapes philippinarum	Manila clam; Japanese carpet shell	Bays and protected coasts, intertidal to very shallow subtidal; sand, mud/gravel; 5–10 cm
Arctica islandica	Ocean quahog	Open ocean shelf to depths of 300 m or more; medium to fine-grain sand, sandy mud, silty sand; 5–10 cm
Anadara granosa	Blood cockle	Mid-intertidal to subtidal (1–2 m water depth) zones; soft mud bordering mangrove swamp forests; does not burrow to any depth
Saxidomus gigantea	Butter clam	Estuarine, low intertidal, subtidal to 40 m; sand/gravel; up to 30 cm
Panopea generosa	Pacific geoduck	Protected bays, subtidal to 110 m but intertidal in north of range; sand, sand/mud; up to 100 cm
Siliqua patula	Pacific razor clam	Open coast, intertidal surf to 10 m; sand; 30–100 cm
Tridacna spp.	Giant clam	Shallow sunlit, fully saline waters of Indo-Pacific coral reefs

From Broom (1985); Heslinga (1989); Malouf and Bricelj (1989); Manzi and Castagna (1989). Habitat is given first, followed by preferred substrate type and burial depth.

Gulf of St. Lawrence to Cape Hatteras, North Carolina (Naidu & Roberts 2006), while the bay scallop *Argopecten irradians* extends from Cape Cod, Massachusetts, to southern Texas (Brand 2006). The calico scallop *Argopecten gibbus*, closely related to *A. irradians*, is also found in the western North Atlantic and the Gulf of Mexico, but in deeper waters offshore (Brand 2006). Another species in the same genus, the Chilean scallop *Argopecten purpuratus*, occurs from Nicaragua (12°N) southwards as far as Valparaiso, Chile (33°S), a distance of more than 5300 km. The weathervane scallop *Patinopecten caurinus* extends all the way from Alaska to Central California, but distribution is patchy along its range and no extensive beds occur (Lauzier & Bourne 2006). On the western side of the North Pacific there are several species that are fished commercially. The yesso scallop *Mizuhopecten yessoensis* is a cold water species that is distributed on both coasts of Japan from northern Honshu to Hokkaido. It is also found on the coasts of Korea and Russia, extending northwards to Sakhalin and the Sea of Okhotsk (Ivin *et al.* 2006). The zhikong scallop *Chlamys farreri* is a subtropical species and is found from North China (40°N) to Fujian Province (25°N); it also inhabits the waters of Japan and Korea (Guo & Luo 2006). *Amusium balloti* and *Amusium pleuronectes* are found in tropical and subtropical waters of the Indo-Pacific. The former is found in Queensland, Australia, and along the north and west coasts, while the latter is recorded from the Indian Ocean, South China Sea, Indo-China, Japan, the Philippines, New Guinea, Java, Indonesia and in northern and western Australia (Brand 2006). The range of the commercial scallop *Pecten fumatus* is confined to the south and south-east coasts of Australia including Tasmania, while *Pecten novaezealandiae* is found only in New Zealand, occurring sporadically around the whole coastline (Marsden & Bull 2006).

Most scallop species are found at depths between 10 and 100 m in sheltered bays and open coast sites (Table 3.3). All scallops secrete a byssus when young but most lose the byssus soon after metamorphosis and recess on sand or gravel bottoms. Species that retain

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Table 3.3 Habitat type and typical densities (where available) of some commercially important species of scallop.

Species	Common name(s)	Habitat
Pecten maximus	Great or king scallop	Just below low water mark to ~150 m, most common at 20–45 m; in clean firm sand, fine or sandy gravel, sometimes with mud; recesses; densities 1–3 m^{-2}
Pecten fumatus	Commercial scallop	Sheltered as well as exposed locations (1–120 m); soft sand or muddy sediments; recesses
Pecten novaezelandiae	New Zealand scallop	Semi-estuarine and coastal waters, low tide to 60 m; soft mud, shell gravel, coarse sand; recesses; <1/5 m^2
Mizuhopecten yessoensis	Japanese scallop; yesso scallop	In sheltered bays at depths 20–25 m; sand and gravel; <1 individual m^{-2}
Placopecten magellanicus	Sea or giant scallop	Low tide to 10–100 m depths; mud, sand, pebbles, rocks and even boulders; 2–4 m^{-2}
Aequipecten opercularis	Queen scallop; queenie	At depths 20–100 m; same substrates as *P. maximus*; does not recess
Patinopecten caurinus	Weathervane scallop	At depths 10–300 m; sand or muddy bottoms; recesses; <1 individual m^{-2}
Chlamys islandica	Iceland scallop	At depths 10–250 m, usually <100 m; attached to sand, gravel, shell and stones; does not recess, attached by byssus; densities 10–70 m^{-2}
Chlamys farreri	Zhikong scallop	Intertidal region to 60 m; attached by byssus to rocks and gravel; densities 2–4 m^{-2}
Argopecten purpuratus	Peruvian or Chilean scallop	Between 5 and 40 m in sheltered bays; sand is preferred substrate, but also found in mud and on rocks; recesses; densities 10–40 individuals m^{-2}
Argopecten gibbus	Calico scallop	Shallow subtidal to 370 m, usually 20–50 m; hard sandy bottom; recesses; ~40 m^{-2}
Argopecten irradians	Bay scallop	Protected bays and estuaries at depths <10 m; young attach by byssus to stones, algae, shell; adults on mud bottoms among eelgrass *Zostera*; sometimes recess in winter; 0.2–4.4 m^{-2}
Argopecten circularis	Catarina scallop	Shallow waters from 6 to 35 m; very varied substrate, for example shell, eelgrass, algae, coral, gravel mixed with sand or mud; ~5 m^{-2}

From Blake and Moyer (1991); Brand (1991, 2006); Blake and Shumway (2006); Guo and Luo (2006); Ivin *et al.* (2006); Lauzier and Bourne (2006); Marsden and Bull (2006); Naidu and Roberts (2006); Strand and Parsons (2006).

the byssus throughout life need a firm substrate such as pebbles, rocks, shell or boulders. Scallops are unique among bivalves in their ability to 'swim' (Chapter 2). They use this primarily to escape predation and for habitat selection (Jenkins *et al.* 2003 and references therein). *P. maximus* that are normally recessed in sandy or sandy mud substrates swim more frequently and disperse widely when placed on hard substrates. In contrast, when placed in sand little or no swimming occurs (Baird 1958). Similarly, when the bay scallop *A. irradians* was released on sand at a distance of 25 cm from a bed of *Zostera* – its natural habitat – it swam towards the grass bed irrespective of the bed's direction relative to the scallop's facing direction (Hamilton & Koch 1996). However, when placed at greater distances from the grass bed (>50 cm) scallop movement was random, which suggests that orientated behaviour in this species is based on visual information, and possibly chemical stimuli also. It is highly unlikely that swimming is used for efficient long-distance movement (Brand 2006). Results from tagging experiments on *P. magellanicus* showed that 52% of recaptures were within 5 km, 77% were within 10 km and 94% were within 25 km of the release point, and that

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movements were related to direction and velocity of water currents (Melvin *et al.* 1985). Similar results have been observed for *P. maximus* and the yesso scallop, *M. yessoensis* (references in Brand 2006).

Factors affecting geographic distribution

As mentioned earlier temperature and salinity not only set limits on the spatial distribution of bivalves but also affect every aspect of biology including feeding, reproduction, growth, respiration, osmoregulation and parasite–disease interactions (see details in Chapters 4–7 and 11). When it comes to distribution on a large geographic scale it is generally recognised that temperature plays a more important role than salinity. However, in coastal and estuarine regions salinity is probably the most important limiting factor, particularly for oyster populations. The synergistic effect of temperature and salinity, acting in concert with other environmental variables such as water depth, substrate type, food availability, water turbidity and the occurrence of competitors, predators and disease, can have more profound consequences than either factor acting alone. Geographical distribution is also governed by hydrographic barriers to larval dispersal, such as oceanic currents, confluences, gyres and surface water stratification.

Physical factors

Temperature, salinity and hydrographic features

Most marine bivalves live within a temperature range from −3 to 44°C (Vernberg & Vernberg 1972). Within this range the degree of temperature tolerance is species-specific, and within individual species early embryos and larvae have a narrower temperature tolerance than adults. In addition, the temperature required for spawning is invariably higher than the minimum temperature required for growth. All of these factors set limits on the natural distribution of individual species on both regional and local scales. A few pertinent examples will make this clear. The Patagonian scallop *Psychrochlamys* (*Zygochlamys*) *patagonica* occurs in the south-west Atlantic Ocean between 36 and 48°S at depths of 70–120 m. The scallop's northern limit (36–37°S) is largely determined by abrupt changes in temperature associated with the Brazil–Malvinas/Falklands Confluence, where water reaches temperatures beyond the species' tolerance limit, causing poor and irregular recruitment, low growth rates and high natural mortality (Gutiérrez *et al.* 2008). The scallop *P. magellanicus* is a cold water species with a temperature optimum of about 10°C, and an upper lethal temperature between 20 and 24°C, depending on acclimation temperature. At the northern end of its range (Gulf of St. Lawrence) the scallop inhabits shallow depths where the water is warmest. The distribution of the species in this part of its range is largely determined by low summer temperatures, which fail to reach the spawning threshold for the species, or which prolong larval development so that recruitment fails. At the southern end of its range (Cape Hatteras, North Carolina) the scallop occurs in much deeper water, usually greater than 55 m, where the water remains cold, separated from the warmer upper layers by a thermocline (Brand 2006 and references therein). Cape Hatteras, until relatively recently, was also the historical southern limit for the mussel *M. edulis*; the northward moving warm water Gulf Stream meets the southward moving cold Labrador current in the region of Cape Hatteras (35.25°N), and provides a temperature barrier for the distribution and survival of mussel larvae south of this point. However, since 1960 due to increasing water and air temperatures (see later section on climate change), the southern limit has

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shifted north to Lewes (38.5°N) by approximately 350 km (Jones *et al.* 2010). At the southern part of the range high water and air temperatures cause mass mortality events, while along the more northerly portion mortality is caused by high temperatures during aerial exposure. Ultimately, water temperatures in excess of thermal tolerances have caused contraction of the mussel's biogeographic range. Still staying with mussels, the range of *M. trossulus* once extended from Baja California north to Alaska and west to Japan. However, when the Mediterranean mussel *M. galloprovincialis* was introduced into southern California in the early 1990s the mussel was able to completely displace the native *M. trossulus* throughout the southern portion of its range. Fields *et al.* (2006) investigated whether biochemical adaptation to temperature might potentially play a role in invasion success. An examination of cytosolic malate dehydrogenase (cMDH), an enzyme known to exhibit distinct patterns of temperature adaptation in kinetic properties, showed that a minor change in structure permits *M. galloprovincialis* cMDH to function at warmer temperatures and may be a part of a broad suite of molecular adaptations that has allowed this species to displace its congener throughout the warmer part of *M. trossulus*' original range in North America and Japan. Interestingly, over the past decade the poleward movement of *M. galloprovincialis* has shown a reversal concomitant with a cooling phase of the Pacific Decadal Oscillation,[1] an important driver of climate (Hilbish *et al.* 2010).

The open ocean typically has surface salinities between 32 and 37 psu, with an average of 35 psu. In contrast, estuaries and bays are subject to pronounced salinity fluctuations because of evaporation, rainfall and inflow from rivers. Oysters are euryhaline (i.e. able to survive in a wide range of salinities) and so it is not surprising that they are successful colonisers of estuarine as well as fully saline waters (Table 3.1). *C. virginica* normally lives in waters of 5–40 psu with an optimum salinity in the range of 14–28 psu (references in Shumway 1996). Many mussels, in particular *Mytilus* spp., are also euryhaline, with an extremely wide marine and estuarine distribution and a salinity tolerance that has been reported to range between 4 and 40 psu. In the northern Baltic, *M. trossulus* is living at the margin of its salinity tolerance (4 psu), and although dwarfed by the low-salinity conditions, the species is very abundant in this area (Westerbom *et al.* 2002). The mussel *P. viridis*, which has a more extensive global distribution than its congener *P. perna*, has driven out the latter from its natural beds on the Venezuelan coast. One of the main reasons for its extraordinary invasive ability is its wide thermal and salinity tolerance compared to *P. perna* (Figure 3.3). The species can survive in salinities as low as 20 psu (optimal range 27–33 psu) and temperatures as low as 12°C, and as high as 39°C, the latter albeit for short periods (optimal range 15–32.5°C) (Figure 3.4; Rajagopal *et al.* 2006). Many clam species are euryhaline. For example, the estuarine habitat in which *M. arenaria* lives is constantly exposed to changes in salinity from about 10 to 25 psu, mainly as a result of freshwater run-off. Consequently, the adult salinity tolerance range is between 4 and 33 psu. Figure 3.5 shows experimentally determined temperature and salinity tolerance ranges for adults and larvae of seven North American clam species. Not surprisingly, the data concur with regional and local distribution patterns for these species (Table 3.2). The narrower tolerance of larvae compared to adults is also highlighted in Figure 3.5. In contrast, most scallop species live in fully saline waters and are unable to colonise low-salinity waters. For example, *P. maximus* and *A. opercularis* have similar geographic distributions in western Europe and both species extend as far as, but not into, the low-salinity (5–6 psu) waters of the Baltic. Experiments have confirmed that *A. opercularis* cannot tolerate low salinity; 16–28 psu was lethal after a 24 h experimental exposure, depending on temperature and size of scallop (Paul 1980). In contrast, *A. irradians*, living in shallow bays and estuaries (Table 3.3) in salinities ranging between 10 and 38 psu, is one of the few euryhaline scallop species (Brand 2006 and references therein).

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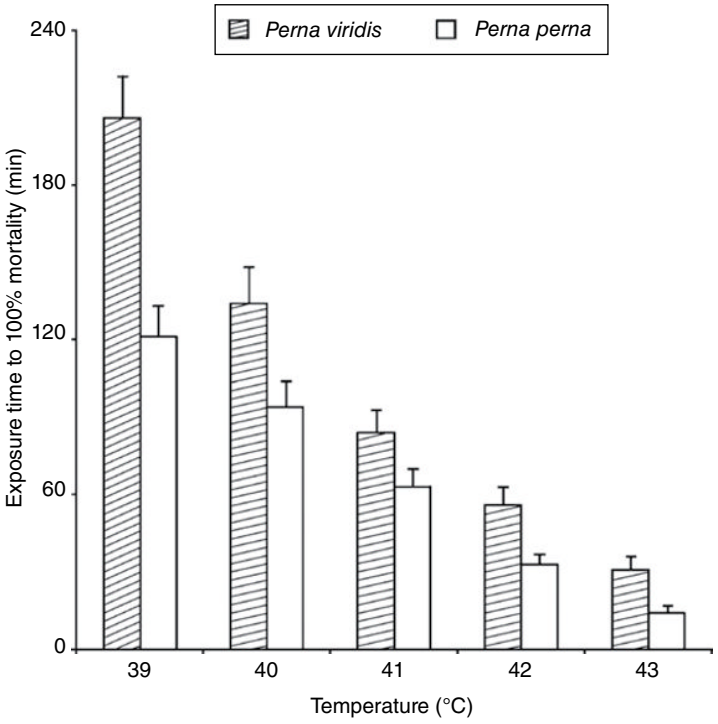


Figure 3.3 Exposure time required for 100% mortality of *Perna viridis* and *Perna perna* at several high temperatures. Mortality data are expressed as mean ± SD ($n = 36$) of six replicate experiments ($n = 6$ in each experiment). The criterion for mortality of mussels was valve gaping with no response of exposed mantle tissues to external stimuli.
From Rajagopal *et al.* (2006). Reproduced with permission of Springer Science + Business Media.

Besides rising air and water temperatures, climate change may also entail increases in precipitation, and estuarine species in particular may be exposed to increasing hypoosmotic stress due to decreasing salinities (Somero 2012). Researchers are starting now to evaluate the potential impacts of hypoosmotic stress on species' distribution ranges. In a recent study Tomanek *et al.* (2012) examined the proteomic responses to hyposaline stress in *M. trossulus* and *M. galloprovincialis*, whose ranges overlap on the west coast of North America (see earlier). Mussels were exposed to short time periods (4 h) of hyposaline stress, followed by a recovery period to mimic conditions typical for bays and coastal areas experiencing heavy freshwater input, with a quick return to full salinity with incoming tides and mixing with full-strength seawater. The differences in protein abundances in gill tissue suggested that *M. trossulus* was able to respond to a greater hyposaline challenge (24.5 psu) than *M. galloprovincialis* (29.8 psu). These differences, in a scenario of reduced coastal salinities, may enable *M. trossulus* to cope with greater hyposaline stress, and outcompete *M. galloprovincialis* in the southern part of the *M. trossulus* range, thereby preventing *M. galloprovincialis* from expanding northwards.

Gene expression screening is also being used to assess physiological responses of *M. californianus* to environmental variation across both large (Place *et al.* 2008) and small (Gracey *et al.* 2008; Connor & Gracey 2011) spatial scales.

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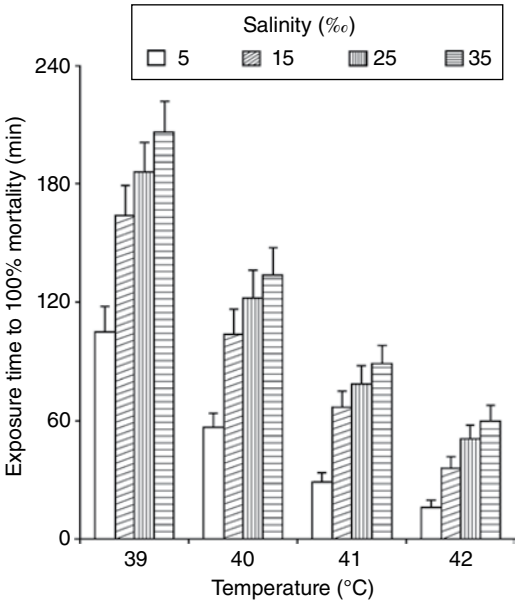


Figure 3.4 Exposure time required for 100% mortality of *Perna viridis* subjected to the combined effect of salinity and temperature. Mortality data are expressed as mean ± SD ($n = 36$) of six replicate experiments ($n = 6$ in each experiment).
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Factors affecting local distribution

It is not surprising that temperature and salinity, which play a key role in geographic distribution, are also important in determining species distribution on a local scale. For subtidal species additional factors such as water depth, substrate type, currents, turbidity, as well as predation and competition play an important role. Upper distributional limits in the intertidal zone are believed to be determined primarily by desiccation and temperature stress, while lower limits are usually established by species interactions (Petes *et al.* 2007 and references therein). For burrowing bivalves, additional physical factors such as substrate type and oxygen concentration come into play, while predation and competition once again are important biological factors. Needless to say, anthropogenic factors such as water-borne pollutants, introduced species and disease can also be significant in determining local distribution patterns.

Physical factors

Temperature

Animals in the intertidal area of the shore have to cope with being out of water at regular intervals. For animals in the high intertidal, emersion times are longest, and consequently these individuals are often subjected to temperature extremes and desiccation. Upper distribution limits for a species are set by its ability to tolerate such extremes by various physiological mechanisms.

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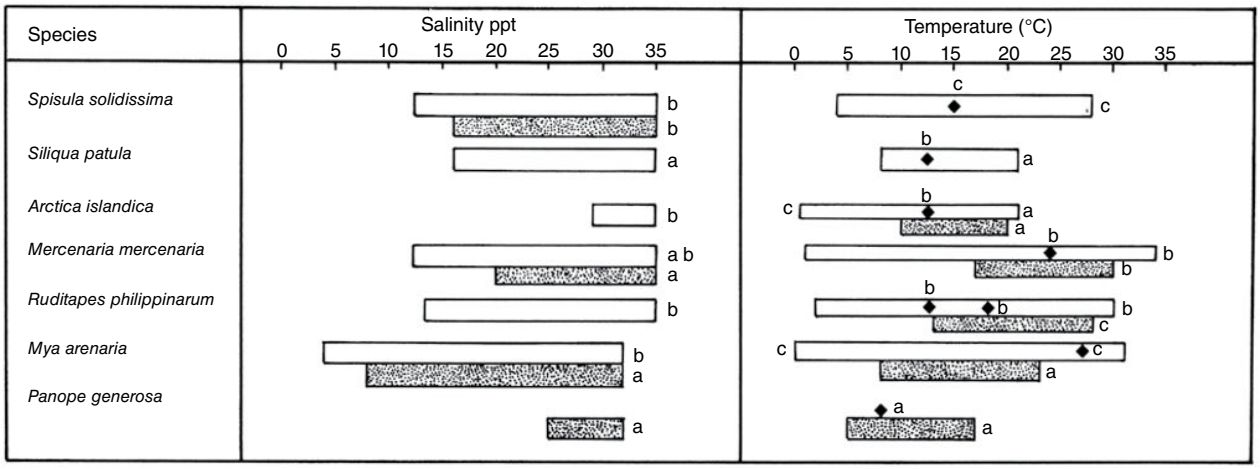


Figure 3.5 Experimentally determined tolerance ranges and observed environmental limits of temperature and salinity for adults and larvae (stippled) of seven clam species. (a) experimentally determined; (b) approximate limits observed in nature; (c) observed in nature or used in culture (not necessarily limits); approximate minimum spawning temperatures (♦) are also shown. See text for geographic distribution. *Siliqua patula* is found from Alaska to northern California.
Adapted from Malouf and Bricelj (1989). Reproduced with permission of Elsevier.

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In view of the enormous amount known about the ecology and physiology of mussels the main focus here will be on *Mytilus* spp. and the impacts of thermal stress on them in the rocky intertidal zone, a habitat that has been described as among the most physically harsh environments on earth (Tomanek & Helmuth 2002). When mussels are submerged their body temperature is close to that of the surrounding water. But during aerial exposure at low tide, body temperatures are driven by the interactions of solar radiation, cloud cover, wind speed, and air and ground temperatures. Consequently, body temperatures can be considerably higher than those experienced during submersion, and can vary substantially from surrounding air and substrate temperatures (Helmuth 1999).

The availability of data logger technology has now made it possible to measure body temperatures of intertidal organisms in the field. Helmuth & Hofmann (2001) continuously monitored temperature at a site in central California for a period of 2 years, using loggers designed to mimic the thermal characteristics of the mussel *M. californianus* (Figure 3.6). Model mussel temperatures were recorded on both a horizontal and a vertical north-facing microsite, and in an adjacent tidepool. Mussels at each microsite were periodically measured for levels of heat shock proteins (Hsp 70), a measure of thermal stress. Their roles in the stress response are to assist misfolded proteins to attain or regain their native states and prevent heat-damaged protein accumulation in cells, thereby preventing the formation of cytotoxic aggregates; heat shock proteins are also induced by sublethal concentrations of a variety of environmental pollutants, salinity fluctuations, hypoxic conditions and pathogens (Werner & Hinton 1999; Werner 2004; reviewed by Fabbri *et al.* 2008 and Clark & Peck 2009; see Dimitriadis *et al.* 2012 and Schmidt *et al.* 2012, for information on other thermal stress biomarkers). Mussel temperatures were consistently higher on the horizontal surface than on the vertical surface and differences between these sites were reflected in the amount of Hsp70. One interesting finding of their study was that seasonal peaks in extreme (acute) high temperatures did not always coincide with peaks in average daily maxima (chronic



Figure 3.6 A living mussel, *Mytilus californianus* (left), an unmodified Onset TidbiT logger (middle), and a 'robomussel' (right) moulded from polyester resin and containing a TidbiT logger thermally matched to the characteristics of a living mussel.
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high temperatures) (Figure 3.7). A subsequent study showed that thermal stress may depend not only on exposure to temperature extremes but also to the thermal history of the temperature signal (Helmuth 2002). For example, on days where daily maximum temperatures increase gradually, mussels may be able to acclimate better to temperature extremes, compared to days where temperature maxima are preceded by cool days (Figure 3.7). In the same study, deployment of loggers at multiple sites showed that each site had a unique thermal signature due to interactions of terrestrial climate, tidal cycle and wave exposure, but significant within-site differences, due to tidal height and substratum angle, were also recorded. See Helmuth (2002) and Fitzhenry *et al.* (2004) for potential pitfalls on logger use, and Lima *et al.* (2011) for information on different types of biomimetic data loggers. Other factors that have a bearing on an individual's response to thermal stress include size, shape, mass and colour of individuals (Helmuth 2002), vertical position in the sediment (Jost & Helmuth 2007), geographic location (Fields *et al.* 2012), and interaction with stressors, for example anoxia (Ueda *et al.* 2009), reduced food supply (Schneider *et al.* 2010) and pollutants (Lannig *et al.* 2006; Metzger *et al.* 2012).

In cold conditions mobile intertidal species can hide in rock crevices or migrate to deeper water, to avoid freezing. But sessile bivalves, often exposed to sub-zero temperatures during winter, do not have such protection. In north-east Canada temperatures can drop to −35°C in winter. Mussels (*M. trossulus*) survive such low temperatures even when their tissue temperatures are as low as −10°C (Williams 1970). As much as 60% of their extracellular fluid (ECF) is frozen at this temperature. The unfrozen ECF becomes more concentrated with solutes, and this process draws water by osmosis out of cells, thus lowering the intracellular freezing point. The high osmotic concentration of the ECF places an osmotic stress on the cells that can damage membranes and enzymes. This damage can be minimized through the production of cryoprotectants, for example the amino acids glycine, alanine and proline, as well as end products of anaerobic metabolism, such as lactate, succinate and

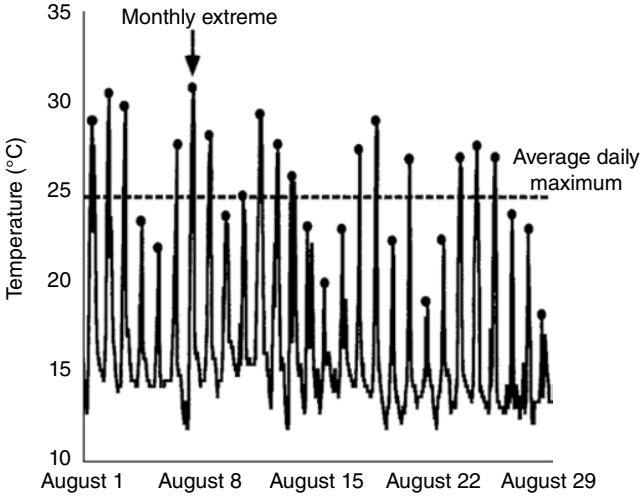


Figure 3.7 Example of fluctuations in temperature experienced over 1 month (August 1999) at the horizontal microsite. Daily maxima were calculated from temperature data collected every 5–10 min. The highest daily maximum was recorded as the monthly extreme ('acute') high temperature at each site. The average of the daily maxima was calculated as a measure of 'chronic' high-temperature exposure. Similarly, average daily minima and monthly minima were calculated.
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strombine (Loomis *et al.* 1988; Loomis & Zinser 2001) and glucose (Gionet *et al.* 2009). Calcium also acts as a cryoprotectant by binding to cell membranes and reducing cell damage during freezing either by physical stabilization of the membrane against mechanical disruption caused by cell shrinkage, or by prevention of the denaturation of membrane compounds (Ansart & Vernon 2003). Another mechanism to avoid intracellular ice formation – an invariably lethal process – is the production of ice-nucleating proteins that are secreted into the ECF and act to induce and control extracellular ice formation. These proteins reduce undercooling from the range −15 to −20°C to the range −5 to −10°C. *G. demissa*, a freeze-tolerant, saltmarsh mussel that is regularly exposed to sub-zero winter temperatures for extended periods of time during low tides, lacks such proteins, but instead it utilizes at least one strain of ice-nucleating bacteria, *Pseudomonas fulva*, from seawater (Loomis & Zinser 2001). Ice formation occurs in the pallial fluid, which does not supercool to temperatures lower than −1°C before nucleation occurs; the mussel can survive temperatures as low as −14°C (McCorkle 2009 and references therein).

Various factors affect the ability of bivalves to tolerate cold exposure at low tide in winter. For example, high winter mortality of the notata strain of the hard clam *M. mercenaria*, in the north-west Atlantic is mainly a result of the animal's inability to accumulate sufficient lipid reserves, and to synthesize enough cryoprotectant glucose, in comparison to the native *M. mercenaria* (Gionet *et al.* 2009). Major differences in triglyceride (TAG) metabolism during overwintering were observed between the cold-adapted mussel, *M. edulis*, and the warmer-water oyster species, *C. virginica* (Pernet *et al.* 2007). Mussels used TAG stores for energy metabolism or reproductive processes during winter, while oysters did not accumulate large TAG stores prior to overwintering. Mussel TAG contained high levels of 20:5n–3 compared to levels in oysters, and this difference may help to counteract the effect of low temperature by reducing the melting point of TAG, thus increasing the availability of storage fats at low temperature. The physiological responses of bivalves to temperature change are dealt with in more detail in Chapter 7.

Salinity

Bivalves on fully marine shores experience normal (~35 psu) salinity most of the time. Tide pools high on the shore experience wide fluctuations in salinity through evaporative water loss or freshwater inflow. But, for the majority of the shore, these effects are minor. Estuaries, however, place significant limits on species distributions. Here mean salinity decreases and salinity variation increases with distance upstream, and both these factors have deleterious effects on bivalve distribution with the result that species diversity is significantly less in estuaries than on fully marine shores. Oysters show a remarkable tolerance to low salinity and salinity variation. For example, the optimum salinity range for *C. virginica* is about 14–28 psu but this species can survive a salinity of 2 psu for a month, or even freshwater for several days when water temperatures are low. At very low salinity recruitment is poor and growth is slow. High salinities of 35–40 psu are also tolerated, but oysters usually show poor growth and do not reproduce (references in Shumway 1996). Surprisingly, juveniles have the same salinity range as adults, and a similar physiological response to suboptimal salinities. The Pacific oyster *C. gigas* is also euryhaline, with an optimal salinity range of 20–25 psu, although the oyster can occur at salinities below 10 psu, and will survive at salinities in excess of 35 psu, although it is unlikely to breed (Helm *et al.* 2004). A number of candidate genes as potential markers of tolerance to hypoosmotic stress have been recently identified in *C. gigas* (Zhao *et al.* 2012).

The mussel *M. edulis* is found from fully marine conditions to salinities as low as 4–5 psu, while the lowest salinities tolerated by *M. galloprovincialis* and *M. californianus* are 12 and

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17 psu, respectively (references in Bayne *et al.* 1976). For mussels, habitat salinity may determine the outcome of competition between native and invasive species. For example, *Brachidontes pharaonis* has increasingly been observed in intertidal habitats in the Mediterranean Sea, where it is outcompeting the indigenous mussel, *Mytilaster minimus*. Sarà and de Pirro (2011) investigated the response of the two species to varying salinity and found that the native species adapted to a very narrow salinity range, as found in the superficial waters of the Mediterranean Sea, showed clear signs of stress (measured as an increase in heart rate) at salinities in the range of 37–45 psu. However, the invasive mussel displayed signs of stress only at salinities over 45 psu, and survived salinities as high as 75 psu, indicating that this species has the potential to invade not just the warm and salty Mediterranean Sea basin but also transitional coastal environments such as brackish and hyperhaline habitats.

Wave exposure

Wave exposure, through both wave force and changes in immersion patterns, has a powerful influence on patterns of zonation and abundance on rocky shores. Lack of tolerance to high wave forces may limit species composition in this habitat, but because intertidal zonation patterns are driven by emersion time, wave action tends to extend biological zones vertically (upshore) by effectively decreasing the frequency and duration of emersion. High wave action will therefore cause a point on the shore to behave as if it is effectively lower than its stillwater tidal height (Gilman *et al.* 2006 and references therein). A method for measuring effective shore level (ESL) has been used to compare the effects of wave action on immersion patterns within multiple sites on the Pacific coast of North America (Harley & Helmuth 2003; Gilman *et al.* 2006).

The bivalves on wave-exposed shores are primarily mussels (*Mytilus* and *Modiolus*), often forming dense, single-species zones. Mussels on these shores are subjected to high water velocities from breaking waves, often as high as 25 m s^{-1} when waves interact with the local topography of a shore (Denny *et al.* 2003). Intuitively, one might expect that when higher water velocities are encountered mussels would produce more byssal threads to increase strength of attachment. However, this is not the case, as shown by Moeser *et al.* (2006), who reported that in flume experiments mussels (*M. edulis*) significantly reduced thread production at water velocities above 15 cm s^{-1}. Similar findings were reported in a subsequent study on four mussel species: *M. trossulus*, *M. galloprovincialis*, *M. californianus* and *Modious moldiolus* (Carrington *et al.* 2008). For all four species, velocities above 20 cm s^{-1} visibly hindered the mussel's ability to extend its foot beyond the margin of the shell, a posture that must be held for several minutes in order to mould and attach a new thread to the substrate. Even in the exposed shore species, *M. californianus*, velocities above 30 cm s^{-1}precluded thread formation. Carrington *et al.* (2008) found that flow was greatly ameliorated within mussel aggregations, ranging from 0.1 to 10% of free-stream velocity, thus explaining why mussels can persist on shores with water flows in excess of their physiological limits.

Wave action also has a controlling influence on mussel bed communities by causing dislodgement through lift and drag, especially when mussel beds are dense and firmly packed, as on the majority of wave-exposed shores (Figure 3.1). When dislodgement occurs new space is created for colonisation. The risk of dislodgement in *M. edulis* increases with flow speed and mussel size and decreases with mussel tenacity, or attachment strength; the latter varies twofold during the year, but this cycle is not aligned precisely with seasonal patterns of wave velocity (Carrington 2002). A recent study has shown that mussels

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(*M. galloprovincialis*) on rocky shores allocating resources to reduce risk of dislodgement (smaller, thicker shell, stronger byssal threads) instead of to growth and reproduction (Babarro & Carrington 2013). Several studies have used a mechanistic model to predict frequency and severity of mussel dislodgement. For example, using inputs of wave height and mussel attachment strength on wave-exposed shores on Rhode island, United States, over a 3-year period, the model correctly predicted strong dislodgement events during hurricane season (August to October) when large waves coincide with relatively weak mussel attachment (Carrington *et al.* 2009). The mortality rate from wave dislodgement was approximately 30%, a value similar to the mortality rate from temperature stress.

Mussel aggregations provide a habitat for a diverse assemblage of organisms, some of which attach directly to the mussel shells (epibionts). Mussels with protruding epibionts, for example kelp and barnacles, are more likely to become dislodged due to higher drag-induced loading (O'Connor *et al.* 2006). When mussels were artificially fouled, attachment strength increased by 7%, and by 17% during winter (Garner & Litvaitis 2013). Attachment strength and position within mussel beds were measured in two mussel species: *M. edulis* and *M. galloprovincialis* (Schneider *et al.* 2005). Results indicated that *M. edulis* moved more frequently and more quickly to the exterior of simulated mussel beds than did *M. galloprovincialis*. Strength of attachment was weaker in *M. edulis* and this, coupled with large hydrodynamic forces at the bed exterior, led to higher dislodgement rates, and ultimately higher mortality, in *M. edulis* than in *M. galloprovincialis*. In areas where the two species hybridise, such as south-west England and the Atlantic coasts of Ireland, movement behaviour could play a significant role in driving patterns of selection by exposing mussels to different conditions of wave exposure (Schneider *et al.* 2005). However, a survey of 20 sites on Irish coasts has indicated no apparent advantage for the *M. galloprovincialis* genotype at exposed shore locations (Gosling *et al.* 2008).

Other factors

A glance at Tables 3.1, 3.2 and 3.3 will show that most bivalves have a 'preference' for a particular substrate. Oysters prefer hard rock, shell or sand bottoms and will not settle on muddy bottoms. The majority of scallops occur on hard substrates of gravel and coarse to fine sand. The association of scallops with this type of substrate is so strong that high-resolution acoustic bottom discrimination techniques are used to provide more precise estimates of scallop abundances (Brand 2006 and references therein). Such substrates are typical of areas with a strong current flow. Clams with their burrowing lifestyle have a preference for softer substrates of sand or mud, or sand/mud mixtures. Surveys of densities and abundances of the hard clam, *M. mercenaria*, in Chesapeake Bay, United States, reported highest numbers in sand (68%), moderate numbers in shell (11%) and mud (21%), and fewest numbers in anoxic mud (<1%), indicating that substrate type is a significant factor in the distribution of this species in the bay (Mann *et al.* 2005).

Muddy bottoms are characteristic of areas with poor current flow. Overlying water tends to be turbid due to suspended material of fine insoluble particles, either inorganic (clay, silt and sand) or organic (industrial or domestic waste). Oxidation of organic waste just above the sediment can reduce oxygen levels, especially in summer when water temperatures are high. For example, anoxic water events in conjunction with high temperatures in summer are believed to be one of the causes of decline in natural oyster (*C. virginica*) reefs in Mobile Bay, Alabama, United States (Ueda *et al.* 2009). Anoxic conditions are a major source of mortality for scallop spat (*M. yessoensis*) when they move from byssal attachment sites to life on the seabed (Yamamoto 1960). Survival is increased if alternative sites, such as algae

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or bryozoans that are raised off the bottom, are available. It is clear that a lack of such sites is a major factor in determining local distribution of this species. High turbidity is not detrimental to all bivalves. For example, evidence from both laboratory and field trials show that the surf clam *Spisula subtruncata* grows well in environments with a high sediment load. This is because the species is extremely efficient in selectively rejecting sediment particles so that only a small fraction of filtered algae is lost in pseudofaeces (Kiørboe & Møhlenberg 1981). In contrast, in another clam, *M. mercenaria*, algal ingestion decreases significantly with increasing sediment load (Bricelj & Malouf 1984), thus explaining the low abundance of this species on muddy bottoms (see earlier).

The importance of large-scale ocean currents in the global distribution of bivalves has already been dealt with. Locally, areas with strong currents usually provide favourable feeding conditions for bivalves. However, very strong currents can have an inhibitory effect on feeding and, consequently, growth. This has been demonstrated experimentally in the scallops *P. magellanicus*, *A. irradians* and *C. islandica* (Brand 2006 and references therein). In addition, strong currents may prevent larval settlement and byssal attachment of spat, ultimately resulting in local variability in recruitment. More data are needed to assess the importance of high water velocity as a factor in the local distribution of bivalves.

Finally, physical factors, apart from wave action, that effectively remove animal and plant communities from the substrate play a minor role in influencing abundance, that is patchiness at the local level. These disturbance factors include scouring by ice, storms that overturn boulders, wave-propelled logs and fishing gear. Most of the information on the effects of, and recovery from, disturbance come from studies on *Mytilus* beds and their associated flora and fauna on exposed shore sites on the Pacific and north-east Atlantic coasts of North America (Seed & Suchanek 1992 for review; Svane & Ompi 1993; Wootton 1993; Beukema & Cadee 1996; Carroll & Highsmith 1996; Hunt & Scheibling 1998; Hunt & Scheibling 2001; Bertness *et al.* 2002; Guichard *et al.* 2003; Calcagno *et al.* 2012). Minchinton *et al.* (1997) monitored the recovery of intertidal algae and sessile macrofauna after a rare occurrence of scouring sea ice denuded the intertidal area of an exposed rocky shore in Nova Scotia, Canada. They found that barnacle cover was restored soon after the ice-scour, macroalgae cover in about 2 years, but that it took considerably longer (4–6 years) for the mussel beds to recover. Similar results have been reported by Brosnan and Crumrine (1994), who trampled 250 experimental plots in the intertidal zone for a period of 1 year and then monitored recovery. The algal–barnacle community recovered in the year following trampling but mussel beds had not recovered in the 2 years following cessation of trampling.

Wave or log damage occurs mostly during the winter and is typically responsible for removing 1–5% of *M. californianus* cover per month on exposed shores (Paine & Levin 1981). The initial size of disturbance gaps can range from single-mussel size to areas as large as 60 m^2. Subsequent enlargement of the gap (as much as 5000%) may occur, especially during winter months, primarily due to weaker byssal thread attachments (Witman & Suchanek 1984).

Fishing methods can affect bivalve abundance directly by causing significant mortality, and, indirectly, by causing shell damage. For example, large numbers of razor clams (*Ensis*) were either killed or damaged by dredging operations in a sandy bay in Scotland (Eleftheriou & Robertson 1992; Robinson & Richardson 1998). Damage to shell valves slows down the clam's escape digging response, and thereby renders it more vulnerable to predatory attacks by crabs and fish. In Spain, mussel seed from intertidal exposed rocky shores is the method most used by farmers to seed ropes in mussel culture areas (Peteiro *et al.* 2007). This practice, while legal, must have a detrimental effect on mussel beds and their community structure, although to date there is no documented evidence of damage.

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Biological factors

Just as humans greatly appreciate the delicate flavour of bivalves so also do a whole range of other organisms from groups as diverse as fish, birds, mammals, crustaceans, echinoderms, flatworms and even other molluscs. Bivalves are usually preyed upon by several of these, which operate at specific times of the year, and which generally focus on the smaller size classes. Predators are probably the single most important source of natural mortality in bivalve molluscs and have the potential to influence population size structure in addition to overall abundance and local distribution patterns.

In this section the main predators of bivalves will be dealt with, along with major pests, fouling organisms and competitors. Most of the information comes from studies in intertidal and shallow water environments. For the sake of simplicity the groups of mussels, oysters, clams and scallops will be treated separately.

Predators, pests and competitors

Mussels

Gastropods are significant predators of mussels worldwide. The dogwhelk *Nucella lapillus* is widely distributed on exposed shores in northern Europe and on the east coast of North America, where it feeds extensively on barnacles and small mussels. Predation is often seasonal with whelks remaining aggregated in pools and crevices over the wintertime. However, numbers on mussel beds on the low and mid shore start to increase in the spring, and densities as high as 300 whelks m^{-2} have been recorded over the summer months in north-east England (Seed 1969). Profitability (energy assimilated from a food item relative to handling time) for dogwhelks feeding on mussels increases with prey size (Hughes & de Dunkin 1984). Yet whelks prefer mussels smaller than the largest available. Hughes (1986) suggests that dogwhelks choose mussels with the maximum average profitability in the face of competition from other dogwhelks that are attracted to the predator by olfactory stimuli from the damaged prey. The whelk uses the radula to drill a small hole through the thinnest part of the shell around the umbone or adductor muscle insertion regions (Seed 1976), or though the shell area overlying the glycogen-rich digestive gland (Hughes & de Dunkin 1984). Prior to drilling, the whelk softens the area using a secretion from the foot. The proboscis is inserted through the hole and the flesh of the prey is rasped away by the radula, and then devoured. Alternatively, a more efficient mechanism that is used by experienced whelks is where the predator inserts its proboscis through the valve gape and induces muscular paralysis by injecting toxins. Feeding rates (drilling plus ingestion times) peak during the summer, but as water temperatures fall through the autumn the time needed for ingestion lengthens, more than tripling the total handling time (Miller 2013). Prey handling time per mussel is generally in the range of 2–3 days, which agrees well with results from laboratory experiments that showed that an adult whelk can consume about two mussels (1–3 cm shell length) per week during the summer (Seed 1969). Although this level of consumption may appear small, the high density of foraging whelks makes a serious impact on mussel coverage on exposed shores. For example, on rocky intertidal sites in Alaska, United States, where *Nucella lima* occurred at densities of greater than 100 m^{-2}, Carroll and Highsmith (1996) estimated that the whelk can eliminate 60–90% of mussels (*M. trossulus*) at a given site in one season. Preference for mussels, as opposed to barnacles, appears to be fixed in early life, that is adult whelks transferred from sites with no mussel cover to those with a high coverage of mussels largely ignore mussels, preferring to feast on barnacles (Wieters & Navarrete 1998). Mussels 'fight' back by ensnaring and immobilising whelks in their byssus threads. An ensnared whelk can be overturned, thus arresting the drilling process

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Figure 3.8 Mussels, *Mytilus edulis*, attach a number of byssus threads to the body whorl of the predator gastropod *Nucella lapillus*. The threads are retracted, thus flipping the predator over and immobilizing it, thereby exposing the whelk to crab predation.
Photograph by Peter Petraitis, University of Pennsylvania, Philadelphia, United States. Reproduced with permission.

(Figure 3.8). Whatever the fate of the mussel, the whelk, once ensnared, is trapped and exposed to crab predation (Petraitis 1987; Davenport *et al.* 1998; Chiu *et al.* 2011). On the west coast of the United States, *Nucella canaliculata* and *Nucella emarginata* are major predators of mussels but seem to favour the thinner shelled species, *M. galloprovincialis*, as opposed to the thicker shelled and less nutritious *M. californianus* (Suchanek 1981). Other predatory gastropods such as *Ocenebra poulsoni*, *Acanthina sopirata*, *Ceratostoma nuttalli* and *Jaton festivus* also feed on *Mytilus* (Shaw *et al.* 1988).

Starfish are also important predators influencing the distribution and abundance of mussels on the lower shore and in the sublittoral zone. Starfish predate on mussels and other bivalves either by using force or by secreting an anaesthetic from their stomach that numbs the bivalve and causes it to gape. The starfish then extrudes its stomach through its mouth into the shell opening and digests the prey. There are numerous studies of the starfish–bivalve interaction on rocky shores, primarily because of ease of observation and manipulation. Such studies have led to a greater understanding of the causes of zonation, and have provided additional evidence on size and spatial refuges in bivalve populations (Dame 2012). On the Pacific coast of the United States the mussel *M. californianus* exists as a well-defined band on rocky intertidal shores. The starfish *Pisaster ochraceus* is a major predator of this mussel (Figure 3.9). In a series of classic studies, removal of starfish over a 10-year period produced marked changes in zonation patterns, that is there was a notable downward shift in the lower limit of mussel distribution of about 2 m through redistribution of adults and normal settlement of mussel larvae (Paine 1974). Consequently, the starfish has been regarded as a 'keystone' predator, one which through its feeding activities exerts a disproportionate influence on community structure, in this case setting the lower limits of mussel distribution. In a keystone predator-dominated system other invertebrate predators have minor effects on community structure, but in the absence of the keystone predator, such species may adopt a major role in the altered system (Navarrete & Menge 1996).

Until recently, the accepted explanation for the distinct zonation patterns on wave-exposed rocky shores has been that dense populations of sedentary organisms, such as mussels, form

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Figure 3.9 The sea star *Pisaster ochraceus* predating on mussels, *Mytilus californianus*, on the US Pacific coast.
Photograph by David Cowles http://rosario.wallawalla.edu/inverts. Reproduced with permission.

in static prey refuges above the reach of natural predators. Robles *et al.* (2009) showed that prey refuges are not in fact static. On the west coast experimental alteration of starfish (*P. ochraceus*) densities caused the downward extension of the lower boundaries of the mussel *M. californianus*, while experimental increases in starfish densities caused the upward recession of the lower boundary well into the zone presumed to be a spatial refuge for mussels from predation. As small mussel prey are depleted by starfish over time, larger mussels are attacked, including those mussels that would otherwise represent the lower boundary of their distribution.

In northern Europe, *Asterias rubens* is a serious predator of *M. edulis*. This starfish aggregates seasonally on mussel beds in large numbers, sometimes as high as 450 m^{-2}, often completely destroying local mussel populations (Dare 1982). In contrast to the results of laboratory-based experiments, *A. rubens* shows no size selectivity when feeding in the field. The solid structure of interconnected mussels forming the bed, however, restricts predation to only those mussels situated at the bed surface, thus providing a refuge from predation for smaller mussels deeper down (Dolmer 1998). Not all mussels are equally susceptible to starfish predation. About 70% of *M. edulis* of North Sea origin were able to resist *A. rubens*, whereas all Baltic mussels, presumably *M. trossulus*, were opened within 1 hour (Norberg & Tedengren 1995; see also Lowen *et al.* 2013). *M. edulis* cultured in close vicinity to *A. rubens* was significantly smaller in shell length, height and width but had significantly larger posterior adductor muscles, thicker shell and more meat/shell volume. These morphological changes have an adaptive value in that predator-exposed mussels have a significantly higher survival rate than unexposed mussels (Reimer & Tedengren 1996). Behavioural changes were also evident; predator-exposed mussels in the laboratory formed larger aggregates, migrated less and sought structural refuges more often (Reimer & Tedengren 1997). Chemical aspects of epibionts, such as barnacles, algae, sponges and hydrozoans, on mussel (*M. edulis*) shells also have the potential to modify the top–down control by starfish (*A. rubens*) by changing or masking prey properties the starfish cues upon, or by producing their own repellants (Laudien & Wahl 2004). This may explain why, in an earlier study, *A. rubens* was reported to prefer clean subtidal mussels over barnacle-overgrown intertidal ones (Saier 2001).

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Crabs (*Cancer*, *Carcinus* and *Pachygrapsus*) are also significant predators of mussels on the lower shore and sublittoral zone. Their effect on mussel abundance is seasonal, with reduced predation in winter when crabs migrate offshore. Results from laboratory and field experiments show that crabs employ size selection of prey, with the upper size limit that can be opened being directly related to the size of the crab (references in Seed 1976). Crabs will almost always choose small-sized prey when offered a range of sizes. It is handling time rather than the energetic costs of handling, estimated as a mere 2% of corresponding gains, that is the basis on which foraging crabs select their prey (Rovero *et al.* 2000). During the handling period the crab is at risk from other predators, competitors and even claw damage. Small mussels are therefore particularly vulnerable to predation as they are easily crushed by most size classes of crabs. A mussel must attain a shell length of at least 45 mm before it is relatively safe from crab predation. Once again, mussels show several defence mechanisms. In laboratory experiments *M. edulis* increases byssus volume in response to water-borne cues from *Cancer pagurus* and *Carcinus maenas* (Cote 1995; Leonard *et al.* 1999). Similar findings were reported when mussels, for example *P. viridis*, were exposed to the crab *Thalamita danae* that had recently consumed conspecifics (Chiu *et al.* 2011). In addition, mussels subjected to heavy predation develop thicker and more robust shells in response not just to crabs but also to the broken shells of other mussels (Leonard *et al.* 1999; see also Freeman & Byers 2006, and comments from Rawson *et al.* 2007 and Freeman & Byers 2007). Similar effects have also been reported in mussels subjected to heavy whelk predation (Smith & Jennings 2000). A behavioural strategy in response to crab predation has been reported in the Wadden Sea, Germany, where oysters (*C. gigas*) have invaded native mussel (*M. edulis*) beds (Eschweiler & Christensen 2011). Mussels subjected to direct contact with crabs (*C. maenas*) migrated from the top of the oyster reef to interspaces at the bottom of the reef, where they showed significantly reduced growth rates and conditions than mussels on the top of the reef. Mussels experience a trade-off between survival and food supply, preferring to take refuge from predation even when this decreases growth and condition.

Several bird species are predators of mussels. The main ones in western Europe are the oystercatcher *Haematopus ostralegus*, the common eider duck *Somateria mollissima* and the herring gull *Larus argentatus*, although the latter only feeds on small mussels on newly established beds. Of these, the most significant predator is the oystercatcher (Figure 3.10). Predation is seasonal, with birds switching in the spring from mussels (and cockles) to deep-living clams such as *Scrobicularia plana* and *Macoma balthica*, and back to surface bivalves in autumn in order to maximise intake rate (Zwarts *et al.* 1996a). The birds cannot survive if their diet is restricted to one or two prey species; they need to switch between three or four, and have to roam over feeding areas measuring at least some tens of square kilometres. Oystercatchers open mussels by stabbing into gaping mussels or prising open closed ones, or by hammering a hole in either the dorsal or ventral shell (Goss-Custard *et al.* 1993). They invariably select thin-shelled mussels to hammer through because they are easier to crack than thick-shelled mussels; it is the thickness of the prismatic layer that largely determines the vulnerability of mussel shells (Le Rossignol *et al.* 2011). All birds show size selection within the prey species; this is because flesh content increases more steeply with prey size than handling time (Zwarts *et al.* 1996b). Removal of the largest mussels may reduce protected refuge for younger mussels, but may also allow younger mussels to grow at a faster rate, although, as already mentioned, gulls preferentially prey on small mussels (Goss-Custard *et al.* 1996).

Different diving duck species, including eiders (*Somateria* spp.), scoters (*Melanitta* spp.) and scaups (*Aythya* spp.), also predate extensively on mussels. In the case of eiders, mussels often constitute as much as 60% of their diet (Nehls & Ruth 1994). Eiders select mussels of smaller than optimal size, because this minimizes shell ingestion, even though larger

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Figure 3.10 The American oystercatcher, *Haematopus palliatus*, a significant predator of bivalves, eating a clam.
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available prey would provide greater net energy gain per prey item (Hamilton *et al.* 1999). In the process of zoning in on their prey the ducks may remove whole mussel clumps, thus causing mussel mortality over and above that produced by direct predation (Raffaelli *et al.* 1990). Mussel farms, with their very high densities of small thin-shelled mussels, can be foraging hot spots for diving ducks, particularly during spring and autumn when birds are building up their energy reserves for reproduction, migration or overwintering. For example, in spring 2011 in Baie de Chaleurs, Quebec, Canada, all mussel growers were severely hit by scoter predation losing almost all their collectors and over 30% of their 1–2-year-old mussels on ropes (Varennes *et al.* 2013). Acoustic and visual deterrents have been tried with little success (Dionne *et al.* 2006). Currently, in Canada, the United States and Europe, the only effective method that provides a complete and long-term control of bird predation in culture facilities is the use of exclusion nets deployed around longlines and rafts of suspended mussel ropes (Varennes *et al.* 2013).

Numerous studies have been undertaken to provide actual data on the impact of oystercatchers and other bird predators on commercial mussel beds. Analysis of faeces or regurgitated pellets provides information on the sizes of mussels selected by the bird species. In the laboratory the ash-free dry weights (AFDW) of different sizes of mussels are determined in order to calculate the biomass eliminated (Hilgerloh 1999). In a sheltered bay in the Wadden Sea, Netherlands, mussel biomass was estimated at 1300 g AFDW m^{-2}. Birds annually removed 30% of the standing stock (Nehls *et al.* 1997). Eiders were by far the most important predators and consumed 346 g AFDW m^{-2} (27%); these were followed by oystercatchers with 28 g AFDW m^{-2} and herring gulls with 3.6 g AFDW m^{-2}. However, as other predators were absent, mussel production was sufficiently high to sustain such a high predation rate. In a separate study on tidal flats on the German coast, annual consumption of mussels in an area of 5.2 km^2 representing 311 t AFDW by these same predators was 165 t AFDW (Hilgerloh 1997). This time, however, the highest proportion of total consumption was by oystercatchers (54%), while eiders consumed 39% and herring gulls 7%.

Other birds that feed on mussels include knots, *Calidris* spp. (Alerstam *et al.* 1992), and crows, *Corvus* spp. (Berrow *et al.* 1992a). Indeed crows are significant predators of mussels

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in the intertidal zone and show several interesting adaptations. The birds frequently cache mussels during low tide, and recover them during high tide some 2–3 days later. This behaviour is believed to be a response to short-term, daily fluctuations in food availability (Berrow *et al.* 1992a). In order to break them open the crows drop mussels and other hard-shelled prey onto hard surfaces, for example road or rocky shore (Berrow *et al.* 1992b). This behaviour peaks during October–February and usually involves only large-sized mussels, no doubt an adaptation to food shortages in winter.

On the west coast of North America the sea otter *Enhydra lutris* is an important predator of *M. californianus*. This species removes large clumps of mussels, which it sorts and consumes on the sea surface by pounding the mussels on a flat stone on its chest, or against other mussels. So, although sea otters are selective in terms of the size of prey they consume, they have profound effects on all size classes of mussels (Seed & Suchanek 1992). In addition, otters have substantial, indirect effects on the biomass of mussel bed–associated communities. The total biomass of species associated with mussel beds was found to be more than three times higher where otters were absent (Singh *et al.* 2013). Other predators of mussels include sea urchins (*Strongylocentrotus droebachiensis*), lobsters (*Panulirus interruptus* and *Homarus americanus*), flatfish (*Platichthys flesus*, *Pleuronectes platessa* and *Limanda limanda*) and seals, walruses and turtles (see Seed & Suchanek 1992 for references).

The most common pests of bottom-dwelling mussels are shell-burrowing sponges (*Cliona* spp.), polychaetes (*Polydora* spp.) and pea crabs (*Pinnotheres* spp.). The detrimental effects of pea crabs and boring polychaetes are described in Chapter 11, while those of boring sponges are covered here in the section on oysters.

Bivalves provide an excellent substrate for the settlement of many fouling organisms. Fouling appears to be a significant cause of mortality in intertidal mussels mainly due to dislodgement caused by the increased weight, especially by barnacles and seaweed. Fouling is a particular problem in suspended mussel culture and almost 100 invertebrate species, including gastropods, crustaceans, bivalves, polychaetes, ascidians, sponges and hydroids have been identified on mussel ropes (Hickman 1992). These organisms cause reduced growth and productivity through competition for space, but are not a major cause of mortality in suspended culture.

Mussels are the most prominent competitors for space in mid to low-shore areas on gently sloping rocky shores, but on steeper shores mussels tend to be replaced by barnacles or algae. Generally where two species coexist there is competition but rarely elimination of one by the other. There are a few notable exceptions. *M. galloprovincialis* was accidentally introduced on the west coast of South Africa in the late 1970s, where it has progressively replaced the indigenous, slower-growing mussel, *Aulacomya ater*, from wave-exposed shores (Branch & Steffani 2004). In the 1980s *M. galloprovincialis* rapidly spread onto the south and east coasts, where it competes with the indigenous mussel, *P. perna*. *M. galloprovincialis* is immune to the trematode parasites that are common in *P. perna* and that reduce both growth rates and reproductive output by castrating infected females (Calvo-Ugarteburu & McQuaid 1998). *M. galloprovincialis* has not yet completely replaced *P. perna*. Instead, the two species are spatially segregated with *P. perna* dominating the low shore and *M. galloprovincialis* the high shore, with an overlap zone between the two (Robinson *et al.* 2005; Rius & McQuaid 2006). *M. galloprovincialis* has had little effect on another indigenous mussel, *Choromytilus meridionalis*, because the latter favours silted areas whereas *M. galloprovincialis* is excluded by silt. Relative to the three native species *M. galloprovincialis* has a faster growth, a higher reproductive output and a higher tolerance to exposure to air (Figure 3.11). Another example of interspecific competition, and one that has been dealt with in an earlier section, is the contribution of *M. galloprovincialis* to the

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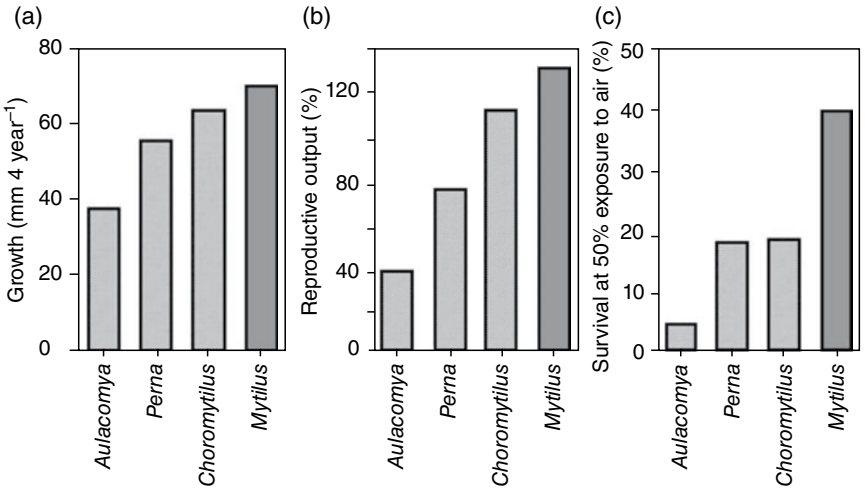


Figure 3.11 The performance of three indigenous mussels, *Aulacomya ater*, *Perna perna* and *Choromytilus meridionalis*, relative to *Mytilus galloprovincialis*: (a) growth rate (mm in the first 4 years); (b) total annual reproductive output as a percentage of body mass; (c) survival rate after 24 months at a shore height experiencing 50% exposure to air per tidal cycle.
Data from Hockey and Van Erkom Schurink (1992); Van Erkom Schurink and Griffiths (1993). From Branch and Steffani (2004). Reproduced with permission from Elsevier.

displacement of *M. trossulus* along much of its historic range in southern California (Shinen & Morgan 2009). Other examples of interspecific competition are those between *M. californianus* and the seapalm *Postelsia palmaeformis* on the Pacific coast (Dayton 1973; Blanchette 1996), and between *M. galloprovincialis* and the indigenous limpet *Scutellastra argenvillei* on the west coast of South Africa (Steffani & Branch 2005). In general, however, intraspecific competition for space is a more serious problem than interspecific competition in that heavy spatfall of mussels onto adult beds can cause the underlying mussels to suffocate, thus loosening the entire population from the rock surface (Seed 1976).

Oysters

Gastropods are also the main predators of oysters. For *C. virginica* these are the drills *Urosalpinx cinerea*, *Eupleura caudata* and *Stamonita haemastoma*. *U. cinerea* inhabits the intertidal and sublittoral zone to a maximum depth of 15 m along the east coast of North America, and has been introduced with oysters onto the west coast, along with the Japanese oyster drill, *Ocenebra inornata*, where they both prey on the Olympia oyster *O. lurida* (Buhle & Ruesink 2009). *E. caudata* has much the same distribution as *U. cinerea* but is not as abundant. *S. haemastoma* is found along the south-eastern and Gulf coasts of the United States. Like *Nucella* these species use their radula to drill, but in between drilling they 'soften' the shell using acidic secretions from the foot. An adult oyster drill is capable of consuming up to three small oysters per week. While *S. haemastoma* is a significant predator of oysters, especially spat, if presented with a choice it will select mussels in preference to oyster spat (see White & Wilson 1996 for further details on the biology of oyster drills). Another gastropod predator is the lightning whelk, *Busycon contrarium*, which has been responsible for serious oyster mortality (as high as 80%) in Florida. The predator attacks oysters, and also clams, by chipping at the shell margin and wedging the valves apart with its foot so that it can insert its proboscis (Menzel & Nichy 1958; Nichy & Menzel 1960).

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In Europe *Ocenebra erinacea and U. cinerea* (introduced from the United States) are the main predatory gastropods. The latter, and other species of *Urosalpinx*, feed mainly on oysters but also attack mussels, cockles and barnacles, and in the United States clam species can be added to this list (Hancock 1960). *Ocenebra* is a much less serious predator of oysters than *Urosalpinx* as it appears to prefer mussels to oysters.

Several different methods are used to try and control gastropod predators on oyster beds. In the past a certain sum of money was paid to fishermen for every *Urosalpinx*, *Ocenebra* and *Crepidula* (a competitor of oysters; see later) that was collected on oysters beds in England (Hancock 1960). This strategy was later replaced by physical methods such as trapping with baited traps or burying, neither of which is really effective as control methods (White & Wilson 1996). Chemical methods have been tried, for example copper barriers around oyster beds, and pesticides. The former does not deter pelagic larvae of predator species from settling on oysters, and the latter kill not only the predator but also other benthic organisms, so their use is tightly regulated. Biological methods, where either parasites or predators of drills are used, have so far been ineffective. Overall, destroying oyster drill egg capsules appears to be much more effective than removing adults (White 2007). The drills lay their eggs in the cracks and crevices of clusters of oyster shells, and can be removed using a screwdriver. It is important to time this control measure with the egg-laying cycle, usually between April and July, for *U. cinerea* and *O. inornata* at sites on the Pacific coast, United States. Catching adults just before egg-laying is another effective control measure, but it is a very time-consuming and labour-intensive process (Heimbigner 2012). See the Ruesink Lab link on research in progress on oyster drill control (http://depts.washington.edu/jlrlab/oysterdrills.php).

Several crab species cause significant mortality of spat and juvenile oysters. On the Atlantic and Gulf coasts of the United States the main predators of *C. virginica* are the stone crab *Menippe mercenaria*, the mud crab *Panopeus herbstii* and the blue crab *Callinectes sapidus*. Stone crabs possess large and heavy claws (chelae) and thus are capable of crushing even market-size oysters. Typical annual consumption is about 200 oysters per crab (Menzel & Hopkins 1956). *P. herbstii* is a constant and serious threat for oyster farmers because of its wide salinity tolerance (10–34 psu), the large tooth on the major crushing chela that allows it to open oysters of a larger size than other crab species of similar carapace width and the fact that it does not migrate offshore in winter (Bisker & Castagna 1987). This species can consume as many as 20 oysters per day. Similar consumption rates have been reported for the blue crab *C. sapidus* preying on *C. virginica* (Menzel & Hopkins 1956), although the Suminoe oyster *C. ariankensis*, under consideration for introduction into Chesapeake Bay, United States, shows greater susceptibility due to this predator due to a lower shell compression strength value (64%) compared to *C. virginica* (Bishop & Peterson 2006; Newell *et al.* 2007). A detailed study of the foraging behaviour and predator–prey dynamics of *C. sapidus* has been published by Eggleston (1990). Predation of juvenile (*C. virginica*) spat by mud crabs is a major contributor to early juvenile spat mortality, thereby hindering natural recruitment and restoration efforts in Chesapeake Bay, United States (Chapter 8). In laboratory experiments the flat mud crab, *Eurypanopeus depressus*, consumed nearly 40% of the spat offered, whereas the white-fingered mud crab, *Rhithropanopeus harrisii*, consumed less than 10%; for a size range of 1–29 mm spat, approximately 75% of spat preyed on were less than 8 mm (Kulp *et al.* 2011). An interesting experiment in which the red rock crab, *Cancer productus*, was offered four prey items, the oysters *C. gigas* and *O. lurida*, and the oyster drills *U. cinerea* and *O. inornata* (Grason & Miner 2012), is an example of intraguild predation, where crabs are predators of drills and compete with them for oyster prey. Crabs consumed drills and oysters at approximately the same rate when only one type of prey (either oyster or drill) was offered.

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However, when crabs were allowed to choose among three prey items (the two drill species and juvenile *C. gigas*) they showed a strong preference for oysters over drills.

Like mussels, the oyster *C. virginica* dramatically shifts from lateral shell growth to shell thickening in the presence of its major predator, the oyster drill *U. cinerea* (Lord & Whitlatch 2012). Similar results were reported for this oyster when exposed to the mud crab *R. harrisii* as for the shell strength of *C. virginica* and the introduced Siminoe oyster, *Crassostrea ariakensis*, exposed to the blue crab *C. sapidus*, although the shell of *C. ariakensis* remained 57% weaker than the shell of *C. virginica* (Newell *et al.* 2007). However, ocean acidification negatively affects the shell calcification process (see later), and therefore bivalves may lose the ability to protect themselves adequately against predators (Amaral *et al.* 2012).

Other predators of oysters include shrimp (Pit & Southgate 2003; Weerman *et al.* 2014), oystercatchers (Tuckwell & Nol 1997a, b), starfish, fish (Anderson & Connell 1999; Brown *et al.* 2008) and polyclad flatworms. Polyclad larvae (*Stylochus* spp.) settle in high densities onto oyster (*C. virginica*) beds, where they grow rapidly to adult size (25–50 mm length). The worms enter oyster spat through the gaping valves, eat the flesh and are responsible for high mortality, especially under crowded culture conditions (Provenzano 1961; Newell *et al.* 2007).

Similar to mussels the common pests of oysters are estuarine sponges (*Cliona* spp.), polychaetes (*Polydora* spp.) and pea crabs (*Pinnotheres* spp.); the last two are covered in Chapter 11. Boring sponges excavate into the shell probably using a chemo-mechanical mechanism, that is chemical dissolution of the shell coupled with mechanical dislodgement of shell fragments (Hatch 1980). The oyster shell comes to enclose the body of the sponge, except for papillae that extend from the shell to the outside environment. This pest greatly weakens the shell, impairs feeding and probably makes the oyster more susceptible to predation. The energy used in the continual effort by the oyster to repair its shell may also have implications for somatic growth and gametogenesis, although Rosell *et al.* (1999) have found no evidence for this in infested populations of the European flat oyster *O. edulis*. In pearl oysters (*Pinctada* spp.) pearl production is seriously affected because infected oysters expend more energy on depositing thickened nacre to protect themselves from the invading sponge instead of depositing nacre on previously inserted pearl nuclei, thus resulting in defective pearls (Bower 2001). Fortunately, in the eastern US boring sponge (*Cliona celeta*) numbers are generally controlled because they are consumed by a range of predators such as molluscs, crustaceans, polychaetes and echinoderms (Guida 1976). However, further north in New Brunswick, Canada, the sponge is an ongoing threat to the quality of oysters (*C. virginica*), with 25–30% of off-bottom cultured oysters showing severe infections (Carver *et al.* 2010). The authors found that a 6 min brine dip (>90% NaCl saturation) was completely effective at eliminating the sponge without harming the oysters.

Organisms that compete with oysters for space and food include algae, sponges, bryozoans, anemones, polychaetes, molluscs, arthropods and even oysters themselves, for example competitive interactions between the native Sydney rock oyster, *Saccostrea glomerata*, and the introduced species, *C. gigas*, on the east coast of Australia (Krassoi *et al.* 2008). Some of these could also be regarded as fouling organisms. Restricted space for settlement and overgrowth of the shell are the most common effects of competitors on oysters. But, even when oysters are not killed, competitors/foulers cause reduced survival and growth (Zajac *et al.* 1989). Current practices for dealing with biofouling in the cultured Pacific oyster, *C. gigas*, in British Columbia, Canada, include removal of fouling organisms by hand, pressure-washing or short-term beach planting to allow daily desiccation (Dunham & Marshall 2012). A novel method whereby expanded clay aggregate is added to culture enclosures is very effective in reducing biofouling (see later).

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In Europe the most important competitor is the exotic slipper limpet, *Crepidula* spp., introduced from the United States in the mid 1800s (see Minchin *et al.* 1995 for historical account). The limpets settle around the same time as oysters and grow more rapidly than oyster spat, causing high mortality (~60%) through overgrowth of the oyster shell (MacKenzie 1970). In addition, the continuous production of faeces and pseudofaeces on the bottom leads to an accumulation of mud, making the substrate unsuitable for oyster settlement. A theoretical biodeposition rate of more than 6.0×106 t year^{-1} has been estimated for the Bay of Mont-Saint-Michel, Western English Channel, adding to the natural suspension-matter deposition rate, which is estimated at 1.5×106 t year^{-1} (Blanchard 2009). The Bay is an important site for oyster (*O. edulis* and *C. gigas*) cultivation but because *Crepidula fornicata* is continuing to spread, with an estimated increase in biomass of about 9000 t year^{-1} since its introduction in the 1970s, the situation will inevitably worsen in the coming decades.

Clams

Crabs are a serious predator of most species of clam. They can sense their buried prey at a distance and can dig them up if they are not buried deep enough. They gain access to the flesh by crushing small individuals, chipping the valve margins of large individuals or forcing the valves apart (Gibbons 1984). A wide variety of crab species prey on clams (Table 3.4) and a comprehensive picture of the foraging behaviour, patterns of predation and habitats used by blue crabs, in particular *C. sapidus*, have emerged over the past two decades (Skilleter 1994; Ebersole & Kennedy 1995; Micheli 1997a, b; Micheli & Peterson 1999; Polyakov *et al.* 2007; Romano *et al.* 2011; Long & Hines 2012; Whitton *et al.* 2012). Other crustaceans such as shrimp and lobster also prey on clams (Ejdung *et al.* 2009; Maire *et al.* 2010). In one locality in Sweden the shrimp *Crangon crangon* consumed 36% and 68% of the annual production of the hard clam *M. mercenaria* and the cockle *Cerastoderma edule*, respectively (Møller & Rosenberg 1983). Some species of snapping (*Alpheus* spp.) and mantis (*Squilla* spp.) shrimp can even crush *M. mercenaria* as big as 25 mm shell length (Beal 1983). The American lobster *Homarus americanus* digs for hard clams and uses its crusher claw to crack the shell (Herrick 1911).

Gastropod predators of clams tend to be the same species that prey on mussels and oysters, that is whelks (*Busycon* and *Murex* spp.) on hard clams, *Buccinum undatum* on cockles (*C. edule*) and oyster drills (*U. cinerea* and *E. caudata* on hard clams, and *Ocenebra japonica* on the Manilla clam *R. philippinarum*). Other predators include polychaetes (Hiddink *et al.* 2002) starfish (Doering 1982), octopus (Mather *et al.* 2012), various fish (Fisher *et al.* 2011; Dame 2012 and references) and bird (Caldow *et al.* 2007; Lewis *et al.* 2007; Tulp *et al.* 2010) species, and marine mammals such as sea otters (Wolt *et al.* 2012).

The burrowing habit of clams gives them a certain amount of protection from some predators. For example, oystercatchers that feed on the clam *M. balthica* and the ragworm *Nereis diversicolor*, both highly profitable prey, find it more difficult to locate buried clams than the less cryptic but mobile ragworms (Ens *et al.* 1996). But the moon snail (*Neverita lewisii*) with its powerful wedge-shaped food is well adapted to burrowing deeply, and only the geoduck *Panopea abrupta* burrows deep enough (100 cm) to avoid this bird predator completely (Peitso *et al.* 1994). When clams (*M. balthica*) were exposed to effluent from crabs (*C. maenas*) feeding on conspecifics, they doubled their burial depth over a period of a several days (Griffiths & Richardson 2006). It is clear that clams use chemical signals to detect predatory crabs, and predators in turn depend on chemical cues to locate clam prey (Smee & Weissburg 2006 and references therein). However, their burrowing habit also poses problems as portions of the exposed siphons are regularly eaten by fish, walrus and occasionally by birds (Gibbons & Blogoslawski 1989 for references; Dame 2012). In some

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Table 3.4 Species of crab that prey on clams in North America.

Crab	Clam species	Geographic range of crab	Typical predation rates
Blue crab, *Callinectes sapidus*	*Mercenaria mercenaria*, *Macoma balthica*, *Rangia cuneata*, *Mya arenaria*	Cape Cod, USA to Uruquay, South America	*M. mercenaria*: 308 clams (≤40 mm shell length) crab^{-1} day^{-1}
Green crab, *Carcinus maenas*	*M. arenaria*, *M. mercenaria*	Nova Scotia to New Jersey	In Europe the crab (carapace width 25–75 mm) consumes three oysters (up to 66 mm shell length) day^{-1} or 36 mussels (up to 45 mm shell length) day^{-1}
Rock crab, *Cancer irroratus*	*M. mercenaria*	Labrador to South Carolina	29, 8–10 mm clams crab^{-1} (carapace width 55 mm) h^{-1}; 30, 1 mm clams crab^{-1} (carapace width 7 mm) h^{-1}
Black-clawed crab, *Cancer productus*, Dungeness crab, *Cancer magister*; Graceful crab, *Cancer gracilis*	*Ruditapes philippinarum*	Pacific coast of North America	*C. magister*: 130, 2–4 mm clams crab^{-1} (carapace width 17 mm) day^{-1}; 10, 9–12 clams crab^{-1} (carapace width 17 mm) day^{-1}
Stone crab, *Menippe mercenaria*; Mud crabs, *Dyspanopeus sayi*, *Panopeus herbstii*, *Europanopeus depressus*, *Rhithropanopeus harrissi*	*M. mercenaria*	East coast USA	*D. sayi*: At 25°C 115, 3 mm clams crab^{-1} day^{-1} (carapace width 19–21 mm) to 22, 7 mm clams crab^{-1} day^{-1}; at 10°C between 45 (3 mm clams) to 11 (7 mm clams) day^{-1}

Data from Carriker (1951); Ebersole and Kennedy (1995); Eggleston *et al.* (1992); Gibbons (1984); Gibbons and Blogoslawski (1989); MacKenzie (1977); Smith and Langdon (1998); http://wdfw.wa.gov/ais/carcinus_maenas/#feeding.

fish species, for example sculpins *Leptocottus armatus* and *Myoxocephalus polyacanthocephalu*, siphon tissue is a significant food source, comprising as much as 25–30% of their gut contents (Meyer & Byers 2005). Siphon nipping may have been selected as a remarkable chemical defence mechanism in the butter clam *Saxidomus gigantea* (Kvitek 1991). This species sequesters a highly potent neurotoxin in its siphons to which the siphon-nipping fish predator, *L. armatus*, has developed an aversion. The fish shows no such aversion to toxic littleneck clams, *Protothaca staminea*, which, unlike *S. gigantea*, retain the toxin in their visceral mass. A few species, for example gaper clams, *Tresus* spp., have armoured plates on their siphons as a physical defence. The plates also serve as a substrate for a wide variety of sessile invertebrates and plants, which may play a role in camouflaging the clam (Stout 1970). Vegetation such as seagrass cover also significantly reduces siphon-cropping and predation on clams (Irlandi 1994). In the case of predators such as sea otters and walrus only deep-burrowing clams have spatial refuge (Kvitek *et al.* 1988). Both of these predators eat large quantities of clams and other bivalves, and because of their abundance and large size may also be responsible for large-scale disruption of soft-bottom communities. Other predator-induced defences are changes such as an increase in shell strength (Neo & Todd 2011a) or the development of scaly projections (scutes) on the shell (Han *et al.* 2008).

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Behavioural changes such as reducing pumping time in the presence of predators in order to decrease prey chemical cues (Delavan & Webster 2012), or squirting water through the exhalant siphon to disorientate the predator (Neo & Todd 2011b) are used by some clam species.

Attempts to control clam predators include many of the methods already described. Mechanical methods such as the use of starfish mops, suction dredges, traps and hand collection have all been tried with little success (Gibbons & Blogoslawski 1989). Chemical methods, such as copper sulphate, quicklime and insecticides, can be used as dips, incorporated in sand and heavy oils, spread over bivalve areas, or used as barriers to protect planted beds. But, as these chemicals are also harmful to endemic species as well as predators, their use is firmly regulated. Gibbons and Castagna (1985) have used the toadfish *Opsanus tau*, along with crushed stone aggregate, to biologically control crab predation on juvenile *M. mercenaria*. The use of off-bottom culture, or on-bottom culture using gravel aggregate and nets, seems to be the most successful way to exclude and isolate predators from juvenile clams (Flimlin & Beal 1993; Leavitt & Burt 2000 review; see also Chapter 9).

Pests of clams include the boring polychaete *Polydora ciliata* (Chapter 11) and the boring sponge *Cliona* spp. (see section on oysters earlier). The nemertean *Malacobdella grossa* has been found within the mantle cavity of several clam species on North American and European coasts. Numbers can be as high as 21 small worms per clam, and some species have infestation rates as high as 80% (Porter 1964; Ropes & Merrill 1967). The worms, while aesthetically displeasing to human consumers, cause no harm to their host. However, pea crabs (*Pinnotheres* spp.), which also inhabit the mantle cavity, are believed to cause gill damage in clams and other bivalves (Chapter 11).

Infaunal clams are affected by few or no biofouling organisms as they are generally not exposed to settling larvae (Dürr & Watson 2010). However, biofouling can be a significant cause of mortality in juvenile clams. Various species of Vorticellidae, Entoprocta and blue green algae attach to shells, often suffocating newly settled clams. The amphipod *Corophium cylindricum* and the sea squirt *Molgula manhattensis* are also fouling pests of post-set *M. mercenaria* (Gibbons & Blogoslawski 1989). Biofouling can also be a significant cause of mortality in clams grown in suspended culture. Removal of fouling organisms by hand is time-consuming and inefficient, while mechanical treatments requiring specialized equipment are labour-intensive. The cost of biofouling control has been estimated to account for 5–15% of production costs (Fitridge *et al.* 2012). A new inexpensive, environmental-friendly method, whereby Hydroton Grow Rocks™ (lightweight ceramic pellets) are added to culture trays, is showing great promise as an antifouling treatment for clams and oysters (Dunham & Marshall 2012; Marshall & Dunham 2013), and will presumable be tested on other bivalves in the future.

There are few reports on the effect of competition for space or resources between species of clam. There is some evidence that dense assemblages of the gem clam *Gemma gemma* (Sanders *et al.* 1962) or the deep-dwelling ghost shrimp *Callianassa californiensis* (Peterson 1977) can limit recruitment of the hard clam *M. mercenaria* and the butter clam *Saxidomus nuttalli*, respectively. The presence of other large infaunal species, or dense plantings of the same species, can retard clam growth and, even in some cases, increase mortality (references in Gibbons & Blogoslawski 1989).

Scallops

The most important predators of scallops are starfish, followed by crabs, lobsters, gastropods, sea anemones, octopus and fish (Table 3.5). Isotopic analysis has been used to identify gastropod and starfish species as the main predators of the Patagonian scallop *Zygochlamys patagonica* (Botto *et al.* 2006). Spat and juvenile scallops are very vulnerable to predation

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Table 3.5 Predators of a selection of scallop species. Predation is usually highest on spat and juvenile stages.

Scallop species	Geographic range	Predator	References
Pecten maximus	Norway to southern Spain	Crabs, lobsters, starfish and fish	Brand (2006)
Pecten fumatus	South and south-east coasts of Australia and Tasmania	Crabs and starfish	Heasman *et al.* (1998)
Pecten novaezelandiae	New Zealand coasts	Starfish, fish, octopus and hermit crabs	Marsden and Bull (2006)
Chlamys islandica	Circumpolar in the northern hemisphere	Starfish, cod and eider ducks	Strand and Parsons (2006)
Chlamys tehuelcha; Tehuelche scallop	East coasts of Brazil and Argentina	Starfish, gastropods, octopus and ratfish	Ciocco *et al.* (2006)
Argopecten irradians	Atlantic coast of North America	Starfish, gastropods, crabs, fish and birds	Rhodes (1991)
Argopecten purpuratus	Central America to Chile	Starfish, crabs, fish, anemones, gastropods, octopus and lobsters	López *et al.* (2000)
Mizuhopecten yessoensis	Coasts of Japan, Korea and Russia	Starfish, crabs, sea urchins and octopus	Kosaka and Ito (2006)
Amusium balloti; Saucer scallop	Tropical waters of the Indo-Pacific	Turtles and slipper lobsters	Gwyther *et al.* (1991)

Common names of species in Table 3.3.

(Magnesen & Redmond 2012; Lefcheck *et al.* 2014), and in areas of high predator abundance this may limit scallop distribution (Brand 2006 and references therein). In culture operations the presence of an alternative prey, for example mussels, may offer a protective refuge to seeded scallops on the seabed (Wong *et al.* 2005). In fishery areas, fishing gear, such as dredges and trawls, degrades habitats and subsequently increases the vulnerability of juvenile scallops to predation by removing epifauna and structural features such as rocks and boulders from the sea floor (Talman *et al.* 2004; see also Wong & Barbeau 2005). Vulnerability of young scallops to predation varies between species, due to different behavioural and morphological adaptations. For example, where *C. islandica* and *P. maximus* live together in the same habitat, *C. islandica* is more heavily preyed upon by plaice and flounder. This is due to its greater tendency to remain bysally attached, and also because of its weaker swimming escape response (Naidu & Meron 1986). In contrast, *P. maximus* spat as small as 4 mm shell height can vacate byssal attachment sites to recess on the seabed (Minchin 1992). On the other hand, the thin and fragile shell of attached *P. maximus* spat makes this species more susceptible to a wider range and size of predators than same-size *A. opercularis* that have a stronger shell (Brand 2006). Byssal attachment can in some cases provide a spatial refuge from benthic predators. The bay scallop *A. irradians* in the wild attaches to shoots of the eelgrass *Zostera marina*. In field experiments Pohle *et al.* (1991) found that 10–15 mm spat tethered to eelgrass 20–35 cm above the bottom experienced significantly reduced mortality compared to those placed on the sediment surface (Figure 3.12). When this species detaches from eelgrass there is a critical window of high predatory risk between 15 and 40 mm, after which it attains a size refuge from predation. In general, vulnerability to predation tends to decrease when spat leave sites of byssus attachment and start to recess on the seabed (Buestel & Dao 1979).

In the laboratory it seems that adults larger than a critical shell height of 70 mm are virtually immune from predation, particularly from crabs (Elner & Jamieson 1979; Lake *et al.* 1987).

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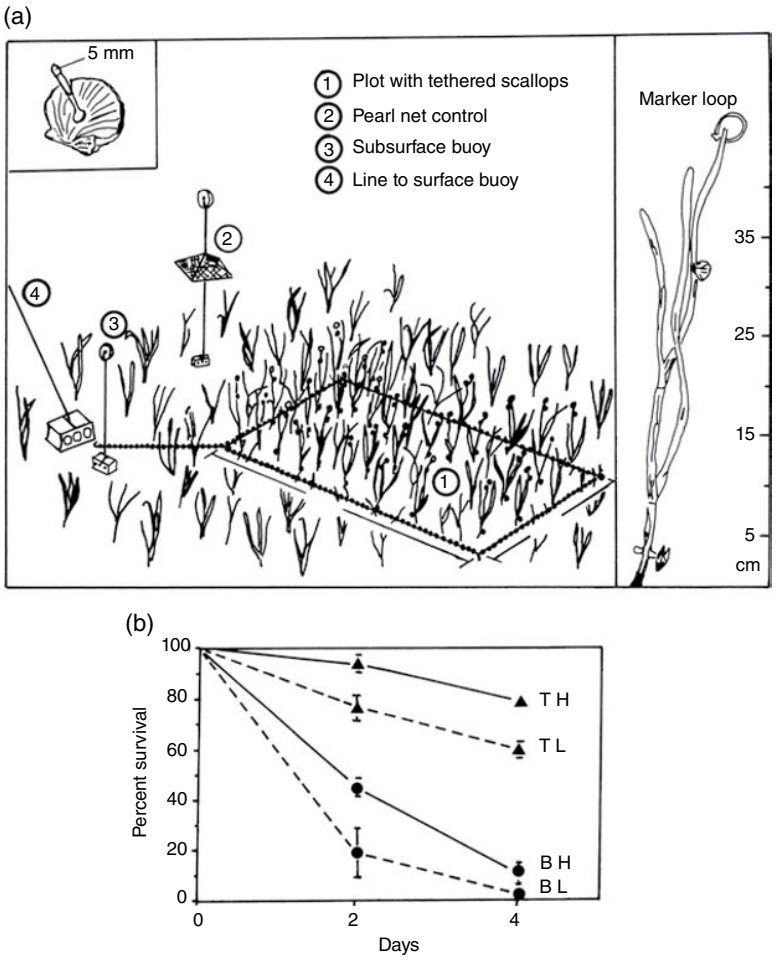


Figure 3.12 (a) Drawing of a typical treatment plot used in scallop (*Argopecten irradians*) field tethering experiments. (1), plot with tethered scallops; (2), pearl net control; (3), subsurface buoy; (4), line to surface buoy. Also shown is the approximate vertical height of tethered scallops on an eelgrass shoot (inset at right), and a scallop with a plastic tether glued to its shell (inset in upper left corner). (b) Mean percentage survival over 4 days of juvenile *A. irradians* tethered to eelgrass in Lake Montauk, Long Island, United States, at the base of the shoots (B, bottom) and in the upper canopy (T, top), less than 5 cm and 20–35 cm above bottom respectively, in plots of low (L) and high (H) density eelgrass. Error bars represent the standard error for three plots.
From Pohle *et al.* (1991). Reproduced with permission from Inter-Research, Germany.

This size must be less in the field for species with a recessing habit and strong swimming escape response, as both of these are effective mechanisms for evading predation. For non-visual predators like starfish, however, recessing affords little protection for scallops.

The escape response, either jumping or swimming, is elicited when the mantle tentacles of the scallop are touched by a predator. When presented with different decapod consumers, the saucer scallop *A. balloti* showed a consistent and vigourous swimming response in contact with the slipper lobster *Thenus orientalis*, the blue swimmer crab *Portunus pelagicus* and the coral crab *Charybdis cruciata* (Himmelman *et al.* 2009). This was not a generalized response to decapods as the scallop showed a weak response to the red portunid crab *Portunus rubromarginatus*, indicating that it was in contact with predators that elicited the

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strong escape response. The ability to differentiate between potential predators and harmless species has an obvious adaptive value because it minimises feeding interruptions, and thus energy wastage (Brand 1991). Escape responses of 3–4 m in the field, and more than 10 m under experimental conditions, have been recorded (Wilkens 2006 and references therein). Interestingly, wild scallops (*A. purpuratus*) show a stronger escape response than cultured scallops, a finding that has implications for scallop restocking programmes (Brokordt *et al.* 2006).

In an elegant series of field experiments Barbeau and colleagues have studied in detail the predatory effects of starfish, *Asterias* spp. and the rock crab *Cancer irroratus* on juvenile *P. magellanicus*, in Lundeburg Bay, Nova Scotia. Scallop survival was assessed using different size classes of scallops tethered at different sites and seasons, and in different densities of surrounding scallops and predators. Tethered scallops were much more susceptible to sea star than to crab predation (Barbeau *et al.* 1994). This is because once encountered free scallops close their valves and are likely to be captured and consumed by crabs. In contrast, in sea star–scallop encounters free scallops readily escape by swimming or jumping, and therefore the probability of sea stars capturing free scallops is low (Figures 3.13 and 3.14). Both starfish and crab predation increased significantly with temperature (4–15°C range) due to the decreased effectiveness of the scallops' escape response (Barbeau & Scheibling 1994b). Predation rate also increased significantly with scallop density, but predator density did not. Thus, predators displayed a functional response (increased their individual consumption rate of prey) as opposed to an aggregative (attracted to the prey and increase their local density) response (Barbeau *et al.* 1998 and references therein). But predation rate in crabs increased linearly with prey density (positively density-dependent) while in starfish predation rate increased but at a decelerating rate until a plateau was reached (density-independent). These results indicate that crabs are more likely to have a greater impact than sea stars on seeded populations of scallops in bottom-culture operations.

Pests of scallops are mostly the same as those mentioned for other bivalve groups: burrowing polychaetes (*Polydora* spp.), sponges (*Cliona* spp.) and pea crabs (McGladdery *et al.* 2006).

Scallop shells often harbour a variety of fouling organisms, including algae, barnacles, tube worms, sponges, hydrozoans, bryozoans and other molluscs. Heavy fouling can increase weight and drag on the scallop shell and thus hamper swimming ability. Winter and Hamilton (1985) have estimated that 6 g of epifauna could cause almost a 30% decrease in the distance travelled by *A. irradians*. In some cases, however, fouling may have a beneficial effect. For example, epizoic sponges on the upper valve of various species of *Chlamys* greatly inhibit predation by starfish. The sponge reduces adhesion of the starfish tube-feet but tactile camouflage may also be involved (references in Brand 2006). In addition, distasteful chemicals in the sponge may deter fish predators (Pitcher & Butler 1987). Fouling is a significant problem in suspended scallop culture, and various methods, for example high-pressure water hoses, antifouling chemicals, saline dips and air-drying have been used with limited success. More recently, there has been increasing interest in the use of biological control methods. For example, sea urchins and hermit crabs were shown to reduce fouling loads on pearl nets by up to 50% in suspended culture of *P. maximus* in the Irish Sea (Ross *et al.* 2004). In addition, the sea urchins could be exploited as part of an integrated culture system.

Competition occurs for scallops mainly at the settlement stage when they may compete, for example, with high densities of mussel spat for space and food. Once settled, either bysally attached or on the bottom, it would appear that scallops do not occur in sufficiently high densities for competition to play an important role.

Before concluding this section it should be pointed out that bivalve mortality during the planktonic larval stage is high, and Thorson (1950) has suggested that predation may be the

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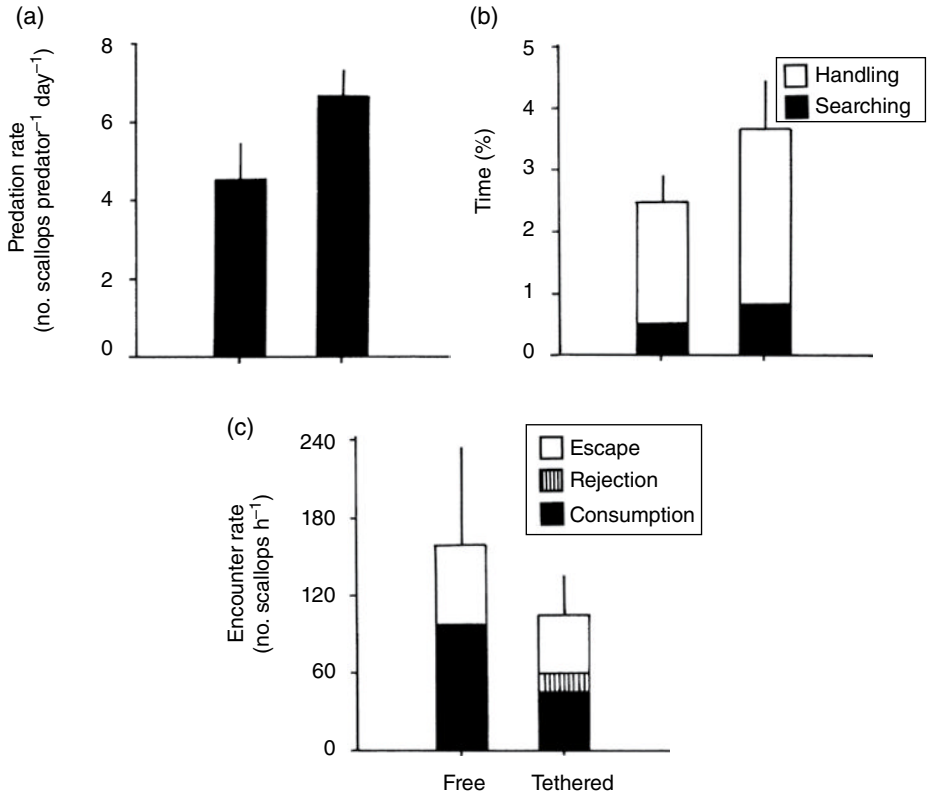


Figure 3.13 Laboratory experiments examining the effect of tethering scallops (*Placopecten magellanicus*) on predation by crabs (*Cancer irroratus*). (a) Predation rate per crab; (b) percent foraging time (searching + handling time) of crabs; and (c) encounter rate between crabs and scallops (different outcomes of encounter have different shadings). The probability of capture upon encounter was calculated as (number of consumptions + rejections)/number of encounters, and the probability of consumption upon capture as number of consumptions/(number of consumptions + rejections). Crabs were offered either free or tethered scallops. Observation time in (b) and (c) = 420 min tank^{-1}. Mean shown for all variates, error bars = SE (for foraging time in (b) and for encounter time in (c)), and number of replicate tanks = 3.
From Barbeau and Scheibling (1994a). Reproduced with permission from Inter-Research, Germany.

single most important cause. Possible predators of larvae include sea squirts, ctenophores, sea anemones, barnacles, and larvae of various crustacean, echinoderm and fish species (Dame 2012). The inadvertent consumption of larvae by filtering adults – of its own and other species – also contributes to mortality.

Climate change

The word climate is usually defined as the 'average weather' in a place, and includes patterns of temperature, precipitation, humidity, wind and seasons. But our climate is changing at a rate faster than any seen in the last 2000 years (http://www.ecy.wa.gov/about.html). The change is due to rising levels of carbon dioxide and other heat-trapping gases in the atmosphere. Greenhouse gases act like a blanket around Earth, causing it to warm, a phenomenon referred to as global warming. Another consequence of rising atmospheric CO_2 levels is increasing ocean acidity, which is predicted to double over the next 100 years in an

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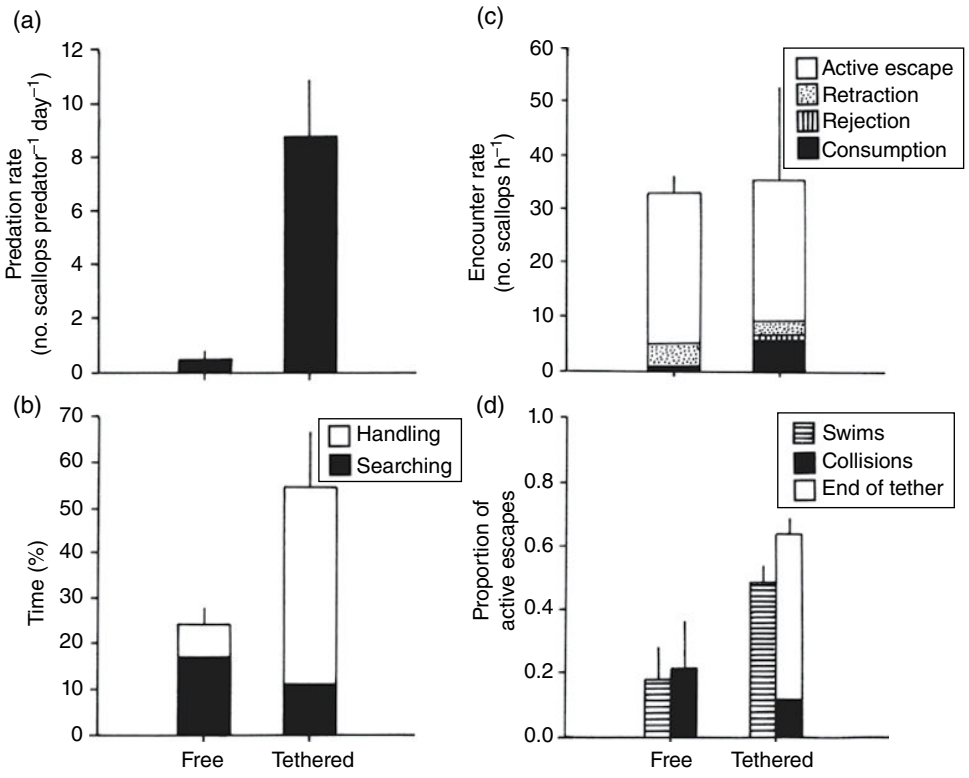


Figure 3.14 Laboratory experiments examining the effect of tethering scallops (*Placopecten magellanicus*) on predation by sea stars (*Asterias vulgaris*). (a) Predation rate per sea star; (b) percent foraging time (searching + handling time) of sea stars; (c) encounter rate between sea stars and scallops (different outcomes of encounter have different shadings); and (d) proportion of active escapes in which scallops swam (active escapes = swims + jumps) and in which scallops collided with the tank walls or reached the end of their tether. The probability of capture upon encounter was calculated as (number of consumptions + rejections)/number of encounters, and the probability of consumption upon capture as number of consumptions/(number of consumptions + rejections). In (c) the numbers of free scallops rejected and consumed are 0.1 and 0.4 scallops h^{-1}, respectively. Sea stars were offered either free or tethered scallops. Observation time in (b), (c) and (d) = 510 min $tank^{-1}$. Mean shown for all variates, error bars = SE (for foraging time in (b) and for encounter rate in (c)), and number of replicate tanks = 3. From Barbeau and Scheibling (1994a). Reproduced with permission from Inter-Research, Germany.

uncontrolled emission scenario (Fauville *et al.* 2013). Over the past century Earth's average temperature has risen by 1.4°F and is forecast to rise another 2–11.5°F over the next 100 years (http://www.epa.gov/). Wide-ranging impacts of rising temperatures include rising sea levels; melting snow and ice; more extreme heat events, fires and drought; and more extreme storms, rainfall and floods (see http://www.ecy.wa.gov/about.html and accompanying links). And humans, through activities such as burning fossil fuels, deforestation, industrial processes and some agricultural practices, are largely responsible. The last of the Intergovernmental Panel for Climate Change (IPCC) report was recently published, and the message, succinctly synthesized by Hickman (2014) in *The Guardian* newspaper, is clear:

> *Climate change is real. We are to blame. It will get worse if we fail to act. The solutions are available and affordable. But time is short.*

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So what are the consequences of climate change for marine ecosystems? Mora *et al.* (2013), using global climate models, have shown that in the next 100 years the entire world's ocean surface will be simultaneously impacted by varying intensities of ocean warming, acidification, oxygen depletion or shortfalls in productivity. In contrast, only a very small fraction of the world's ocean surface, mostly in polar regions, will experience increased oxygenation and productivity, and almost nowhere will there be cooling or pH increase. From a compiled list of 32 marine habitats and biodiversity hot spots they found that all would experience simultaneous exposure to changes in multiple biogeochemical parameters, which will demand multiple physiological adjustments from marine biota. However, regional-scale differences in response to climate change can often be more relevant than global averages. For example, a study of sea surface temperature (SST) change in 63 global Large Marine Ecosystems (LMEs) over a 50-year period (1957–2006) revealed strong regional variation, with the Subarctic Gyre, European Seas and East Asian Seas warming at 2–4 times the global mean rate (Belkin 2009). The most rapid warming was observed in the land-locked or semi-enclosed European and East Asian seas (Baltic Sea and North Sea, Black Sea, Japan Sea/East Sea and East China Sea), and also over the Newfoundland–Labrador Shelf. The Subarctic Gyre warming is likely caused by natural variability in relation to the North Atlantic Oscillation, a climatic phenomenon that varies over time but has no particular periodicity.

Potential and observed impacts of climate change on marine bivalves

Climate warming

One major response to warming is a shift in distribution, usually poleward, as species move to occupy areas within their metabolic temperature tolerances (Root *et al.* 2003). As already mentioned, over the last 50 years the southern limit of the mussel *M. edulis* on the east coast of the United States has shifted northwards by more than 300 km (Jones *et al.* 2010). Seasonal air and water temperatures along this coast have significantly risen since 1960, and the biogeographic contraction appears to be caused by intolerance of adults to high summer temperatures (see also Zippay & Helmuth 2012). In the same time frame, *M. californianus* along the Strait of Juan de Fuca near Washington State has decreased its vertical distribution by over 50% over the past 52 years (Harley 2011). At Victoria in the Strait summer average daily maxima have risen at a rate of 0.654°C per decade since 1950, which corresponds to an increase of 3.4°C over 52 years. These data provide strong evidence that the change in vertical distribution is linked to global warming. So, is it possible to determine the potential impact of climate change on species distributions?

Geographic ranges of species are determined by climatic conditions and this forms the basis of bioclimatic models, which use associations between aspects of climate and species' occurrences to estimate the conditions that are suitable to maintain viable populations (Araújo & Peterson 2012). Mechanistic bioclimatic models use a species' physiological tolerances to heat, cold and so on to predict the species' range, while in empirical modelling a number of climatic variables such as maximum and minimum temperatures and precipitation are measured for many different locations and statistically compared to the occurrence of the focal species at these locations (Jeschke & Strayer 2008). Empirical models, which do not take species' physiological tolerances into account, yield the climatic range limits of the species' distribution and allow prediction of range shifts due to climate change. Most bioclimatic models do not explicitely consider biotic interactions, such as predation and competition between species, or limitations to dispersal, and assume that species lack

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sufficient plasticity to adapt to environments beyond those currently occupied (Jeschke & Strayer 2008; Fuller *et al.* 2010). The ecological effects of climate change can only be predicted if there is an understanding of the physiology of the species in its natural environment. Evidence based on temperature indicates that species' distributions are driven both by short-term exposure to lethal conditions and by repeated or longer-term exposures leading to energetic failures. The relationship between physiological performance, for example scope for growth (SFG), and body temperature can be depicted as a thermal performance curve (Figure 3.15). Woodin *et al.* (2013) define the difference between lethal and sublethal exposure limits as the transient event margin (TEM), the range of environmental conditions below the short-term lethal limit that a species may endure on a transient basis, but which would lead to mortality over longer time spans. The magnitude of the disparity (TEM) between performance and tolerance temperature thresholds relative to environmental variance determines the likelihood of failure of bioclimatic model predictions (Woodin *et al.* 2013). To define TEM, both CTmax SFG and LTmax data are necessary, but in the majority of datasets only LTmax estimates are available. Jones *et al.* (2010) used a mechanistic biogeographic model for *M. edulis* on the Atlantic coast of North America based on LTmax. Physiological limits were compared against environmental temperatures and the biogeographic distribution of the species was accurately predicted. The hindcast using the model also successfully predicted the historical changes in distribution over half a decade already described earlier. But, when the same was applied to Europe it failed to predict the distribution of *M. edulis*, that is its distribution in Europe was 50% less than that predicted by the model. However, when an energetics model was applied to Europe the predicted distribution

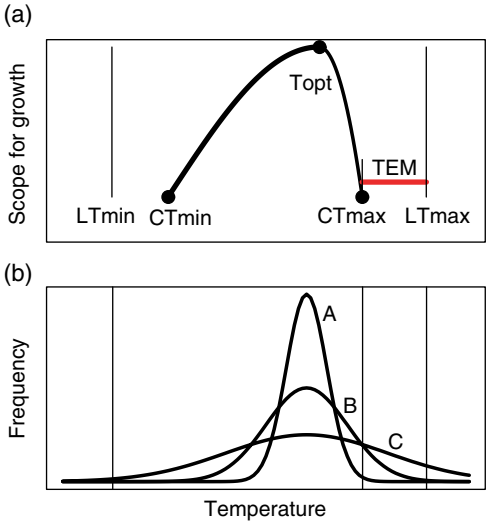


Figure 3.15 (a) Relation between performance as scope for growth (SFG) and temperature. Lethal limits are LTmin and LTmax. Performance limits are the critical minimum temperature (CTmin) and critical maximum temperature (CTmax), the body temperature at any point in space and time is Tbody and the optimal temperature is Topt. The upper transient event margin (TEM) = LTmax – CTmax. Thermal response curves vary from species to species but all typically display some optimum temperature (Topt) at which performance is maximized, as well as critical minima (CTmin) and critical maxima(CTmax), beyond which mortality and/or reproductive failure occur (Helmuth *et al.* 2010). (b) Temperature versus frequency for environments A, B and C. TEM from (a) is expressed on yearly environmental variance curves for three environments, all with the same mean environmental temperature, but differing in variance.
From Woodin *et al.* (2013). © John Wiley & Sons/CC-BY-3.0.

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was close to the actual distribution of the species on western European coasts, highlighting the need for caution in applying the same model across large geographic distances (see details in Woodin *et al.* 2013). For more information on forecasting/hindcasting biographic responses of intertidal bivalves to climate change see Helmuth *et al.* (2006), Wethey and Woodin (2008), Barnes *et al.* (2010), Somero (2011), Zippay and Helmuth (2012) and Dunphy *et al.* (2013).

Apart from the impact on bivalve biogeographic distribution there is evidence that global warming can impact on interspecific (Petes *et al.* 2007; Kordas *et al.* 2011) and predator–prey interactions (Freitas *et al.* 2007); growth (Petes *et al.* 2007; Beukema *et al.* 2009), the timing (Philippart *et al.* 2003; Beukema *et al.* 2009) and magnitude (Kimmel & Newell 2007) of recruitment; the immune response (Matozzo & Marin 2011) and species invasion success (Firth *et al.* 2011; Zippay & Helmuth 2012), and potentially capture fisheries and aquaculture (Allison *et al.* 2009; Cochrane *et al.* 2009; Brander 2010).

Ocean acidification

Increasing levels of atmospheric CO_2 lead to increased levels of dissolved CO_2 in the oceans, which creates carbonic acid (H_2CO_3), most of which quickly dissociates into a hydrogen ion (H^+) and bicarbonate (HCO_3^-), which can further dissociate into carbonate (CO_3^{2-}) and hydrogen ions ($2H^+$). Some of the carbonate ions already in the ocean combine with some of the hydrogen ions to form further bicarbonate, thereby reducing the availability of carbonate ions that are necessary for marine calcifying organisms such as corals, foraminiferans, echinoderms, crustaceans and molluscs, to produce their $CaCO_3$ shells and skeletons (Fabry *et al.* 2008).

Since the industrial revolution, surface ocean pH has decreased by 0.1 units on the logarithmic pH scale, representing an approximately 25% decrease. By 2100, a pH decline of 0.15–0.31 is predicted; the degree of change depends on whether we adopt a concerted rapid CO_2 mitigation effort or a 'business as usual' attitude (Mora *et al.* 2013).

To date, while the vast majority of studies (>90%) on the potential effects of ocean acidification are laboratory-based, there are a few field studies using natural gradients or CO_2-rich environments, or large-scale field studies using mesocosms that can provide insights into the short- and long-term responses at the ecosystem level (Dupont & Pörtner 2013 and references therein). Laboratory studies show that numerous oyster larval species show developmental delay, decreased calcification and altered shell ultrastructure in response to low pH (Gazeau *et al.* 2007, 2011; Kurihara 2008; Talmage & Gobler 2011; Dineshram *et al.* 2013; Timmins-Schiffman *et al.* 2013). In some of these studies delayed larval growth resulted in poor recruitment (Parker *et al.* 2010; Barton *et al.* 2012). To illustrate, Timmins-Schiffman *et al.* (2013) characterized the effects of elevated levels of pCO_2 on size, calcification and early larval development in the oyster *C. gigas*. Larvae were exposed to ambient (400 ppm) and two elevated pCO_2 levels (700 and 1000 ppm; based on projections for the coming century) for 72 h following fertilization. Development in the larvae raised at high pCO_2 was delayed by 3 days post fertilization, which caused them to be smaller and have less calcified material than controls, findings consistent with other studies on oysters (see earlier references). The pCO_2 700 ppm level had no adverse effects on oyster development. In contrast, an approximately similar pCO_2 level (750 ppm) significantly depressed survival, development, growth and lipid synthesis in hard clam *M. mercenaria* and bay scallop *A. irradians* larvae after about 3 weeks exposure (Talmage & Gobler 2011), illustrating differential interspecific responses to similar pCO_2 levels (see also Bressan *et al.* 2014).

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Elevated pCO_2 on bivalves impact the escape response in scallops (Schalkhausser *et al.* 2013), byssal attachment in mussels (O'Donnell *et al.* 2013), cellular systems involved in the immune response in mussels (Bibby *et al.* 2008; but see Ellis *et al*. 2015) and expression of genes involved in energy and protein metabolism in mussels (Hüning *et al.* 2013).

Several studies have examined the combined effects of warming and acification on bivalve physiology (Findlay *et al.* 2008; Parker *et al.* 2010; Talmage & Gobler 2011; Hiebenthal *et al.* 2013). For example, gametes, embryos, larvae and spat of the Sydney rock oyster *S. glomerata* and the Pacific oyster *C. gigas* were exposed to four pCO_2 levels, one ambient (375 ppm) and three elevated (600, 750 and 1000 ppm), and four temperatures, one elevated (30°C) and three natural spawning temperatures (18, 22 and 26 °C); these were selected on the basis of IPCC projections for 2100. Elevated pCO_2 and temperature caused reduced fertilization of gametes, reduced development and increased abnormality of D-larvae and reduced growth of larvae and spat of both species, with greater impacts on *S. glomerata*, once again highlighting differential interspecific responses to environmental stressors (Parker *et al.* 2010). If oceans continue to acidify and warm at predicted rates, *C. gigas* could become the more dominant species along the south-east coast of Australia, further recruiting into estuaries that are currently dominated by the native *S. glomerata*.

Hypoxia

Another serious consequence of global warming that has gained attention only in the last few years is the decrease in dissolved O_2 content of the world's oceans (Keeling *et al.* 2010). Warming of surface waters reduces their capacity to take up oxygen, intensifies stratification and reduces oxygen transport into deeper waters. In coastal and inland waters nutrient input from waste water and agriculture exacerbates the problem. Anoxic areas, known as dead zones, currently make up less than 2% of the world's ocean volume but it is predicted that global warming could cause dead zones to grow by a factor of ten or more by the year 2100. Notable dead zones include the Gulf of Mexico, the east China Sea, and in European waters, the Adriatic Sea, the German Bight, the Baltic Sea and parts of the Black Sea (Diaz & Rosenberg 2008). However, it has been demonstrated that decreased nutrient loading strongly decreases the probabily of hypoxic events. The north-west Black Sea represents a relatively rare example of how a major reduction in nutrient loading can dramatically mitigate coastal hypoxia (Mee *et al.* 2005; Kemp *et al.* 2009). With the loss of fertilizer subsidies from the former Soviet Union in the late 1980s nutrient loading decreased two- to fourfold, so that by the mid 1990s hypoxia had disappeared and benthic assemblages had begun to recolonize.

There is growing awareness that low oxygen regions of the ocean are also acidified (Meire *et al.* 2013; Melzner *et al.* 2013). While the effects of hypoxia on marine bivalves are well documented (see Chapter 7) the combined effects of low oxygen and acidification are largely unknown. The consequences of these factors on early development has recently been assessed in two economically important bivalves: the bay scallop *A. irradians* and the hard clam *M. mercenaria* (Gobler *et al.* 2014). In larval scallops experimental and naturally occurring acidification (pH 7.4–7.6) reduced survival (by >50%) while low oxygen (30–50 mM) inhibited growth and metamorphosis (by >50%). In early life stage clams hypoxic waters led to 30% higher mortality while acidified waters significantly reduced growth (by 60%). The combined effect of these stressors was more severe than any one individual stressor. Therefore, these two stressors should be considered together when assessing how marine organisms respond to these conditions today and under future climate change scenarios (Gobler *et al.* 2014).

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It's very important to understand that climate change is not just another issue in this complicated world of proliferating issues. Climate change is THE issue which, unchecked, will swamp all other issues.

Ross Gelbspan, author of *Boiling Point*, a book on global warming.

Note

1 Pacific Decadal Oscillation: detected as warm or cool surface waters in the Pacific Ocean north of 20°N and, depending on the phase of the oscillation, may regionally ameliorate or counter global climate trends (Stenseth *et al*. 2003).

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4 How bivalves feed

Introduction

Primitive protobranch bivalves such as *Nucula* feed primarily by means of ciliated fleshy extensions of the labial palps. These extend into the substrate and collect particles that are carried on to the labial palps for sorting, prior to ingestion. Thus the gills are primarily respiratory organs. In contrast some protobranchs, such as *Solemya velum*, ingest suspended algae and are regarded as crude suspension-feeders. Such species rely on chemoautotrophic bacteria in their gills for their main nutrient supply, with phytoplankton from suspension-feeding as a supplementary source (see later). The vast majority of bivalves use the gills for feeding and these have become greatly enlarged to deal with their secondary derived role. This method of feeding is called suspension- or filter-feeding because the gills with their different ciliary tracts remove suspended particles from the water pumped through the mantle cavity. The gills divide the mantle cavity into inhalant and exhalant chambers (Figure 2.10a, Chapter 2). The water that enters through the inhalant opening or siphon is driven from the inhalant to the exhalant chambers by cilia on the gills and mantle surface, and exits by the exhalant opening or siphon. Both openings possess a muscular velum, the inner fold of the mantle, which regulates water flow through the mantle cavity. A full description and illustration of gill structure and ciliation is provided in Chapter 2.

Because there is much less published information on deposit-feeding bivalves (reviewed in Ward & Shumway 2004 and Dame 2012) this chapter will focus on feeding, digestion and absorption in suspension-feeding bivalves, and the various mechanisms that they employ to control the quantity and quality of their diet. Such mechanisms include the following:

1. Varying the rate at which water is passed over the gills, and the rate and efficiency with which particles are removed from the feeding current
2. Sorting of edible seston from material of low nutritional value
3. Regulating the volume of material processed and ingested
4. Modulating the digestion and absorption process, through control of the rate and passage of material through the gut, sorting in the stomach and changes in digestive enzymes.

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Filtration rate

Filtration or pumping rate is defined as the volume of water flowing through the gill in a unit of time. The clearance rate (CR) is that volume of water completely cleared of particles per unit of time. When all particles presented to the gill are cleared from suspension, then the CR is the same as the filtration rate.

The two processes, clearance and filtration, are independently controlled in bivalves. This makes absolute sense in view of the dual function of the gill in feeding and respiration. Bivalves are capable of altering the size of the gill ostia and the gill itself, and changing the beat frequency and pattern of the latero-frontal cilia to accommodate differing particle concentrations in the incoming water. However, the ventilation current created by the lateral cilia is unaffected by particle concentration, and thus gas exchange is unimpaired (Bayne *et al.* 1976). The general mantle surface is believed to play a significant role in gas exchange.

Estimation of filtration and clearance rates

Filtration or pumping rate in bivalves is determined using direct methods, where the exhaled water is physically separated from the surrounding water and is collected and measured. Direct methods are most successful when applied to those species where the inhalant and exhalant currents can be easily separated. The set-up for a direct method is illustrated in Figure 4.1. A bivalve is placed in a tank that is divided into two chambers C_1 and C_2 by a membrane with a slit to accommodate the bivalve and to separate its inhalant and exhalant siphons. A shunt connects the two chambers and this is closed at the start of the experiment. As the animal pumps water from C_1 to C_2 the water level in C_2 is monitored with a laser beam striking a mirror that is fixed to a floating ping-pong ball. The mirror reflects the laser beam onto a scale at about 8 m distance from the mirror. A deflection of 1 cm on the scale represents a 0.1 mm change in the water level in C_2. Indirect methods are used to determine CRs. The bivalve(s) is placed in a known volume of water containing a suspension of particles for a set period of time. The particles are usually algae cells, organic and inorganic

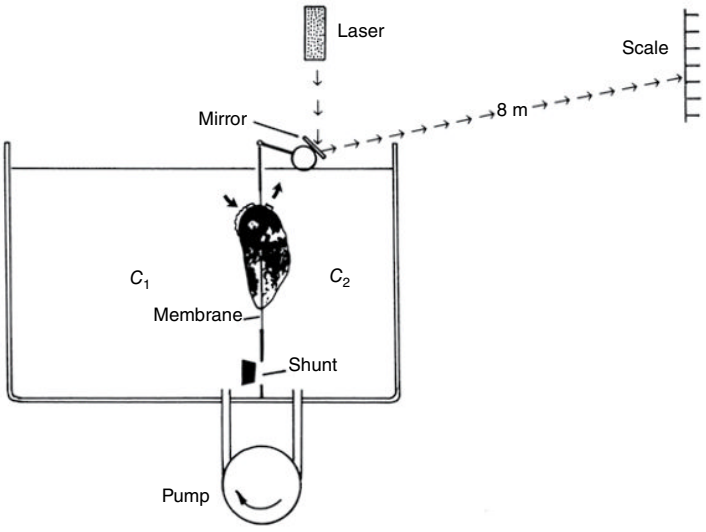


Figure 4.1 Experimental set-up for direct measurement of filtration rates in bivalves. From Jørgensen *et al.* (1986). Reproduced with permission from Inter-Research.

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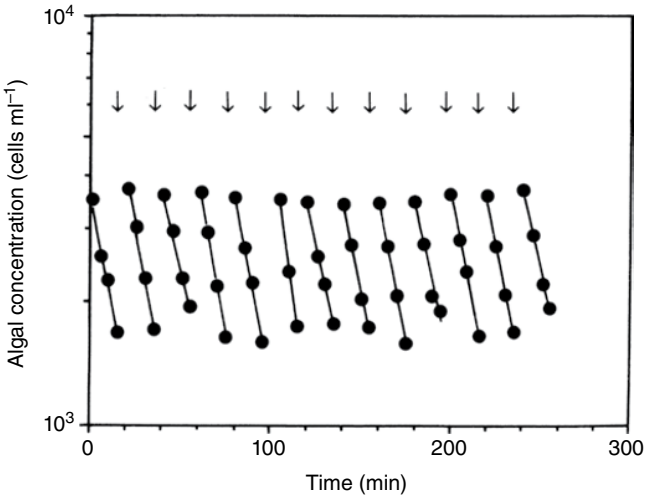


Figure 4.2 Reduction of algal cell concentration during an experiment to measure clearance and filtration rates in mussels (*Mytilus edulis*). Arrows indicate additions of algal suspension. The slope of lines gives the mussels' filtration rate.
From Clausen and Riisgård (1996). Reproduced with permission from Inter-Research.

powders, or even bacteria. Filtration results in a decrease in particle concentration over time. This is one disadvantage of the method, but this can be overcome by adding new particles to maintain concentration within a certain range. The reduction in the number of particles (algae) as a function of time is tracked either by measuring the fluorescence or by taking water samples at fixed time intervals and measuring particle concentration using an electronic particle counter (Pleissner *et al.* 2013). CR is determined from the exponential decrease (e.g. verified as a straight line in a semi-log plot, Figure 4.2) in algal concentration as a function of time using the equation

$$\mathrm{CR} = \left(\frac{V}{nt}\right)\ln\left(\frac{C_0}{C_t}\right)$$

where C_0 and C_t = particle concentration at time 0 and t, V is the volume of water and n is the number of animals. The slope of the lines expresses the experimental animals' filtration rate (Figure 4.2).

There are several disadvantages associated with closed systems, such as accumulation of excretory products, reduction in oxygen concentration and declining particle concentration, all of which can, to varying degrees, alter normal filtration behaviour. These problems have been largely overcome using the flow-through chamber (FTC) method (Figure 4.3). The CR is determined by using the equation

$$\mathrm{CR} = \mathrm{Fl} \times \frac{C_\mathrm{i} - C_\mathrm{o}}{C_\mathrm{i}}$$

where Fl = flow rate (volume of water flowing through the chamber per unit of time), and C_i and C_o = concentration of suspended algal cells at inlet and outlet of the chamber, respectively. The requirements of this method are that algal cells must be 100% efficiently retained by the gills to reduce underestimation of CR measurement (Riisgård 2001a; Filgueira *et al.* 2006).

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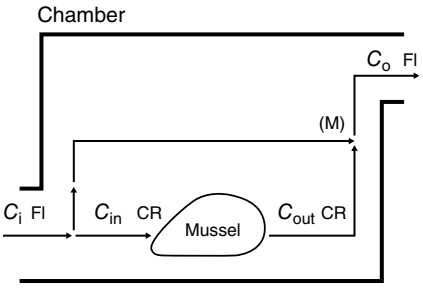


Figure 4.3 Diagram of the flow-through chamber (FTC) method. (M): point of perfect mixing; C_i and C_o: concentration of chamber inflow and outflow, respectively; C_{in} (= C_i) and C_{out} (= 0 for 100% retention) represent the concentration of inhalant and exhalant flows, respectively, while Fl and CR represent the chamber through-flow and clearance rate, respectively.
From Larsen and Riisgård (2011). © Larsen & Riisgård/CC BY 3.0.

This implies the following: (i) no recirculation of water already filtered, (ii) only flow of chamber inlet concentration should enter mussels, and (iii) chamber exit flow is fully mixed at point (M) of Figure 4.3 (Larsen & Riisgård 2011).

CR measurements cannot be easily used in field experiments without disturbing the bivalves. However, CR can be measured from direct collection of biodeposits according to the ratio Cl = (inorganic matter egested as faeces and pseudofaeces)/(total inorganic matter in seawater). The bio-deposition method is easy to set up and has been used successfully for several field studies (see Pouvreau *et al.* 2000; Riisgård 2001a). The method also provides useful information on feeding behaviour in the field (Bayne 2004).

Riisgård (2001a) presented a very comprehensive and stimulating review of the previous example, and seven other methods, highlighting methodological shortcomings and possible misinterpretations of data, and suggesting guidelines to aid in obtaining reliable CR data. This was later followed by a comparison of three of these methods (Petersen *et al.* 2004), which in turn led to a series of comments and debate (Bayne 2004; Petersen 2004). More recently, Filgueira *et al.* (2006) proposed a way for designing experimental chambers, and developed a protocol for evaluating them for CR measurement using the FTC method. A similar approach has recently been applied for the FTC and steady-state methods (Larsen & Riisgård 2011). The recent development of the direct *InEx* technique, which allows *in situ* sampling of inhalant and exhalant currents from undisturbed suspension-feeders, may provide more reliable estimates of pumping rate and CR (Yahel *et al.* 2005).

Filtration rate, body size and interspecific variation

Water pumping rate and thus feeding capacity scales closely with gill area and size in bivalves. The allometric relationship between filtration rate (F; l h^{-1}) and tissue dry weight (W; g) is described by the equation

$$F = aW^b$$

The constants a and b are fitted parameters, a represents the filtration rate of an individual of unit body weight (or length) and b is the power to which increase in size raises that rate. The exponent b usually lies between 0.67 and 1.00, indicating proportionality between surface area and body weight. Generally speaking, weight-specific filtration rates decline with increase in body size. For example, in the hard clam *Meretrix lusoria*, a commercially important species cultured in Taiwan, Chien and Hsu (2006) found that clam size was

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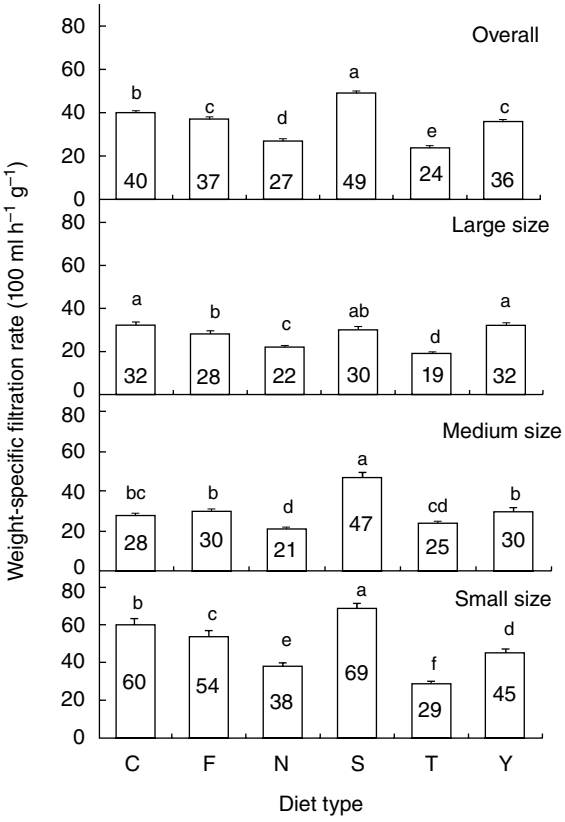


Figure 4.4 Mean filtration rate of three size classes (small: 7–11.9 g total wet body weight; medium: 12–17.9 g; large: 18–26 g) of hard clams (*Meretrix lusoria*) receiving six diets (C: commercial hard clam feed, F: fishmeal, N: microalgae *Nannochloropsis oculata*, S: soybean meal, T: microalgae *Tetraselmis chui* and Y: bread yeast). Numbers indicate weight-specific filtration rate values, and different letters indicate significant differences ($p < 0.05$).
From Chien and Hsu (2006). Reprinted with permission from the National Shellfisheries Association–BioOne.

inversely correlated with weight-specific filtration rate (Figure 4.4). The decline in filtration rate with size occurs at a faster rate than metabolic rate, and this causes a decline in growth efficiency, which limits the ultimate body size of bivalve species (see Chapter 6).

There is considerable variation in filtration rates both within and between species (Table 4.1). While some of this variation represents genuine differences, some can be attributed to the differing experimental conditions employed in the different studies. Bricelj and Shumway (1991) suggested that as filtration rates are extremely sensitive to changes in food quality and quantity, interspecific comparisons are best carried out from studies that employ identical experimental protocols on a wide variety of bivalve species. Riisgård and Larsen (1995) and Riisgård (2001a) have pointed out the importance of performing filtration experiments under optimal conditions; for example, using algae concentrations similar to those to which the bivalve is adapted in nature. Suboptimal conditions, for example unnaturally high algae concentrations, can result in partial valve closure, leading to a reduction in filtration rate (Jørgensen *et al.* 1988). In addition, Jones *et al.* (1992) have suggested that for population comparisons, where a size range is used to determine the pumping rate, it should be determined for individuals, or collectively for animals with a very small size range. This is because water-pumping rates vary considerably with time for any single individual.

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Table 4.1 Filtration rates measured by different methods under optimal laboratory conditions.

Suction method	
Møhlenberg and Riisgård (1979)	
Cardium echinatum	$F = 4.22\,W^{0.62}$
Cardium edule	$F = 11.60\,W^{0.70}$
Mytilus edulis	$F = 7.45\,W^{0.66}$
Modiolus modiolus	$F = 6.00\,W^{0.75}$
Arctica islandica	$F = 5.55\,W^{0.62}$
Photoaquarium method	
Riisgård and Møhlenberg (1979)	
M. edulis	$F = 7.37\,W^{0.72}$
Clearance method	
Griffiths (1980)	
Choromytilus meridionalis	$F = 5.37\,W^{0.60}$
Berry and Schleyer (1983)	
Perna perna	$F = 8.85\,W^{0.66}$
Riisgård (1988)	
Crassostrea virginica	$F = 6.79\,W^{0.73}$
Geukensia demissa	$F = 6.15\,W^{0.83}$
Replacement method	
Coughlan and Ansell (1964)	
Mercenaria mercenaria	$F = 2.5\,W^{0.78}$
Thermistor method	
Meyhöfer (1985)	
Clinocardium nuttalii	$F = 3.1\,W^{0.80}$
Mytilus californianus	$F = 7.9\,W^{0.72}$
Chlamys hasata	$F = 8.7\,W^{0.94}$

From Riisgård (2001a). Reproduced with permission from Inter-Research.
Filtration rates (F, $l\,h^{-1}$) as a function of size (W, g body dry wt) are presented as allometric equations $F = aW^b$. For clarity temperature, size range and so on have been omitted.

Particle concentration

Pumping rates are largely dependent on the concentration of particulate material in the medium. In laboratory experiments Wilson and Seed (1974) have shown that mussel pumping rate rapidly decreased or stopped altogether when particle-free seawater was used. They concluded that particulate material present in natural seawater, in the form of plankton and fine detritus, stimulates and maintains pumping. Mussels do not filter in very dilute suspensions, and this undoubtedly conserves energy during periods when particulate food is scarce, as in winter months. In order for filtration to start particle concentration must reach a critical threshold level. In *Mytilus edulis* this is about 50 cells μl^{-1} for the algae *Phaeodactylum tricornutum*, and filtration rate remains high and constant (Figure 4.5a) until particle concentration reaches about 700 cells μl^{-1} (Foster-Smith 1975). However, results from a later study (Riisgård 1991) showed that mussels filter maximally at much lower particle concentrations than those given earlier. For example, mussels fed on a concentration of 3–10 cells μl^{-1} (*Rhodomonas baltica*) filtered maximally, but when the concentration was elevated above 15 cells μl^{-1} the mussels reduced siphon opening and valve gape,[1] or complete valve closure, which ultimately led to a decline in filtration rate. Riisgård and Randløv (1981) reported similar results using *P. tricornutum.* In recent years chlorophyll α concentration is used as a proxy for algal biomass. This is because it is coloured, is specific to, and is shared amongst all primary producers (Huot *et al.* 2007). For *Rhodomonas salina*, a particle concentration of 6000 cells ml^{-1} is equivalent to 7.5 µg chl α l^{-1} (Pleissner *et al.* 2013).

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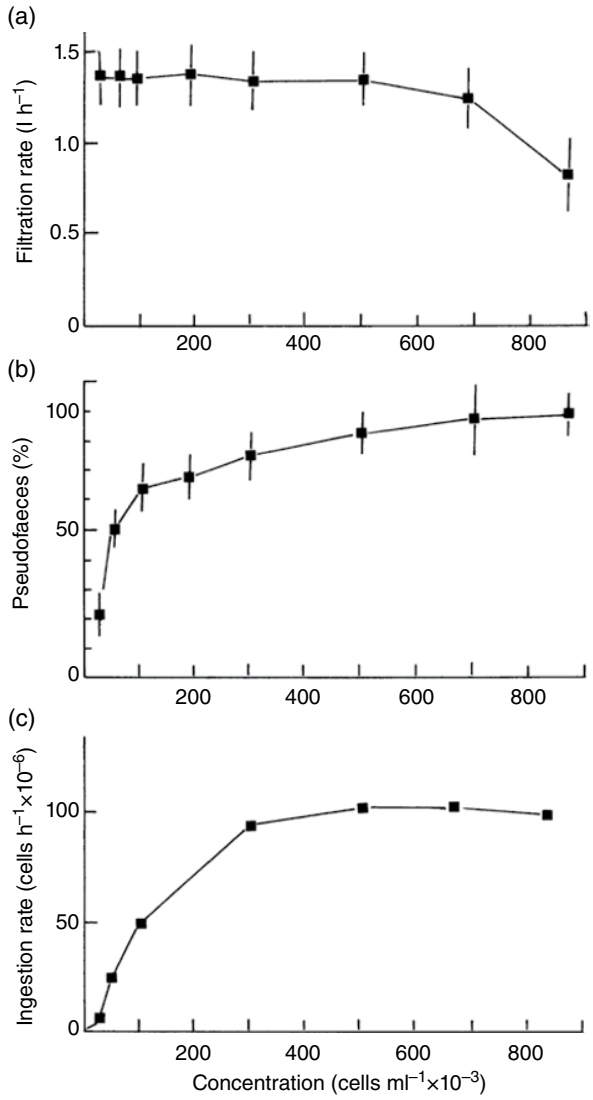


Figure 4.5 (a) Filtration rate, (b) percentage of material rejected before ingestion and (c) rates of ingestion in *Mytilus edulis* with increasing concentrations of the algae *Phaeodactylum tricornutum*. From Foster-Smith (1975). Reproduced with permission from Elsevier.

Apart from *M. edulis*, other species, for example the scallop *Pecten maximus*, filter at low particle concentrations (Strohmeier *et al.* 2009). Thus, bivalves may be adapted to exploit their filtration capacities at low algal concentrations, presumably a frequent occurrence in near-bottom water (Riisgård *et al.* 2003; see also Riisgård *et al.* 2006). It is therefore possible that the generally low growth rates obtained in laboratory studies may be due to the use of unnaturally high concentrations that lead to valve closure, reduced metabolism and reduced biosynthesis/growth.

As particle concentration increases the rate of pseudofaeces (material cleared from suspension but rejected before ingestion) production rises. In the mussel *M. edulis*, pseudofaeces production rises rapidly when algae concentration is increased from 50 to 100 cells μl^{-1}, and increases gradually at higher concentrations (Figure 4.5b). Subtracting the rate of

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pseudofaeces production from filtration rate gives the true ingestion rate (Figure 4.5c). For example, in *M. edulis* Foster-Smith (1975) found that ingestion rate gradually increased up to a concentration of 300 cells μl^{-1} and then remained constant up to 800 cells μl^{-1}. Thus, this species maintains a constant filtration rate over a wide range of cell concentrations, but rapidly increases pseudofaecal production to control ingestion ration. On the other hand, the clam *Venerupis pullastra* achieves the same result by decreasing its filtration rate at elevated particle concentrations, thus producing less pseudofaeces, but all the while maintaining a fairly consistent ingestion rate (Foster-Smith 1975).

Temperature

The effect of temperature on bivalve filtration rates has been well documented (Kittner & Riisgård 2005 and references therein). In the scallop *Argopecten irradians concentricus*, filtration rates are fairly constant over a temperature range of 10–26°C but are markedly reduced at 5°C. As metabolic expenditure (measured by O_2 consumption) increases with rising temperatures *A. irradians* needs a concomitant increase in food levels in order to avoid rapid weight loss at high temperatures. Filtration rates in another scallop, *Aequipecten opercularis*, are independent of temperature over the range 10–20°C but drop markedly at 5°C. Unlike *A. irradians*, oxygen consumption remains constant over this temperature range, allowing conservation of energy at higher temperatures (McLusky 1973). This strategy was also displayed by *M. edulis* (Bayne *et al.* 1976), although a more recent study found no evidence for temperature compensation in this species (Kittner & Riisgård 2005).

Temperature acclimation in filtration rates has been demonstrated for a variety of bivalve species. Filtration rates in the clam *Hiatella arctica* sampled in north-east Greenland increased with increasing temperature from less than −1°C to 4–8°C (Petersen *et al.* 2003). At higher temperatures, filtration ceased and the clams closed their valves. In specimens of the same species, sampled from temperate waters on the west coast of Sweden, rates increased with increasing temperature from 0 to 19–20°C. Filtration in *H. arctica* is adapted to constant low temperatures, for example in Polar regions, by having a lower minimum temperature of activity than found in individuals from temperate regions. Interestingly, lack of temperature compensation by increased filtration rates at low temperature is not limiting for growth at the prevailing food concentration levels. Another study investigated filtration rates in two species, *Crassostrea virginica* and *M. edulis* collected in eastern Canada and maintained in cold water (0, 4 or 9°C) over a 63-day period (Comeau *et al.* 2008). Experiments were carried out in a closed system initially inoculated with 10 000 algal cells ml^{-1}. For *C. virginica* the percentage of animals clearing the phytoplankton declined from peak values of 50% at 9°C to no animals filtering at 0°C, while for *M. edulis* the values ranged from 100% at 9°C to 17% at 0°C (Figure 4.6). Unlike *C. virginica*, *M. edulis* became progressively more tolerant to low temperatures over the 63-day period. The authors suggest that this puts the former species at a disadvantage in relation to seasonal 9°C in eastern Canadian waters. However, they caution that further experiments should be carried out to determine how changes in food quality or availability might influence CR in *C. virginica* at low temperatures.

Salinity

Mussels are euryhaline, being able to survive in salinities ranging from 4 to 5 psu to fully marine conditions. When filtration rates were measured in Baltic (15 psu) and North Sea (30 psu) mussels Theede (1963) observed that despite differing salinity, filtration rates were

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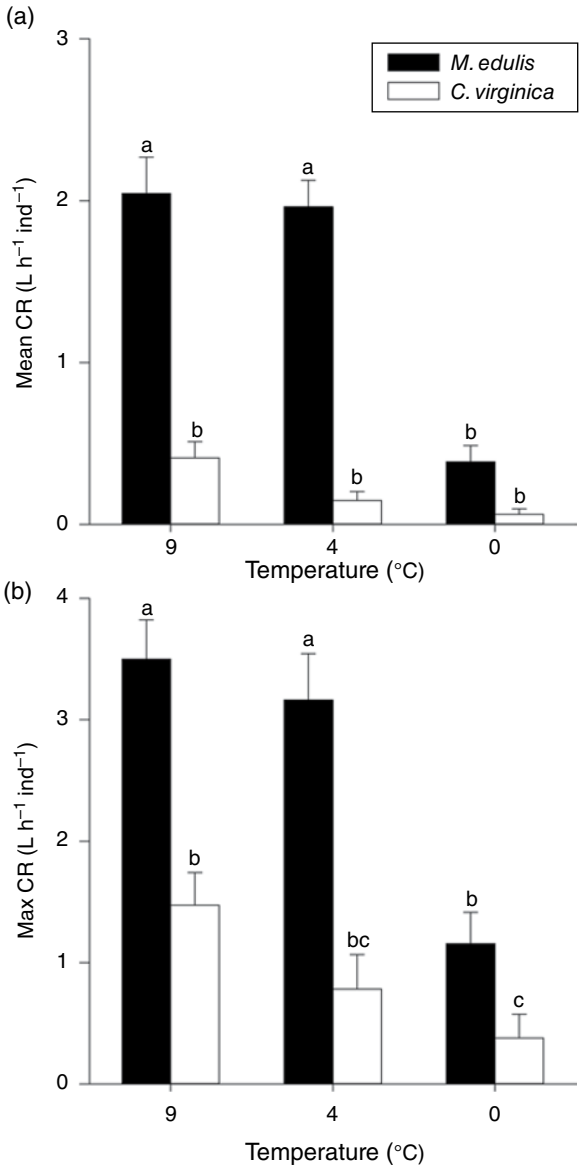


Figure 4.6 (a) Mean and (b) max clearance rate (CR) as a function of species (*Mytilus edulis, Crassostrea virginica*) and temperature (0, 4 and 9°C). Error bars represent one standard error of the mean. Different letters indicate significant differences ($p < 0.05$).
From Comeau *et al.* (2008). © 2008 Fisheries and Oceans, Canada. Reproduced with permission.

similar for the two populations. However, when mussels were transferred to either higher or lower salinity filtration rate was markedly reduced although given adequate time, on the order of weeks, acclimation to the new salinity can occur (Bayne *et al.* 1976). For example, when mussels were transferred from 34 to 18 psu filtration rate, which was initially depressed, it took more than 7 weeks to return to the control value (Bøhle 1972). During this period of acclimation, the length of which depends on the extent of salinity change, complete metabolic compensation takes place, culminating in a recovery of respiration and filtration rates to control values.

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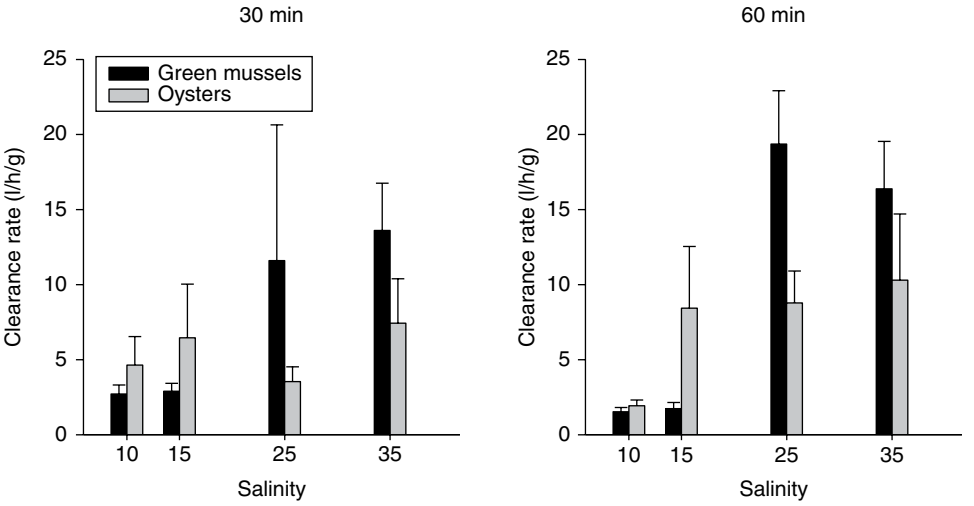


Figure 4.7 Mean clearance rates of five pooled replicates for oysters (*Crassostrea virginica*) and green mussels (*Perna viridis*) at 30 and 60 min. Bars represent standard errors.
From McFarland *et al.* (2013). © McFarland, Donaghy & Volety/CC BY 2.0.

The scallop *Argopecten purpuratus* has been introduced in the south of Chile as an alternative culture species. Navarro and Gonzalez (1998) investigated how decreasing salinity, similar to values observed during periods of heavy rain in winter in the south of Chile, might affect CRs in this species. Scallops were acclimated for a week at the experimental salinities 30, 27, 24, 21 and 18 psu.

CRs were high at 27 and 30 psu, but were significantly reduced at lower salinities. Scope for growth (Chapter 6) was greatly affected by low salinities, with positive values only between 27 and 30 psu, indicating that sites within this range are optimal for culture of this species. Another example studied the effect of different salinities (10, 15, 25 and 35 psu) on CRs in the eastern oyster *C. virginica* and the green mussel *Perna viridis*, a recent invader to Florida, United StatesA (McFarland *et al.* 2013). At 25 and 35 psu CRs in mussels were 2–3 times higher than those of oysters, but unlike native oysters, mussel CRs decreased by an order of magnitude at the lower salinities, and mussels increasingly closed their valves over time so that within 120 h of exposure all were either closed or dead (Figure 4.7). Their results suggest that this recent invader may be salinity-limited, providing *C. virginica* with a refuge from competition in estuaries that experience acute periods of low salinity.

Other factors

Marine bivalves orientate to maximise their filtration capacities and exploit their food supply. Increased water flow not only brings more food but also stimulates the animal to feed more rapidly. Initially, CR increases with flow speed as increasing flow compensates for seston[2] depletion around the bivalve. For example, in the clam *Ruditapes decussatus*, Sobral and Widdows (2000) observed a maximum CR of 2.5 $l h^{-1}$ per individual (0.3 g dry tissue mass) at current velocities up to 8 $cm s^{-1}$. CR declined, however, with increasing current speed, especially above 17 $cm s^{-1}$. At high flow speeds there is a build-up of a pressure differential between inhalant and exhalant apertures that interferes with filtration, and ultimately results in decreased growth rates (Wildish & Kristmanson 1988). The effect of flow speed on CR can vary both between and even within species. For example, in another

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clam species, the infaunal cockle *Cerastoderma edule*, CR was not significantly affected by changes in current speed, at least between 5 and 35 cm s^{-1} (Widdows & Navarro 2007). Until recently, the effect of flow on CRs in beds of conspecifics has largely been ignored. In a bivalve bed re-filtration takes place due to the formation of a seston-depleted layer above the bed (Jonsson *et al.* 2005), potentially reducing individual CR. Flow speed is likely to be an important factor in influencing CR over dense beds, as increasing flow rates will replenish seston-depleted water and reduce re-filtration (Jones *et al.* 2011 and references therein). In the infaunal clam *Austrovenus stutchburyi*, individual CR was significantly ($p<0.01$) greater at the high flow speed (0.88 $l h^{-1}$ per individual), compared to the medium and low flow speeds (0.34–0.54 $l h^{-1}$ per individual). With increasing bed density, individual CR was significantly ($p<0.001$) reduced; at low densities CR ranged from 0.6 to 1.4 $l h^{-1}$ per individual, but at high densities it ranged from 0.1 to 0.8 $l h^{-1}$ per individual (Jonsson *et al.* 2005).

Chemical stimuli also affect filtration rates in shellfish. The scallop *Placopecten magellanicus* increases its filtration rate in response to metabolites from the diatom *Chaetoceros muelleri*. The stimulus saturates at a low concentration of diatom extract equivalent to 5 cells μl^{-1} (Ward *et al.* 1992). These authors have suggested that chemical cues from phytoplankton are important factors that allow scallops to adjust their feeding rates in the wild.

Dodgson (1928) observed that under similar temperature conditions mussels cleared water more rapidly in September–October than in February–March, most likely related to different food levels in the water at different times of the year. In the eastern oyster *C. virginica* Li *et al.* (2012) reported not just seasonal variation but also a diel cycle in CRs, with a maximum weight-specific CR of 2.21 $l h^{-1} g^{-1}$ occurring around midnight, and a minimum CR of 0.32 $l h^{-1} g^{-1}$ at 07:40 h, coincident with the lowest concentration of dissolved oxygen in the water.

Factors that have a negative impact on bivalve filtration rates are heavy metals (Liu *et al.* 2014), biotoxins (May *et al.* 2010; Contreras *et al.* 2011) and loess, flocculent clay that is sprayed in the vicinity of aquaculture sites to mitigate the effect of harmful algal blooms (Shumway *et al.* 2003).

Control of filtration rate

There are two opposing schools of thought on whether filtration rate is physiologically controlled. Most of the supporting evidence is based on laboratory studies on the mussel *M. edulis*. The most widely held belief is that the process is under physiological control, and that mussels regulate pumping rate according to both the availability and composition of suspended seston (Bayne 1998; Hawkins *et al.* 1998a; Hawkins *et al.* 1999). The alternative less popular view, first mooted by Jørgensen in 1966 and elaborated on since then in various publications (see also Jørgensen 1990, 1996; Riisgård 2001b, and replies from Bayne 2001; Cranford 2001; Widdows 2001), is that accumulation of food particles on the gills is not regulated according to the nutritional needs of the individual, but is an automatic process basically determined by the capacity of the pump, the concentration of food particles in the water and the efficiency with which these particles are retained on the gills. Riisgård *et al.* (2003, 2011, 2014) have argued that these different points of view are mainly due to inconsistencies in methodological measurements of filtration, and that most of the reported experiments have been conducted in laboratories using high algal concentrations that are much higher than those encountered by mussels in the field. In addition, Riisgård and Larsen (2007) maintain that the effect of temperature on filtration rate is purely mechanical, controlled by viscosity, a parameter inversely related to water temperature. The debate continues.

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Energy costs

Suspension-feeders must filter large volumes of water to meet their food requirements. Because of life in a nutritionally dilute environment they possess low-energy pumps that continuously pump the surrounding water through gills that are efficient in retaining small food particles (Riisgård & Larsen 1995; Clausen & Riisgård 1996). However, published estimates of the metabolic cost of pumping are somewhat variable. Several studies have inferred high-energy costs in a wide selection of species. For example, in *M. edulis* and *M. californianus* Bayne and Newell (1983) estimated that 24% of an ingested ration of algae cells represented the cost of feeding, of which digestion and assimilation accounted for only 4–6%. A later study estimated the energetic costs of digestion to be about 17% of energy expenditure in *M. edulis* (Widdows & Hawkins 1989). In contrast, low values (1–3% of total metabolic expenditure) have also been reported (Bernard & Noakes 1990; Jørgensen 1990; Hawkins & Bayne 1992; Riisgård & Larsen 1995). In a later study the energy costs of filter feeding were evaluated using the ratio of useful pumping power (P) and total metabolic power expenditure R_t (Riisgård & Larsen 2000a). This ratio, referred to as the overall pump efficiency P/R_t, was estimated for various ciliary-feeders, including bivalves. Useful pump work constituted 0.3–4% of the total metabolic energy expenditure, with a value of just over 1% for *M. edulis*. However, the authors argue that measures of efficiency other than overall pump efficiency may be appropriate when estimating the energy costs of filter feeding. If the costs of powering bands of lateral cilia and their associated cells (R_p) are considered instead, then a minimum efficiency would be $R_p/R_t = 6.6\%$, rather than the 1% calculated earlier for *M. edulis*. Furthermore, if the ratio of gill to metabolic rate $R_g/R_t = 19\%$ is used it is clear that the energetic costs of filter feeding are significant (Riisgård & Larsen 2000a).

Particle processing on the gills, labial palps and in the stomach

Over the past two decades considerable progress has been made in understanding particle-processing mechanisms in suspension-feeding bivalves, and to a much lesser extent in deposit-feeders. Several complementary techniques have made this possible. Video endoscopy (Figure 4.8) uses an optical insertion tube inserted into the mantle that permits

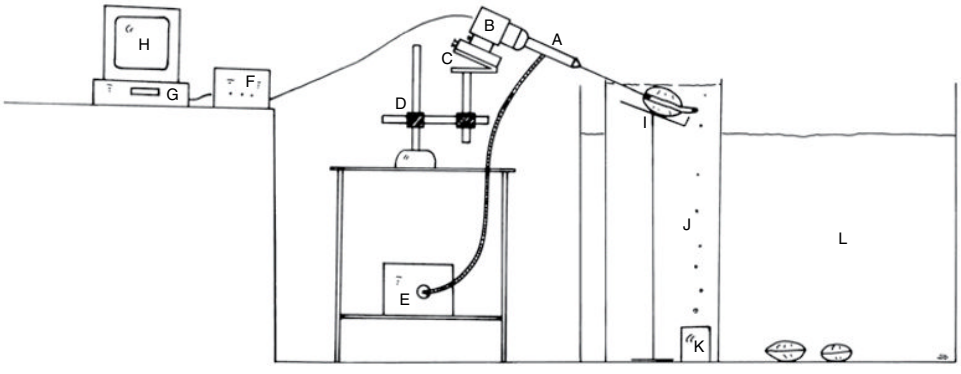


Figure 4.8 Equipment used for endoscopic observations. (A) Endoscope, (B) video camera, (C) micro-manipulator, (D) adjustable stand, (E) light source, (F) camera control box, (G) Hi-8 video cassette recorder, (H) monitor, (I) inclinable specimen stand, (J) bucket, (K) water-circulating pump and (L) aquarium. From Beninger *et al.* (1997b). Reproduced with permission from Inter-Research.

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direct *in vivo* observations of whole intact structures, for example gills and labial palps, in relatively undisturbed animals (Ward *et al.* 1991; Beninger *et al.* 1992). Combined with fluid sampling and subsequent chemical and histochemical analyses, histology and mucocyte (mucus-secreting cell) mapping, endoscopy has been responsible for a major leap forward in our understanding of the filter-feeding process in bivalves. Another technique, confocal laser scanning microscopy (CLSM), has the ability to provide high-resolution images from selected depths. Images are taken point by point and reconstructed with a computer to produce 3D reconstructions of complex objects. CLSM has been used to good effect to examine the spatial relationships, structure and movement of cilia and cirri on living gill filaments (Silverman *et al.* 1999; Stanton 2012).

Gills first appear at the pediveliger larval stage, and their structural and functional complexity increases until the juvenile stage has been reached. Studying the development of gills has led to new insights about the evolution of these structures and about the phylogenetic relationships among bivalve taxa (Cannuel & Beninger 2006; Beninger & Decottignies 2008; Cannuel *et al.* 2009). The simplest gill structure is the homorhabdic filibranch type found only in mussels (Figure 4.9a). Scallops also have this gill type but the gill filaments are differentiated into principal and ordinary filaments, so the gill type is termed heterorhabdic filibranch (Figure 4.9b). The majority of bivalves possess the slightly more complex eulamellibranch structure (Figure 4.9c). The pseudolamellibranch type is only found in oysters (Figure 4.9d). In some bivalves, for example scallops and oysters, the surface area of the gill filaments is greatly increased by folds or plicae (Figure 4.9b and d). Particle processing has been studied in detail in these four different gill types using representative species (see references later): the mussel *M. edulis* (homorhabdic filibranch), the scallops *P. magellanicus* and *P. maximus* (plicate heterorhabdic filibranch), the clam species *Spisula solidissima* and *Mya arenaria* (eulamellibranch) and the oyster *C. virginica* (plicate heterorhabdic pseudolamellibranch).

Bivalves employ a hydromechanical and mucociliary mechanism of particle transport. Lateral cilia, more often referred to as cirri, on the gill filaments maintain a flow of water through the mantle cavity and the gills. The water is filtered at the entrance to the interfilament spaces (leading to the ostia in the eulamellibranch gill) by the latero-frontal cirri, which strain particles from the water and throw them onto the frontal surface of the filaments. Cilia on the abfrontal surface, facing the excurrent flow, do not participate in pumping water, primarily because of their small size and sparse density, and also because of associated high mucocyte densities (Dufour & Beninger 2001). There are two main types of frontal ciliary tracts: lateral tracts of fine cilia and median tracts of coarse cilia (see Figure 2.10b and c, Chapter 2). The filaments, particularly the frontal surfaces, are well supplied with mucocytes. The particles are trapped in a fine mucus layer (raft) overlying the frontal cilia and transported towards the ventral ciliated particle grooves (Beninger *et al.* 1997a). There the material is incorporated into mucus strings that are transported along the grooves towards the labial palps (Figure 4.10). In the ventral gill particle groove material is never in suspension, even at low particle concentration (Ward *et al.* 1993). This mechanism of particle processing is seen in homorhabdic filibranchs (mussels) and eulamellibranchs (clams), that is the vast majority of bivalve species. However, heterorhabdic filibranchs (scallops) employ a different strategy (Beninger *et al.* 2004; Beninger & Le Pennec 2006). Their gills do not have a ventral food groove so particles drawn into the pallial cavity move towards the dorsal region of the gill, and are deflected from the ordinary filaments into the plical troughs. A principal filament (PF) occupies each plical trough (Figure 4.9b). Material exits the plical troughs and is incorporated into a mucus slurry in the dorsal ciliated tracts and is transported to the labial palps. The transport mechanism is hydrodynamic but the medium is a mucus slurry. At high particle

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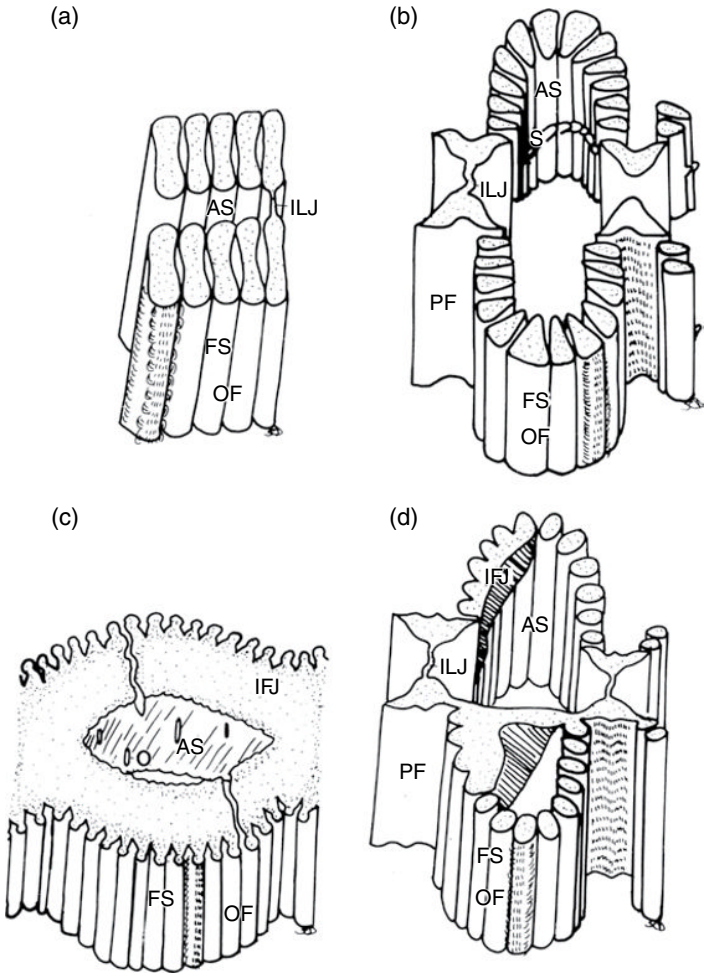


Figure 4.9 Transverse sections through the demibranchs of the principal gill types in suspension-feeding bivalves. (a) Homorhabdic filibranch. Ordinary filaments (OF) connected by interlamellar junctions (ILJ). (b) Heterorhabdic filibranch. Principal filaments (PF) and OF, joined by ILJ and ciliated spurs (S). (c) Homorhabdic eulamellibranch. OF joined by interfilament junctions (IFJ). O: ostia. (d) Heterorhabdic pseudolamellibranch. Principal filaments and ordinary filaments, joined by ILJ and IFJ. AS, abfrontl surface; FS, frontal surface.
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concentrations particles embedded in viscous mucus are ejected from the PFs onto the ordinary filament plicae, and then directed to ventral ciliated tracts. The ventral tract appears to be a rejection route that is activated when the ingestive or handling capacity of the scallop is saturated. The scallop gill therefore exhibits a division of labour, with the PFs involved in feeding and the ordinary filaments concerned with cleaning (Beninger *et al.* 1992, 1993). Pseudolamellibranchs (oysters) employ yet another strategy. Most particles captured on the frontal surfaces of ordinary filaments are directed to the ventral grooves and travel embedded in a mucus string towards the palps. Some particles, however, move from the ordinary filaments into the plical troughs. From there they are transported into dorsal ciliated grooves, and move anteriorly suspended in a slurry (Ward *et al.* 1994).

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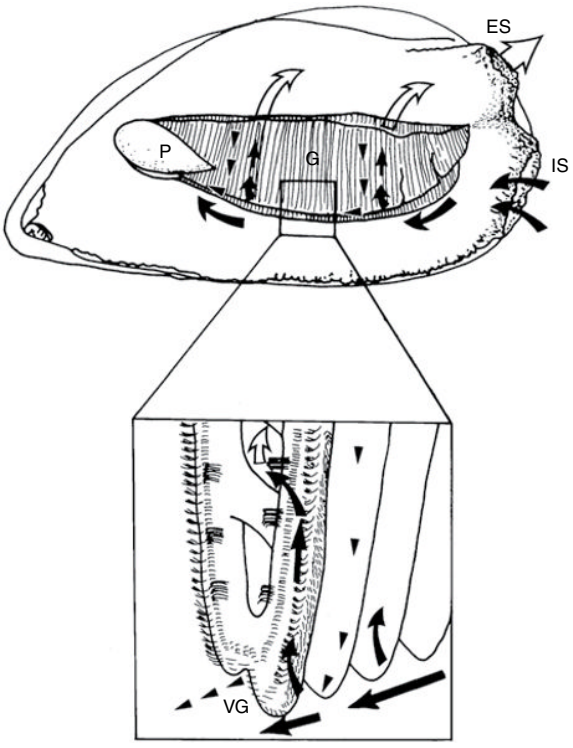


Figure 4.10 Principal pathway of current flow and particle transport on the homorhabdic filibranch gill of *Mytilus edulis*. Water enters the animal through the inhalant siphon (IS). The frontal surface of the gill (G) is exposed to an antero-posterior flow at the ventral margin, and to a ventro-dorsal flow on the rest of the frontal surface (solid arrows). Water exits the gill from the abfrontal region, out through the exhalant siphon ((ES); open arrows). Arrowheads represent particle transport to the ventral particle groove (VG); (P) labial palps.
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The gills, however, are not mere sieves but are able to control the rate at which suspended material is removed from the surrounding water, either by altering the angle of ciliary beat or by altering gill dimensions (interfilament width, ostial area, cross-sectional area of water tubes) through contraction of gill muscles (Medler & Silverman 2001).

Selective particle capture

The capture mechanism in suspension-feeding bivalves involves particle encounter and retention on the gills. Strong selection of particles at this stage should improve the quality of material, which subsequently undergoes particle sorting processes, potentially increasing the efficiency of pre- and postingestive particle sorting (Ward & Shumway 2004).

Capture efficiency in relation to particle size has been determined in a wide variety of bivalves using both direct and indirect methods (see Ward & Shumway 2004 and references therein; Yahel *et al.* 2009). Bivalve larvae show a high retention rate for particles in the size range 1.4–4 μm diameter (Tezuka *et al.* 2009 and references therein), while most adults retain particles of 3–4 μm diameter with an efficiency of 100% and retain particles of 1 μm diameter with a reduced efficiency of as much as 50% (Shumway *et al.* 1985; but see Strohmeier *et al.* 2012). Mussels can retain motile flagellates as small as 1–2 μm diameter,

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but can also remove bacteria (0.3–1.0 µm) from suspension, albeit with low efficiency. The mussel *Geukensia demissa*, for example, retains particles as small as 0.4–0.6 µm with 86% of its efficiency for larger particle capture. This ability is related to the narrow space (~0.5 µm) between the latero-frontal cilia (Wright *et al.* 1982). The mussel *Perna perna* also shows high retention efficiency (RE) for particles of this size and therefore both of these species seem specifically adapted to exploit the bacterial size-fraction. Lower capture efficiency of large particles compared to small particles has been reported in oysters. For example, *Crassostrea gigas* captures the diatom *Nitzschia closterium*, preferentially when compared to phytoplankton of larger diameter; the inflexible spicules of the diatom frustule may facilitate this differential retention (Bougrier *et al.* 1997). Defossez and Hawkins (1997) have suggested that preferential rejection of larger particles as pseudofaeces could have an adaptive value if larger particles are on average less nutritious than smaller particles. In contrast, the lower limit for effective retention of particles in scallops is about 5–7 µm and therefore bacterioplankton is not available as a food source (see later). But scallops are capable of ingesting relatively large particles; 10–350 µm diameter particles were described from the gut contents of *Placocopecten magellanicus* (Shumway *et al.* 1987) and particles up to 950 µm from *Patinopecten yessoensis* (Mikulich & Tsikhon-Lukanina 1981).

RE can also depend on particle shape and motility, and on qualitative factors such as particle stickiness, electrostatic charge (Hernroth *et al.* 2000) or molecular excretions (e.g. living versus dead diatoms; Beninger *et al.* 2008), as well as lectin (mucopolysaccharide) secretion on the gills (Pales Espinosa *et al.* 2009; Jing *et al.* 2011). Environmental factors may also play a role. At low tide the rock pool clam, *Venerupis corrugatus*, retained particles of 5–9 µm with efficiencies of 70–100%, while at high tide particles of 8–13 µm were retained with efficiencies between 75 and 100% (Stenton-Dozey & Brown 1992). The shift in capture efficiency coincided with an increase in the size range of particles that contained the highest organic content. Results of a recent study have shown that mussels (*M. edulis*) can modulate RE in relation to seasonal shifts in the composition of particles (Strohmeier *et al.* 2012). RE progressively increased from small to large particles with a maximum RE (80–100%) at 30–35 µm particle size. Temporal changes in seston size distribution towards a dominance of smaller particles coincided with a decrease in the RE maxima to 14–64% and 12–86% for particles between 7 and 15 µm, respectively. Unlike Stenton-Dozey and Brown (1992), these authors did not analyse the organic content of particles in the different size ranges. Results from both studies clearly indicate the capacity of bivalves to vary RE in response to exogenous trophic resources. Moreover, the evidence contradicts the traditional view that RE increases non-linearly with increasing particle size to a maximum at approximately 4–7 µm (see earlier), a size threshold that has become engrained within the bivalve ecophysiology literature (see Strohmeier *et al.* 2012 for discussion).

It is generally believed that the latero-frontal cilia or cirri play a key role in bivalve feeding (Silverman *et al.* 1999). In *M. edulis*, for example, the arrangement forms a filter with a mesh size of 2.7 × 0.6 µm, which would clearly explain the high retention of 1–2 µm diameter particles recorded for this species (Møhlenberg & Riisgård 1979). Indeed, species with well-developed latero-frontal cirri show 90% RE of particles in the size range 2.0–3.5 µm, whereas species with either short (*Ostrea*) or undeveloped latero-frontal cirri (some scallop species) show only 50% efficiencies in this size range (Jørgensen 1990). In species lacking latero-frontal cirri it seems likely that oscillatory currents produced by the lateral cilia are responsible for the transfer of suspended material onto the frontal surface of the filaments (references in Jørgensen 1990). Ward *et al.* (1998a) contend, however, that it is the gill filaments themselves that are the main capture units, and not the latero-frontal cirri. They argue that the latter still play an important role in particle capture by producing vortices that redirect particles and flow away from the inter-filamentary spaces towards the frontal

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surfaces of filaments (but see Riisgård & Larsen 2000b, 2010). Rather than acting as mechanical sieves (see earlier) the latero-frontal cirri may act as solid paddles and function in a manner very similar to the fine setules of small aquatic organisms. We are still some ways from a clear understanding of particle capture mechanisms in suspension-feeding bivalves. A series of informative video clips on particle capture on bivalve gills, produced by Evan Ward and colleagues at the Department of Marine Sciences, University of Connecticut, may be accessed at http://web.uconn.edu/jevanward/video.htm.

Preingestive particle processing

Preingestive sorting leads to the formation of pseudofaeces – material cleared from suspension but rejected before ingestion. It should be emphasized that production of pseudofaeces relies on two very different processes: (i) ingestion volume control, in which case the pseudofaeces may contain nutrient-rich particles, and (ii) qualitative and/or size selection, in which the pseudofaeces are enriched in 'undesirable' particles. Similar to the process of selective particle capture discussed earlier, strong selection of particles at the post-capture stage in the feeding process would enhance the quality of material ingested, thereby increasing the efficiency of postingestive particle sorting in the stomach (Ward & Shumway 2004).

The role of mucus

A variety of different techniques have clearly demonstrated that mucus plays a key role in all aspects of particle processing, and that different types of mucus are used for different functions on gills, labial palps, mouth and mantle (Beninger & St-Jean 1997a, b; Beninger *et al.* 2005). Generally, high-viscosity mucus, secreted by acid mucopolysaccharide (AMPS) mucocytes, is used for particle transport on surfaces exposed directly to inhalant flow (e.g. in the ventral groove, on the frontal surfaces of ordinary filaments) or on surfaces leading to areas exposed directly to flow; it is also used in rejection pathways as well as initial transport on the gill for a subsequent 'decision' (pertinent only to mussels and clams with a single ventral particle groove). Transport in these cases is counter to the current flow, and such highly viscous mucus greatly facilitates particle transport. 'Counter' is defined as an angle approximately 0–90° with the current flow. Lower-viscosity mucus, secreted by mixed mucopolysaccharide (MMPS) mucocytes, is used when particle transport is on an enclosed or semi-enclosed surface, such as the dorsal tracts and PFs, that is particles destined for ingestion and moving with the current flow. Neutral MPS mucocytes are found in areas where low-viscosity mucus is important, for example the ridged areas of the labial palps (Beninger & St-Jean 1997a, b). Although mucus is an important element in suspension feeding there are no data on the energetic costs of its production. However, the costs are unlikely to be high as mucus produced in feeding is either reabsorbed or ingested, and the animal is generally in adequate energy balance to support the costs of mucus produced for rejection (Beninger *et al.* 1993).

Labial palps and pseudofaeces production

The labial palps are paired, fleshy leaf-shaped structures on either side of the mouth. The inner surface of each palp faces the gill, and is folded into numerous ridges that carry a complicated series of ciliary tracts and various types of mucocytes. The outer surfaces of the palps are smooth, and between the inner and outer surfaces there is muscular-connective tissue. The palps may reach posteriorly for about one-third, for example in the mussel *Mytilus chilensis*, to three-quarters of the length of the gills, for example in the taca clam

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Mulinia edulis (Garrido *et al.* 2012). But individual species can alter the size of labial palps (and gills), which could be a response to changes in food quantity and quality (Honkoop *et al.* 2003 and references therein).

Bivalve gill type dictates the nature of particle processing on the labial palps. On the homorhabdic filibranch gill in mussels (Beninger & St-Jean 1997b; Garrido *et al.* 2012) all captured particles move ventrally into the ventral gill particle groove, even when particle concentrations are high, and proceed anteriorly embedded in a viscous mucus cord towards the palps. The dorsal canal of the gill is not involved in particle accumulation and transport. It should be emphasized at this point that little, if any, selection takes place on the gill, possibly due to is simple, non-plicate structure (Ward *et al.* 1998b). The outer and inner palps enclose the anterior gill region (Figure 4.11); the outer demibranch is applied to the ridged surface of the outer palp and the inner demibranch to the ridged inner palp surface. The gill rests against a specialised region of the palp called the dorsal fold. This is a smooth, non-ridged flap of densely ciliated tissue that covers approximately half of the ridged surface of each palp (Figure 4.12). Its ventral margin is unattached to the underlying ridges, forming an antero-posterior ciliated tract called the palp particle groove. This part of the dorsal fold is contractile, allowing it to cover or expose areas of the ridged palp surface. If the ingestive/handling capacity of the gill is not overloaded, a mucus particle cord arriving in the gill

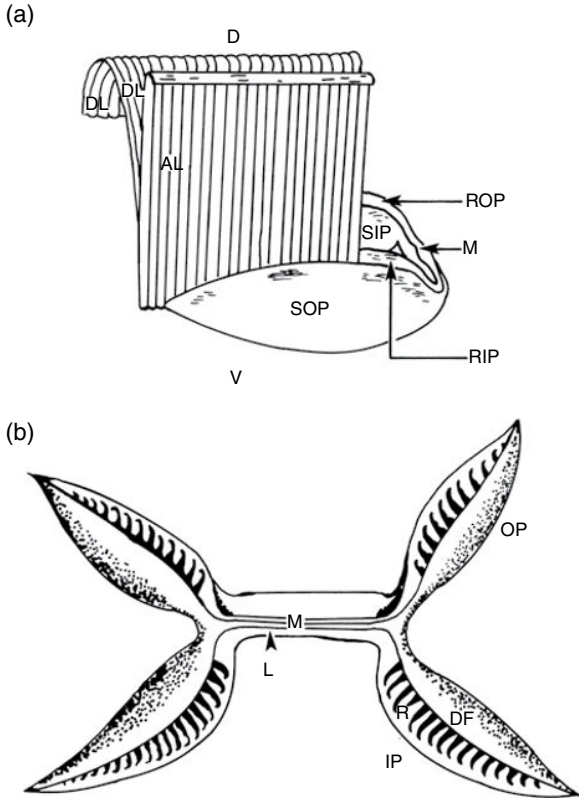


Figure 4.11 Arrangement of the gill, labial palps and mouth in *Mytilus edulis*. AL, ascending lamella of gill; D, dorsal; DL, descending lamella; M, mouth; RIP, ridged surface of inner palp; ROP, ridged surface of outer palp; SIP, smooth surface of inner palp; SOP, smooth surface of outer palp; V, ventral. From Beninger and St-Jean (1997a). Reproduced with permission of Springer Science and Business Media.

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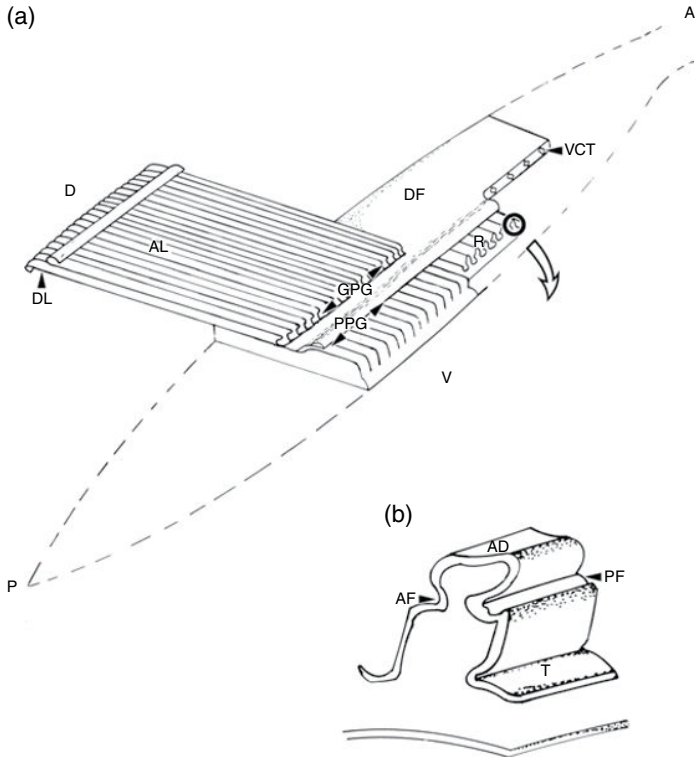


Figure 4.12 Drawing of ridged surface of a single labial palp in *Mytilus edulis*. A, anterior; AL, ascending lamella of gill; D, dorsal; DF, dorsal fold; DL, descending lamella of gill; GPG, gill particle groove; P, posterior; PPG, palp particle groove; R, ridges; V, ventral; VCT, vestigial ciliated tracts (derived from the fusion of the epithelium of the palp crests with that of the dorsal fold). From Beninger and St-Jean (1997a). Reproduced with permission of Springer Science and Business Media.

particle groove is pulled out by cilia on the dorsal fold and directed ventrally to the palp particle groove. The mucus particle cord is subjected to mechanical stress through kneeding of palp ridges in the palp particle groove and to biochemical stress through mucus secretion. Consequently, the cord disintegrates and the material becomes less viscous and more flocculent. Some of this material travels anteriorly, presumably for ingestion, while some enters the palp troughs, exiting at the palp ventral margin for eventual rejection. When the ingestive/handling capacity is overloaded the dorsal fold retracts, thus preventing the mucus chord from entering the palp particle groove. Instead the cord is moved to the ventral margins of the palps for rejection. Thus, the dorsal fold, which is unique to homorhabdic filibranchs, determines whether material from the gill is destined for ingestion or rejection. Recently, Garrido *et al.* (2012) observed that the mucus particle chord may be transferred directly from the gill ventral groove to the mantle wall without the participation of the palps, in which case no selection or ingestion takes place.

In eulamellibranchs (clams) the palps, however, do not have a dorsal fold and the inner and outer palps of each pair are fused along their dorsal margins. The inner and outer palps are thus a functional unit with the ridged surface of the inner and outer palp accepting material from the inner and outer demibranchs, respectively. The eulamellibranch gill is similar to the homorhabdic filibranch gill in that the dorsal groove plays no significant role in the accumulation or transport of food particles; so all captured particles move into the

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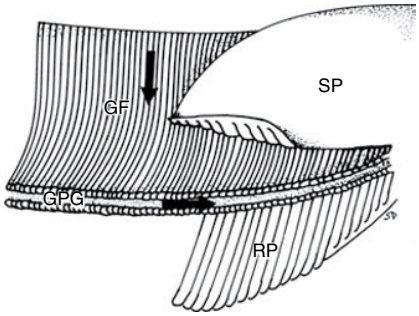


Figure 4.13 Position of the eulamellibranch gill and labial palps in *Mya arenaria*. Arrows indicate direction of mucus particle masses along gill filaments (GF) and in gill particle groove (GPG). RP, ridged palp surface; SP, smooth palp surface.
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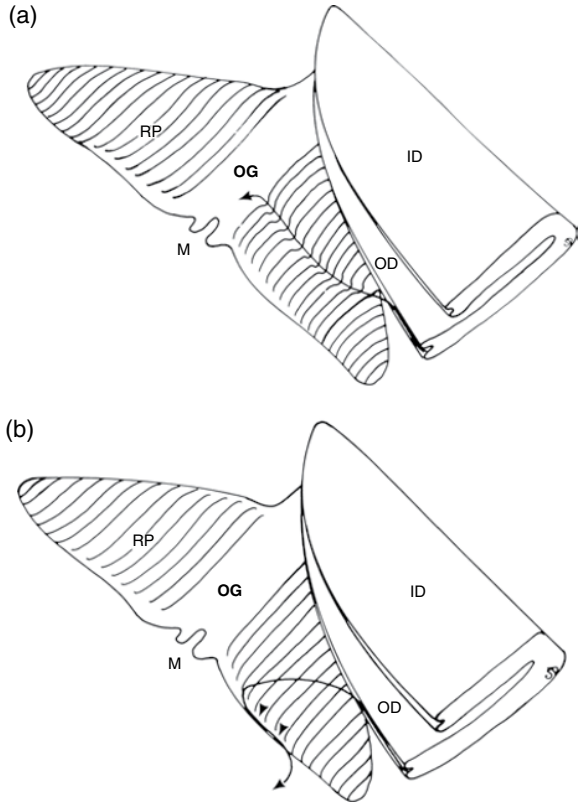


Figure 4.14 Drawings of labial palps and gill of *Mya arenaria* showing mucus particle trajectories during ingestion and rejection. Labial palps are spread to reveal ridged surface. (a) Direction of mucus particle masses destined for ingestion along ridged palp surface (RP). (b) Direction of rejected mucus particle masses along ridged palp surface. Arrowheads represent small mucus particle masses exiting troughs and joining mucus cord on the palp ventral margin. ID, inner demibranch; M, mouth; OD, outer demibranch; OG, oral groove.
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ventral particle groove and proceed embedded in a cohesive mucus cord towards the palps (Figure 4.13). There the cord detaches from the gill onto the ridged surface of the palps. Material for ingestion passes over the palp crests at right angles to the ridges (Figure 4.14a). *En route* it is subjected to mechanical forces applied by the apposition and grinding motion of both palps. Consequently, the integrity of the cord is destroyed and all material in the oral region is in the form of a mucus particle slurry (Ward *et al.* 1994; Beninger *et al.* 1995). After a prolonged period of feeding, mucus particle cords from the gill are instead transported obliquely over the palp crests to the palp ventral margin for rejection as pseudofaeces (Figure 4.14b). Similar to homorhabdic filibranchs, the mucus particle chord may be transferred directly from the gill ventral groove to the mantle wall without the participation of the palps (Garrido *et al.* 2012).

Ward *et al.* (1994) have given a very comprehensive account of particle processing on the labial palps of the pseudolamellibranch oyster *C. virginica*. Labial palp structure in pseudolamellibranchs is similar to that in eulamellibranchs. As already mentioned, particles on the gills of pseudolamellibranchs are transported in the dorsal and ventral grooves towards the labial palps. Particles in the dorsal tracts are transported in a slurry towards the gill–palp junction. They leave this junction by one (or more) of four routes (Figure 4.15a):

1. Into the oral groove on the palp, and onwards to the mouth
2. Onto the ridged sorting surface of the palp
3. Onto the smooth ciliated tract on the palp edge
4. Onto the anterior margin of each demibranch for posterior transport.

Cilia on the palp ridges are organised into different functional tracts: rejection tracts that transport particles to the palp margins, oral acceptance tracts that transport particles towards the mouth and resorting tracts that either reject or accept particles. The route that particles take is highly dependent on ambient particle concentration. Under most conditions particles are transported along the oral groove, or onto the ridged sorting surfaces of the palps. However, at high particle concentrations the rate of particle capture by the gills exceeds the animal's ingestive capacity, or the sorting capacity of the palps, and consequently the gill–palp junction becomes overloaded. Then, particles are rapidly removed via the smooth ciliated tract from where they are either carried onto the ridged palp surface, or rejected off the edge of the palp as pseudofaeces. When particle concentrations increase even more, particles are removed from the gill–palp junction onto the ventral ciliated indentation (continuation of ventral groove) on the anterior margin of each demibranch.

Particles in the ventral grooves are carried anteriorly in mucus strings until they reach a point where anteriorly directed ciliary action stops. At these points (marked X in Figure 4.15b) the mucus-bound particle strings coalesce, forming balls with any particles being moved posteriorly along the ventral indentation. If large enough, these balls are picked up by the highly mobile palp tips and transported onto the ridged palp surface, resulting in a continuous particle string from the gills to the palps. Alternatively, the balls are sometimes swept off the gill by water currents, and rejected as pseudofaeces. The mucus particle strings are fluidised in a manner similar to that already described earlier. The particles released from the mucus strings, together with those from the dorsal grooves, are sorted on the palp ridges, and then either enter the oral groove or are rejected off the palp. At high particle concentrations mucus strings from the ventral food grooves may not enter the palps at all but may be rejected at the gill–palp junction and combine with other rejected material to form pseudofaeces. In pseudolamellibranchs both the gill and the labial palps effect particle sorting, unlike homorhabdic fillibranch and eulamellibranch bivalves.

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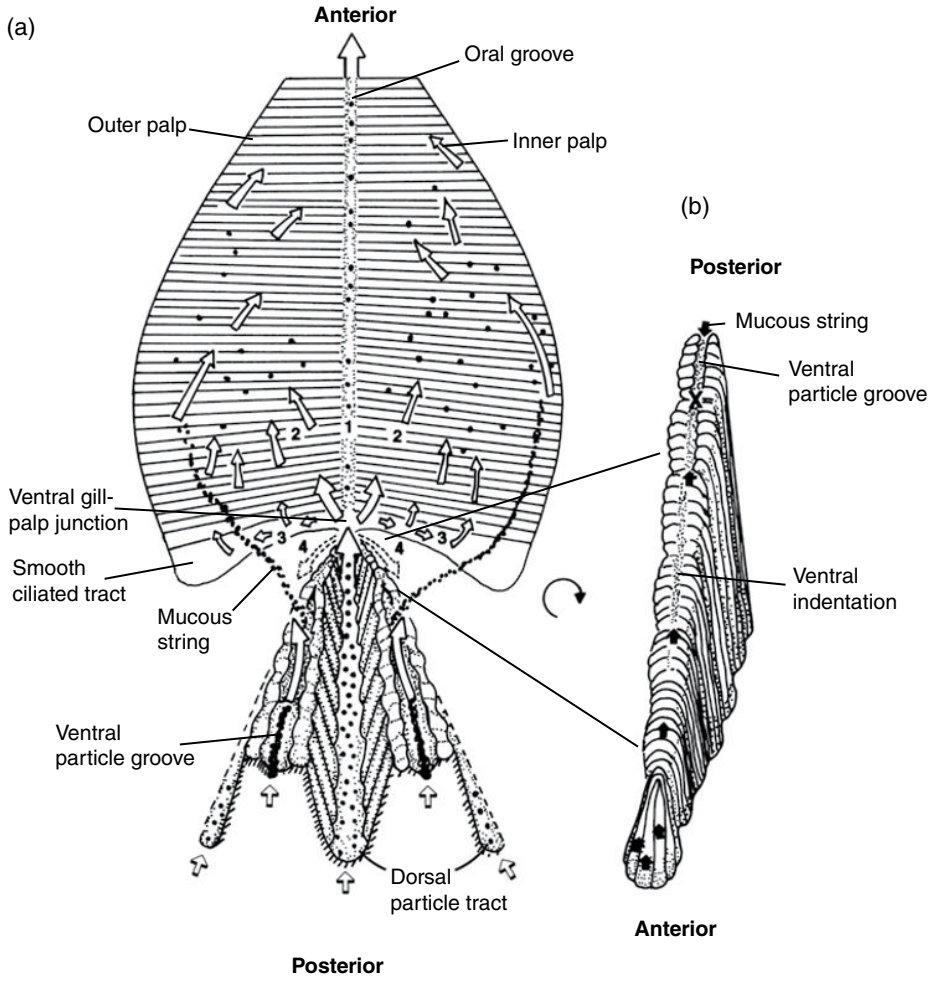


Figure 4.15 Schematic diagram of the anterior portion of two demibranchs of *Crassostrea virginica* and their junction with one pair of labial palps. (a) The labial palps are folded open (not real-life position) to reveal the general direction of particle movement on the smooth ciliated tract and the ridged sorting surface. The movement of particles bound in two mucus strings from the ventral food grooves onto the inner surface of the palps is illustrated, and open arrows indicate the subsequent direction of movement of these particles (solid circles) on the ridged palp surface. The viscosity of the mucus strings is reduced by the mechanical action of the ridged palp surface so that the entrapped particles are dispersed and subsequently sorted. Solid circles and short open arrows indicate the movement of particles from the basal food tracts towards the gill–palp junction. These particles may either move directly into the oral food groove (marked 1), onto the palps (marked 2) or initially along the smooth ciliated tract and then onto the palps (marked 3). Transport of material away from the gill–palp junction via the ventral indentation on each demibranch is shown by broken arrows (marked 4). (b) An enlargement of the anterior termination of one demibranch illustrating how the ventral food groove becomes narrower and shallower until it forms the ventral indentation tract. Note that the orientation of (b) is opposite to (a) to more clearly illustrate the anterior end of the demibranch. Small black arrows indicate the transport of particles posteriorly, away from the gill–palp junction. X marks the point where these posteriorly beating cilia meet the anteriorly beating cilia in the main ventral groove.
From Ward *et al.* (1994), figure 2. Reproduced with permission of the Marine Biological Laboratory, Woods Hole, MA.

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In heterorhabdic filibranchs, for example the scallops *P. magellanicus* and *P. maximus* (Beninger *et al.* 1992, 2004; Beninger & Le Pennec 2006), particles destined for ingestion travel along dorsal tracts on the gill, whereas the ventral tract is concerned solely with material destined for rejection. The ventral tract is not a groove, so pseudofaeces can be produced at any point along its course (Beninger *et al.* 1999). In this gill type, and also in the pseudolamellibranch gill, pseudofaeces are produced by ingestion volume control and by qualitative particle selection (see later). The mantle does not possess cilated rejection tracts, and pseudofaeces are expelled entirely by periodic valve clapping. Material arriving at the palps from the dorsal tracts is in the form of a low-viscosity slurry. When the ingestive capacity of the scallop is not overloaded this material moves to the mouth. Like pseudolamellibranchs, both the gill and the labial palps of heterorhabdic fillibranchs are involved in particle sorting. When overloading occurs this material is rejected off the palps, and is transferred to the mantle until it is ejected at the next valve-clapping episode. Particle processing on the gill and labial palps in the four major gill types described earlier is summarised in Table 4.2.

Pseudofaeces transport

With the exception of heterorhabdic filibranchs (see earlier) pseudofaeces are transported along well-defined rejection tracts on the ciliated mantle surface to the inhalant opening or siphon and periodic, sudden and forceful closure of the shell valves ensures that it is carried

Table 4.2 Summary of post-capture particle processing on the pallial organs of the four main gill types.

		Function		
Gill type	**Species**	**Gill**	**Labial palps**	**Mantle**
Homorhabdic filibranch	*Mytilus edulis*	Indiscriminate transport, ventral groove only	Qualitative selection and pseudofaeces rejection; ingestion volume control	Pseudofaeces rejection
Heterorhabdic filibranch	*Placopecten magellanicus*, *Pecten maximus*	Initial positive selection (dorsal tracts); ingestion volume control and initial negative selection (ventral tracts)	Qualitative selection and pseudofaeces rejection	No rejection function; pseudofaeces expelled from ventral gill margin by shell adduction
Eulamellibranch	*Spisula solidissima*, *Mya arenaria*	Indiscriminate transport; ventral groove only	Qualitative selection and pseudofaeces rejection; ingestion volume control	Pseudofaeces rejection
Pseudolamellibranch	*Crassostrea virginica*, *Ostrea edulis*	Initial positive selection (dorsal tracts),* ingestion volume control and initial negative selection (ventral tracts)	Qualitative selection and pseudofaeces rejection; ingestion volume control	Pseudofaeces rejection

From Peter Beninger, MMS, UFR Sciences, Université de Nantes, France.
*For particles small enough to enter the principal filament grooves.

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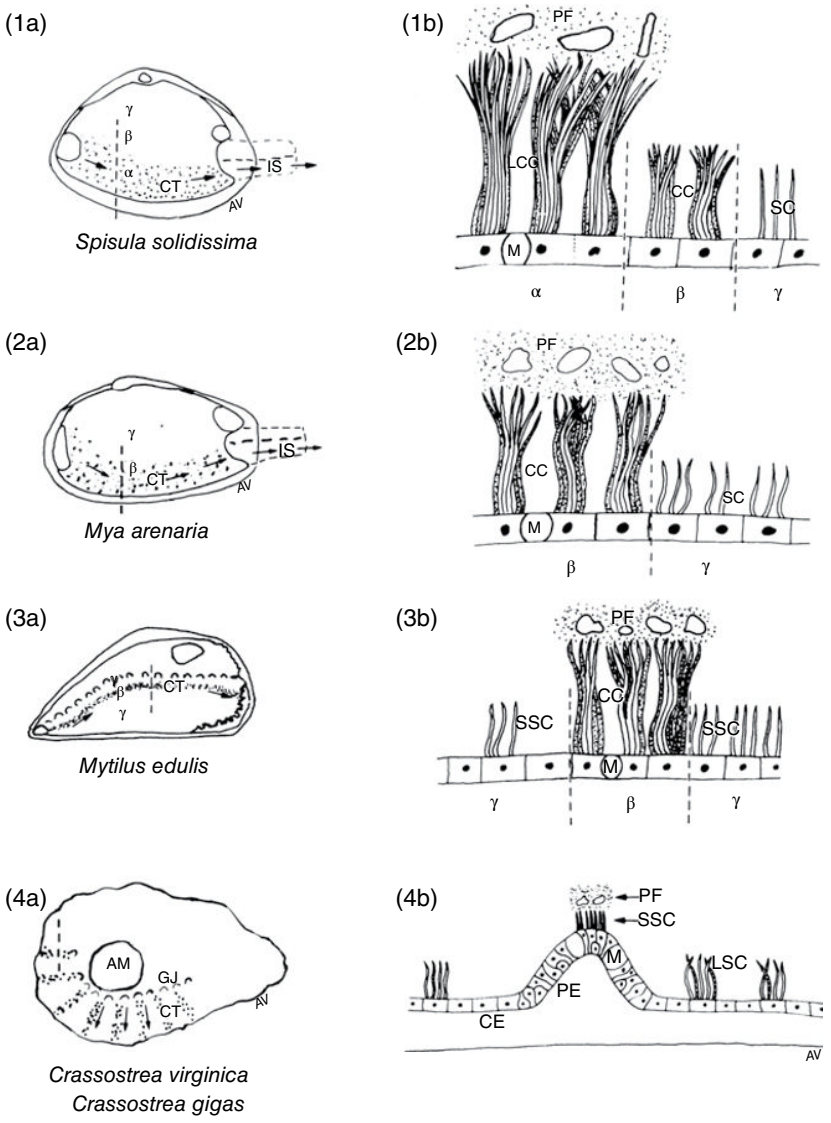


Figure 4.16 Schematic drawings of the distribution and types of cilia involved in pseudofaeces transport on the mantle of *Spisula solidissima*, *Mya arenaria*, *Mytilus edulis*, *Crassostrea virginica* and *Crassostrea gigas*. The first three species possess a ventral gill particle groove and siphons, while the last two possess both ventral and dorsal particle tracts but no siphons. *Spisula solidissima* 1a: Three ciliary bands are arranged in a ventro-dorsal sequence: α, long composite cilia (LCC); β, composite cilia (CC); γ, simple cilia (SC). Arrows show direction of pseudofaeces transport; dashed line shows plane of section for 1b in which transport of pseudofaeces (PF) atop the LCC cilia of the mantle (M) rejection tract is illustrated. *Mya arenaria* 2a: two ciliary bands are found: β (CC) and γ (SC). Arrows show direction of pseudofaeces transport; dashed line shows plane of section for 2b in which transport of pseudofaeces atop the CC cilia of the rejection tract is illustrated. *Mytilus edulis* 3a: Three parallel ciliary bands extend dorso-ventrally: short simple cilia (γ), compound cilia (β) and short simple cilia (γ). Arrows show direction of pseudofaeces transport; dashed line shows plane of section for 3b in which transport of pseudofaeces atop the CC cilia of the rejection tract is illustrated. *Crassostrea virginica*, *Crassostrea gigas* 4a: Ciliated tracts of mantle pseudofaeces rejection ridges, showing direction of pseudofaeces transport (arrows). AM, adductor muscle; GJ, gill junction with mantle. Dashed line shows plane of section for 4b in which short simple cilia (SSC) on rejection ridge, and isolated tufts of long simple cilia (LSC) in inter-ridge region are illustrated.
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away from the animal in the exhalant current. In bivalves with a gill ventral particle groove, that is homorhabdic filibranchs, the tracts are characterised by composite cilia that are extraordinarily long compared to those on the general mantle surface (Beninger & Venoit 1999; Beninger *et al.* 1999). These effectively elevate and isolate the pseudofaeces from the rest of the mantle surface. In addition, high-viscosity mucus secreted in the tracts firmly binds the rejected material and anchors it to the cilia, thus ensuring counter-current transport to the inhalant siphon (see Figures 4.16 and 4.17). In contrast, transport of pseudofaeces in pseudolamellibranchs is effected by short simple cilia on the top of specialised radial ridges on the mantle (Figure 4.16(4a)). It is the ridges, rather than specific cilia types, that elevate the pseudofaeces from the general mantle surface. In all species with mantle rejection tracts, the tracts are positioned away from the gill ventral particle groove (Beninger *et al.* 1999). This is to ensure that the pseudofaeces travelling in an antero-posterior direction do not entangle with the mucus strings moving in the opposite direction along the ventral gill particle groove. In eulamellibranchs, which possess small gills and a ventral particle groove that only extends one-third to one-half the distance between the hinge and the ventral margin of the shell, the tracts are situated in the most ventral part of the mantle (Figure 4.16(1a) and (2a)). In *M. edulis*, however, where the gill extends almost to the mantle edge the tracts are situated along the middle of the mantle (Figure 4.16(3a)). In pseudolamellibranchs, where under high particle load mucus strings may be ejected from the gill ventral particle groove at any point, the radial arrangement of tracts over the entire inhalant portion of the mantle facilitates the rejection process (Figure 4.16(4a)).

Particle selection

Bivalve food consists of a variety of suspended particles such as bacteria, phytoplankton, microzooplankton, detritus, but also dissolved organic matter (DOM) such as amino acids and sugars (see later). However, this nutritious material is in suspension along with silt, which is usually present in much higher concentrations than the food particles. To compensate for the dilution of organic material in suspension, bivalves are able to preferentially select nutritive particles, such as algae, and reject particles of poor nutritive value in their pseudofaeces (Newell & Jordan 1983; MacDonald & Ward 1994; Hawkins *et al.* 1996, 1998a; Ward & MacDonald 1996; Barillé *et al.* 1997; Navarro & Widdows 1997). Kiørboe and Møhlenberg (1981) determined the efficiency with which 10 species of filter-feeding bivalves from temperate latitudes separated the diatom *P. tricornutum* from silt when their concentrations were 10–30 cells μl^{-1} and 10–20 $mg\,l^{-1}$, respectively. All species were able to ingest the diatom selectively and reject the silt. Selection efficiency (SE) was expressed as the ratio of chlorophyll *a* in the suspension offered and pseudofaeces rejected by undisturbed filtering bivalves. This ratio varied considerably and was significantly correlated with the size of the labial palps and turbidity of the environment (Figure 4.18). For example, the mussel *M. edulis* from the Wadden Sea had relatively larger palps and a higher SE than mussels from the Øresund, and they suggested that this could be an adaptation to high particulate load in the Wadden Sea (Figure 4.18).

Bivalves are also capable of discriminating between similar-sized algae cells in their diet (Shumway *et al.* 1985). When mixed cell suspensions of the dinoflagellate *Prorocentrum minimum*, the diatom *P. tricornutum* and the crypyomonad flagellate *Chroomonas salina* were fed to bivalves, the oyster *Ostrea edulis* preferentially cleared *P. minimum*, while the clam *Ensis ensis* and scallop species *P. magellanicus* and *Arctica islandica* consistently rejected this species. Shumway *et al.* (1997) reported similar results for other species of scallop. The site of selection differed between the different species. In *O. edulis* preferential selection took place on the gills, while in *E. ensis*, *P. magellanicus* and *A. islandica* the

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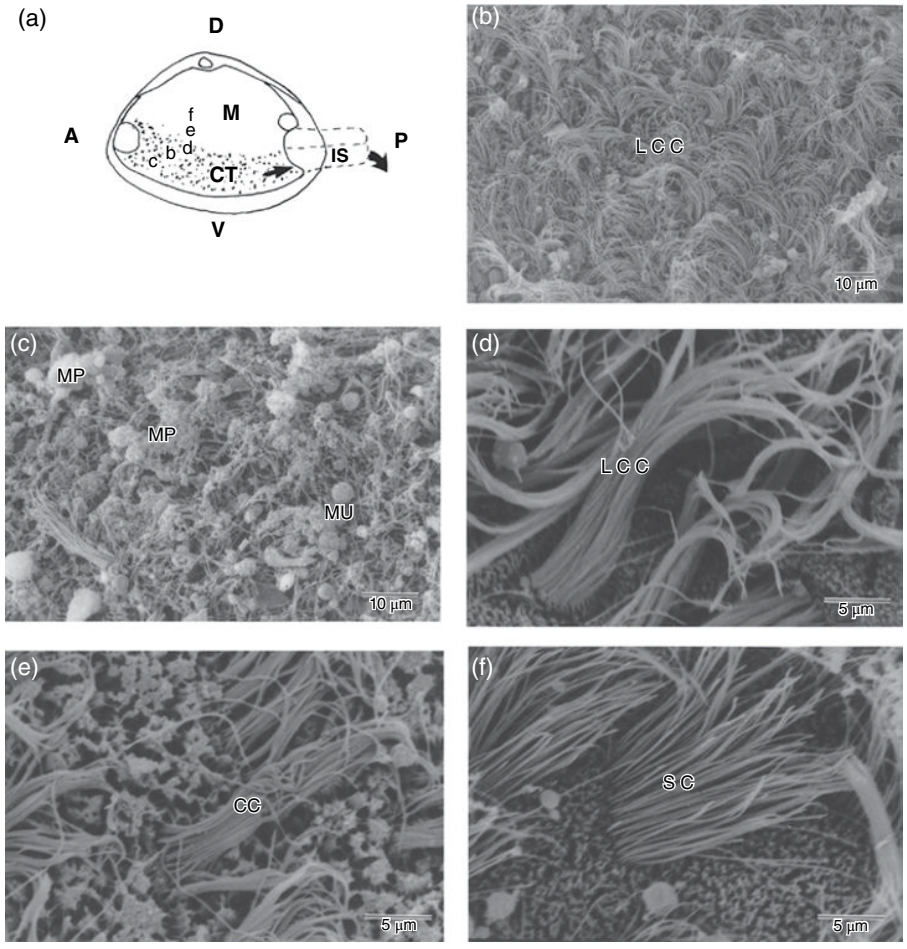


Figure 4.17 Mantle ciliation in *Spisula solidissima*. (a) Location of ciliated mantle rejection tract (CT) in relation to general mantle surface (M) and inhalant siphon (IS). A, D, P and V: anterior, dorsal, posterior and ventral orientations, respectively. Specimen oriented as in subsequent micrographs. Lower case letters (b–f) designate locations of corresponding scanning electron microscopy (SEM) images; large arrows show direction of pseudofaeces transport. (b) Dense long composite cilia (LCC) cover of mantle rejection tract. (c) Mucus particle masses (MP) and mucus balls (MU) representing dehydrated residue of mucus particle raft characteristic of mucociliary transport in ciliated mantle rejection tract. (d) Detail of cilia within ciliated mantle rejection tract, showing them to be of the long composite cilia type (LCC). (e) Detail of cilia on the intermediate band of the general mantle surface, showing them to be of the composite cilia type (CC). (f) Detail of cilia in the dorsal region of the general mantle surface, showing them to be of the simple cilia (SC) type.
From Beninger *et al.* (1999). Reproduced with permission from Inter-Research.

labial palps were the site of preingestive selection. Preferential selection on the gills has also been shown for other oyster species, but in the mussel *Mytilus trossulus* it is the labial palps that play the major role in particle sorting (see earlier and Ward *et al.* 1998a). The dimensions of nutrient particles can determine the site of selection. For example, when oysters (*C. gigas*) were fed on suspensions of the elongated diatoms *Pleurosigma planctonicum* and *Rhizosolenia setigera*, only those *P. planctonicum* that orientated in a dorsoventral position could enter the PFs for delivery to the dorsal acceptance tract, whereas *R. setigera* was prevented from entering the PF because of its curved and twisted shape (Cognie *et al.* 2003).

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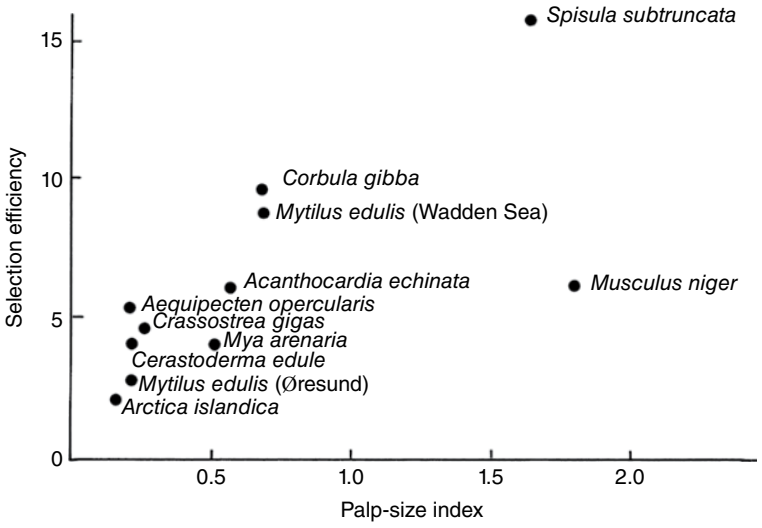


Figure 4.18 Plot of selection efficiency (relative chlorophyll *a* concentration in inhalant water/relative chlorophyll *a* concentration in pseudofaeces) against palp-size index (palp area relative to clearance) in bivalves filtering a mixture of algae and silt. Wadden Sea *Mytilus edulis*, which are exposed to high concentrations of particulate matter, have larger labial palps than mussels from Øresund, where the concentration of particulate matter is considerably lower.
From Jørgensen (1990). Reproduced with permission from Inter-Research.

These results show that particles within the size range naturally encountered by *C. gigas* in the field may only be subjected to qualitative selection on the gill if their shape and dimensions allow them to enter the PF. Consequently, for particles unable to enter the PF, the site of selection is the labial palps, rather than the gill. The cellular status of diatoms may also be an important determinant of selection and ingestion in this species. Beninger *et al.* (2008) found that oysters were able to discriminate between intact and naturally dead (empty) cells. Although each possessed a perifrustular envelope, the dead cells lacked intracellular organic content. Small molecules such as sugars and amino acids leaking out of living cells may act as a signal of diatom 'quality' for *C. gigas*. Interestingly, it is the perifrustular envelope of diatoms, and not their functioning cellular status, that acts as a quality cue for selection in the scallop *P. maximus* (Beninger & Decottignies 2005). The mechanism of selection is at least partially mediated by interactions between lectins in gill mucus and food particles (Pales Espinosa *et al.* 2009). Lectins are a group of carbohydrate-binding proteins that specifically and reversibly bind to sugar molecules covering living cells. To date, specific microalgal-binding lectins have been identified in the mucus produced by feeding organs in the mussel *M. edulis* (Pales Espinosa *et al.* 2010a, b) and the oyster *C. virginica* (Pales Espinosa *et al.* 2009, 2010c). The physico-chemical surface properties of particles, such as charge and wettability, may also contribute to the particle selection process (Rosa *et al.* 2013).

But how is particle selection affected by variations in the quantity and quality of suspended food? Studies have shown that the SE of the cockle *C. edule* increased with the organic quality of the seston to about a maximum of 40% organic content, and then decreased at higher-quality contents. Food quality is usually measured in terms of particulate organic matter (POM) or chlorophyll content (Ward & Shumway 2004). When chlorophyll-containing particles were used there was a decrease in SE similar to that observed for organic matter (Figure 4.19; Urrutia *et al.* 1996, 2001). However, when organic content fell below 40%, SE

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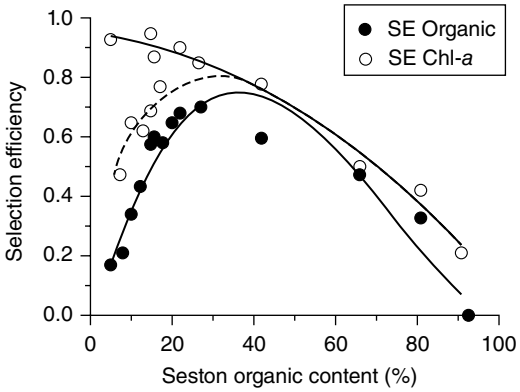


Figure 4.19 Relationships between preingestive selection efficiency (SE) and organic content of the seston (POM/TPM) obtained from published studies on the cockle *Cerastoderma edule*. Solid symbols (●) indicate SE for organic matter from studies that used prepared diets. Open symbols (○) indicate SE for chlorophyll-containing particles from studies that used natural seston (– – –) and prepared diets (—). Higher values indicate higher selection for chlorophyll-containing or organic particles.
From Ward and Shumway (2004). Reproduced with permission from Elsevier.

for chlorophyll-containing particles either increased or decreased with decreasing organic content (Figure 4.19). Ward and Shumway (2004) suggest that the decrease in SE in response to increasing organic content may be due to limitations of the selection process in bivalves. They contend that as the proportion of non-organic particles increases with increasing organic content of the seston, selectively rejecting the few poor-quality particles may be difficult. This, taken together with the reduction in the amount of rejected material that occurs with increasing organic content, causes a drop in SE. The decrease in organic SE in response to decreasing organic content of the seston may also be due to limitations of the selection process, because of a less heterogeneous mix at low organic content. Alternatively, proportionally more pseudofaeces are produced when bivalves are exposed to seston of low quality, and mucus represents the main organic component of this rejected material. The presence of this mucus could be responsible for the decrease observed in SE when the quality of seston is low (Urrutia *et al.* 1996). A later study did indeed show this to be the case in the cockle *C. edule* (Urrutia *et al.* 2001).

Ward and Shumway (2004) have examined the effect of changing seston concentration on SE and found that in a number of studies on different species SE for organic material and chlorophyll-containing particles increased slightly with increasing seston loads between 1 and 15 mg l^{-1}. However, at concentrations greater than 20 mg l^{-1} there was great variability in response, probably due to interspecific differences, and the different diets employed in the different studies. The authors suggest that it is filtration rate or the product of seston quantity and CR, rather than seston quantity *per se*, that affects SE. This is because the concentration of seston to which a bivalve is exposed is less important than the quantity of material actually captured and processed by the gill and labial palps (Ward & Shumway 2004; see also Hawkins *et al.* 1996, 1998b).

Postingestive particle processing

Particles in suspension enter the mouth from an oral groove at the base of the labial palps and travel down the oesophagus into the stomach where the final stage in particle selection takes place. The coordinated ciliary beat of the stomach and intestinal epithelia cause the crystalline style, which projects across the floor of the stomach, to rotate against the

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gastric shield. The rotating style, together with the gastric shield teeth, grinds and crushes particulate material, breaking apart large particles and particle aggregates. In the process the style is abraded and dissolved, thereby releasing digestive enzymes. Ingested particles are mixed with the liberated digestive enzymes from the crystalline style. During the mixing and extracellular digestive processes the stomach contents come under the influence of ciliary tracts that cover large areas of the stomach. These tracts have fine ridges and grooves and act as sorting areas. Finer particles and digested matter are kept in suspension by cilia at the crests of the ridges, and this material is continually swept towards the digestive gland, which opens into the stomach via several ducts. Larger particles, and also small dense particles such as sand grains, are segregated out and channelled into the intestine along a deep rejection groove on the floor of the stomach. From the intestinal groove this material is transported to the midgut where it mixes with other undigested material and is incorporated into faecal pellets. Deposit-feeders display some modifications that are most likely related to the ingestion of larger, coarser material of low organic content, for example a large caecum that serves as a temporary store for particles entering the stomach, a more robust crystalline style, a larger gastric shield with massive teeth and in some species reduced sorting areas on the stomach walls (references in Ward & Shumway 2004).

Compared to the process of selective particle capture and preingestive particle sorting described earlier, there is much less published information on postingestive selection. The process may occur either by the retention of some particles longer than others in the stomach so that extracellular digestion has more time to act, or by directing some particles to the digestive gland for intracellular digestion. In both cases gut retention time (GRT) can be used to indicate whether sorting has occurred (Brillant & MacDonald 2000 and references therein; reviewed by Ward & Shumway 2004). The assumption is that bivalves retain the higher-quality particles for further processing (long GRT) and reject the poorer-quality or indigestible fraction of the diet to the intestine (short GRT). One of the earliest studies on postingestive selection found that when six bivalves were fed three species of phytoplankton, *P. minimum*, *P. tricornutum* and *C. salina*, faecal analysis by flow cytometry showed that differential absorption, that is postingestive selection, in the gut occurred (Shumway *et al.* 1985). Three bivalve species preferentially ingested *P. minimum* and *C. salina* but only the latter was efficiently digested as a significantly higher proportion of whole *P. minimum* cells was egested in the faeces. One species (*C. virginica*) ingested all phytoplankton species in the same proportions but egested significantly more whole *P. minimum* and *P. tricornutum* in the faeces. The study indicated that selection was most likely based on qualitative factors as the phytoplankton species were of similar size, and also highlighted that large interspecific differences exist in the selection process. A more recent study showed that the scallop *P. magellanicus* could distinguish between particles of different sizes and densities, retaining larger particles (20 μm) longer than smaller ones (5 μm), and lighter ones longer than denser ones (Brillant & MacDonald 2000). The authors suggested that the ability to reject small, dense particles might benefit the scallop by reducing the amount of time spent in attempting to digest poor-quality particles such as silt. A subsequent study, again using *P. magellanicus*, tested its ability to sort a mixture of organic (^{14}C-labelled *P. minimum*) and inorganic (^{51}Cr-labelled polystyrene beads) particles of similar size and shape (Brillant & MacDonald 2002). The ^{14}C/^{51}Cr ratios were compared from stomach, digestive gland and faeces over a 5 h period, and the results showed that a higher proportion of *P. minimum* was transported to the digestive gland, while a higher proportion of beads was egested in faeces, indicating that the scallop was able to sort organic from inorganic particles. When scallops were presented with a mixture of protein-coated and uncoated beads, they retained the former in the gut longer than uncoated beads, indicating that scallops could discriminate between physically identical particles solely on the basis of surface chemistry. This species

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also has the ability to sort living from dead material on the basis of chemical properties. Scallops were fed a mixture of radiolabelled live and heat-killed *Chlorella* spp., which has a thick cell wall that remains physically intact following heat treatment while the carbon, nitrogen and chlorophyll α content declines. Live cells were retained significantly longer than heat-killed cells, an ability that would enhance the digestive efficiency of this species by reducing the amount of time expended in digesting nutrient-poor material (Brillant & MacDonald 2003).

The alimentary canal and digestive process

The mouth and the oesophagus, which leads into the stomach, have a ciliated epithelial lining that is well supplied with mucocytes that secrete both acid and neutral mucopolysaccharides, even when the animal in not feeding (Beninger *et al.* 1991). The oesophagus does not have a digestive function, merely serving to propel material along ciliated tracts towards the stomach. The stomach, digestive gland and, to a much lesser extent, the intestine are the main organs of the digestive system. From the stomach there are two pathways for particle processing: an intestinal path and a glandular path. Material destined for the intestine is subjected to extracellular digestion during gut passage, while particles processed via the glandular pathway pass from the stomach to the digestive gland and are subjected to both extracellular digestion in the stomach and intracellular digestion in the digestive gland. Particulate waste from the digestive gland is carried to the intestine and may be subjected there to further extracellular digestion before egestion (Penry 2000, and references therein).

The stomach and extracellular digestion

The stomach is a flattened, oval-shaped sac into which the oesophagus opens at the anterior end and from which the midgut leaves at the posterior end (Figure 2.13). Purchon (1957) presents very detailed information on stomach structure in a wide variety of bivalves. The crystalline style is secreted by the stomach and is largely composed of protein, at least some of which is bound to sugars such as glucose, mannose and galactose. But how these substances form the solid gel of the style is not yet known. The style sac secretes a variety of enzymes, for example amylase, cellulase, agarase, laminarinase, protease and lipase, which are incorporated into the crystalline style as it is being secreted (Alyakrinskaya 2001; Tizon *et al.* 2013). Style-associated bacteria have been reported from several bivalve species, as far back as 1882, and it has been suggested that they may play a major role in extracellular digestion, although this has never been verified (Husmann *et al.* 2010). The stomach wall is also important in digestive enzyme secretion, and it is also likely that some enzymes from the digestive gland enter the lumen of the stomach and act extracellularly.

The following enzymes have been reported from bivalve stomachs (Reid 1968; Mathers 1973a, b; additional references in Bayne *et al.* 1976): esterases that are important in lipid digestion; acid and alkaline phosphatases that are believed to play a role in absorption and phagocytosis of material from the stomach; and endopeptidases, for example trypsin, that break down proteins. High to moderate activity of carbohydrate-splitting enzymes such as α-amylase, α- and β-glucosidase, β-galactosidase, maltase, chitinase and cellulases have also been reported. Chitin is a structural component in diatoms and cellulose is a common component of algal cell walls. Not surprisingly, the activity of cellulases is particularly high in bivalve stomachs.

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The crystalline style is not a permanent structure in the stomach. The size of the style has been reported to vary systematically with the tidal cycle. The maximum size corresponds to the time when there is most food in the stomach, that is at high tide when the animal is feeding (Alyakrinskaya 2001). Morton (1971) found that in the oyster *O. edulis*, the volume of the crystalline style decreased from 25 to 3–4 mm^3 during each tidal cycle, although Langton (1972) reported a much smaller decrease in volume for the same species. Alternatively, Morton (1970, 1971) has suggested that feeding in bivalves is rhythmic, and correlated with this could be a rhythmic digestion pattern. He proposed that feeding occurs over the high tide period and during the period of ebb and low tide extracellular digestion in the stomach takes place. Ingested material is not passed to the digestive gland until the next high tide period. However, the evidence for rhythmic digestion has not been forthcoming as other investigators have since shown that material once ingested is rapidly passed to the digestive gland for intracellular digestion (see Owen 1974 for references).

The digestive gland and intracellular digestion

The principal (primary) ducts of the digestive gland branch into smaller secondary ducts that ultimately lead into blind-ending digestive tubules (see Figure 2.14, Chapter 2). Within these ducts there is a continuous two-way flow: materials enter the gland for intracellular digestion and absorption and wastes leave en route to the stomach and intestine. These tubules comprise two cell types: digestive cells and basophil secretory cells. Digestive cells are the most abundant type. They are columnar and vacuolated and are responsible for intracellular digestion of food. The free surface of the digestive cell is extended into microvilli and the cytoplasm is characterised by the presence of numerous cytoplasmic vesicle types (Figure 4.20a). Particulate matter is taken up at the base of the microvilli by pinocytosis and is initially stored within large vesicles called phagosomes. Digestion takes place within large vesicles called lysosomes that contain hydrolytic enzymes. The end products of digestion are released directly into the haemolymph system and waste products are contained in residual bodies within the digestive cells. The cells eventually rupture and the waste material is swept along the ciliated secondary and primary ducts of the digestive gland towards the stomach, and ultimately to the intestine.

Basophilic secretory cells are pyramidal in shape (Figure 4.20b) and like digestive cells they also have microvilli on the free surface. Much of the cytoplasm is filled with rough endoplasmic reticulum and Golgi bodies, which indicates that these cells play an extensive role in protein synthesis. The exact role of secretory cells is unclear; they may play a role in enzyme secretion for extracellular digestion (Weinstein 1995). A third cell type, the flagellated basophil cell, has been identified and described in the oyster *C. virginica* (Weinstein 1995). This cell is columnar and possesses a single long flagellum, but lacks the conspicuous organelles found in digestive and secretory cells. Although flagellated cells are as numerous as secretory cells their role in digestion has not so far been elucidated. Lack of mitotic activity in both types of basophil cell makes it unlikely that they serve as stem cells for digestive cells (Weinstein 1995). Dimitriadis *et al.* (2004) provide additional information on cytochemical and histochemical aspects of digestive gland cells.

It is thus clear that the digestive gland cells have a finite lifespan after which they disintegrate and are replaced. Various workers have suggested that there is a well-defined rhythm to the cycle of changes taking place in the digestive tubules, and some have linked this with tidal periodicity (Owen 1972; Mathers 1976; additional references in Beninger & Le Pennec 1991; Zaldibar *et al.* 2004, 2008). Owen (1972) has proposed that at low tide, when food is not available, the tubules are in 'holding phase'. When the animals are covered by the tide and feeding starts, food material rapidly reaches the tubules and is taken up by

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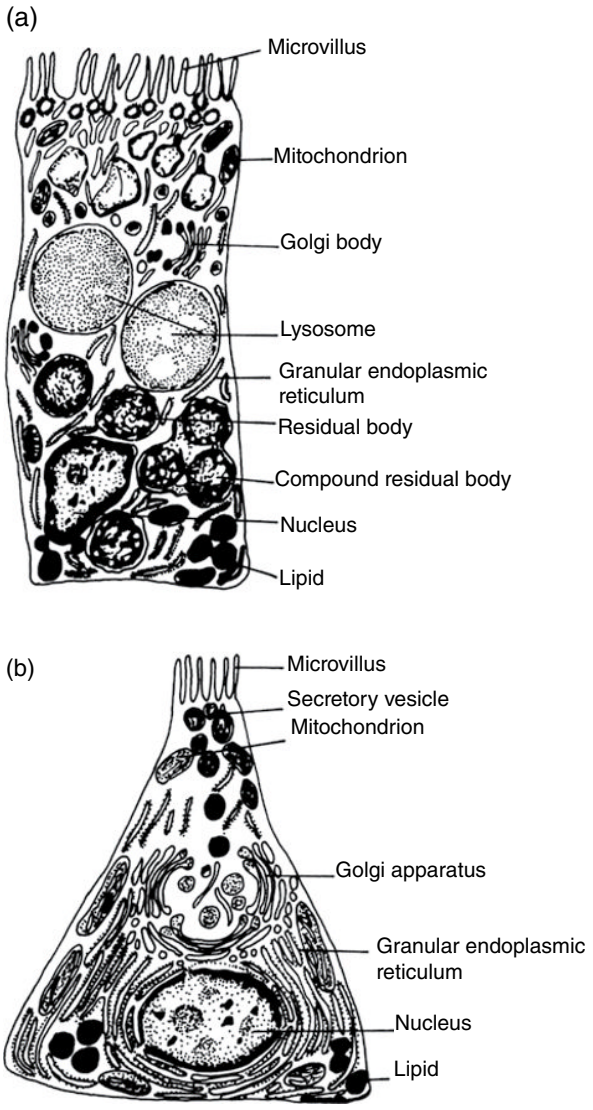


Figure 4.20 Diagram of a bivalve (a) digestive and (b) basophil secretory cell. From Owen (1972). Reprinted with permission from Science Reviews 2000 Ltd.

endocytosis for intracellular digestion. Breakdown and regeneration of the digestive cells then follows and the cells are then in the 'holding phase' for the next high tide. However, in many species no such synchronised structural changes have been observed (Langdon & Newell 1996). In addition to short-term changes, Le Pennec *et al.* (2001) observed long-term cytological and cytochemical changes, related mostly to lipid content, in the digestive gland of the scallop *P. maximus*. These changes were associated with nutrition and reproductive effort, and shell growth. While the adductor muscle serves as an energy reserve for gametogenesis, the digestive gland also participates in this effort, at least as a relay organ when adductor muscle reserves have been depleted (Le Pennec *et al.* 2001) and during periods of physiological stress (Bayne *et al.* 1976). The digestive gland also plays an important role in the uptake of contaminants (Chapter 2).

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Table 4.3 Carbohydrate, fat and protein-splitting enzymes in the digestive gland of bivalves.

Enzyme	Substrate	References
α-Amylase	Starch, glycogen	Teo and Sabapathy (1990)*; Sabapathy and Teo (1992)*
Cellulases	Cellulose	Crosby and Reid (1971); Teo and Sabapathy (1990)
Laminarinase	Laminarin	Wojtowicz (1972)
Maltase	Maltose	Mathers (1973a); Teo and Sabapathy (1990); Teo *et al.* (1990)*
Sucrase	Sucrose	
Trehalase	Trehalose	Teo and Lim (1991, 1993)*
α-Glucosidase	Sucrose Maltose Trehalose	Teo and Sabapathy (1990)
β-Glucosidase	Cellobiose	Kristensen (1972)
β-N-Acetyl-glucosaminidase	Chitobiose	Sumner (1969)
Lipase	Large fat molecules	Mathers (1973b)
Esterase	Small fat molecules	Mathers (1973b)
Chymotrypsin-like	Protein, peptides	Teo and Sabapathy (1990); Le Chevalier *et al.* (1995)*
Trypsins	Peptides	Reid (1968); Reid and Rauchert (1970, 1972, 1976)
Cathepsins	Peptides	

*Studies in which biochemical characterisation has been carried out.

A wide range of enzymes has been recorded from the digestive tubules of bivalves (Table 4.3). Much of the work on both stomach and digestive gland enzymes has been either qualitative or semi-quantitative, providing little indication of levels of enzyme activity. However, some carbohydrate-splitting enzymes and proteases have been purified and characterised from the digestive glands of *P. viridis* and *P. maximus* (see Table 4.3 for references). The substrates for these enzymes have been identified from a range of algae, fungi and bacteria (Kristensen 1972).

Mathers (1972) has shown that when ^{14}C-labelled algae cells were fed to oysters within 10 min of commencement of feeding the cells had been broken down by extracellular digestion in the stomach, passed to the digestive gland and digestive cells had phagocytosed the breakdown products. After 90 min waste material appeared in the ciliated gutter of the primary ducts, thus lending support for a two-way movement of material in the primary ducts of the digestive gland. The length of this cycle varies among bivalve species but is generally on the order of 2–20 h (references in Ward & Shumway 2004). At high seston concentrations the digestive tubules can reach saturation, giving rise to a greater proportion of material being transported directly to the intestine without being subjected to intracellular digestion, ultimately resulting in reduced absorption efficiency (see later).

The intestine

Rejected particles from the stomach as well as waste material from the digestive gland pass into the intestine. The rejected particles are not necessarily lost as wandering haemocytes that migrate freely into the gut from the haemolymph stream may phagocytose them, digest them and transport them around the body. These cells, which also phagocytose diatoms and other food particles in the stomach, are therefore important in intracellular digestion and also play a unique role in the transport of food. The intestine is also active in extracellular digestion. Mathers (1973a, b) reported the following enzymes from the midgut wall

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(first part of intestine) of the oysters *O. edulis* and *Crassostrea angulata*: α-amylase, maltase, trehalase, cellobiase and various glucosidases. Esterases, acid and alkaline phosphatases, chitinase and endopeptidases have also been reported from the midgut of a range of bivalves (Reid 1968). The intestine terminates in an anus, and faeces in the form of faecal pellets are swept away through the exhalant opening.

The intestine is also involved in the transfer of nutrients to other organs. For example, Beninger *et al.* (2003) demonstrated the transport of ferritin from the intestinal lumen to the intestinal epithelial cells, from there to the gonad acini and finally to developing oocytes in the female portion of the gonad of the scallop *P. maximus*. This transfer is facilitated in scallops by the close association between gonad and intestine, and also because the gonad–intestinal complex is well separated from the other organs. However, in bivalves the digestive system and gonad are generally closely associated and intertwined so that transfer of nutrients from digestive epithelia to developing oocytes may be a general feature of the Bivalvia (Beninger *et al.* 2003).

It is interesting to note that in the evolution of bivalves there is an increasing tendency for digestion to become extracellular, and for the intestine to function in digestion and absorption. Extracellular digestion reduces the cell volume that must be devoted to intracellular digestion, and also reduces the time taken to obtain absorbable products from the diet. The midgut functioning as a region of digestion and absorption allows more efficient utilisation of food, as under optimum feeding conditions much of the ingested material is passed undigested through the stomach to the intestine (Reid 1968).

Bivalve diet

Bivalves in the wild feed on phytoplankton of a wide range of sizes and palatability, but detritus, microzooplankton, bacteria, particle aggregates as well as DOM are also important to varying extents. While it is generally assumed that bivalves rely on phytoplankton from the water column as their main energy source, the other food sources mentioned may represent a valuable supplement when phytoplankton abundance is low. Various studies have shown that there is spatial, seasonal and tidal variation in the concentration and characteristics of seston in coastal waters, particularly in shallow water systems such as estuaries (Huang *et al.* 2003a and references therein). Bayne and Hawkins (1990) have estimated that the quantity commonly varies from less than 3 to greater than 100 total dry $mg^{-1}l^{-1}$, of which 5–80% by mass may be organic. Despite such variability bivalves maintain relatively constant rates of nutrient acquisition by various compensatory adjustments discussed later. The following sections deal with the standard methods used in bivalve diet analysis.

Gut content analysis

This method is the traditional and simplest way to examine what bivalves are ingesting. The gut, usually the stomach, is dissected from each individual, fixed in formalin and the contents identified and quantified under an inverted microscope. Examining total gut contents in large-sized species can be time-consuming but a method that permits quantitative assessment from selected subsamples with acceptable precision is now available (Hernández *et al.* 2005). Shumway *et al.* (1987) examined the gut contents of *P. magellanicus* throughout the year from a shallow-water (~20 m depth) and a deep-water (~180 m depth) location. A total of 27 species of algae, ranging in size from about 10 to 350 μm, were identified, as well as considerable amounts of detritus and bacteria (Tables 4.4 and 4.5). Benthic and pelagic food species were equally well represented in shallow-water (inshore) scallops, but benthic

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Table 4.4 Gut contents of inshore scallops *Placopecten magellanicus*.

Species	Category	Habitat	Size (μm)
Nitzschia spp.	B	Bn	25–150
Navicula spp.	B	Bn	8–240
Pleurosigma spp.	B	Bn	200
Thalassiothrix spp.	B	Bn	50 (chain)
Amphora spp.	B	Bn	10–30
Licmophora spp.	B	Bn	25–180
Acnanthes spp.	B	Bn	40–90
Pinnularia spp.	B	Bn	40–80
Surirella spp.	B	Bn	15–25
Cylindrotheca closterium	B	Bn	80–100
Protogonyaulax resting cyst	D	Bn	35–40
Unidentified cyst		Bn	25–35
Melosira spp.	B	Bn	30–55 (chain)
Striatella spp.	B	Bn	40–50
Coscinodiscus spp.	B	Bn/P	40–180
Ditylum brightwellii	B	P	50–150
Protoperidinium spp.	D	P	60–70
Eucampia zoodiacus	B	P	40–75 (chain)
Peridinium spp.	D	P	20–30
Prorocentrum micans	D	P	45–55
Skeletonema costatum	B	P	30–50 (chain)
Dinophysis acuminata	D	P	50–60
Dinophysis spp.	D	P	32–60
Thalassiosira rotula	B	P	20 (chain)
Thalassiosira nordenskioldii	B	P	20 (chain)
Thalassiosira spp.	B	P	10–200

Adapted from Shumway *et al.* (1987). Reprinted with permission from the National Shellfisheries Association–BioOne.

Miscellaneous: Silicoflagellate strew; pollen grains (30–40 μm); green filamentous algae (>1000 μm); ciliates; zooplankton tests; bacteria; detritus; unidentified, non-pigmented still active forms: (3 μm), multiflagellate (10 μm) and ciliated mass (40–200 μm). Category B = Bacillariophyceae (diatoms); category D = Dinophyceae (dinoflagellates). Benthic (Bn) or pelagic (P) habitat of food species indicated.

species, as might be expected, outnumbered pelagic ones in the deeper-water (offshore) population. Seasonal variations of food items occurred and coincided with bloom periods for the individual algae species. For example, in inshore scallops the most prominent pelagic food items were *Prorocentrum* (October/November; January), *Thalassiosira* spp. (January; March) and *Dinophysis* (October). Similar analyses have been carried out on the mussels *Mytilus galloprovincialis* (Lok *et al.* 2010) and *M. edulis* (Rouillon *et al.* 2005), and the black oyster *Hyotissa hyotis* (Villalejo-Fuerte *et al.* 2005), although for the last two mentioned species seasonal data on phytoplankton composition of the seston at the study sites were also analysed. See also Raby *et al.* (1997) for details on stomach content analysis in bivalve veliger larvae.

In the Shumway *et al.* (1987) study variable quantities of detritus were also found in scallop gut contents. When scallops recess into the substrate the inhalant current is lowered to at least the level of the sediment surface and benthic material can be more easily drawn into the mantle cavity (Brand 1991). Vigorous clapping of the shell valves helps to resuspend the surface sediments. The importance of this food item in their diet has been highlighted by the results from a laboratory-based study on *P. magellanicus* (Cranford & Grant 1990). When diets of cultured phytoplankton (*Isochrysis* aff. *galbana* and *Chaetoceros gracilis* Schütt),

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Table 4.5 Gut contents of offshore scallops *Placopecten magellanicus*.

Species	Category	Habitat	Size (μm)
Melosira spp.	B	Bn	50 (chain)
Protogonyaulax resting cyst	D	Bn	35–40
Navicula spp.	B	Bn	60–350
Nitzschia spp.	B	Bn	110
Thalassiothrix spp.	B	Bn	50 (chain)
Acnanthes spp.	B	Bn	40–90
Amphora spp.	B	Bn	10–30
Pleurosigma spp.	B	Bn	280
Licmophora spp.	B	Bn	120–180
Pinnularia spp.	B	Bn	70–100
Surirella spp.	B	Bn	15–25
Unidentified dinoflagellate cyst	D	Bn	25–35
Coscinodiscus spp.	B	P/Bn	50–160
Prorocentrum micans	D	P	45–55
Dinophysis spp.	D	P	32–60
Thalassiosira spp.	B	P	35–50
Eucampia zoodiacus	B	P	100 (chain)
Ditylum brightwellii	B	P	150
Ditylum brightwellii resting spore	B	P	40

Adapted from Shumway *et al.* (1987). Reprinted with permission from the National Shellfisheries Association–BioOne.
Miscellaneous: Pollen grains (30–40 μm); zooplankton tests (100–250 μm); bacteria; detritus; unidentified, non-pigmented still active forms: uniflagellate (3 μm), multiflagellate (10 μm) and ciliated mass (70–300 μm).
Abbreviations as in Table 4.4.

kelp powder and resuspended sediment were fed to this species the results demonstrated that, although phytoplankton is the primary food source, detritus particles, which constitute a significant fraction of suspended POM, do contribute to energy gains during periods when phytoplankton is less available (see later). For example, results using stable isotopes (see later) showed that detritus from the marsh-grass *Spartina alterniflora* represented a significant source of nutrients for the ribbed mussel *G. demissa*, providing 50% of the carbon in mussel tissues when they were sampled close to the marsh and 30% when they were close to the sea (Langdon & Newell 1990; see also Huang *et al.* 2003b). Similarly, the mussels *M. galloprovincialis* and *Aulacomya ater* used kelp-derived detritus as their main source of carbon (50%) and nitrogen (65%) (Bustamante & Branch 1996). Detritus is also an important food item for the Japanese scallop *P. yessoensis* (Aya & Kudo 2007), the oyster *C. gigas* (Riera & Richard 1996; Decottignies *et al.* 2007a) and the clam *Pinna nobilis* (Davenport *et al.* 2011). The extent to which detritus is exploited as a food item has probably more to do with sorting mechanisms on the gill than on the particular life habit of the species concerned.

Stable isotope analysis

One problem with gut content analysis is that the method only provides estimates of the percentages of various materials ingested, all of which may not be assimilated. Therefore the recent developments of techniques such as isotope ratios and fatty acid profile analysis allow us to trace long-term (weeks to months) dietary patterns in bivalves in the field. Fatty acid analysis provides information on the lipids characterizing food items and consumers (see later), while stable isotope analysis uses carbon and nitrogen as signatures to trace the

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origin of nutrients contributing to growth in marine bivalves (Navarro *et al.* 2009 and references therein). In isotope analysis the ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N are expressed in δ notation with respect to deviations from standard reference material. The ratio of stable isotopes of nitrogen ($\delta\ ^{15}N$) can be used to estimate trophic position (e.g. intertidal versus subtidal) because the $\delta\ ^{15}N$ of a consumer is typically enriched by 3–4% relative to its diet, whereas the ratio of carbon isotopes ($\delta\ ^{13}C$), used to identify primary food sources, changes little as carbon moves through food webs (Bustamante & Branch 1996; Post 2002; but see Dubois *et al.* 2007). Stable isotope studies have demonstrated the importance of phytoplankton (Yokoyama & Ishihi 2003; Marin-Leal *et al.* 2008), microphytobenthose (microscopic algae, mainly diatoms, living on benthic surfaces; Riera *et al.* 1999; Yokoyama & Ishihi 2003; Kang *et al.* 2006; 2009; Pernet *et al.* 2012) and detritus (references earlier) in the diets of suspension-feeding bivalves, including larvae (Baldwin & Newell 1991; Raby *et al.* 1997). As stable isotope analysis typically classifies carbon sources into broad functional groups (phytoplankton versus benthic microalgae) the method has recently been combined with the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), a method that has the potential to identify ingested organisms to the genus or species level. By combining PCR-DGGE short-term (hours) ingestion data with stable isotope longer-term (months) assimilation data a more complete picture of the trophic relationships was provided for mussel populations from a semi-enclosed marine bay in northern Ireland (Maloy *et al.* 2013).

Isotope studies have also shown that spatial, temporal and hydrographic factors (tides, freshwater input) can influence the relative contribution of these different food sources in bivalves' diet (Riera & Richard 1997; Kasai *et al.* 2004; Rossi *et al.* 2004; Kang *et al.* 2006; Cabanellas-Reboredo *et al.* 2009; Allan *et al.* 2010; Marchais *et al.* 2013). Moreover, isotopic studies have shown variable degrees of competition and resource partitioning within and between species. To take an example, the temporal dynamics of carbon and nitrogen isotope ratios of co-occurring suspension-feeders in two shellfish culture areas (north France) were investigated over 2 years to evaluate the interspecific trophic partitioning and relative contributions of different organic matter sources to benthic suspension-feeders'diet (Lefebvre *et al.* 2009). Oysters (*C. gigas*), mussels (*M. edulis*) and cockles (*C. edule*) and two non-bivalves, limpets (*Crepidula fornicata*) and polychaete worms (*Lanice conchilega*), were sampled from an estuarine environment, while oysters and mussels, limpets and worms (*Sabellaria alveolata*) were sampled in a marine environment. In marine systems phytoplankton (POM) represents the major food source for suspension-feeders, while in estuarine systems other organic matter sources (OMS), for example riverine particulate material, microphytobenthos, macroalgal detritus and bacteria, significantly contribute to benthic suspension-feeders (references in Lefebvre *et al.* 2009). In the marine system, whatever the year, all species, with the exception of *S. alveolata*, relied strongly on phytoplankton, especially during phytoplankton blooms (May and July), but in winter when phytoplankton is less abundant, they switched to OMS. In the estuarine system OMS was the major dietary item for all species. But is there competition for food sources between these co-occurring suspension-feeders? The authors suggest that particle capture and selection may be important in limiting competition and may explain why ecosystems can often support large numbers of suspension-feeders. The three bivalve species utilize very efficient selection mechanisms to feed on suspended particles while the polychaetes, although they are suspension-feeders, are less efficient than oysters or mussels in retaining small particles. Polychaetes live close to the sediment and can use their feeding tentacles to exploit macroalgal fragments, the largest component of their diets. The limpet species is a suspension-feeder but absence of particle-sorting mechanisms on the gills means that they filter phytoplankton from the water column but microphytobentos and macroalgae

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fragments are inevitably also part of their diet as they, like polychaetes, live close to the sediment. In contrast, a study by Decottignies *et al.* (2007b) showed that there was significant dietary overlap between *C. gigas* and *C. fornicata*.

Fatty acid profile analysis

Bivalves obtain their lipid requirements from their diet or by synthesis of lipids from dietary protein and carbohydrate precursors. Dietary lipids are broken down into their constituent fatty acids and are incorporated into the consumer's tissues largely unchanged (Howell *et al.* 2003). While most of the different food types that are available to bivalves have many fatty acids in common, there are some specific fatty acids, groups of fatty acids and fatty acid ratios that serve as biomarkers of food sources such as diatoms, flagellates, macroalgae and bacteria. For example, high concentrations of 20:5(*n*–3) and 16:1(*n*–7), a ratio of 16:1(*n*–7)/16:0 close to 1 and the presence of C16 PUFA (polyunsaturated fatty acids), mainly 16:4(*n*–1), are diatom markers. The sum of branched chain and odd fatty acids, as well as 18:1(*n*–7) is a biomarker for bacteria. Principal indicators of dinoflagellates are 18:4(*n*–3) and 22:6(*n*–6), while 18:2(*n*–6), 20:4(*n*–6) and 22:6(*n*–3) are indicative of heterotrophic flagellates and ciliates. Fatty acids 22:6(*n*–3) and 18:1(*n*–9) are abundant in lipids of zooplankton and thus provide an indicator of carnivorous organisms (Silina & Zhukova 2007 and references therein).

Fatty acid composition in digestive gland and soft tissues was investigated in two populations of scallops (*P. yessoensis*) inhabiting sites with similar temperature, salinity and hydrodynamic regimes, but with different bottom sediments (Silina & Zhukova 2007). Lipid and fatty acid composition of scallops were quite different, indicating different food availability in near-bottom water at the muddy and sandy sites. Biomarker analysis showed that scallops inhabiting the muddy bottom site were highly reliant on diatoms as a food source, while scallops from the sandy bottom site were primarily reliant on flagellates, ciliates and invertebrate larvae, findings consistent with the distribution of fatty acids in near-bottom water POM at the study sites. Fatty acid analysis has also been used to assess the influence of spatial (Xu & Yang 2007; Allan *et al.* 2010) and seasonal (Prato *et al.* 2010; Ezgeta-Balić *et al.* 2012) variability on the diet of bivalves. A combination of fatty acid profile analysis and stable isotope analysis has been used in some of these studies (Xu & Yang 2007; Allan *et al.* 2010).

Zooplankton, bacteria, dissolved organic matter and marine aggregates as food sources

Davenport *et al.* (2000) were the first to report that mussels (*M. edulis*) can capture and ingest zooplanktonic and benthic animals, for example crustacean and bivalve larvae in the 0.2–2 mm size range (mesozooplankton). Gastric processing of the prey is rapid (<40 min at 15–20°C), which probably explains why the phenomenon had not been previously reported. In laboratory experiments approximately 90% of clam larvae (*Ruditapes philippinarum*) offered to mussels was ingested and fully digested (Lehane & Davenport 2004). Stomach content analysis of farmed mussels showed that a large size range of larvae were ingested year-round, suggesting that mussels significantly reduce numbers of bivalve larvae in nearby waters. The phenomenon has also been reported in other bivalves (Alfaro 2006; Troost *et al.* 2009; Davenport *et al.* 2011; Peharda *et al.* 2012). Besides bivalve larvae, other zooplankton, for example copepods, and crustacean, ascidian and gastropod larvae, are ingested by adult filter-feeding bivalves (Nielsen & Maar 2007; Davenport *et al.* 2011; Peharda *et al.* 2012; Ordóñez *et al.* 2013). Recently, because of bivalves' ability to consume

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mesozooplankton, Webb *et al.* (2013) suggested that bivalves could act as biological agents in the control of parasitic sea lice at salmon farms. Results of experiments showed that bivalves ingest sea lice larvae, regardless of phytoplankton presence or absence, and that large individuals consumed a significantly greater proportion of larvae than small individuals.

There is increasing interest in the role of bacteria in bivalve nutrition. For example, the role of probiotics, beneficial bacteria that override pathogens by producing inhibitory substances or preventing pathogenic colonization in the host, are increasingly used as dietary supplements in bivalve hatcheries (Chapter 9). In the wild, bacterial concentrations can be high (4×10^6 bacteria ml^{-1}) and can reach an average of 20% of planktonic primary production (Langdon & Newell 1990; Prieur *et al.* 1990), although a more recent study puts this figure even higher at 32% (Huang *et al.* 2003b). As most bacteria are smaller than 1 μm, RE on the gills is generally low. For example, in experiments using free, unattached bacteria, the oyster *C. gigas* had an RE of 5% compared with an efficiency of 16% for the mussel *G. demissa* (Langdon & Newell 1990). Assuming a 20% RE for most bivalves and a bacterial production of 20% of phytoplankton production, bacteria probably only meet less than 4% of a bivalve's nutritional needs (Prieur *et al.* 1990). Langdon and Newell (1990) estimated that unattached and attached bacteria during summer contributed just 5.5% to the metabolic carbon requirements of *C. virginica*, but could provide 31% of carbon requirements in *G. demissa*. Because the C/N ratio of bacteria is lower than that of phytoplankton (3.5 compared with 6.6) bacteria may better meet the nitrogen needs of bivalves. Langdon and Newell (1990) have shown that during the summer the estimated contributions of unattached and attached bacteria in meeting metabolic nitrogen requirements were 27% for *C. virginica* and 71% for *G. demissa*. However, these authors point out that it is only in certain environments such as eutrophic estuaries, kelp beds and marshes that bacterial concentrations are sufficiently large to contribute significantly to the nutrition of suspension-feeding bivalves. In open coastal and oceanic waters bacteria are generally less abundant and therefore are unlikely to be nutritionally significant for bivalves.

Marine DOM is a complex mixture of thousands of different molecules and is a major reservoir of non-living organic matter (Benner 2006). DOM, defined as the fraction passing through a filter of pore size of 0.22 μm, is the largest oceanic carbon pool in seawater, mainly consisting of amino acids and carbohydrates. There is some evidence that bivalves can actively transport DOM across the gills (references in Stewart & Bamford 1975) and utilise it as a nutritional supplement. More recently, DOM has been shown to influence uptake and bioconcentration of a variety of contaminants in bivalves (Bejarano *et al.* 2005 and references therein). Because of the complexity of DOM, bivalve uptake studies have focused on the uptake of dissolved free amino acids (DFAA) and carbohydrates. Manahan *et al.* (1982) demonstrated that *M. edulis* rapidly removed up to 94% of naturally occurring amino acids from seawater, and they estimated that uptake of amino acids at ambient concentrations in seawater could meet 34% of the metabolic requirements in this species (Manahan *et al.* 1983). Other studies (cited in Hawkins & Bayne 1992) have downgraded this figure to as low as 10%. A more recent study has examined the uptake of radiolabelled glucose and two amino acids in the mussel *P. viridis*, and found that mussels accumulated carbon predominantly from POM with less than 0.2% coming from dissolved organic carbon (Pan & Wang 2004). Although these figures are low DOM could serve as a potential energy source for bivalves when other sources of nutrition are least abundant. It may be that bacteria take up DOM more efficiently than bivalves, thus reducing its concentration to a low level (references in Pan & Wang 2004). It should be kept in mind that all of these studies have been conducted in the laboratory, and so far none have been undertaken to test DFAA uptake in the field. See Chapter 7 on the role of FAA in osmotic regulation.

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Cells between 0.2 and 2 µm are referred to as picoplankton, and as seen earlier in relation to bacteria, most bivalves cannot efficiently capture and ingest particles in this size range. However, picoplankton is often concentrated into marine aggregates that are tens to hundreds times larger than picoplankton size (Waite *et al.* 2000). Aggregates, often referred to as marine snow, are made up of organic particles (plankton, diatoms, faecal matter) and inorganic particles such as sand. The aggregate is held together by transparent extracellular polysaccharides (TEP), a sugary mucus exuded as waste products by phytoplankton, bacteria and a variety of suspension-feeders, including bivalves (Heinonen *et al.* 2007; Li *et al.* 2008). The aggregates, formed in the upper water column, eventually sink to the ocean floor. Kach and Ward (2008) have shown that suspension-feeding bivalves can ingest picoplankton-sized particles embedded within aggregates, which suggests that picoplankton, presented in this way, may be an important food source. The increasing use of nanotechnology means that there is an increasing risk of nanoparticles (1–100 nm) entering the aquatic environment with potential toxicological consequences for animals and plants. When 100 nm nanoparticles were embedded within aggregates and presented to mussels (*M. edulis*) and oysters (*C. virginica*) the particles were ingested and transported to the digestive gland, a finding that has implications for the accumulation of nanoparticles in bivalves and their transfer to higher trophic levels including humans (Ward & Kach 2009; see Canesi *et al.* 2012 for review). Marine aggregates also serve as potential reservoirs of bivalve pathogens (QPX, *Vibrio* spp., *Escherichia coli*) (Lyons *et al.* 2005, 2007).

Symbiotic nutrition

Another source of nutrition for bivalves, but one that is only exploited by a few species, is the utilisation of the autotrophic pathways of symbiotic algae to provide an additional carbon source. All 10 species of giant clams (genus *Tridacna*) living on coral reefs, a nutrient-poor environment, contain symbiotic zooxanthellae (genus *Symbiodinium*), which reside intercellularly within a special tubular system in the exposed mantle tissue connected to the stomach. Juvenile clams in the wild obtain the symbionts from the water column through filter-feeding. When clams gape the mantle is extruded and the zooxanthellae are exposed to sunlight, thus allowing photosynthesis to take place. The clams utilize the photosynthetic products as nutrients, rather than the zooxanthellae themselves (Dame 2012 and references therein). Thus, giant clams obtain their nutrition by a combination of heterotrophic and autotrophic pathways, which probably explains why they have faster growth rates than other bivalves. Of concern to clam seed producers is the low symbiosis rate observed in juvenile *Tridacna*, apparently due to the procedures used in hatcheries to sterilize seawater (Kurihara *et al.* 2013). A few studies have investigated the relative contribution of zooxanthellae photosynthesis to respiratory cabon demand in *Tridacna maxima*. In small clams (10 mg dry tissue weight) filter-feeding provided about 65% of the total carbon needed for respiration and growth, whereas large clams (10 g) acquire only 34% of their carbon from this source (Klumpp *et al.* 1992). In addition, zooxanthellae also conserved and recycled all nitrogenous end products within *Tridacna gigas*, giving clams a nutritional advantage over nonsymbiotic bivalves (Hawkins & Klummp 1995; Mingoalicuanan & Lucas 1996).

A second type of symbiosis is the chemo-autotrophic relationship between bivalves and sulphide- and methane-oxidizing bacteria. Chemosymbiosis was first recorded in a few clam species from deep-water hydrothermal vents, but has since been recognized in numerous species of bivalves from a wide variety of anoxic habitats such as sands and mud, mangrove sediments and seagrass beds. The best-studied species are mussels in the genus *Bathymodiolus* that are found in hydrothermal vents in the Pacific and Atlantic oceans.

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The symbionts are intracellular, residing within the epithelial cells of the gill. Bathymodiolid mussels exploit at least three food resources: chemosynthates from endosymbionts, suspended POM and DOM (Riou *et al.* 2010; Dame 2012). Nutrient transfer is probably accomplished through leakage of metabolites from the symbiont to the host, and also lysosomal resorption of dead bacteria as an auxiliary strategy for organic molecule transfer (Kádár *et al.* 2008). Bivalves with endosymbionts rely on them as a carbon source, to varying extents (Le Pennec *et al.* 1995 and references therein). For example, carbon isotope ratios indicated that approximately half the carbon in the littoral bivalve *Lucinella divaricata* was of bacterial origin, meaning that approximately 40% of its metabolic requirements came from endosymbionts. This species has a complete and functional digestive system and the gills, while slightly modified, still function in particle capture and transport. Another bivalve, *Solemya reidi*, which lives in an extremely sulphide-rich habitat in littoral waters, totally lacks a digestive system; 60–80% of its metabolites are derived from bacterial endosymbioants and the remainder originate in DOM. Lastly, the epibenthic *Bathymodiolus thermophilus* shows the least degree of reliance on endosymbionts (see earlier) and, not surprisingly, there are no modifications to either gill or digestive system. With increasing reliance on endosymbionts as a carbon source there is a progessive reduction in the size of the labial palps, stomach and digestive gland until, as seen in *S. reidi* earlier, the digestive system disappears altogether.

A rather unique example of symbiotic nutrition is that displayed by shipworms, for example *Teredo navalis*, and bacteria. The species eats its way into wood, digesting it with the aid of symbiotic cellulolytic nitrogen-fixing bacteria contained in specialized epithelial cells on the gills. Unlike other species, *T. navalis* feeds almost exclusively on wood, although it also filters and digests phytoplankton. In the process of boring, shipworms create numerous burrows in untreated wooden structures, consequently causing extensive and costly damage, especially in the past (Hoppe 2002).

Absorption efficiency

The efficiency by which ingested ration is absorbed is called the absorption efficiency (AE). AE is functionally interrelated with gut capacity, the residence time for food in the gut and the ingestion rate. Altering the rate of ingestion and gut residence time provides a bivalve with a powerful means of adapting to a variable food source (Bayne & Newell 1983). Another mechanism by which AE can be enhanced is through increased synthesis of digestive enzymes. This has the effect of both increasing the rates of enzymatic breakdown of ingested material and creating more space in the gut for ingested material (Bayne *et al.* 1993; Wong & Cheung 2001). Physiological changes that result in increased AE are believed to occur over a period of days, whereas changes in filtration rate and rejection of particles as pseudofaeces occur over much shorter time scales (Bayne *et al.* 1993).

AE is estimated by using the following equation:

$$\mathrm{AE} = \left[\left(\frac{I-F}{1-F}\right)\times I\right]\times 100\%$$

where I is the percentage of organic material in the food offered and F the percentage of organic material in the faeces (Conover 1966). Details on methods of determining AE are provided by Ren *et al.* (2006) and Navarro *et al.* (2009).

The influence of food type on AE is fundamental to the prediction of production, and is of particular relevance in bivalve culture operations. The effect of diet on assimilation

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efficiencies in the mussel *Perna canaliculus* was investigated using different ^{14}C-labelled phytoplankton (three diatoms, three flagellates and three dinoflagellates) species found in a mussel-farming ecosystem in New Zealand (Ren *et al.* 2006). AE varied with algal species but was significantly higher when mussels were fed dinoflagellates (85%) compared with diatoms (62%) and flagellates (78%). While AE of dinoflagellates and flagellates increased with gut passage time (GPT[3]) there was a negative correlation with the diatom diet. The cell walls of diatoms are rigid and resistant to enzymatic digestion and physical breakdown, and diatoms also contain significant amounts of inorganic matter. Therefore, there would be little benefit in withholding diatoms in the gut for more efficient digestion. A more detailed study of AE and corresponding digestive processes has been carried out on the cockle *C. edule* fed variable concentration diets of phytoplankton (*I. galbana*) and organic detritus from salt marsh cordgrass *Spartina maritima* (Navarro *et al.* 2009). The detritus diet was assimilated with a high efficiency of 65%, when values of ^{14}C were considered, whereas the value was 82% for phytoplankton (Figure 4.21). While the value for phytoplankton is in line with several AE values reported in the literature, the detrital value is considerably higher than values reported for mussels (14%) and oysters (3%) fed salt marsh detritus (Langdon & Newell 1990). Such discrepancies may be due to differences in detrital quality or variable physiological condition of bivalves induced by environmental factors. Digestive responses in cockles to increasing phytoplankton availability included faster processing of food particles coupled to improved enzyme activity in the digestive gland leading to increased gut performance. In contrast, utilization of detritus was constrained by its resistance in the short term (2 days acclimation) to digestion, and the magnitude of metabolic faecal losses by cockles on detrital diets. However, the increased gut fullness, achieved through a longer

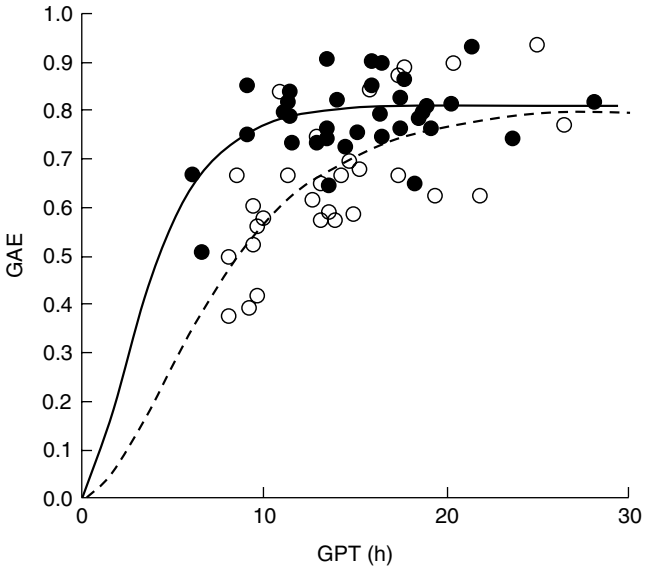


Figure 4.21 Gross absorption efficiency (GAE) in the cockle *Cerastoderma edule* as a function of GPT (h). Solid symbols and continuous line: phytoplankton diets; open symbols and dashed line: detritus diets. Fitted parameters (± asymptotic standard error) were: phytoplankton diets: $Y_{max} = 0.8125 \pm 0.0182$, $B2 = 0.4166 \pm 0.0600$, $R^2 = 0.183$, $p < 0.001$; detritus diets: $Y_{max} = 0.8040 \pm 0.0507$, $B2 = 0.2450 \pm 0.0314$, $R^2 = 0.460$, $p < 0.001$. GAE, using ^{14}C-labelled diets, is calculated as GAE = (^{14}C ingested – ^{14}C egested)/^{14}C ingested, while AE (net absorption efficiency, NAE) is commonly determined by the Conover method (Enrique Navarro, personal communication, February 2014).
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period of acclimation (12 days) compensated for reduced digestibility, allowing more detritus to be processed, thereby cancelling out the effects of reduced passage time, and ultimately leading to improved AE (Navarro *et al.* 2009). Not all algae species show efficiencies as high as that reported for *I. galbana*. For example, in *A. irradians concentricus* Peirson (1983) observed a low AE value of 17% for *Chlorella autotrophica*, and in *C. virginica*, *Tetraselmis suecica* was digested and absorbed with an efficiency of only 6% (Romberger & Epifanio 1981). These low AE values have been attributed to the thick indigestible cell walls of these chlorophyte species.

Besides the studies of Ren *et al.* (2006) and Navarro *et al.* (2009) already mentioned, active regulation of AE has also been reported in other bivalves. When mussels (*M. edulis*) were fed diets of different organic content, those on a diet of low organic content increased their filtration rate, rejected a higher proportion of filtered material as pseudofaeces and increased the efficiency with which filtered matter of higher organic content was selected (Bayne *et al.* 1993). In an earlier study Bayne and Hawkins (1990) observed that elevated rates of ingestion were accompanied by unchanging GPT so that total gut content increased, helping to maintain organic gut content independent of changes in either food quality or quantity. Similar findings have been reported for the scallop *P. magellanicus* (Cranford *et al.* 1998) and the oysters *C. gigas* and *Saccostrea glomerata* (Bayne 2002 and references therein).

Other factors besides diet influence AE. In general, AE has been found to increase with the organic content of the ingested matter (Navarro *et al.* 1996; Grant *et al.* 1997; Cranford *et al.* 1998; Hawkins *et al.* 1998b, 1999; MacDonald *et al.* 1998; Rueda & Smaal 2004), while increasing food quantity causes a decrease in AE. For example, a fourfold increase in the density of the alga *Thalassiosira weissflogii* from 3 to12 cells μl^{-1} caused a reduction in AE from 90 to 65% in *A. irradians* (Kuenster 1988). Temperature and salinity also affect AE as demonstrated by Enríquez-Ocaña *et al.* (2012), who used a combination of four temperatures (23, 26, 29 and 32°C) and four salinities (25, 30, 35 and 40 psu) to evaluate their effect in the mangrove oyster *Crassostrea corteziensis*. The highest AE value (65%; range 43–65%) was for the combination 29°C and 35 psu. Habitat also has been shown to influence AE. A comparison of cultured and intertidal mussels (*M. galloprovincialis*) showed that higher values of AE were systematically recorded for cultivated mussels, which may be related to habitat differences in terms of cycles of food availability and air exposure (Labarta *et al.* 1997). In contrast, Charles and Newell (1997) examining the effect of increasing tidal exposure on mussels (*G. demissa*) found that mussels periodically emersed had AE values between 22 and 26%, which were significantly higher than those of mussels continuously immersed (16%). The authors concluded that mussels compensate for reduced feeding time by exhibiting a higher AE than mussels that are permanently covered by water.

Dynamic Energy Budget (DEB) theory aims to quantify the energetic framework of an individual organism as a dynamic model (Chapter 6), and is increasingly being used to describe the main feeding processes in bivalves. Those wishing to explore how DEB theory has been used to model processes of filtration, ingestion and assimilation might consult the following: Kooijman (2006); Ren (2009); Rosland *et al.* (2009); Saraiva *et al.* (2011) and Lavaud *et al.* (2014).

Effects of bivalve suspension-feeders on the ecosystem

The diverse ways in which suspension-feeding bivalves influence marine ecosystem processes have been thoroughly reviewed by Dame (2012). This section will focus only on the effect that bivalves have on their own habitats through feeding, biodeposition of sediments and cycling of nutrients.

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Bivalve feeding and the plankton community

Suspension-feeding bivalves have a direct impact on phytoplankton communities in coastal waters. Their large grazing potential causes depletion of phytoplankton biomass above the seabed, but also increases phytoplankton productivity by recycling of inorganic nutrients from suspended POM (see later), thereby exerting both top–down and bottom–up control on phytoplankton.

The grazing impacts of bivalves on natural plankton assemblages was studied in two estuarine embayments on Long Island, New York, over 2–6 month periods. The total grazing pressure (per cent bay volume cleared per day) on phytoplankton by bivalves was substantial, sometimes approaching or exceeding the daily flushing rate of the embayment (Lonsdale *et al.* 2009). Other examples of top–down control are provided by Ogilvie *et al.* (2003), Newell *et al.* (2007), Grant *et al.* (2008), Maar *et al.* (2008), Petersen *et al.* (2008) and Dame (2012), with the most dramatic examples in areas following the rapid population growth of an invasive bivalve species (see Newell 2004). Another effect of bivalve feeding is that turbidity is reduced through removal of phytoplankton and other particles from the water column (Figure 4.22). This has the knock-on effect of increased light penetration to the sediment, which in turn can potentially enhance the growth of seagrasses and benthic algae; the former provide a heterogeneous habitat for sessile and mobile fauna, while the latter are an important food source for benthic herbivores, which in turn are a food item for fish (Newell 2004). One downside of increased transmission of light at the sediment surface is enhanced growth of macroalgal species, some of which may restrict water flow and cause sediment hypoxia when they decay (Rafaelli *et al.* 1998).

Bivalves also have the potential to influence zooplankton communities. They do so directly by consuming zooplankton (see earlier) and by competing with zooplankton for phytoplankton resources. Bivalves can in fact outcompete zooplankton because they overwinter as adults and are able to start feeding as soon as water temperatures increase in the spring. Copepods, a dominant component of zooplankton, have to rebuild their population in the spring and as most of the phytoplankton is then being grazed by bivalves, copepod populations can diminish as their fecundity relies on this food source (Newell 2004).

Biodeposition by bivalves

Waste products from bivalves are excreted in dissolved form, or released as faeces and pseudofaeces. The high variation in assimilation efficiency shown by bivalves (see earlier), as well as the substantial amounts of POM in pseudofaeces, means that large amounts of undigested particulate organic nitrogen (PON) and phosphorus (P) are transferred to the sediment surface as biodeposits (faeces and pseudofaeces). Biodeposition by bivalve filter-feeders can be substantial, especially in suspended mussel culture systems. For example, a single raft in the Galician rias, Spain, may produce up to 129–190 kg (dry weight) day^{-1} of biodeposits, which translates into an annual input per raft of 69 t of sediment, 5220 kg of carbon (C) and 620 kg of nitrogen (N) to the seabed (Otero *et al.* 2009 and references therein). Analysis of mussel biodeposits shows that they are relatively rich in C, N and P. Biodeposits may gradually be incorporated into the sediment or resuspended, depending on the velocity of bottom water currents (references in Newell 2004). Increasing levels of organic input into sediment can have a dramatic effect on benthic faunal communities. For example, soft sediment communities, which are typically dominated by large filter-feeders, are replaced firstly by smaller and greater numbers of deposit-feeders, then polychaetes, shifting then to nematodes, and finally culminating in anoxic conditions (McKindsey *et al.* 2006 and

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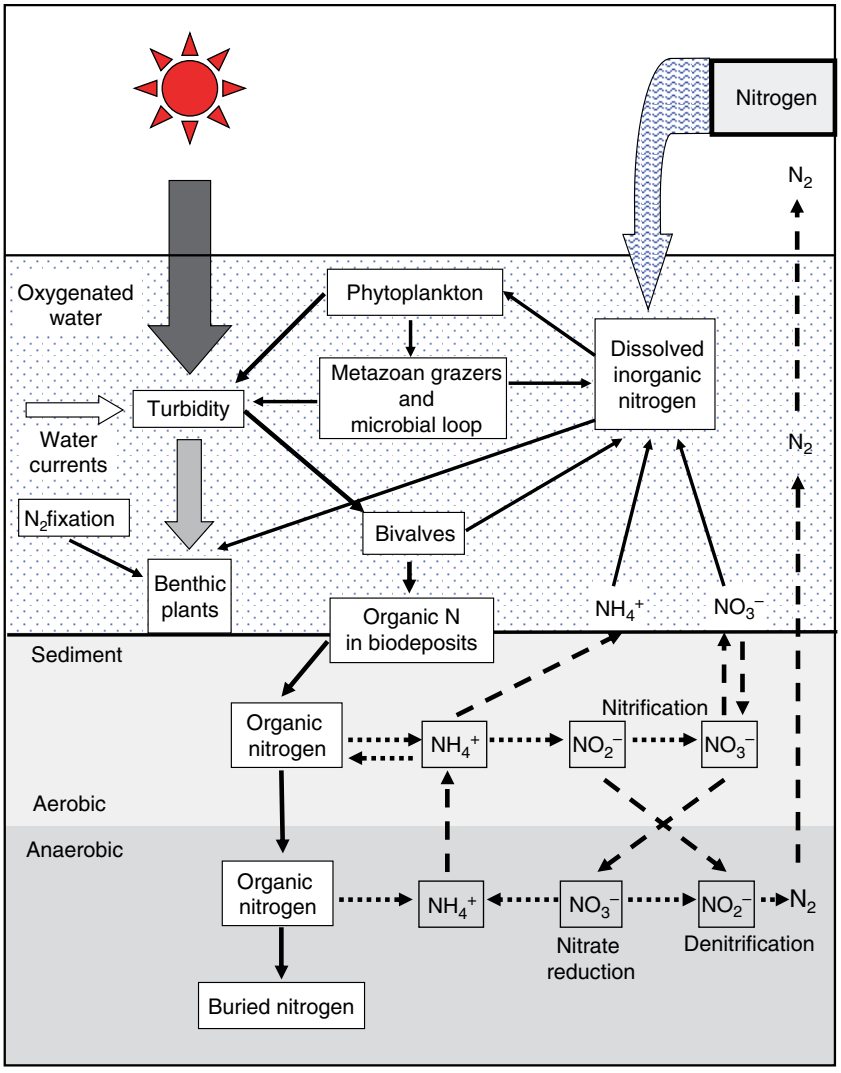


Figure 4.22 The nitrogen (N) cycle in a bivalve suspension-feeder–dominated ecosystem. Bivalves remove organic and inorganic particles from the water column and transfer undigested particulate matter in their biodeposits to the sediment surface. Burial of N throughout the sediment leads to removal of N from the water column. Within the aerobic sediment layers microbial-mediated nitrification occurs, which, when linked to denitrification in the underlying anaerobic sediment, leads to the further loss of N as N_2 gas. Bivalve grazing on phytoplankton leads to a reduction in turbidity, which enhances the growth of benthic plants (see text for more details). Solid lines indicate movement of materials; dashed lines indicate diffusion of materials; dotted lines indicate microbial-mediated reactions.
From Newell *et al.* (2002). Reproduced with permission of the Association for the Sciences of Limnology and Oceanography, Inc.

references therein). The development of anoxic sediments can lead to increased hydrogen suphide to levels that are toxic to benthic organisms (Diaz & Rosenberg 1995). In contrast to the scenario described, Dittmann (1990) reported that biodeposition from mussel beds in the North Sea actually lead to an enhanced and more diverse invertebrate assemblage. See McKindsey *et al.* (2006) for a detailed discussion on the direct and indirect effects of biodeposition on benthic assemblages.

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The role of bivalves in nutrient cycling

The elements C, N and P that are excreted by bivalves and regenerated from their biodeposits are subsequently recycled back to the water column to support further phytoplankton production. This section, which is heavily reliant on information by Newell *et al.* (2002) and Newell (2004), will focus on N, which undergoes numerous transformations, as well as state changes as it cycles through the environment (Dame 2012). Bivalves obtain their N not just from phytoplankton but also from N-rich bacteria and flagellates. When bivalve biodeposits settle to the sediment surface any remaining PON undergoes bacterial degradation, which can lead to some NH_4^+ being regenerated to the water column (Figure 4.22). This form of N is preferentially taken up by photoplankton. This is an example of a positive feedback loop in which both bivalves and phytoplankton benefit from the process (Dame 2012). Measured rates of NH_4^+ flux can be high, ranging from approximately 1 to 5 mmol N m^{-2} h^{-1} in bivalve populations. N that is not bacterially metabolized becomes buried in the accumulating sediments. If there is sufficient oxygen in the sediments then aerobic denitrifying bacteria can oxidize N compounds to NO_2^- and NO_3^-, some of which diffuses out of the sediment into the water column's pool of dissolved inorganic nitrogen (DIN) and some which diffuses down into the underlying anaerobic sediments. Here denitrifying bacteria reduce NO_2^- and NO_3^- to N_2 gas. This gas, which is in a form unavailable for phytoplankton production, passes to the atmosphere. Where there is an active benthic microalgal community they limit nutrient release to the water column by assimilating inorganic nitrogen released by bacterial decomposition. But when N requirement exceeds that supplied from the water column and regenerated from the sediment, the plants can convert N_2 to NH_4^+, a process called nitrogen fixation.

Those wishing to explore regeneration of other biologically essential elements, such as carbon, phosphorous and silicon, would be well advised to read the recent, very comprehensive review by Dame (2012).

Notes

1 In mussels exhalant siphon area, and to a lesser extent valve gape, constitutes the best proxy of filtration rate (Newell *et al.* 2001; Maire *et al.* 2007)
2 Organisms and non-living material swimming or floating in a water body.
3 Gut passage time (GPT) is defined as the time at which 90% of the cumulative defaecation of an element is recovered, assuming 100% recovery at 96 h (Wang & Fisher 1996).

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5 Reproduction, settlement and recruitment

Introduction

The reproductive system in bivalves is extremely simple (see Chapter 2). The paired gonads are made up of branching tubules, and gametes are budded off the epithelial lining of the tubules. The tubules unite to form ducts that lead into larger ducts, which eventually terminate in a short gonoduct. In primitive bivalves the gonoduct opens into the kidneys, and eggs and sperm exit through the kidney opening (nephridiopore) into the mantle cavity. In most bivalves the gonoduct is no longer associated with the kidneys but opens through independent pores into the mantle cavity close to the nephridiopore. Fertilization is external and the gametes are shed through the exhalant opening of the mantle, except in the case of brooding bivalves.

Sexuality

The majority of bivalves are dioecious, that is the sexes are separate, and there are usually equal numbers of males and females, although the sexes cannot be differentiated on external characters. The standard method for distinguishing the sexes is to fix gonadal tissue and then use histological processing and light microscope examination (see later). Jabbar and Davies (1987) have described a simple colorimetric test for sex determination in mussels that is based on the substantial difference in lipid concentrations between eggs and sperm. In mussels, Mikailov *et al.* (1995) have identified a 'male-associated polypeptide' that is virtually absent in females. Both these methods, while cheaper and less time-consuming than histological processing, involve sacrificing the animals. A preparation of squashes from fine-needle aspirates of mantle tissue is preferable because it can be done on live bivalves (Burton *et al.* 1996). More recently, several bioanalytical techniques have been adopted to determine gender. For example, magnetic resonance imaging (MRI) has been used on live Pacific oysters to identify sex and to follow gonad development without having to open the shells (Davenel *et al.* 2006). A novel reverse

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transcriptase polymerase chain reaction (RT-PCR) method provides a simple diagnostic based on the presence or absence of gender-specific transcripts in mussels (Hines *et al.* 2007). Of all techniques, RT-PCR is currently the most accurate and robust one for absolute gender determination and, unlike the other methods, is applicable to both ripe and spent mussels (Hines *et al.* 2007; Sedik *et al.* 2010).

Scallops are predominantly hermaphrodites, with distinct male and female portions of the gonad. These bivalves exhibit synchronous hermaphroditism, that is the gonad simultaneously produces male and female gametes either within the same tubules or in two distinct zones in the gonad. To prevent self-fertilization male gametes are generally released first, a process known as protandry. In contrast, asynchronous (sequential) hermaphrodites, such as some oyster (*Ostrea, Crassostrea*) or clam (*Mercenaria*) species, are either male or female for one or several annual cycles, after which a sex change occurs. In *Ostrea edulis* when the young oyster reaches sexual maturity the gonad normally develops as a male; after spawning the gonad changes to female and regular alternation may continue throughout life (Walne 1974). In some species, for example *Crassostrea gigas*, the percentage of females increases with age and size (Guo *et al.* 1998). Until recently, the prevailing view was that sex in oysters was determined by environmental factors such as food supply and water temperature (Coe 1936), or that hormones might be the major controlling mechanism (Wang & Croll 2004; Chávez-Villalba *et al.* 2011). However, Guo *et al.* (1998) suggested that a single major gene with two genotypes controls sex in *C. gigas*, with a dominant M allele for male maturation and a protandric F allele. FM oysters are fixed males, that is they do not change sex while FF oysters may mature as either males or females depending on other genes and/or environmental effects. An alternative 3-genotype model has also been proposed that features two types of females, a protandric FM and a fixed female FF (for details see Hedrick & Hedgecock 2010). A number of genes that may play a role in sex determination and differentiation have recently been identified in oysters (Naimi *et al.* 2009; Yu *et al.* 2011; Dheilly *et al.* 2012).

Clams are mostly dioecious but two types of sexual pattern other than separate sexes have been recognised, asynchronous and simultaneous hermaphroditism. The quahog clam *Mercenaria mercenaria* become sexually mature before they are 1 year old, developing first as males but changing to an equal sex ratio in the second year (Menzel 1989). On the other hand, giant clams, *Tridacna*, are both male and female at the same time; sperm are released first, followed by eggs some hours later. There is no evidence that giant clams are capable of producing viable offspring through self-fertilization (Heslinga 1989).

Mussels (*Mytilus*) are dioecious but exhibit two unusual phenomena. They have two types of mitochondrial DNA (mtDNA) with separate transmission routes, one through the eggs (F genome) and the other through the sperm (M genome), a process called doubly uniparental inheritance (DUI; Zouros *et al.* 1994). Female mussels typically inherit their F genome only from their mother, but they transmit this F genome to both sons and daughters. Male zygotes inherit their mtDNA from both parents, but they sort the mixture of mitochondrial genomes present so that the M genome inherited from their father becomes established in the germ line (Breton *et al.* 2007). The other feature is that it is the female parent that determines the sex ratio among offspring of a pair mating (Saavedra *et al.* 1997). The coupling of sex and mtDNA transmission in mussels raised the possibility that sex ratio determination might be under the control of the mtDNA of the female parent. But this is not the case; it is the mother's nuclear rather than mtDNA genotype that exercises control (Kenchington *et al.* 2002). Currently, DUI has been identified in more than 30 bivalve species, both marine and freshwater (Theologidis *et al.* 2008). Very recently it has been identified in protobranchs, the most basal extant group within the Bivalvia, which suggests that DUI might have evolved much earlier in bivalve evolution than previously thought (Boyle & Etter 2013). For further information on DUI see Kenchington *et al.* (2009).

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Gametogenesis

Bivalves undergo an annual reproductive cycle that involves a period of gametogenesis, followed by a single, an extended or even several spawning events, which in turn is followed by a period of gonad reconstitution (see later).

In spermatogenesis, primary spermatogonia undergo repeated mitotic divisions to produce secondary spermatogonia, which undergo meiosis to become spermatocytes. These produce spermatids that differentiate into flagellated spermatazoa that measure 25–60 μm in length (more details in Franco *et al.* 2008). Oogenesis follows a similar pattern to spermatogenesis in that primary oogonia undergo repeated mitosis to give secondary oogonia that enter meiosis but are then arrested at the prophase stage of meiosis I. The remaining meiotic stages are completed at fertilization. The oocytes then undergo a period of vitellogenesis, which involves the accumulation of lipid globules and small quantities of glycogen (see Chung 2007 for details). The deposition of these reserves is accompanied by an increase in oocyte size, reaching 70 μm in the mussel *Mytilus edulis* (de Zwann & Mathieu 1992), and 15–120 μm in scallops, depending on the species (Beninger & Le Pennec 2006). Lysis (atresia) of oocytes is usually observed at the end of vitellogenesis but previtellogenic oocytes may be affected due to the presence of hydrolytic enzymes released from lysed oocytes. It is believed that lysed oocytes provide metabolic substrates for energy production at a time when energy reserves in adductor muscle are at their lowest (Beninger & Le Pennec 2006).

Reproductive cycles

Methods of assessment

The principle method for assessing the reproductive cycle in bivalves is based on histological preparations of gonad. Usually, animals for analysis are collected over a 1–2-year period at monthly or bi-monthly intervals. About 10–20 individuals are randomly sampled, and back in the laboratory, shell and soft tissue measurements may be taken before a small section (2–3 mm^3) from the middle area of the gonad is dissected. The tissue for histology is fixed in a standard fixative such as 10% formalin, dehydrated in alcohol and after dehydration it is embedded in paraffin. Sections (5–7 μm thickness) are prepared from each individual and stained with haematoxylin–eosin or an equivalent stain. The proportion of developing, ripe, spawning and spent individuals in each sample can then be estimated. By multiplying the number of individuals at each development stage by the numerical ranking of that stage, and dividing the result by the total number of individuals in the sample, a mean gonad index (GI) is arrived at for each sampling interval. The GI increases during gametogenesis and decreases during spawning. Gonad classification schemes, too numerous to mention, abound in the literature. One such scheme for the dioecious Iceland scallop *Chlamys islandica* (Thorarinsdóttir 1993; Figure 5.1) is presented as follows:

Stage I. *Maturing* (*recovering*). First recognisable stage of recovery after spawning. Gonad growing, flabby and containing much free water. Follicles becoming larger and denser, connective tissue between them. Lumina filling with growing oocytes 20–30 μm in diameter. Genital ducts losing circular configuration.

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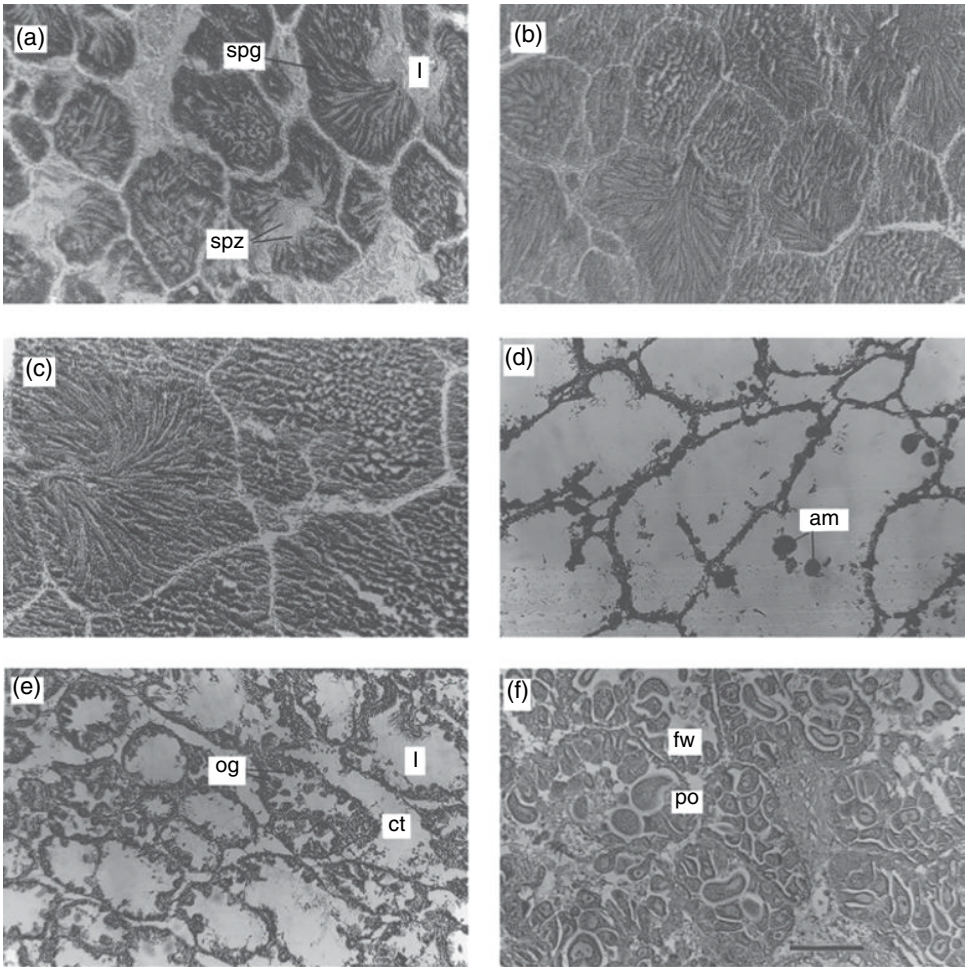


Figure 5.1 Photomicrographs of transverse sections through male and female gonads of the scallop *Chlamys islandica* at various stages in gamete development. (a) Male, stage II, maturing. (b) Male, stage III, maturing. (c) Male, stage IVA, mature. (d) Male, stage IVC, spent. (e) Female, stage II, maturing. (f) Female, stage IVA, mature. am, amoebocytes; ct, connective tissue; fw, follicle wall; l, lumen of follicle; og, oogonia; po, polygonal ova; spg, spermatogonia; spz, spermatozoa. Bar is 100 μm. From Thorarinsdóttir (1993). Reproduced with permission of Elsevier.

Stage II. *Maturing* (*filling*). Gonad still larger and thicker, colouring brighter. Still flabby but containing little free water. Follicles larger and closer together. Radially arranged spermatazoa in male follicles, lumen of females contains half-grown oocytes, many of them attached to the follicle wall. Little connective tissue.

Stage III. *Maturing* (*half-full*). Gonad again larger and thicker, containing very little free water. Follicles becoming packed together. Lumina becoming packed with spermatazoa or fully grown oocytes 50–60 μm in diameter. Reduction in connective tissue.

Stage IVA. *Mature* (*full*). Gonad has gained maximum size and contains no free water. Follicles highly coloured, and closely packed, testis cream-coloured, ovary usually orange to brick red. Male follicles packed to periphery with spermatazoa. Sperm heads measuring about 3 μm. Follicle walls extremely thin.

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Female follicles crowded with polygonal or hexagonal oocytes. Connective tissue restricted to alimentary canal and gonoducts, which appear flattened.

Stage IVB. *Spawning*. Gonads retain differentiation. Tissue becoming dull, flabby and containing much free water depending on the number of follicles emptied. Zones containing un-spawned follicles exhibit ripe germ cells and are in stage IV of development. Follicles of varying degrees of spawning are crowded by phagocytes. In some sections ova or sperm may be seen in ciliated ducts.

Stage IVC. *Spent*. Gonad considerably shrunken in volume, generally dull, flaccid and fawn-coloured, containing much free water. Follicles empty, retaining few residual germ cells. Amoebocytes attack un-spawned gametes, sometimes seen as cellular debris. In late stages some reorganisation of follicles and early gametogenic stages are apparent.

Gonad classification schemes, however, tend to be subjective because they are based on the qualitative characterization of overall morphology of the gonads and not on quantitative analysis of the gametes. One such quantitative method expresses the weight of the gonad as a percentage of the total soft tissue weight for each individual, to give a mean gonadosomatic index (GSI) for each sampling interval (Figure 5.2). Seasonal changes in GSI reflect accumulation and release of gonad material as well as utilization of stored energy reserves (Toro *et al.* 2002). Both wet and dry tissue weights can be used, but the latter are preferable because water content varies seasonally and between different tissues. This method is particularly suitable for scallops where the gonad is anatomically distinct and therefore can be easily removed intact (Barber & Blake 2006). Alternatively, in oysters where the gonad is fused with the digestive gland, a GSI, which compares areas of gametes (GA) relative to the areas of the total visceral mass (VMA), can be estimated. Image analysis software is

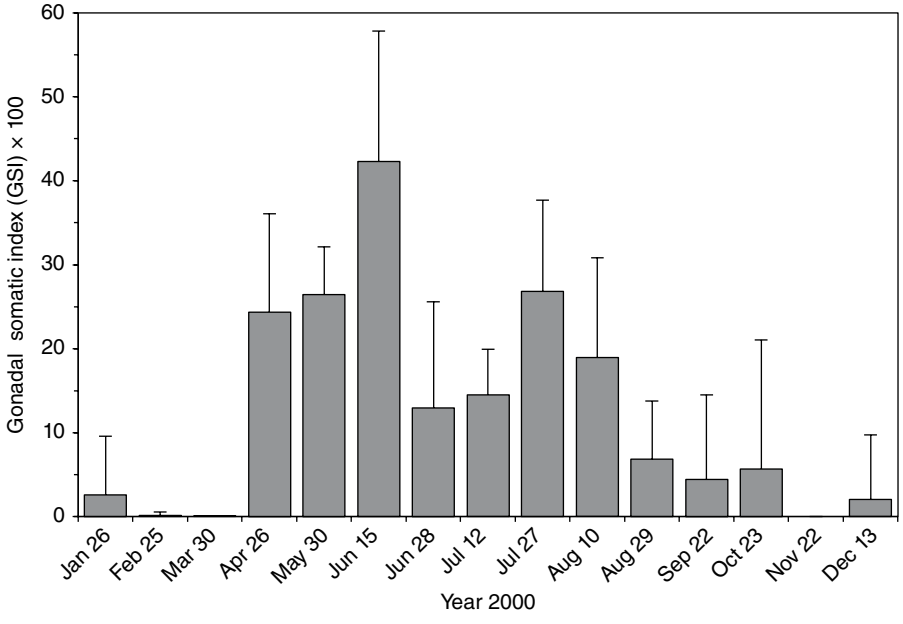


Figure 5.2 Seasonal variation in gonadal somatic index of the Pacific oyster *Crassostrea gigas*, oysters raised in Goseong Bay. Korea. Each bar represents the monthly mean GSI with the standard deviation as a vertical line.
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used to measure both these areas on digitized histological sections from each individual. GSI is calculated as follows:

$$\mathrm{GSI}(\%) = \left(\frac{\mathrm{GA}\ \mu\mathrm{m}^2}{\mathrm{VMA}\ \mu\mathrm{m}^2} \right) \times 100$$

and individual GSI means are averaged for each sampling date. This measure was developed and utilized by Dutertre *et al.* (2009) to examine the effect of temperature and seston quantity and quality on reproduction of farmed oysters, *C. gigas*, on the west coast of France. Another quantitative technique is stereology (Weibel & Gomez 1962), which is used to measure changes in the volume fractions of different components within the gonad (oocytes, nutritive storage cells and connective tissue) from point counts on test grids applied to histological sections of gonad. An important requirement of the technique is that the sections examined are representative of the whole gonad (Lowe *et al.* 1982). Stereology has been used in mussels to estimate the fractional area of the mantle composed of gametes (%GVF; Toro *et al.* 2002; Doherty *et al.* 2009). To illustrate, Doherty *et al.* (2009) examined tissue sections with an Olympus CX41 compound microscope interfaced with a PC and Olympus DP soft image analysis software©. For each slide a test grid of 30 points measuring 180 × 165 mm was applied to 10 fields of view (400×). The percentage of the mantle volume occupied by gametes was calculated from the relationship between the number of pixels occupied by the gametes and the total pixels in the field for a number of individuals for each sampling interval. GVF varies between 0% for a reproductively inactive individual and 100% for an individual in peak reproductive condition, and also provides a measure of the relative maturity of the gonad (Toro *et al.* 2002). As gametogenesis proceeds there is an increase in the volume of the gonad with a concomitant increase in oocyte size. Indeed, Lango-Reynoso *et al.* (2000) proposed a quantitative reproductive scale for oysters based on oocyte diameter, which they suggested would complement the traditional qualitative method of staging. Image analysis is the technique most often used to measure oocyte area and/or diameter (clams: Aragón-Noriega *et al.* 2007 and Kang *et al.* 2009; mussels: Doherty *et al.* 2009; oysters: Arcos *et al.* 2009 and Dutertre *et al.* 2010). Stereology has been used to measure oocyte volume fraction (OVF) – the percentage of ovary volume occupied by oocytes – and can also provide different size frequency distributions of developing oocytes (Pazos *et al.* 1996). In addition, enzyme-linked immunosorbent assays (ELISA) have been used to quantify eggs, and ultimately to estimate reproductive output (fecundity), in a variety of bivalves (see Long *et al.* 2008 for references). More recently, MRI has been used to assess seasonal changes in gonad development of a number of bivalves (Smith & Reddy 2012 and references therein), and Corporeau *et al.* (2012) have used a proteomic approach to identify several proteins in oysters that were differentially accumulated according to oocyte quality.

All methods of assessing gametogenesis have their advantages and disadvantages and therefore the optimum strategy is to employ at least one qualitative (histology) and one quantitative (GSI, GVF, gonad weight and OVF) method.

Annual cycles

The reproductive cycle in bivalves involves several stages: growth and ripening of gametes, spawning and gonad redevelopment. Understandably, there is enormous variation in the timing and duration of each stage both within and between species. Therefore, in this section a few appropriate examples will be described to illustrate the breadth of variation.

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Mussels in the genus *Mytilus* are widely distributed throughout the cooler waters of the northern and southern hemispheres (Chapter 3). *M. edulis*, which can mature in its first year (Duinker *et al.* 2008), usually commences gonad redevelopment in October or November in the northern hemisphere and the process continues over the winter months so that by February the gonads are ripe. Partial spawning occurs in the spring and a second period of gametogenesis takes place over the summer months, which culminates in spawning in early autumn. When feeding conditions are favourable, such as on the low shore, the number of gametes spawned in spring and autumn is comparable. This is not the case under suboptimal conditions, for example the high shore, when fewer gametes are shed in the autumn. There are a number of variations in this pattern; some populations exhibit a single short spawning period of a few weeks while others, for example at Strangford Lough, Ireland, have a spawning period from March to September, which is sometimes extended into the winter months (Seed & Brown 1975). Even at a single location there are differences in spawning patterns. For example, in Killary Harbour, on the west coast of Ireland, wild mussels partially spawn in early spring but spawn completely in summer, while rope-cultured mussels spawn twice over the summer months (Rodhouse *et al.* 1986).

Differences in spawning patterns have been reported between coexisting species of mussel. The indigenous mussel *Perna perna* and the invasive mussel *Mytilus galloprovincialis* show different patterns on the South African south coast, with the former spawning in summer and early winter, and the latter in summer and autumn (Zardi *et al.* 2007). However, not all cohabiting mussel species show different spawning cycles (see Maloy *et al.* 2003; Barber *et al.* 2005; Doherty *et al.* 2009). Mussels are therefore good examples of bivalves with a flexible reproductive strategy, adjusting their cycle according to prevailing environmental conditions (Table 5.1). For a detailed listing of spawning periods in *Mytilus* and other mussel species see Seed (1976).

The oyster *O. edulis* inhabits waters from Norway to the Mediterranean and Adriatic seas. The gonad remains dormant in the male or female phase over the winter months but development resumes once spring arrives (Walne 1974). The age at sexual maturity is usually 3 years (~50 mm shell length). In Britain spawning occurs from the end of June to the beginning of July, but in warmer waters several spawnings occur between May and October (Lubet 1994). Another oyster, *C. gigas*, originates in the Pacific but was introduced into Europe in the 1970s, and wild populations are now found from Germany to southern Portugal (Cardosa *et al.* 2007). Unlike *O. edulis*, gametogenesis proceeds over the winter months, accelerates in spring and several intensive spawnings occur between July and September, providing temperatures exceed 20–22°C. As there are areas in Europe where these temperatures are seldom if ever reached in summer, *C. gigas* must be artificially conditioned and spawned in the hatchery (methods in Chapter 9). Otherwise, gametes are resorbed *in situ* in autumn, and the metabolites thus recovered are stored in the form of glycogen, which is then used to sustain growth over the winter months (Lubet 1994). Over the past decade or so wild populations have been reported in England, Ireland, Norway and Sweden, most likely due to the influence of global warming (Miossec *et al.* 2009).

In Europe the two clam species *Ruditapes decussatus* and *Ruditapes philippinarum* (the latter introduced from the Philippines) show interspecific differences in breeding cycles (Laruelle *et al.* 1994). When the two species were compared from the Bay of Brest, Brittany, France, *R. philippinarum* showed a more extended breeding period and a greater number of spawning events than *R. decussatus*. The latter spawned mainly in early July and in late August, while the former partially spawned in late May and in July, and completely in mid August to mid September (Table 5.1). Inter-site variations in breeding pattern were observed for each species, which appeared to be linked with environmental differences in temperature

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Table 5.1 Spawning periods in a selection of bivalve species.

Species	Locality	Spawning period	Reference
Mussels			
Mytilus edulis	Sognefjord, west Norway	April–May; late June; sometimes September	Duinker *et al.* (2008)
Mytilus galloprovincialis	Galicia, Spain	Spring to summer	Cáceres–Martínez and Figueras (1998)
Mytilus trossulus	Alaska, USA	All summer	Blanchard and Feder (1997)
Aulacomya ater	Chile, South America	June–January	Jaramillo and Navarro (1995)
Perna canaliculus	North Island, New Zealand	June–December	Alfaro *et al.* (2001)
Modiolus barbatus	Adriatic Sea, Croatia	June–August	Mladineo *et al.* (2007)
Oysters			
Ostrea edulis	Murcia, Spain	Spring–early summer	Cano *et al.* (1997)
	UK	June–July	Walne (1974)
Crassostrea gigas	Galicia, Spain	June–July; October	Ruiz *et al.* (1992)
Crassostrea virginica	Long Island, USA	July–August	Zarnoch and Schreibman (2012)
Saccostrea glomerata	New South Wales, Australia	March–May	Dove and O'Connor (2012)
Pinctada margaritifera	French Polynesia	Throughout year	Pouvreau *et al.* (2000)
Clams			
Ruditapes decussatus	South coast of Ireland	September	Xie and Burnell (1995)
	Bay of Brest, France	July–October	Laruelle *et al.* (1994)
Ruditapes philippinarum	South coast of Ireland	August	Xie and Burnell (1995)
	Bay of Brest, France	Partial late May and July; complete mid August–mid September	Laruelle *et al.* (1994)
	Korea	May–December	Kang *et al.* (2007)
Mercenaria mercenaria	New York, USA	June–September	Doall *et al.* (2008)
	Florida, USA	February–March and September–October, but partial year round	Dalton and Menzel (1983)
Mya arenaria	Washington, USA	May–August	Porter (1974)
	Maryland, USA	May–June and October–December	Pfitzenmeyer (1965)
Spisula solidissima	New Jersey, USA	October–November	Chintala and Grassle (1995)
Arctica islandica	Iceland	Year round; intense June–August	Thorarinsdóttir (2000)
Panopea globosa	California, USA	January–February	Aragón-Noriega *et al.* (2007)
Scallops			
Pecten maximus	Isle of Man, UK	Partial April–May; complete July–August	Ansell *et al.* (1991)
Placopecten magellanicus	Iles de la Madeline, Canada	September	Giguere *et al.* (1994)
	Newfoundland, Canada	September–October; sometimes spring	DiBacco *et al.* (1995)
	New Jersey, USA	October–December	MacDonald and Thompson (1988)
Pecten fumatus	South-east Australia	June–November	Gwyther *et al.* (1991)
Pecten novaezelandiae	New Zealand	August–April	Bull (1991)
Patinopecten yessoensis	Japan	March–April	Ito (1991)

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Table 5.1 (Continued)

Species	Locality	Spawning period	Reference
Argopecten irradians	Massachusetts, USA	May–September	Rhodes (1991)
Argopecten gibbus	Bermuda	February–June,	Sarkis *et al.* (2006)
Adamussium colbecki	Rothera, Antarctica	September–October	Tyler *et al.* (2003)
Chlamys islandica	West Iceland	End June–beginning of July	Thorarinsdóttir (1993)
Chlamys varia	West coast of Ireland	May–June and July–August	Ansell *et al.* (1991)
Aequipecten opercularis	Isle of Man, UK	Spring, and autumn	Ansell *et al.* (1991)
	Faroes	June	Parsons *et al.* (1991)
Chlamys glabra	Aegean Sea, Greece	March–April	Lykakis and Kalathakis (1991)
Chlamys farreri	Northern China	May–June and late September–October	Lou (1991)
Chlamys nobilis	Southern China seas	April–May, August–September	Lou (1991)

and food supply. In these two species sexual maturation begins at a small size; in *R. decussatus* it is between 10 and 21 mm shell length (Lucas 1968), while in *R. philippinarum* it starts at a shell length of 5 mm, when clams are still less than 1 year old (Holland & Chew 1974). The hard clam *M. mercenaria* usually produces gametes before it is 1 year old (Menzel 1989), and in the surf clam *Spisula solidissima* sexual maturity occurs even earlier, within 3 months of settlement at a size of about 5 mm (Chintala & Grassle 1995).

The king scallop *Pecten maximus* is distributed from northern Norway south to the Iberian peninsula (Chapter 3), and inhabits a more stable environment than *Mytilus*, as it is found from the infra-littoral to 100 m depths (Ansell *et al.* 1991). In the British Isles *P. maximus* shows two peaks of spawning: a partial spawning in spring (April or May), and a more complete autumn spawning (July or early August). Juvenile scallops only spawn in autumn. In Galway Bay on the West Coast of Ireland, scallops show the same bimodal spawning pattern but, in addition, a low level of spawning continues throughout the summer months. At Loch Creran on the West Coast of Scotland there is one main summer spawning, but this is not closely synchronous in all individuals, and there may also be considerable variation in the timing of the spawning period from year to year. In contrast, further south in the Bay of St. Brieuc on the northern coast of Brittany, scallops show a unique highly synchronous maturation of the population in the spring, leading to a massive spawning in July, with secondary spawning until the end of August (Ansell *et al.* 1991). However, scallops from the closely located Bay of Brest show little synchrony between individuals and there are repeated cycles of gamete maturation throughout the year, with much resorption of poorer-quality gametes during the winter months (references in Brand 2006). The age of sexual maturity varies between scallop species; it is 71 days in *Amusium gibbus* (20 mm shell length), 1 year in *Argopecten irradians* (55–60 mm shell length) and 3–5 years in *C. islandica* (35–40 mm shell length; references in Barber & Blake 2006).

Factors controlling reproduction

The control of reproduction involves the complex interplay between exogenous factors like temperature, food, salinity and light, and endogenous factors such as neuroendocrine cycles and genotype. The role of these factors in the initiation and duration of gametogenesis – a

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process that extends over several months – most likely differs from their role in the timing and synchronisation of spawning, a much briefer event (Seed & Suchanek 1992). Therefore, in the following section gametogenesis and spawning are treated separately.

Exogenous regulation of gametogenesis

Temperature and food supply

Temperature is the single exogenous factor that is most often cited as influencing gametogenesis in bivalves. Bayne (1975) reported a linear relationship between the rate of gametogenesis in the mussel *M. edulis* and the rate of temperature change measured as day degrees. This was clearly demonstrated in broodstock *C. gigas* maintained between 16 and 22°C in which the speed of oocyte development increased significantly with temperature although the growing speed decreased in oysters maintained at 25°C compared to those maintained at 22°C. This was perhaps related to a lower feeding rate at the higher temperature, thereby resulting in an energy balance that limited the allocation of energy for gametogenesis (Chávez-Villalba *et al.* 2002). Sastry and Blake (1971) have shown that temperature influences the initiation of oocyte growth by regulating the transfer of nutrient reserves to the gonads (Figure 5.3). Other authors (Lubet & Aloui 1987) have suggested that a 'temperature window' exists for each species, the limits of which are set by the upper and lower lethal temperatures (LT_{50}) for the species. Inside this window gametogenesis occurs over a certain optimum temperature range. For example, they found that the upper and lower lethal temperatures for *M. edulis* were 23 and 0°C, respectively, and the optimum temperature for gametogenesis was between 2 and 15°C. An increase in temperature within this range does not affect the speed of gametogenesis, except at temperatures close to the lower LT_{50}.

For many species, food rather than temperature is the major factor determining the timing of gametogenesis. This is not surprising as the process is energy-demanding and therefore dependent on available food supply, stored energy reserves or both. Sastry (1966, 1968, 1970) and Sastry and Blake (1971) have examined these relationships in *A. irradians*. Scallops at different stages in their gametogenic cycle were held without food and exposed to various temperatures. In early gametogenic stages starvation at 10, 20 and 30 °C resulted in a decrease in digestive gland tissue, GI and also resorption of oogonia. At a later reproductive stage scallops with minimal reserves released gametes at 25 and 30°C, but at 15 and 20°C a decrease in digestive gland tissue and GI along with resorption of oocytes occurred. Scallops in the resting stage showed a decrease in digestive gland tissue and GI and failed to initiate gametogenesis at all experimental temperatures. Abundant food supply is thus necessary for gametogenesis, as pre-stored energy reserves are not sufficient by themselves. However, once minimal gonad reserves have accumulated gametes develop to maturity. But the deposition of minimal gonad reserves in this species is, as seen earlier, also temperature-dependent; below 20°C *A. irradians* failed to accumulate gonad reserves and therefore did not initiate gametogenesis. These results emphasise the importance of food supply in the control of gametogenesis, but also highlight the complex interplay between food supply and temperature in this species. Food quality is also an important consideration in gonad maturation. Navarro *et al.* (2000) found that the highest percentage of ripe scallops occurred in individuals fed with an algal diet supplemented with a lipid emulsion of essential fatty acids (see Chapter 9), at conditioning temperatures of 16 and 20°C (68% at 16°C and 42% at 20°C), while lower percentages were obtained with pure microalgae and with microalgae supplemented with carbohydrate.

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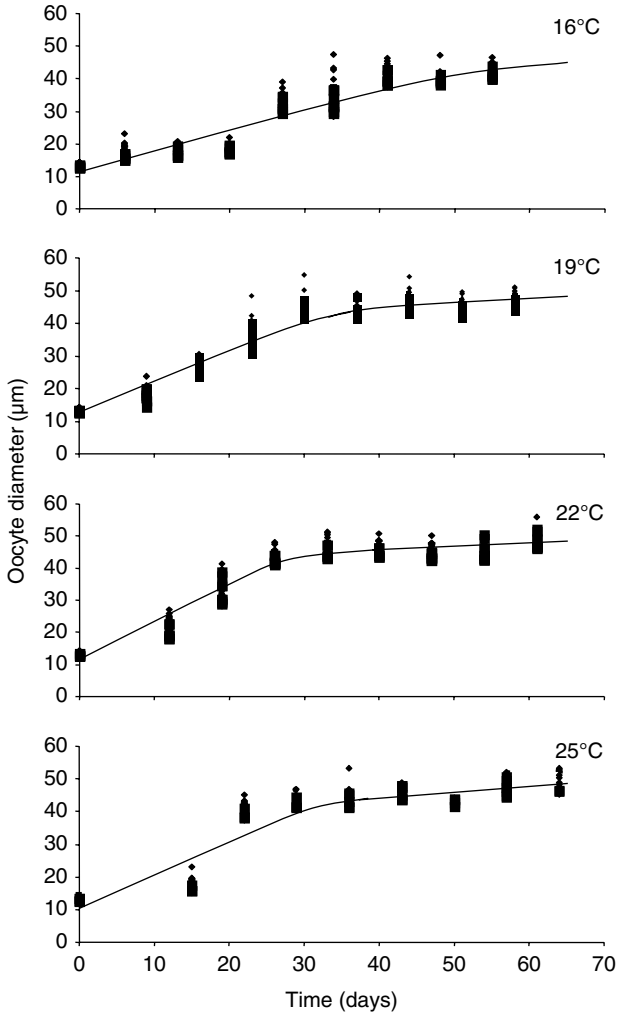


Figure 5.3 Oocyte diameter evolution (µm) according to the conditioning time for each tested temperature. The point series (scatterplots) correspond to the upper decile of data from the oocyte diameter measurements, and the curves represent the fittings of the logistic growth model for the Pacific oyster *Crassostrea gigas*.
From Chávez-Villalba *et al.* (2002). Reproduced with permission of Elsevier.

There is little information on factors such as salinity, lunar phase or tides on bivalve gametogenesis. In a series of experiments Loosanoff (1953) noted that adult *Crassostrea virginica* held in salinities of ≤5 psu did not show normal gametogenesis but at ≥7.5 psu gamete development was normal in both males and female oysters. With regard to the effect of photoperiod Sastry (1979) has reported that in Massachusetts, United States, gametogenesis in *A. irradians* is initiated during the spring, a time of increasing day length, and maturity is attained in midsummer when day length is maximal. But further south in North Carolina gametogenesis commences in midsummer and maturation and spawning correlate with decreasing day length of late to early autumn. These differences could, however, be ascribed to the different temperature and food regimes experienced by these two populations. More recently, Fabioux *et al.* (2005) have shown that under conditions of optimal food, temperature and/or photoperiod drive the reproductive internal clock of *C. gigas*, in

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particular for the regulation of gonial proliferation and germ cell maturation. Modifying these parameters led to the complete modification of the timing of gametogenesis, making it possible to obtain ripe oysters, and consequently ripe gametes, for an extended period throughout the year.

Endogenous regulation of gametogenesis

In bivalves, the reproductive cycle is controlled by interactions taking place between ganglion neurosecretions and steroids. Neurosecretory cells, mostly located in the cerebral ganglia of the central nervous system, secrete neuropeptides that exert various physiological effects on the gonad (see de Zwann & Mathieu 1992). One of these is the peptide hormone gonadotropin-releasing hormone (GnRH), which in humans regulates reproduction indirectly by stimulating secretion of the follicle-stimulating hormone (FSH) or luteinizing hormone (LH) from the anterior pituitary gland. In bivalves, however, GnRH and other GnRH-like peptides seem to have a direct effect on the gonad, by stimulating gonial mitosis, although the exact mechanism has not yet been elucidated (Morishita *et al.* 2010). Other neuropeptides, for example Ala-Pro-Gly-Trp amide (APGWamide) and Phe-Met-Arg-Phe amide (FMRFamide) also play a putative role in mollusc reproduction (Pani *et al.* 2005; reviewed in Morishita *et al.* 2010). In the oyster *C. gigas*, insulin-like peptides are involved in gonadal tubule rebuilding in the winter as well as the development of germinal cells in the spring and maturation of gametes in the summer (Gricourt *et al.* 2006). The monamine serotonin (5-hydroxytryptamine or 5-HT) is another molecule that acts as a neurohormone in modulating spawning (see later) and the process of oocyte maturation in bivalve gonad (references in Garnerot *et al.* 2006). The activity of neurosecretory cells is low during the resting phase of gametogenesis, progressively increases in synchrony with the developing gonad and reaches a maximum just before spawning (Figure 5.4). This pattern is mirrored by the activity of aspartate transcarbamylase (ACTase), an enzyme involved in nucleotide synthesis, which in the clam *Mya arenaria* is high at developing and ripe stages of gametogenesis, and low at the spawning and spent stages. ATCase activity measurement could be a good method for assessing gametogenesis and possibly the effects of endocrine disrupters (pesticides, polychlorinated biphenyls (PCBs) and phthalates) in exposed populations of bivalves in the field (Etchian *et al.* 2004).

A number of analytical techniques such as chromatography, radioimmunoassays (RIA) and ELISA have been used to identify and quantify steroids in molluscan tissue. Vertebrate

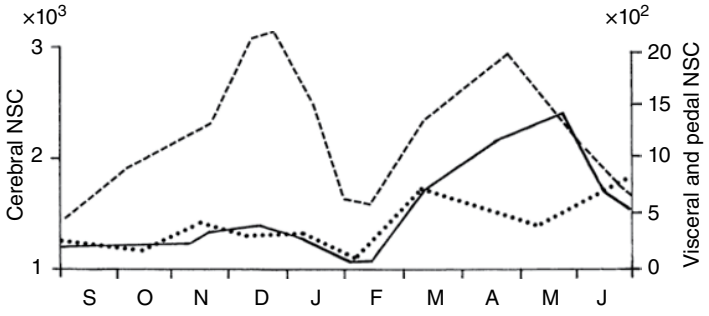


Figure 5.4 Variation of active neurosecretory cell (NSC) numbers in ganglia of the mussel *Mytilus edulis*, during the annual reproductive cycle. Cerebral ganglia, broken line; pedal ganglia, unbroken line; visceral ganglia, dotted line.
From de Zwann and Mathieu (1992). Reproduced with permission of Elsevier.

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sex steroids such as testosterone, estradiol-17β and progesterone have now been identified in several mollusc species, and fluctuations in levels are correlated with the sexual maturation cycle in a variety of bivalves, suggesting that sex steroids may play an important role in reproductive regulation (see Croll & Wang 2007 for references). Injection of these sex steroids stimulated both oogenesis and spermatogenesis in the scallop, while injection of estradiol stimulated vitellogenesis in the oyster *C. gigas*. There is also evidence that sex steroids may play a role in the determination of gender and sex ratios (see review by Lafont & Mathieu 2007). Most of the required enzymes for production of sex steroids from cholesterol have also been identified in molluscs, as well as the presence of estrogen receptors, molecules that mediate the effects of steroids (Croll & Wang 2007; Lafont & Mathieu 2007).

Exogenous regulation of spawning

Ciliary movement and muscular contraction of the gonoducts cause gamete release into the water column. Water temperature is the factor most frequently implicated in the initiation of spawning in wild, laboratory and hatchery (see Chapter 9) populations. In the field many species of scallop spawn when water temperatures are either increasing or decreasing (Table 5.1). For example, the cool water species, *C. islandica*, spawns with increasing water temperature in the spring (8–10°C), while the warmer water species, *Argopecten gibbus*, spawns when water temperatures are decreasing (<22.5°C). For *A. irradians* the northern subspecies spawns when water temperatures are increasing, while the southern one spawns when water temperatures are decreasing (references in Barber & Blake 2006). Field observations and laboratory experiments show that the clam *Macoma balthica* spawns in spring when temperatures gradually reach 8.3°C. Using an extensive long-term data set (1973–2001) Philippart *et al.* (2003) found that between 1973 and 2001 (Period I), spring temperatures significantly increased at a rate of 0.07°C year^{-1}. Between 1986 and 2001 (Period II) the rate of increase was also significant at 0.11°C year^{-1}. The day number at which bivalve spawning took place showed a significant declining trend with a rate of 0.44 days year^{-1} for Period I and 0.99 days year^{-1} in Period II. In other words, the day of the year that water temperatures reached the threshold value of 8.3°C was 112 in 1972 (Period I) but was considerably sooner, day 95, in 2001 (Period II). Advancement of spawning time in *M. balthica* is most likely due to global warming (Philippart *et al.* 2003).

Physical stimulation by jarring or scraping the shell, pulling or cutting the byssus threads can stimulate spawning in *M. edulis*. Seed and Suchanek (1992) suggest that these are the types of environmental cues received during storms that could also signal the presence of storm-generated patches of bare rock onto which mussel spat can settle. Other natural stimuli include salinity change, lunar phase and tidal fluctuations (Barber & Blake 2006 and references therein). Once spawning is initiated the presence of gametes in the water provides a powerful chemical stimulus to other ripe individuals, and thereby enhances the chances of fertilization.

Endogenous regulation of spawning

Spawning in bivalves is chemically regulated, both by environmental chemical cues, for example spawning of same species individuals, and by internal chemical mediators. The scallop osphradium (Chapter 2) has been shown to produce neurosecretions that are axonally transported to the gonad, hence suggesting a role both in the detection of broadcast pheromones and in the production of neurosecretions that are stored in the visceral ganglion, from which they are released at spawning (Beninger *et al.* 1995). The monoamine serotonin

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(5-HT) as well as prostaglandins PGE_2 and $PGF_{2\alpha}$) play key roles as mediators in the release of gametes. Injection of 5-HT (10^{-6} to 10^{-4} M) into fresh sections of the female gonad in the scallop *Argopecten purpuratus* increased the number of ooyctes released and these numbers increased with the duration of incubation (Martínez *et al.* 2000). PGE_2 (10^{-6} M) also increased oocyte release, but $PGF_{2\alpha}$ did not affect the process. Both 5-HT and the two prostaglandins also increased the rupture rate of oocytes from the germinal vesicles. Steroid hormones also play an important role in the spawning process. When ripe male and female scallop gonads were injected with 17β-estradiol, testosterone and progesterone Wang and Croll (2006) found that administration of estradiol to both sexes, and testosterone to males, increased the intensity of spawning; progesterone, however, decreased the proportion of individuals that spawned. In the same study injections of estradiol promoted 5-HT-induced spawning in both sexes, while testosterone potentiated spawning in males only; progesterone inhibited 5-HT-induced spawning in females but potentiated it in males. These findings could lead to the development of more efficient methods for inducing spawning in hatchery bivalves (Croll & Wang 2007).

Annual storage cycle

Gametogenesis is an energy-demanding process. During the annual reproductive cycle nutrients are stored when food supplies are abundant, and gonad activity is minimal. Subsequently, these energy reserves are utilised to meet the energetic requirements of gametogenesis. The main energy reserve for gametogenesis is glygogen, a branched polysaccharide of glucose units, and the storage site varies depending on the species. In scallops the main storage tissue is the adductor muscle, with smaller amounts stored in the digestive gland, mantle and gonad. In the scallop *P. maximus*, the adductor muscle doubles in weight between March and November but declines as the gonad increases in weight over the winter (references in Barber & Blake 2006). In the oyster *C. gigas*, most storage occurs in the labial palps, gonad and surrounding mantle area, while in the mussel *M. edulis*, the main storage area is the mantle where gonad and storage tissue coexist (Berthelin *et al.* 2000).

In *M. edulis* two types of cells are involved in energy storage: adipogranular (ADG) cells, located only in the mantle, accumulate lipids, protein and small amounts of glycogen, and vesicular connective tissue (VCT) cells, in both mantle and labial palps, store large quantities of glycogen. See Hanquet *et al.* (2011) for details on the mechanism of glucose transport into vesicular cells. As mentioned earlier, in addition to glycogen, proteins and lipids are also important energy reserves in bivalve gametogenesis. Table 5.2 shows annual variations in the amounts of these reserves in the gonad of female *P. perna*. Amounts of glycogen and lipid, and to a lesser extent protein, increased in the summer to autumn period (July–November) when GI in this species is low, and decreased over the winter months (December–February) when GI is high (Benomar *et al.* 2010). Proteins are probably used at the final gamete development stages, while the production of mature oocytes needs a store of lipids to produce yolk (references in Benomar *et al.* 2010). Lipid content is now used as a reliable indicator of oocyte and larval quality (Phillips 2002; Gómez-Robles *et al.* 2005; Angel-Dapa *et al.* 2010). Recently, a novel assay that uses a human diabetic glucose meter to measure glycogen has been used to rapidly assess the nutritional quality of seed mussels (*Perna canaliculus*) on commercial farms in New Zealand, thereby reducing losses of seed to the industry (Sim-Smith & Jeffs 2011).

Condition indices (CI) are also useful measurements of the nutritive status of bivalves. These are generally expressed as either wet or dry tissue weight (g) divided by some parameter of the shell such as dry shell weight (g), internal shell capacity (g) or volume (ml); see Crosby

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Table 5.2 Annual variations in the amounts of three biochemical components in the female gonad of the mussel *Perna perna* from Cap Ghir, southwest Morocco, in 1999.

Month	Glycogen (%)	Lipids (%)	Proteins (%)	Total
January	0.44	15.52	38.04	54.00
February	0.33	11.22	28.42	39.95
March	0.22	11.16	32.57	43.95
April	0.58	11.70	26.13	38.10
May	0.84	15.87	28.93	45.63
June	1.69	15.58	22.52	39.79
July	2.82	15.85	23.65	42.31
August	3.39	15.04	21.07	39.49
September				
October	1.85	22.36	42.19	66.39
November	2.18	22.61	48.79	73.57
December	2.14	21.57	31.23	54.92

Adapted from Benomar *et al.* (2010). Reproduced with permission of Cambridge University Press.
No data for September due to abnormal defrosting of samples.

and Gale (1990) for a review and evaluation of different CI methods. One commonly used index is CI = dry soft tissue weight (g)/internal shell cavity volume (ml) × 1000 (Walne 1970). The volume is measured as the total displacement volume of a completely closed shell minus the displacement volume of the shell after removal of the body tissue. A sample size of 50–100 individuals is recommended, to allow for individual variation (Quayle & Newkirk 1989). Using this index Walne (1974) reported that an average sample of the oyster *O. edulis* will have an index of 90–100, while well-conditioned oysters have an index of 120 or more, and oysters in poor condition have an index of 70–80. Condition varies both seasonally and from site to site, being influenced by food levels (Marzec *et al.* 2010), local environmental conditions (Okumuş & Stirling 1998), parasitism (Olivas-Valdez & Cáceres-Martínez 2002; Mercado-Silva 2005) and pollution (Pridmore *et al.* 1990; Roper *et al.* 1991). CI is now used routinely as a biomarker of metal and chemical contamination in the marine environment (Rajkumar 2009 and references therein). On a seasonal level, CI increases during the period of energy storage and gametogenesis, and declines with the main spawning event.

In vertebrates the peptide hormone glucagon controls the release of glucose from glycogen storage cells. Until relatively recently the existence of molluscan glucagon-like peptides was poorly documented. However, Kellner *et al.* (2002), using an immunocytochemical approach, found evidence for the presence of glucagon-like molecules in neurosecretory cells in the cerebral ganglia of *M. edulis*. Using an *in vitro* bioassay they investigated the effect of vertebrate glucagon on glucose metabolism and found that the hormone significantly decreased incorporation of ^{14}C-labelled glucose into glycogen.

Reproductive effort and fecundity

Young bivalves grow fast, converting all available energy into somatic growth; but with increasing size there is a gradual shift from somatic growth into reproduction, so that in the largest animals most production (>90%) is channelled into gamete synthesis (Figure 5.5). Paine (1976) has suggested that this may be the result of selective pressure for early increase in size as a refuge from predation, coupled with selection for high fecundity (egg production) in larger individuals.

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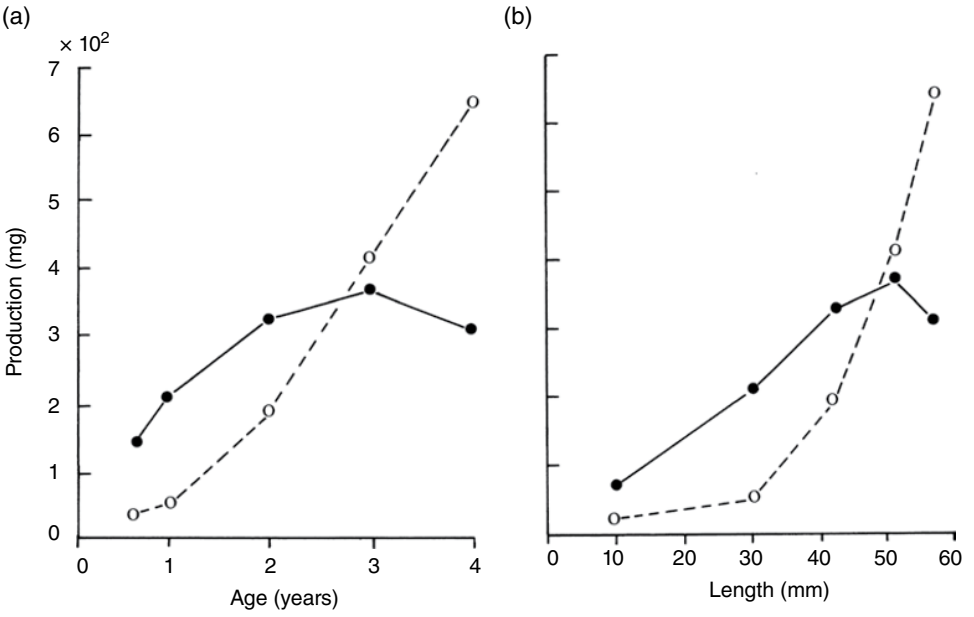


Figure 5.5 Somatic (solid circles) and gamete (open circles) production as a function of (a) age and (b) shell length in low shore mussels (*Mytilus edulis*).
From Rodhouse *et al.* (1986). Reproduced with permission of Springer Science and Business Media.

The term reproductive effort (RE) is used to refer to the level of energy allocated to reproduction and is commonly expressed as a proportion of total production:

$$\mathrm{RE} = \frac{P_g}{(P_g + P_s)}$$

where P_g is gamete production and P_s is somatic production over the same time period, usually 1 year. See Thompson and MacDonald (2006), Royer *et al.* (2008), Cáceres-Puig *et al.* (2009) and Kim and Choi (2012) for methods of measuring RE. There is substantial variation in RE between species (Figure 5.6), most of which has been ascribed to environmental factors such as temperature and food supply. Unusually, some species, for example the scallops *C. islandica* (Vahl 1985) and *A. irradians irradians* (Bricelj & Krause 1992), actually show a decline in RE with age. There is also considerable variation in RE between conspecific populations. For example, RE in 3-year-old mussels in different populations from south-west England ranged between 43 and 95% (Figure 5.7). At Lynher mussels received a richer food supply and experienced little environmental stress compared to the population at Cattewater, which was located near a warm water outfall and was therefore subjected to thermal stress during winter and spring (Bayne & Widdows 1978; Bayne *et al.* 1983). In a laboratory-based study Normand *et al.* (2009) found that triploid oysters (*C. gigas*) had a lower RE relative to diploid individuals. This is not surprising because triploids are partially sterile, diverting less of their energy into gonad development than into somatic growth (see Chapter 10). What was unexpected was that gonadic occupation (a measure of RE) was only 47% less than in diploids, indicating that gonadic occupation is not closely related to eventual gamete numbers because only a fraction of the germ cells appeared to mature into gametes. In another study on *C. gigas*, Huvet *et al.* (2010) reported

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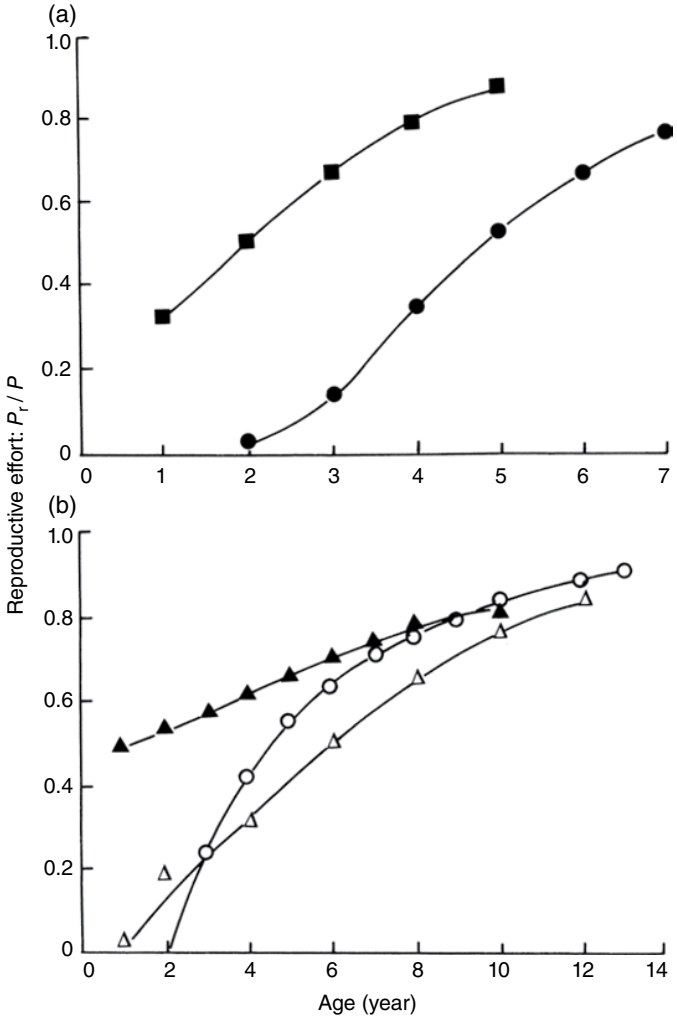


Figure 5.6 Reproductive effort calculated as a proportion of total production in five bivalve species, related to age. (a) ■, *Crassostrea virginica* (Dame 1972, 1976); ●, *Mytilus edulis* (Bayne & Widdows 1978); (b) ▲, *Choromytilus meridionalis* (Griffiths 1980); ○, *Placopecten magellanicus* (R.J. Thompson, unpublished data); Δ, *Ostrea edulis* (Rodhouse 1978).
Adapted from Bayne and Newell (1983). Reproduced with permission of Elsevier.

a negative correlation between RE and resistance to summer mortality (Chapter 11), suggesting that resistant oysters may survive better because they are not as reproductively active as susceptible oysters. In the latter, the high energetic demand of gametogenesis could lead to an energy imbalance that might lead to decreased defences against pathogens during the reproductive season.

Fecundity or reproductive output (RO) is the amount of gametogenic material produced by an individual. RO is measured by first determining the body mass index (BMI mg cm^{-3}), defined as ash-free dry weight divided by cubic shell length for a subsample of individuals, usually female. Each individual in the remainder of the sample is induced to spawn, each in a separate container, and eggs are collected, counted and often measured (Honkoop & van der Meer 1998; see Bayne *et al.* 1983, for related methods of measuring RO). Unsurprisingly, egg number (RO) and BMI are positively correlated (Figure 5.8).

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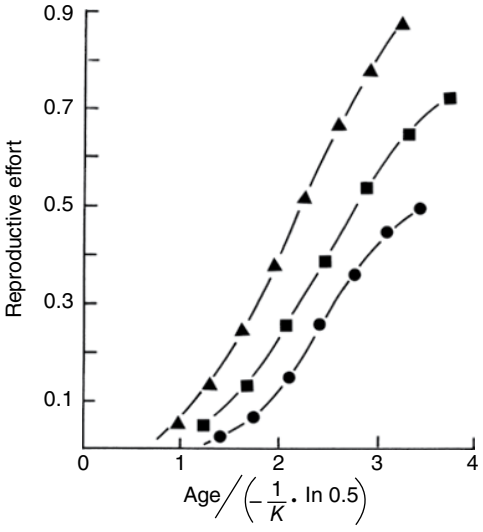


Figure 5.7 Reproductive effort of the mussel *Mytilus edulis* from three populations, related to age, corrected to exclude differences due to different rates of growth. K is the rate constant in the von Bertalanffy growth equation (see Chapter 6) for each site. ▲, Lynher; ■, Mothercombe; ●, Cattewater (all in south-west England).
From Bayne *et al.* (1983). Reproduced with permission of Springer Science and Business Media.

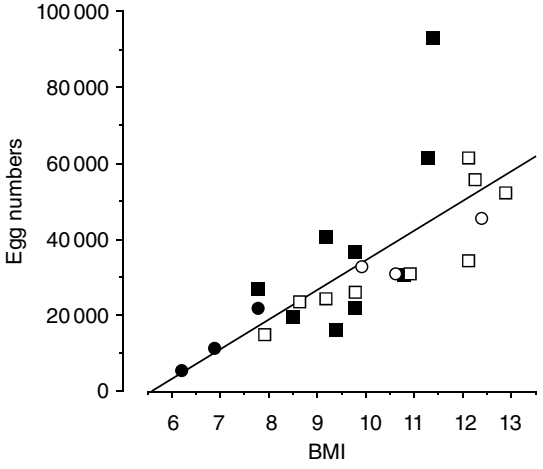


Figure 5.8 Mean egg number produced by a standardized *Macoma balthica* (with a shell length of 15 mm) in relation to body mass index (BMI) values measured just prior to spawning. BMI is ash-free dry mass divided by cubic shell length (mg cm^{-3}). Experimental data obtained in 1994 (■) and 1995 (□) and field-collected data in 1995 (●) and 1996 (○). Line represents the best linear fit: egg number = 7739 × BMI − 43314.
From Honkoop and van der Meer (1997). Reproduced with permission from Inter-Research.

More recently, immunological techniques have been used to quantify RO in several bivalve species (Park *et al.* 2005; Royer *et al.* 2008).

In the mussel *M. edulis* individual females produce between 7 and 40 million eggs, depending on shell length (Thompson 1979). In the brooding oyster species *O. edulis*

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(see later) egg production is much lower, varying from 1.0×10^5 to 1.5×10^6 eggs, depending on age (Walne 1974). But environmental factors also play a role. For example, egg production in the scallop *Placopecten magellanicus* ranged from $3.1–6.6 \times 10^7$ eggs per female at a shallow site (13–20 m) to $1.4–2.4 \times 10^7$ at a deep site (170–180 m), almost a threefold difference, most likely due to lower food availability in deeper water (Barber *et al.* 1988a). Over a 3-year period fecundity was significantly different in oysters in the James River Virginia with annual mean values per female of 5.6, 0.94 and 0.12 million eggs, respectively (Mann *et al.* 1994). The decline in fecundity was correlated with declining average salinity, the stressful effect of which may have reduced the energy available for reproduction in these oysters. On a more serious level, Philippart *et al.* (2003) used a long-term data set to show that with rising seawater temperatures due to global warming, recruitment in *M. balthica* is decreasing due to a decrease in RO, as well as spring advancement of spawning, which increases shrimp predation on early larval stages of the species.

Deleterious effects on the reproductive cycle

The presence of parasites has been correlated with the inhibition or retardation of gametogenesis (details in Chapter 11). The most significant are those belonging to the phylum Haplosporidia (protistans) and class Trematoda (flatworms) in the phylum Platyhelminthes. The protistan *Haplosporium nelsoni* (MSX) causes a lethal disease in the American oyster *C. virginica* on the mid-Atlantic coast of the United States. Oysters with systemic (general) MSX infections had a CI that was 31% lower, and a relative fecundity that was 81% lower than uninfected oysters (Barber *et al.* 1988b), probably due to the effect of the parasite on carbohydrate storage. Selection for resistance to MSX improved gametogenesis, but only in oysters with few parasites (Ford *et al.* 1990). Other protistans, for example *Bonamia ostreae*, *Perkinsus* spp., *Marteilia sydneyi* and Quahog Parasite Unknown, cause loss of condition in infected bivalves (Chapter 11). Bucephalid trematodes are serious parasites of many bivalve species. The larval stages (sporocysts) form a dense branching interwoven network that infiltrates most host organs, especially the gonad. Castration is often the outcome of heavy infestation and is caused by sporocyst consumption of storage tissue, especially glygogen.

Gonadal neoplasia is another condition that affects the reproductive cycle in bivalves. It is characterized by multiplication of germ cells to form gonadal germinomas (tumours), leading to abnormal gametogenesis and reduced fecundity, sometimes ending in the death of the host. An increased incidence has been reported in hybrids between *M. mercenaria* and *M. campechensis*, implicating a genetic component to the disease (Bert *et al.* 1993; Eversole & Heffernan 1995). The reduced fitness of the hybrids presents a good example of how a disease may act as a barrier to gene flow between species.

Various pollutants have also been shown to affect gametogenesis. Exposure to a number of metal and organic contaminants suppresses gamete development and/or enhances gamete breakdown in the mussel *Mytilus*. In addition, low-level hydrocarbon exposure reduces stored energy reserves, although the effect is reversible following depuration (Livingstone & Pipe 1992 for references). Other detrimental effects on the reproductive cycle are covered in previous sections.

Fertilization

Most marine bivalves are broadcast spawners, simultaneously releasing their eggs and sperm into the water column where fertilization takes place. Sperm is discharged in a thin, steady stream through the exhalant opening or exhalant siphon, while eggs are discharged,

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more intermittently, in clouds (Helm *et al.* 2004). Species of *Spisula*, *Mytilus*, *Crassostrea* and *Dreissena*, the freshwater mussel, have served as valuable models for understanding general mechanisms of fertilization (Misamore *et al.* 2006 and references therein). Briefly, enzymes in the head of the sperm (the acrosome) digest an opening through the jelly-like coating and inner vitelline layer of the egg. The plasma membranes of the sperm and egg fuse and the nucleus and mitochondria of the sperm enter the egg cytoplasm (see Chapter 10). Only one sperm nucleus joins with one egg nucleus at fertilization. Polyspermy is prevented by electrical and/or non-electrical changes in the plasma membrane of the egg (details in Gould & Stephano 2003). Meiosis is completed in the egg once fertilization has taken place. One challenging line of research is why egg sizes in broadcast spawners tend to vary among and within species (see Levitan 2006 for review,). Luttikhuizen *et al.* (2011) tested the hypothesis that egg size may be locally tuned to expected ambient sperm concentrations during fertilization. They found that in the clam *M. balthica* adult density, a proxy for sperm concentration, was negatively correlated with egg size in the field, suggesting that sperm limitation may play a role in fertilization success (larger eggs are larger targets for sperm) and in shaping egg size variation, and that locations with high population densities may contribute disproportionately to the next generation.

In oysters, for example *O. edulis*, eggs and sperm are released through the genital ducts close to the exhalant opening in the mantle. When the oyster is functioning as a male the sperm are carried out into the sea, but in the female the eggs come to lie on the surface of the gills in the inhalant region of the mantle. The eggs are fertilised by sperm brought in with the feeding current, and the fertilised eggs develop into shelled larvae before being discharged into the sea (shell length 170–190 μm) for further development (Walne 1974). In the Pacific oyster *C. gigas* the eggs and sperm are released straight into the sea, where fertilization takes place. Brooding is a common and successful strategy for many bivalves, particularly in cold water and food-limited environments such as the deep sea and polar oceans.

Although many scallop species are hermaphrodites, and frequently release both male and female gametes simultaneously, there is no evidence that self-fertilization occurs although where the time interval is short between the release of sperm and eggs, for example 5–10 min in *A. purpuratus*, the self-fertilization rate can be as high 10% (Winkler & Estévez 2003). In *P. maximus* larvae from self-fertilised eggs had significantly reduced growth rates, but not increased mortality, compared to larvae produced through cross-fertilization (Beaumont & Budd 1983). In another species, *Argopecten circularis*, larvae produced through self-fertilization had lower growth and survival rates than those produced from pair mating or mass spawning (Ibarra *et al.* 1995). But in *A. purpuratus* there was no evidence of inbreeding depression in larvae or juveniles of families produced by self-fertilization and pair mating (Winkler & Estévez 2003; but see Martinez *et al.* 2007).

Recently, research has focused on understanding the mechanisms underlying sperm–egg interaction during fertilization. Recognition and adhesion between sperm and egg are crucial for fertilization, and gamete recognition proteins (GRPs) play a central role in this process. The best studied GRPs are bindin in *Crassostrea* and lysin in *Mytilus*, both of which are found in the sperm acrosome, and bind to glycoprotein receptors on the egg cell surface, ultimately bonding with the egg vitelline envelope (Moy *et al.* 2008; McAnlis *et al.* 2010; Wu *et al.* 2011). GRPs evolve rapidly and are species-specific (Riginos *et al.* 2006; Wu *et al.* 2011). Gamete incompatibility can result in a mismatch between egg and sperm recognition proteins and these fertilization failures are sufficient to reproductively isolate even closely related taxa. In addition to maintaining existing species boundaries gamete recognition divergence could also contribute to the origin of new species (Springer & Crespi 2007 and references therein). One way of testing gamete compatibility between species is to compare conspecific and heterospecific fertilization success by pair-mating with no gamete

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competition, and pooling sperm from both species to allow gamete competition. Using two co-occurring closely related taxa, *M. edulis* and *M. galloprovincialis*, Bierne *et al.* (2002) found that interspecific fertilization occurred when there was no gamete choice, but that intraspecific fertilization occurred when gamete choice was available, a situation most likely to occur in the wild. Similar results have been reported for other closely related *Mytilus* (Miranda *et al.* 2010) and *Crassostrea* (Bushek *et al.* 2008) taxa.

Larval development

The fertilised egg rapidly divides to become a ball of cells that begins to swim once cilia appear some 4–5 hours after fertilization. A ciliated trochophore stage (60–80 µm) is reached about 24 h after fertilization (Figure 5.9a). A shell gland begins to secrete the first larval shell, the prodissoconch I. The shell is D-shaped in outline and the larva, now a veliger (100–120 µm shell length; Figure 9.5, Chapter 9), immediately starts to secrete the second larval shell, or prodissoconch II. This shell is secreted by the mantle and exhibits growth lines. Veliger larvae possess a velum, a circular lobe of tissue bearing a ring of cilia, which serves as a swimming and feeding organ (Figure 5.9b). Small particles (1–2 µm diameter) caught by the cilia are swept towards the mouth and onwards into a simple gut. In *M. edulis* this stage lasts several weeks and is characterized by rapid growth from 120 to 250 µm shell length (Bayne 1976). As metamorphosis approaches pigmented eyespots and an extensible ciliated foot appear. The larva, now known as a pediveliger, is between 210 and 300 µm in length and is characterized by the features outlined in Table 5.3. Larviparous flat oysters such as the Chilean *Tiostrea chilensis* and the New Zealand *Tiostrea lutaria* release their larvae when they are almost ready to set (450–490 µm shell length), while the European oyster, *O. edulis* releases its larvae at the much smaller size of 170–190 µm (Helm *et al.* 2004).

Larvae vary in their response to light, gravity and pressure. During the veliger stage larvae are positively phototrophic and are sensitive to pressure. These responses tend to keep the shelled larvae in the surface waters. On the other hand, pediveligers are positively

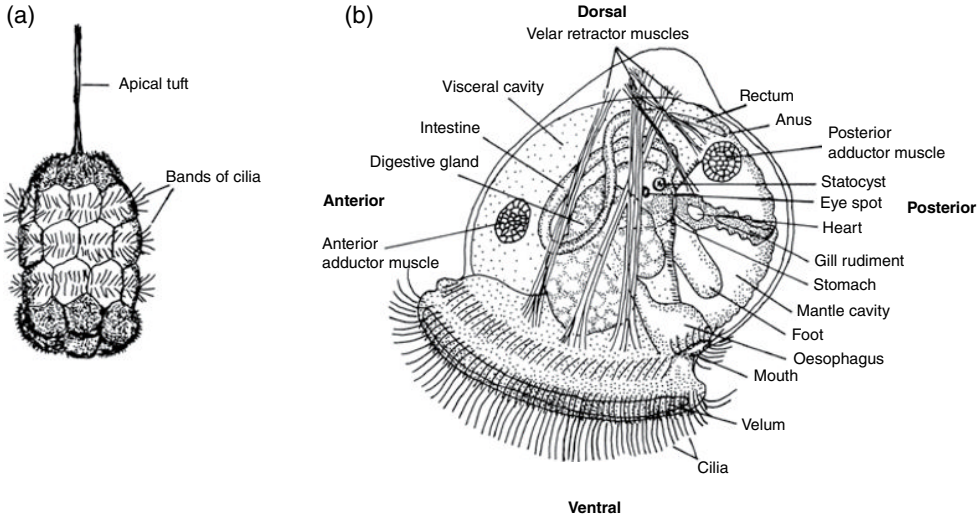


Figure 5.9 (a) Trochophore larva ×400. (b) Veliger (prodissoconch II) larva of the oyster *Crassostrea virginica*, viewed from the left side ×380. Redrawn from Eble and Scro (1996) after Galstoff (1964) and Elston (1980).
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Table 5.3 Morphological features of *Mytilus edulis* pediveliger larvae.

Large velum for swimming and feeding
Ciliated palp that sorts food particles
A few pairs of gill filaments
Mouth, oesophagus, stomach with style sac and large digestive gland, simple intestine
Thin mantle that secretes shell
Foot used in crawling and byssus secretion
Cerebral, pedal and visceral ganglia, sensory pigment spots, pedal statocysts

Adapted from Bayne (1971) and Lutz and Kennish (1992).

geotrophic and insensitive to pressure which encourages them to descend to the bottom in preparation for settlement. In addition, short-term fluctuations in larval abundance are influenced by factors such as temperature, salinity, wind velocity, chlorophyll *a* level and predation, while seasonal fluctuations in abundance are controlled by temperature, the major factor in the timing of bivalve reproduction (Chícharo & Chícharo 2000). Another factor to be considered is predation by adult bivalves on bivalve larvae, a widespread phenomenon called larviphagy, which is believed to reduce numbers of larvae in waters with a high adult bivalve filter feeder biomass (Lehane & Davenport 2004; Troost *et al.* 2009; Chapter 4).

Factors affecting larval growth

The factors important in larval development are, not surprisingly, the same factors influencing bivalve growth in general, namely temperature, salinity and food ration (see Chapter 6 for details). Almost all information on the effects of these factors on growth comes from larval cultures produced in the laboratory.

Temperature and salinity

Temperature is probably the most important factor influencing larval growth as metabolic rate is determined by the water temperature in which they swim (Helm *et al.* 2004). Often, larvae grow much better at temperatures higher than those experienced in the wild. For example, exposure of mussel (*M. galloprovincialis*) larvae to 17°C resulted in poor growth compared to rearing larvae at 20°C or 24°C (Sánchez-Lazo & Martínez-Pita 2012). Growth rate ($\mu m\,day^{-1} \pm SD$) was 6.65 ± 0.02, 9.06 ± 1.2 and 8.71 ± 0.4 at 17, 20 and 24 °C, respectively, with growth rate significantly lower at 17°C than at either of the other two temperatures (Figure 5.10). This strong positive correlation between temperature and larval growth has also been reported for other species (Nair & Appukuttan 2003; Helm *et al.* 2004; Galley *et al.* 2010; Kheder *et al.* 2010). In a recent study Talmage and Gobler (2011) examined the combined effects of temperature and CO_2 on growth and survival of larvae and juveniles of three bivalve species, the rationale being that atmospheric CO_2 concentrations and global temperatures are rising and are expected to continue to rise in the coming decades. They found that increases in temperature and CO_2 each significantly depressed larval development and growth, as well as survival and lipid synthesis, in *M. mercenaria* and *A. irradians*, but not in *C. virginica* and that the effects were additive. Generally, larvae were substantially more vulnerable than juveniles. See Chapter 3 for more details on potential negative consequences of climate change for bivalve populations.

Bayne (1965) measured the effect of salinity on larval growth in *M. edulis* larvae from two populations where the parents experienced different salinity regimes. Larvae from

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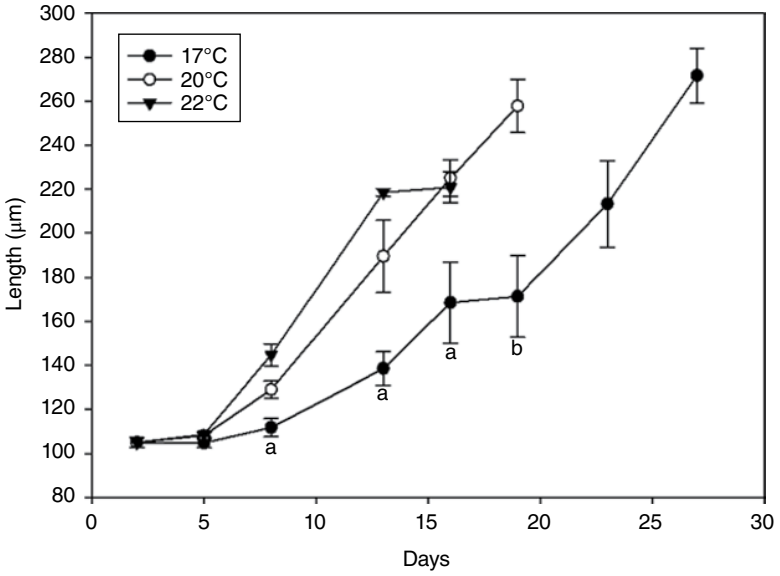


Figure 5.10 Larval growth (mean ± standard error) of *Mytilus galloprovincialis*, reared at three temperatures (17, 20 and 24°C). Letters indicate a significant difference: 'a' indicates that 17°C is different from 20 and 24°C, and 'b' indicates that 17°C is different from 20°C.
From Sánchez-Lazo and Martínez-Pita (2012). Reproduced with permission of John Wiley & Sons.

North Wales, United Kingdom, did not grow at 19 psu and showed retarded growth at 24 psu, but at 30–32 psu growth was normal. In larvae from the Øresund, Denmark, close to the Baltic, growth occurred even at 14 psu, indicating that there is a genetic component to salinity (and probably temperature) tolerance.

It is now more usual to look at the combined effects of temperature and salinity on larval growth (Tettlebach & Rhodes 1981; Robert *et al.* 1988; Dudas & Dower 2006). Generally speaking, as the limits of salinity tolerance are approached for the larvae of a particular species the range of temperature tolerance is markedly narrowed. Shelled larvae also show a greater tolerance to salinity and temperature change than do the embryonic larval stages.

Food ration

About 70% of marine invertebrate species have planktotrophic larvae (originate from small eggs with little yolk). These are produced in huge quantities, and once their feeding apparatus is functional they are totally dependent on the plankton for food. Lecithotrophic larvae develop from large yolky eggs, feed exclusively on the energy reserves within the egg cell and generally have a short larval life. The planktotrophic eggs of bivalves have reserves of lipid, protein and glycogen that fuel the early developmental stages. Feeding commences shortly after the development of the shell and velum. Although the diet of larvae in the wild is not known for certain, they probably depend on a ration of phytoplankton cells, heterotrophic flagellates, amino acids, dissolved organic material (DOM), detritus and bacteria for successful growth and development (Bos *et al.* 2006 and references therein). A novel DNA-based approach has recently been developed to explore qualitative similarities in diets using wild larvae of *O. edulis* and *Mysella* spp. (Maloy *et al.* 2014). Of the sequenced clones, 75% originated from diatoms (Bacillariophyta), a further 16%

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originated from fungi representing the phyla Ascomycota and Basidiomycota, while the remaining sequences belonged to flowering plants (Magnoliophyta), single-celled green algae (Prasinophyceae), potential parasites (Ichthyosporea), dinoflagellates (Dinophyceae) and brown algae (Phaeophyceae). No qualitative difference in diet was observed between the species (see also Maloy *et al.* 2009).

There is an impressive literature on the best hatchery foods for larval growth. Mixed algal diets are beneficial, with a combination of two or three high nutritional value species, including a suitably sized diatom and a flagellate, thereby providing improved rates of larval growth and development compared to single-species diets (see Chapter 9; Helm *et al.* 2004; Galley *et al.* 2010). However, in the wild phytoplankton concentrations are much lower than the concentrations recommended for optimal growth in the laboratory. But it is likely that larvae in the wild may be feeding on a different, or wider, range of phytoplankton species, supplemented by detritus, bacteria and DOM (Chapter 4). Egg quality, in particular the total lipid content of newly spawned eggs or of newly liberated larvae in the case of larviparous species, also has a bearing on subsequent larval performance. For example, growth increment of larvae in the larviparous oyster *O. edulis* in the 4-day period following liberation from adults was significantly correlated with lipid content at the time of release, emphasising the importance of maternally donated reserves during early larval development (Helm *et al.* 2004). Various energetic models that simulate growth in larval to adult stages under various environmental conditions are described in Chapter 6.

Other determinants of larval growth are seawater quality, larval density, silt concentration, pollutants and genetic factors. In northern temperate latitudes seawater quality in the spring is relatively stable due to cool temperatures and short day length. However, as spring progresses into early summer seawater quality can often deteriorate due to intense phytoplankton blooming. Bacteria associated with the blooms or the metabolites, including toxins they produce, probably cause the deterioration in water quality (Helm *et al.* 2004). The problem can be rectified in the hatchery by the use of artificial seawater or chemical treatments. For hatchery production greater larval density may increase spat yields but the increased food demand and metabolic waste production may impact negatively on water quality, and ultimately result in reduced larval growth (Doroudi & Southgare 2000). Turbidity, for example due to silt, can also be a factor in larval growth retardation. However, tolerance to silt concentration appears to be species-specific. For example in *C. virginica* larvae growth was retarded in water carrying $0.75\,g\,l^{-1}$ of silt in suspension, but larvae of *M. mercenaria* grew normally in a concentration of $1.0\,g\,l^{-1}$ (Loosanoff 1962 cited in Bayne (1976)). The embryos and larvae of marine organisms are more sensitive to toxic substances than adults, and so early life stages of bivalves are routinely used in bioassays to assess seawater quality and sediment elutriates. Larvae are incubated in the test water for a fixed period and at constant temperature, and parameters such as EC_{50} (the concentration of contaminant that results in 50% abnormal development or retarded growth) and LC_{50} (median lethal concentration) are calculated (His *et al.* 1999). In larval bioassays growth is significantly retarded by heavy metals, such as mercury, lead, copper, cadmium, nickel and zinc (Beiras & His 1994; Geffard *et al.* 2002; Wang *et al.* 2009; Fathallah *et al.* 2011). An adaptation of the larval toxicity test has recently been made for high-throughput screening of emerging contaminants such as endocrine-disrupting chemicals (EDCs), brominated and perfluorinated compounds (BFRs and PFCs) and pharmaceuticals (Fabbri *et al.* 2014). Finally, Meyer and Manahan (2010) have identified more than 180 candidate genes for differential larval growth in the oyster *C. gigas*. Half of the candidate genes are genes involved in protein metabolism, energy metabolism and regulation of feeding.

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Larval dispersal and population connectivity

The mobile larval dispersal phase in bivalves is the primary mechanism for connecting and replenishing sedentary adult populations. Variation in larval supply into local populations is believed to be an important determinant in the structure and dynamics of adult populations (Shima *et al.* 2010 and references therein). In planktotrophic (feeding) marine bivalves the length of larval life (time to reach ~300 µm shell length) varies between 3 and 5 weeks, depending on environmental factors such as temperature, salinity and food ration (see earlier). The dispersal phase is considerably shorter (hours to days) in lecithotrophic larvae and brooded embryos. During the early embryonic phase of development dispersal is passive, but once feeding starts vertical dispersal is by active swimming, while lateral dispersal is by means of water currents. The main factors affecting vertical distribution of larvae are light, gravity and water flow, and the effects of these vary during the larval lifespan. Larvae are initially photopositive and concentrate in the upper surface waters, but as they approach settlement they become increasingly photonegative and descend into deeper waters where they ultimately settle (Dobretsov & Miron 2001). However, factors that change over various spatio-temporal scales, for example temperature, salinity, tidal phase, season and food availability, are believed to also influence vertical migration patterns (Dekshenieks *et al.* 1996; Knights *et al.* 2006 and references therein). Knights *et al.* (2006) investigated vertical distribution of veliger larvae (*Mytilus*) at a range of depths at different tidal states at two sites on two dates. Larvae were homogeneously distributed throughout the water column during flood tide, whereas they were more densely aggregated in middle and bottom waters during ebb tide. Highest densities were greatest near the bottom during low flow conditions. Interestingly, larval size had no effect on the patterns of vertical distribution of larvae in the water column. There may also be a genetic component to vertical migration, as suggested by Manuel *et al.* (1996), who found significantly different vertical migration patterns and depth distributions in scallop veligers reared under similar conditions but originating from three different populations in North America. There is little evidence that vertical migration may be a behavioural strategy to avoid predation (see Manuel *et al.* 1996).

Several studies indicate that length of larval life – often referred to as propagule duration (PD) – and dispersal distance (DD) are positively correlated in marine invertebrates. Many models have estimated DD using PD coupled with oceanographic models and generally assume that larvae are passively dispersed. To test whether dispersal is passive Shanks (2009) used DD and PD from 67 marine species including bivalves. In their data PD varied from a couple of minutes to several months, and DD varied from less than 1 m to hundreds of kilometres. They plotted PD against DD and unsurprisingly found a positive correlation between PD and DD (Figure 5.11). On the same graph they plotted the distance larvae would be dispersed if they behaved as passive particles in a steady flow of 10–30 cm s^{-1}, and in a more realistic flow field using a Lagrangian particle-tracking model. Overall, they found that all larvae, irrespective of PD, had DDs many times shorter than predicted for passive particles (Figure 5.11). However, the distribution was bimodal: a reasonably tight group represented by species with long PDs and DDs, and a more diffuse and larger group characterized by short DDs and a range of PDs. Thus, there were many species (>60%) with very much shorter DDs (orders of magnitude shorter) than one would predict from their DDs. They achieve this by remaining close to the bottom where currents are much reduced. Larvae that have long PDs (>1 week) and long DDs (>20 km), as found in bivalves, are dispersed into coastal waters, where they remain, close to shore in the coastal boundary layer. Here currents are typically slower and can alternate back and forth due to changes in wind direction (Largier 2003), all of which limit net alongshore transport of larvae, thereby

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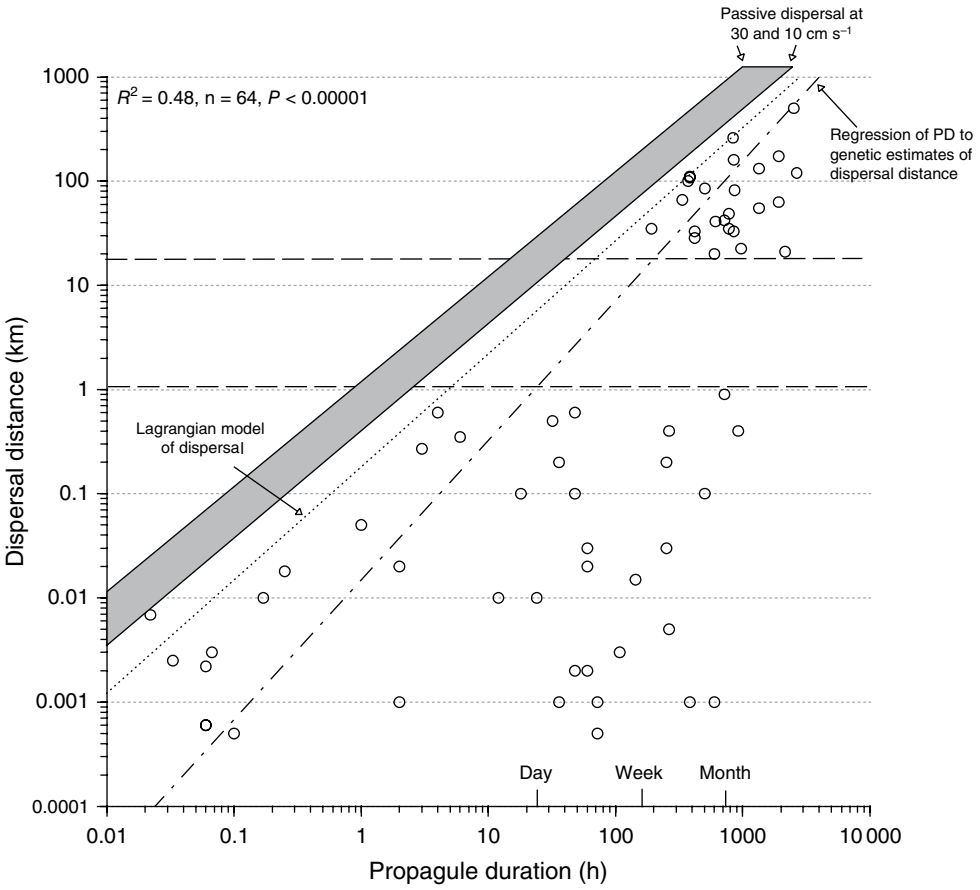


Figure 5.11 Log/log plot of propagule duration (PD) in hours and dispersal distance (DD) in kilometres. Data are from Shanks *et al.* (2003) and table 1 in Shanks (2009). Plotted with the data points are (1) the distance propagules would be dispersed if they behaved as passive particles in a steady flow of 10 and 30 cm s^{-1} (gray shaded zone), (2) the distance passively drifting larvae would disperse as calculated using a Lagrangian model of dispersal (dotted line) (Siegel *et al.* 2003), and (3) dispersal distance estimated from a regression relating PD to DD calculated from genetic data (dot and dash line) (Siegel *et al.* 2003). Statistical results are from a correlation of the log/log data. From Shanks (2009), figure 1. Reproduced with permission of the Marine Biological Laboratory, Woods Hole, MA.

leading to lower than predicted dispersal. Shanks and Brink (2005) consider it makes sense, from an evolutionary perspective, that the larvae of coastal species remain near the coast and their potential settlement sites, rather than allowing themselves to be swept offshore. In summary, larval behaviour plays a critical role in determining DD (Shanks 2009).

Connectivity, the exchange of individuals among marine populations at the pelagic larval stage, is a hot topic in marine ecology, but before patterns of population connectivity can be fully described we need to understand the processes that drive dispersal patterns (Cowen & Sponaugle 2009). Methodological approaches to assess connectivity include applications of physical larval dispersal models, and natural and artificial tags. Lagrangian particle-tracking models and diffusion models of tracer dispersion are just two types of models that are extensively used (Levin 2006). Specific biological traits such as swimming behaviour, mortality

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and growth are usually incorporated into the models, coupling both physics and biology. See Werner *et al.* (2007) for a comprehensive review of coupled biological–physical models. A number of key findings have emerged from modelling larval dispersal. For example, the interaction of various biophysical mechanisms may counter dispersion to varying extents and enable retention of larvae in their natal habitat; populations may therefore be less open than originally believed (Sponaugle *et al.* 2002; Levin 2006; Cowen & Sponaugle 2009). Other key insights are the potential importance of biological, for example larval behaviour (see earlier) and physical factors, for example tidal forces, wind, surface waves, water stratification, turbulence, ocean eddies and coastal topography, in influencing larval dispersal (reviewed in Sponaugle *et al.* 2002; Thomas *et al.* 2012). Physical models in combination with other techniques have also been used to determine how far larvae travel. Genetic data combined with results from a 2D surface circulation model, were used to test the hypothesis that larval dispersal in the *Mytilus* hybrid zone in south-west England is regulated by physical circulation. Larvae dispersed over distances of 30–60 km and the model accurately predicted patterns of dispersal between genetic regions. Other methods for tracking larvae include studying the spread of newly invasive species, using genetic marker data, and using artificial or natural tags. Since 1988, when the invasive mussel *M. galloprovincialis* was introduced into South Africa, DDs over a 4-year period were 12–97 km per year depending on wind direction, but 90% of recruits settled within 5 km of the original site of introduction (McQuaid & Phillips 2000). Genetic isolation-by-distance (IBD) models (Palumbi 2003; Pinsky *et al.* 2010; White *et al.* 2010) and assignment tests (Hogan *et al.* 2012) using genetic marker data (Chapter 10) are also useful methods for estimating larval DDs (but see Weersing & Toonen 2009). IBD is most obvious when comparing populations separated by 2–5 times the mean larval DD. Estimates of this mean range from 100 to 500 km for a selection of bivalves (Kinlan & Gaines 2003). Larval carbonate structures such as shells or otoliths can be marked artificially using: fluorescent dyes such as tetracycline and calcein, elemental tags such as strontium chloride, radioactive isotopes and applied thermal stress marks (reviewed in Thorrold *et al.* 2002; Moran & Marko 2005; Strasser *et al.* 2007; Zacherl *et al.* 2009). Because dilution rates in the sea are too great to yield significant numbers of artificially marked larvae, naturally occurring markers, which mark all larvae exposed to a particular environment, are increasingly being used (Levin 2006). One such method is trace elemental fingerprinting, which assesses larval origins or trajectories, based on the elemental composition of larval tissues or hard parts, which in turn reflects the chemistry of the water in which they were formed (reviewed in Thorrold *et al.* 2007). To illustrate, Becker *et al.* (2007) reared larvae of two mussel (*Mytilus*) species *in situ* at 13 locations spanning 75 km of the Californian coast. Differences in the shell elemental chemistry of the outplanted larvae were sufficient to discriminate among the study sites. Using this regional reference map, they compared the expected larval chemistry for each region to the prodisscoconch shell chemistry of newly settled recruits, assuming that they had developed in the water column during the period of *in situ* larval culturing. They then predicted the natal origin of wild-caught juvenile mussels by using this chemical reference map. The two species exhibit substantially different connectivity patterns. For *M. californianus* most (88%) individuals settling at the 13 sites originated in the northern region with high (87%) self-recruitment, and high (91%) larval importation in the south, clear evidence of asymmetric mixing. *M. galloprovincialis* recruits had more diverse origins from northern, southern and bay areas but substantial (40%) self-recruitment. These differences are probably a reflection of their different susceptibilities to the prevailing southward surface current just offshore of the region; *M. californianus* larvae are released along the open coast, while *M. galloprovincialis* larvae are mainly released into bays. Overall, the results showed that mussel larvae,

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previously thought, from genetic analysis, to be highly dispersed, could be retained within 20–30 km of their natal origin (Becker *et al.* 2007).

Larval identification and abundance estimation

Planktonic larval dispersal and recruitment success largely determine distribution patterns of adult bivalves, and in order to distinguish and study these processes in the field we need tools to identify the larval stages (Hendriks *et al.* 2005 and references therein). This is particularly important for predicting time and location of peak spatfall in culture operations based on natural spat collection (see Chapter 9).

Identification is a difficult task and requires considerable taxonomic experience because of the similar morphology displayed by many species, particularly during the early larval stages. The technique most commonly used is light microscopy, which examines features of the larval shell, for example shape, dimensions and colour patterns, all of which are phenotypically plastic, and thus generally inadequate for definitive species identification (Garland & Zimmer 2002). Scanning electron microscopy of the larval hinge structure is also used but the method is costly, time-consuming and impractical in the field. Recent improvements in image analysis technology have led to the automation of larval shape analysis by computer, thus reducing subjectivity of the method (Tiwari & Gallagher 2003; Hendriks *et al.* 2005; Flyachinskaya & Lesin 2006). However, molecular methods using antibody and oligonucleotide markers are increasingly being used to identify larvae to species, regardless of larval development stage (Garland & Zimmer 2002). Monoclonal antibodies that stain all larval stages and permit the identification of mussel larvae by immunofluorescence assays are now being used to monitor larval abundance in the Galician rías, north-west Spain, the biggest mussel-producing region in Europe (Pérez *et al.* 2009). The development of oligonucleotide probes involves the identification of a nucleotide sequence that is conserved within a species and does not occur in closely related species (see Goffredi *et al.* 2006 for details). Probes specific for the 16S and 18S ribosomal RNA genes have proved useful in identifying larvae in a wide range of marine bivalves (Bell & Grassle 1998; Frischer *et al.* 2000; Larsen *et al.* 2005, 2007; Livi *et al.* 2006; Pradillon *et al.* 2007; Thomas *et al.* 2011). In addition, PCR-RAPD markers (André *et al.* 1999), microsatellites (Morgan & Rogers 2001) and a multiplex polymerase chain reaction (PCR) identification assay based on cytochrome oxidase I (COI) product size (Hare *et al.* 2000) have been used in larval identification.

Many probes have been developed to provide a rapid, reliable and high-throughput method of monitoring temporal and spatial abundance of specific bivalve species in the plankton (Jones *et al.* 2008; Pérez *et al.* 2009; Henzler *et al.* 2010; Quinteiro *et al.* 2011; Thomas *et al.* 2011). Henzler *et al.* (2010), for example, have used a fluorescence *in situ* hybridization (FISH) method, which involves assaying with a fluorescein-labelled species-specific oligonucleotide probe targeted against the 18S rRNA gene in the intact target species; if the target species is present and hybridization is successful, it will fluoresce green under epifluorescent microscopy. They combined the FISH technique, originally developed for quantifying starfish larvae (Mountfort *et al.* 2007) with cell sorting (CS) to identify and rapidly count and sort larvae of several bivalve species in diverse plankton samples. Jones *et al.* (2008) have also developed oligonucleotide probes for mussel, barnacle, polychaete and crab larvae that occur in Monterey Bay, California. The probes were then printed in arrays on nitrocellulose membranes and deployed in a robotic Environmental Sample Processor (ESP) for near real-time *in situ* detection and quantification of specific larvae.

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Settlement, metamorphosis and post-larval dispersal

Settlement

The act of settlement involves descent from the plankton to the sea bottom, followed by a sequence of swimming and crawling behaviour that culminates in attachment once a suitable substrate is chosen. Morphological change (metamorphosis) then follows and this heralds the end of the pelagic larval phase and the start of benthic life. Almost all bivalve larvae become competent to settle in the size range 250–300 μm shell length, although many are able to delay the process until they are considerably larger (see later).

Bivalve larvae are capable of discriminating between different substrates as evidenced by their behaviour prior to attachment. Pascual and Zampatti (1995) have described this behaviour in the oyster *Ostrea puelchana*: 'The pediveliger larva actively explores the substratum, crawling back and forth on its foot over each particle or surface, stopping on certain substrata for variable periods of time, occasionally resuming swimming, and crawling further again until final cementing.' Once cemented, the oyster has no chance of relocation. It is therefore not unexpected that oyster larvae exhibit clear responses to chemical cues during settlement. The most important of these are chemical cues emitted by adult conspecifics, which can lead to gregarious larval settlement and dense persistent reef communities in *Crassostrea* sp. (Tamburri *et al.* 2008 and references therein). This larval settlement pattern benefits group living but has the potential cost of cannibalism by the filter-feeding adults. However, in laboratory flume experiments only a small percentage of settlers (<5%) were cannibalized even when they passed within 1 mm of the inhalant opening or 'gape' (Tamburri *et al.* 2007). The gape, however, was only 5% of the total plane surface area of a typical reef, so a larva settling onto a reef is unlikely to be cannibalized. Not surprisingly, there is considerable interest in elucidating the chemical nature of attractants. Biofilms, thin coats of microorganisms on solid surfaces in the sea (reviewed by Hadfield 2011) and chemicals such as neurotransmitters L-3, 4-dihydroxyphenlyalanine (L-DOPA), 5-hydroxytryptamine (serotonin), epinephrine, norepinephrine and γ-aminobutyric acid (GABA), as well as ammonia (NH_3) and KCl, have been shown to be effective inducers of metamorphosis and settlement for oysters (Wassnig & Southgate 2012 and references therein), mussels (Dobretsov & Qian 2003; Bao *et al.* 2007; Alfaro *et al.* 2011), scallops (Leyton & Riquelme 2008; Mesías-Gansbiller *et al.* 2008) and clams (García-Lavandeira *et al.* 2005). Several of these chemicals are being used as artificial cues to induce larval settlement in the pearl culture industry (Doroudi & Southgate 2002; Yu *et al.* 2008; Wassnig & Southgate 2012). There is also evidence that natural cues, such as extracts from macroalgae, may promote larval settlement in several mussel species (Gribben *et al.* 2011 and references therein). In contrast, a wide variety of biogenic compounds from marine algae and some marine organisms act as repellents to settling larvae, and could be useful as environment-friendly antifouling agents (Bhadury & Wright 2004 and references therein). Physical cues may also be important. Baker (1997) has shown from field and laboratory studies that gravity is the primary settlement cue for *Crassostrea* larvae; larvae preferentially settled on the lower surfaces of shells. This strategy is most advantageous for larvae settling in estuaries with a high sediment load, where the lower surfaces of substrates are likely to be sediment-free.

Mussel larvae settle on a wide variety of substrates, for example rocks and ridged, hard surfaces, filamentous macroalgae, mussel beds and fibrous ropes (Bayne 1964; McGrath *et al.* 1988; McGrath & King 1991; Cáceres–Martínez *et al.* 1994; Lasiak & Barnard 1995; Buchanan & Babcock 1997; Yang *et al.* 2007). A wide range of algal species, as well as

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seagrass, hydroids, bryozoans and empty shells, serve as suitable settlement surfaces for scallop larvae (Cragg 2006 and references therein). Minchin (1992) lists some 47 species of algae to which *P. maximus* spat attach. In *P. magellanicus* levels of settlement were greatly enhanced when collectors were coated with a thin film of chitinous material extracted from hydroids and crustaceans (Harvey *et al.* 1997). Most scallop species attach byssally to their substrate and remain attached for variable lengths of time after metamorphosis (see later). Clams settle on a variety of substrates. For example *M. mercenaria* settles on sand, sand-mud, stone, gravel and combinations of these, once the substrate is firm enough to prevent sinking. The small clam attaches to a pebble or a piece of shell by a single byssus thread and does not lose the ability to attach until it is 7–9 mm long (Menzel 1989). Giant clam spat, *Tridacna* spp., form byssal attachments to coral reef. The main function of the byssus is to maintain the clam in an upright position, thus ensuring a favourable orientation to sunlight for the symbiotic algae contained in the mantle edge (Heslinga 1989). The larger species eventually lose the byssus and displacement is prevented by the weight of the shell valves. The smaller clam species remain strongly byssate throughout life and actively burrow into the reef by mechanical and chemical means (Yonge 1980). However, juvenile organisms associated with soft-bottom sediments may not stay in the areas where initial settlement occurs (see later). For this reason, the term 'settlement' does not necessarily apply to a single event in the life of a soft-bottom species, in contrast to its use for most hard-bottom organisms (Huxham & Richards 2003).

Studies in the field and in laboratory flumes as well as the use of mathematical models have shown that factors such as flow direction and velocity, turbulence intensity and boundary shear stress affect settlement in many benthic invertebrate groups, including bivalves (reviewed in Koehl 2007). Most larvae will settle during relatively calm and low-velocity periods when instantaneous shear stresses are smallest and lull periods are longest (see Crimaldi *et al.* 2002). Such lulls give sufficient time for the larvae to respond to settlement cues, attach and resist re-suspension. In laboratory flumes laser-Doppler velocimetry is used to measure the fluctuating water velocities that would be experienced by larvae on spatial scales of hundreds of microns and temporal scales of fractions of a second (Koehl & Reidenbach 2007). Fuchs and DiBacco (2011) have shown that mussel larvae sink in strong turbulence, a behaviour that in energetic coastal waters could improve their chances of being retained near shallow habitats and settling onto adult mussel beds.

When a pediveliger encounters a surface while swimming the velum is retracted and the larva explores the substrate by means of the foot. After a while the larva may swim off again and this cycle of activity may be repeated many times over a period of several days. Once a suitable substrate has been located the larva stops crawling and begins the process of attachment. In mussel, scallop and clam species attachment is by byssus. In oyster species, however, a drop of 'cement' is squeezed from a gland in the foot, and the larva applies its left valve to the cement, which sets rapidly (Walne 1974). The adhesive cement is an organic–inorganic hybrid but differs in composition from oyster shell (Burkett *et al.* 2010). The cross-linked protein matrix is analogous to barnacle and mussel adhesives (see Chapter 2), but the inorganic $CaCO_3$ crystal form is unique to oysters.

Metamorphosis and post-larval dispersal

Metamorphosis is a critical phase in the life history of a bivalve because the ability to move is lost, and there is massive reorganisation of body parts to suit a sedentary existence. In all bivalves rapid morphological changes take place in the attached larva, now termed a plantigrade (Figure 5.12). The extent of morphological reorganisation varies from species to species. In the oyster *O. edulis* Walne (1974) describes the process as follows: within 48 h

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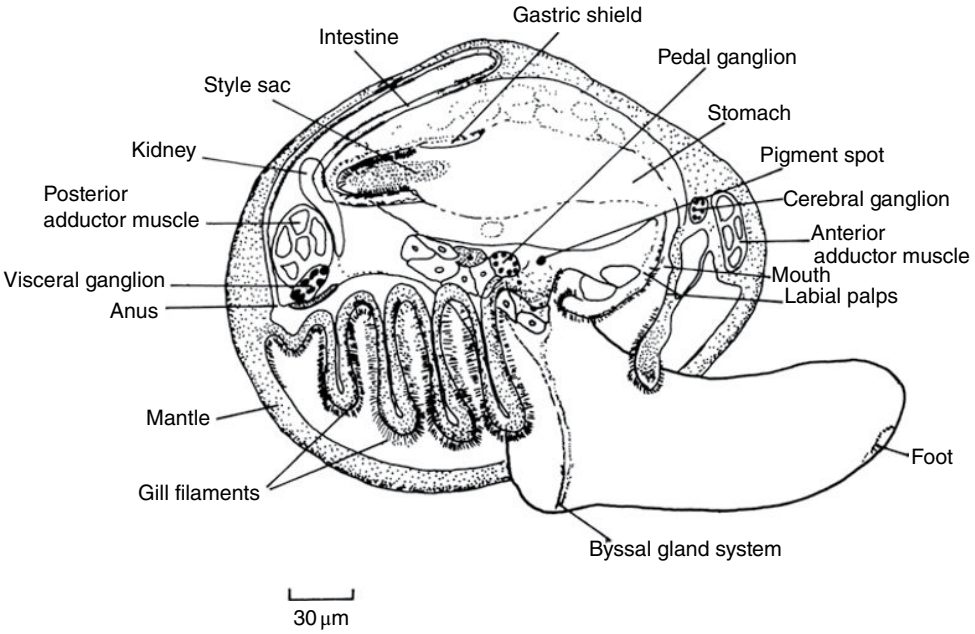


Figure 5.12 Early plantigrade larva of the mussel *Mytilus edulis* immediately after metamorphosis and before secretion of the dissoconch shell has begun. Redrawn from Bayne (1971). Reproduced with permission of Cambridge University Press.

of attachment, velum, foot, eyespot and anterior adductor muscle disappear, the mouth moves through an angle of 90° and the posterior muscle moves centrally. The gills gradually acquire connections between the filaments, assuming the eulamellibranch gill type (Chapter 4). The left attached shell valve starts to form the typical cup shape of the adult shell. The entire process takes about 3 or 4 days, during which time the larva cannot feed, but stored nutrients are used as an energy source. After metamorphosis the adult dissoconch shell is secreted. This shell differs from the larval prodissoconch II shell in pigmentation, ornamentation and mineral content. In addition, a remarkable change in shape takes place so that the dissoconch shell is more similar to the later adult form. The prodissoconch–dissoconch boundary provides a clear morphological trait that is useful in differentiating true juveniles from metamorphosing post-larvae (Lutz & Kennish 1992).

Many bivalves undergo a post-settlement dispersal phase after metamorphosis (Baker & Mann 1997). Behaviours that promote this include active emergence from the sediment to aid re-suspension and water column transport, and release of byssal threads to increase drag and provide lift, thereby enabling post-larvae (<1 mm) and juveniles (1–4 mm) to leave the substrate and float (Lundquist *et al.* 2004 and references therein). Post-metamorphic stages have the ability to drift up to a size of 2.0–2.5 mm (Sigurdsson *et al.* 1976) for distances on the scale of $1–10^4$ m, which probably represents an add-on period of 1–2 months for pelagic dispersal. Baker and Mann (1997) suggest that the role of drifting for post-larvae and juveniles is not so much a dispersal mechanism to colonize new habitats, but instead facilitates an ontogenetic habitat shift. To illustrate, *M. balthica*, the most common and widespread bivalve in the Wadden Sea, undergoes two post-metamorphic migrations through byssus drifting (Hiddink *et al.* 2002). In May the post-larvae (300 μm, 1–3 months old) migrate from the low tidal flats where they settle initially to the nurseries (spring migration) on high, silty tidal flats (Armonies & Hellwig-Armonies 1992). During the following December to

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March period juveniles (3–7 mm length, 8–11 months old) migrate from their nurseries (winter migration) to the low intertidal flats and the subtidal region of the Wadden and North seas (Beukema & de Vlas 1989). Thus, by secondary (and tertiary) dispersal a species may enlarge its distributional range. Laboratory flumes have been used to investigate the effects of hydrodynamics, size and behaviour on dispersal rates of juvenile bivalves (Hunt 2004; Petuha *et al.* 2006; Jennings & Hunt 2009). In the field, dispersal rates and spatial distribution have been assessed using a fluorescent stain for marking juveniles *in vivo*, and a mark-and-recapture methodology (Norrko *et al.* 2001).

Recruitment

Settlement is generally difficult to measure in the field and so it is usually inferred from recruitment data measured some days, or even weeks, after settlement has actually occurred. In most studies of sessile species a positive relationship between the abundances of recruits and settlers has been found (Hunt & Scheibling 1997, review). Some studies have also reported a positive correlation between planktonic larval and recruitment abundances (Chícharo & Chícharo 2001; Dobretsov & Miron 2001). Seed and Suchanek (1992) define recruitment as the process of successful colonisation after some specified period of time defined by the researcher, during which some post-settlement mortality will generally have occurred. Mortality in the days to weeks period after settlement is generally greater than 90% (Gosselin & Qian 1997). The main causal factors are delay of metamorphosis, biological disturbance, physical disturbance and hydrodynamics, physiological stress, competition and predation, the latter being the best-documented cause; other possible causes are developmental abnormalities, insufficient energy reserves, disease and parasitism (Hunt & Scheibling 1997). Johnson and Geller (2006) found that blue mussels (*Mytilus* spp.) settled abundantly where their adults were rare, and suggested physical factors such as removal by waves, and interspecific interactions with the larger *M. californianus*, as causal factors for post-settlement mortality. Not surprisingly, recruitment in many species of marine bivalves has been shown to vary spatially, over scales from metres to tens of kilometres, and from year to year.

Recruitment in the field, for example in clams, can be quantified by taking a delimited grab or fisherman's hand dredge sample from the surface sediment (upper 0.01 m) at a number of sites, and then sieving and counting the number of spat. Using this method Borsa and Millet (1992) reported dramatic spatial and temporal variations in recruitment of clam spat in the lagoon of Thau, south France (Table 5.4). The poor recruitment of some years was attributed to weather conditions that swept larvae into intensive culture zones where the filtering activity of oysters and mussels may have caused massive larval mortality. This has indeed been demonstrated in a field-based study on the cockle *Cerastoderma edule*, where

Table 5.4 Density (individuals m^{-2}) of spat *Ruditapes decussatus* in three locations in the lagoon of Thau, south France from 1986 to 1989.

Year	Location 1	Location 2	Location 3
1986	0	–	–
1987	177	390	107
1988	3	34	3
1989	1	–	–

Adapted from Borsa and Millet (1992). Reproduced with permission of Elsevier.
A zero indicates no recruitment and a dash indicates no data.

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adults reduced the settlement of *C. edule* larvae by 33% in an area immediately surrounding individual adults (André *et al.* 1993). Over dense beds of cockles this figure rose to 75%. Alternatively, core samples (~6 cm diameter) of sediment to a depth of 3 cm along 10-m transect lines may be used (Bowen & Hunt 2009). Using core sampling Honkoop *et al.* (1998) studied recruitment of *M. balthica* from 1973 to 1996 on a 50 km^2 tidal flat area in the Wadden Sea. A substantial part (37%) of the year-to-year variation in recruit densities could be explained by interannual variation in winter temperatures; and the number of adults and the total number of eggs spawned in a certain year were poor predictors of subsequent recruit abundance. See also Philippart *et al.* (2003).

Recruitment can also be quantified by counting numbers of spat on filamentous algae or on artificial substrates. To measure recruitment on filamentous algae subsamples of algae species are examined at regular intervals for spat; the spat are removed, counted and the algal material is dried to constant weight for estimation of settlement density (King *et al.* 1989). Artificial substrata in use include rubberised hair, slate, asbestos, perspex, concrete, mortar, wood, fibreglass, polystyrene, rope and domestic pan scourers (see McGrath *et al.* 1994 for references). Artificial substrates have the advantage over plant material in possessing a relatively constant surface area and textural composition. This makes it easier for recruitment to be quantified over fixed time intervals, unlike natural substrates where the numbers recorded represent cumulative results from recruitment over an unknown period prior to collection (McGrath *et al.* 1994).

Various models have been used to predict or back calculate recruitment. Although planktonic larval abundances are often used to determine recruitment there are several associated methodological difficulties, such as sampling frequency, ability to follow larval, post-larval or juvenile cohorts, and ability to calculate growth and mortality rates (Chícharo & Chícharo 2001). Using an intensive sampling strategy these authors estimated growth and mortality rates in planktonic larval and benthic post-larval/juvenile cohorts of the clam *R. decussatus*. Integrating these data they developed a model that allowed them to estimate future abundances of recruits during the first year of benthic life. Zhang and Hand (2006) used an age-structured projection model based on a large amount of accumulated survey data, age composition data and published mortality data, to back-calculate historic recruitment patterns to enable them to evaluate impacts of alternative harvesting intensities in geoduck clam populations, which support lucrative dive fisheries in Washington State and British Columbia, North America.

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6 Growth

Introduction

Growth in bivalves is usually described in terms of an increase in some dimension of the shell valves. In mussels, for example, length – the maximum distance between the anterior and posterior margins of the shell – is the dimension of choice, whereas in scallops, shell height – the maximum distance between the dorsal (hinge) and ventral shell margins – is used (Figure 2.2). Growth rate can be measured in one of two ways: either the size of the whole organism is related to age (absolute growth), or the rate of growth of one size variable is related to that of another variable (allometric growth).

It should be pointed out at this stage that shell length as an indicator of size is chosen because it is more easily measured than the more appropriate parameter, flesh weight, and also because growth history is recorded in the shell. In most bivalve species shell and flesh weight increase are uncoupled. For example, in the mussel *Mytilus edulis* shell growth is rapid during the spring and summer and slows down over the winter, while flesh weight is subject to seasonal fluctuations associated with the reproductive cycle (Figure 6.1). In experiments designed to uncouple shell and soft tissue growth, Lewis and Cerrato (1997) found that shell growth in the clam, *Mercenaria mercenaria*, was positively correlated with oxygen consumption, but that soft tissue growth was either not correlated or negatively correlated with oxygen consumption and shell growth. These authors suggest that growth line patterns on the shell may be used to reconstruct metabolic rates from field-collected individuals.

Methods of measuring absolute growth

Methods used to measure rates of increase in shell growth include size distribution analysis of single cohorts, analysis of size increment following mark-and-recapture experiments using different labelling techniques, shell growth-ring analysis, internal growth lines analysis

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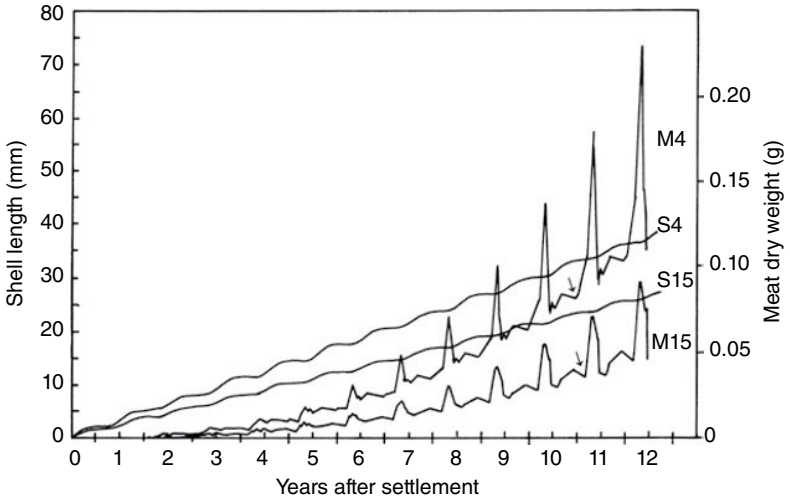


Figure 6.1 Seasonal patterns of shell (S4, S15) and meat (M4, M15) growth over 12 years in the mussel *Mytilus edulis* growing at 4 and 15 m depths in the Baltic Sea. Arrows indicate examples of periods of negative meat growth before gonad build-up in the winter.
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in shell sections, elemental analysis and analysis of stable oxygen isotopes (see Herrmann *et al.* 2009a and references therein).

Length frequency distributions (LFDs)

The biggest advantage of this method is that it measures growth of a population under undisturbed natural conditions. The accuracy of the analysis depends on how well the collected samples reflect the size structure of the actual population. Shell lengths are measured from a random sample of individuals, ideally more than 1500 individuals sampled over a minimum 6-month period (Pauly 1987), and the data are divided into size frequency classes and plotted in the form of a histogram. Size class intervals are normally small, from 1 to 5% of the size of the largest specimen in the population (Cerrato 1980). Where recruitment is seasonal and the life span is short, and there is little variability in individual growth rates, individual year classes can often be identified as distinct modes (Figure 6.2). By following the position of these modes over time the mean growth rate of each year class can be estimated. However, in long-lived species with extended recruitment and variable individual growth rates it is not possible to use size frequency distributions to measure growth rate due to merging of year classes. This is particularly true for older populations where year classes overlap to such an extent that each loses identity because slow growers of one year class overlap with fast growers of the previous year class. Several methods are available that can separate an LFD into groups that are assumed to represent separate cohorts. Early methods were graphical, such as the Bhattacharya plot (Bhattacharya 1967; Pauly & Caddy 1985; Sparre & Venema 1992), which separates an LFD into a series of normal distributions or pseudo-cohorts (Figures 6.3 and 6.4), but these methods have been superseded by programmes such as electronic length frequency analysis (ELEFAN; Pauly 1987), MULTIFAN (Fournier *et al.* 1990), computer-assisted analysis of mixtures (C.A.MAN; Böhning *et al.* 1992) and Shepherd's length composition analysis (SCLA; Shepherd 1987). A number of growth models (see later) have been incorporated into these programmes.

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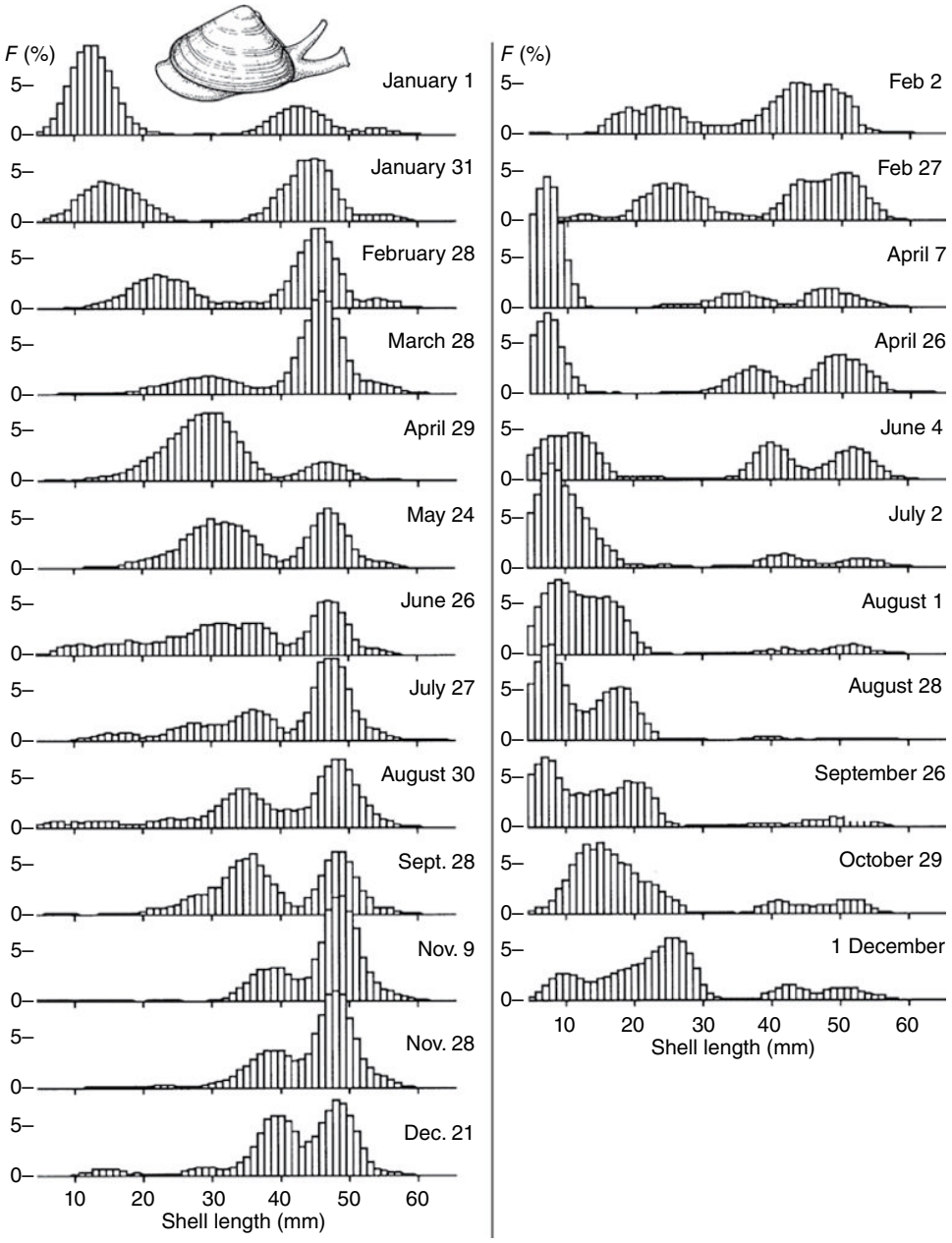


Figure 6.2 Length frequency data for the surf clam *Donax* (*Plebidonax*) *deltoids* from southern Australia. Collection dates are shown on the right of each graph, and sample sizes range from 176 to 712 individuals. The major cohort that appears on January 1 at a modal length of 12.5 mm shell length can be followed over time. For example, on April 29, 4 months later, this cohort has a modal shell length of 30 mm. The numbers in each group are reduced by mortality over time.
From King (2007). Reproduced with permission of John Wiley & Sons.

A growth curve can be estimated from the relative position of the modes of an LFD, the assumption being that the modes represent groups spawned 1 year apart (King 2007). Growth curves may also be constructed from mark-recapture data and analysis of growth checks on the shell (see later). Various equations are used to fit curves to the

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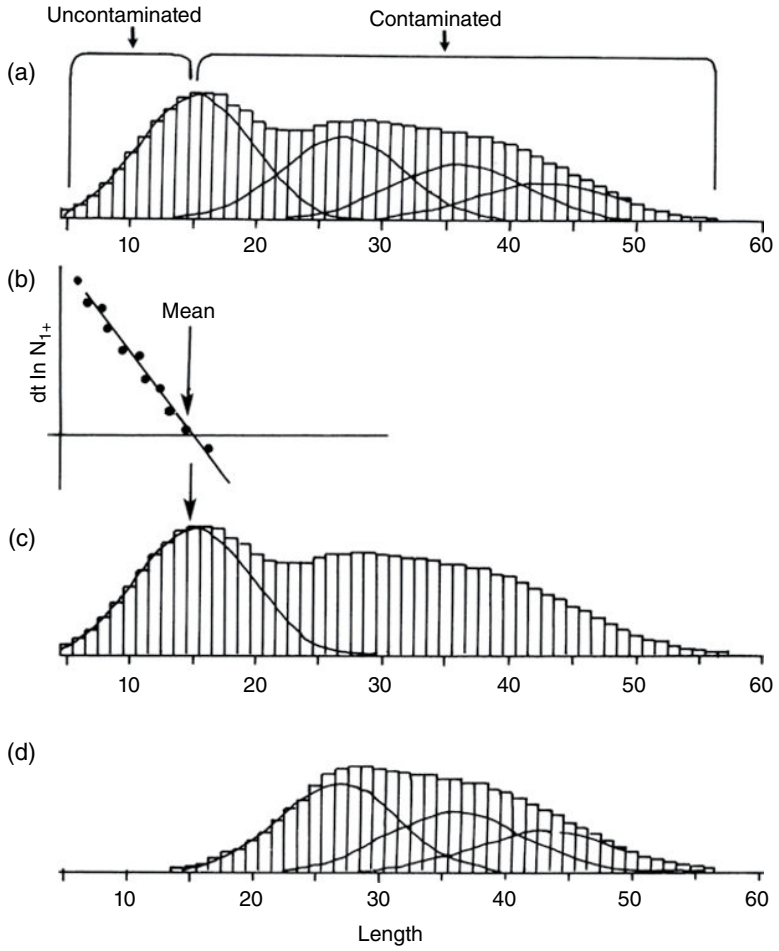


Figure 6.3 The sequences of operation in the use of the Bhattacharya method of separating a length frequency distribution into normal components. The method is based on approximating the assumed normal curve of a length frequency distribution as a parabola, which is then converted to a straight line. (a) The left-hand side of the length frequency distribution is assumed to consist only of individuals in the first pseudo-cohort, and is uncontaminated by individuals in other overlapping pseudo-cohorts to the right. (b) A Bhattacharya plot of $dt(\ln[N])$ against the upper limit of the preceding length class is used to estimate the mean of the first cohort from the point where the straight line crosses the length axis. $dt(\ln[N])$ is the difference between the natural log of the number in one length class and the number in the preceding length class. (c) By working backwards, the linear regression results are used to produce a normal curve that is assumed to contain the total number of individuals in the first pseudo-cohort. (d) The number of individuals in the first cohort is subtracted from the total length frequency distribution. The remaining distribution has a new 'clean' left-hand side, from which a second cohort may be separated. From King (2007). Reproduced with permission of John Wiley & Sons.

growth data but the one that best describes growth in bivalves and in fish is the von Bertalanffy (1938) growth equation:

$$L_t = L_\infty \left(1 - \exp\left[-k(t - t_0)\right]\right)$$

where L_t is the length at age t, L_∞ is the theoretical maximum size attained under specific environmental conditions and k is the growth constant reflecting the rate at which maximum size L_∞ is approached (Figure 6.5). The assumption of equations such as this is that growth is

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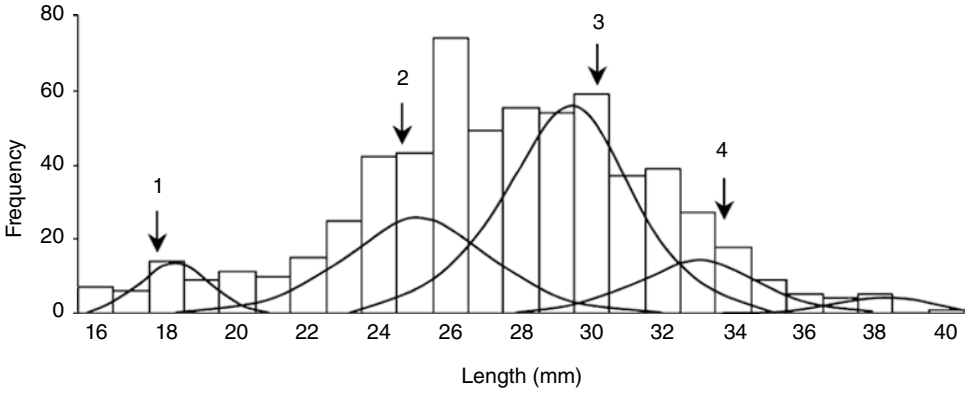


Figure 6.4 Length frequency distribution in a striped venus clam (*Chamelea gallina*) population on the Algarve coast, southern Portugal. Curves represent the components determined by Bhattacharya's method. Arrows indicate the mean length (mm) of each cohort estimated from acetate peels. From Gaspar *et al.* (2004). Reproduced with permission of Oxford University Press.

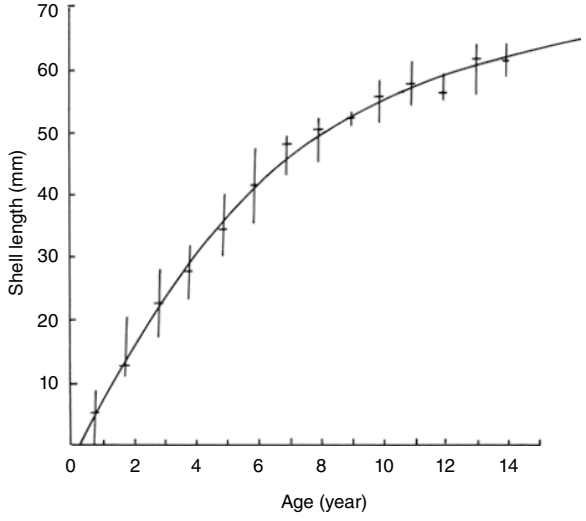


Figure 6.5 A von Bertalanffy growth curve for a sample of mussels *Mytilus edulis* ($N = 417$) from Dungarvan Harbour, south coast of Ireland. The predicted maximum size (L_∞) for this population is 70.74 mm shell length, achieved after considerably more than 14 years of growth; $k = 0.1598$ and $t_0 = 0.423$. The L_∞ value is fairly typical of Irish sheltered shore mussels. Note that, as in most bivalves, growth is rapid during the first years of life and slows down as size increases. From Ottway and Ross (unpublished data).

determinate, that is some maximum attainable size exists for any given population. However, in many bivalves growth may be indeterminate, that is growth continues right through the entire life span (Seed 1980), and because of this some workers use polynomial expressions to describe shell growth in preference to the von Bertalanffy or equivalent growth equations.

A commonly used graphical method of estimating L_∞ and k is a Ford–Walford plot (Walford 1946), where length at age t is plotted against length at $t+1$ years. L_∞ is given where the line of best fit intercepts the 45° angle, or by

$$\frac{y}{1-\text{slope}}$$

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where y is the intercept of the best-fit line on the y-axis. The growth constant is calculated as minus the natural log of the slope (see King 2007 for details).

While the von Bertalanffy equation has been widely used to describe growth in bivalves it does not take into account that many species, for example scallops, exhibit sigmoid (an inflection point on the growth curve) growth at a small size. The Gompertz (1825) equation, which is similar to the von Bertalanffy equation but uses the logarithms of length, has been used to describe growth in many scallop studies (Orensanz *et al.* 1991) and also in slow-growing populations of *Mytilus* (Seed & Suchanek 1992). Neither of these equations takes into account seasonal variations in growth rate. However, the von Bertalanffy model has been modified to produce several growth models that allow for seasonal oscillations in length within each growth year (García-Berthou *et al.* 2012 and references). Somers' (1988) model is highly popular probably because it is available in a large number of software programs including ELEFAN and FAO-ICLARM Stock Assessment Tools (FiSAT; Gayanilo *et al.* 2005).

In addition to information on age-specific growth, size frequency distributions also provide information on recruitment patterns and size-selective mortality caused by predation or intraspecific competition for space (Ma *et al.* 2006; Deval & Göktürk 2008; Herrmann *et al.* 2009b).

Mark-recapture and growth increment analysis (GIA)

Mark-recapture involves marking a number of individuals from a population and returning them to that population. Subsequently, a set number of individuals are captured and the number of marked individuals relative to the number of captured individuals provides an estimate of the population size at the time of marking and release. Marking in bivalves is achieved by filing a notch (1 mm) on the shell, marking with coloured ink, or applying a metal or plastic tag with adhesive. These were common marking techniques in the past but for various reasons, such as tag loss, and growth checks caused by filing, other methods have come to replace them. For example, the fluorescent compounds oxytetracycline (OTC), calcein and alizarin red S (ARS), which are incorporated into the shell, offer a rapid and non-invasive option (Lucas *et al.* 2008; Bownes & McQuaid 2010; van der Geest *et al.* 2011). Of these three compounds OTC was identified as the most useful for industry, producing a bright mark that lasted for at least 10 months (Lucas *et al.* 2008). OTC and calcein have been used in combination to double mark individual shells; specific 'bar codes' could be used to distinguish between different cohorts or spat deployment strategies. The elements strontium and manganese have also been used to mark shells (Fujikura *et al.* 2003; Lartaud *et al.* 2010). In addition to quantification of growth the method provides information on mortality, recruitment, habitat and environmental conditions (Bownes & McQuaid 2010). Van der Geest *et al.* (2011) used calcein to mark the external shell of the intertidal infaunal bivalve, *Loripes lacteus*, and growth rate was expressed as the maximum growth axis between the fluorescent calcein mark at the exterior of the shell and the ventral margin to the nearest 0.01 mm divided by the interval (in days) between calcein administration at t_0 and collection of the shell at t_1 (Figure 6.6).

Comparing the two methods, LFD and GIA from mark-recapture, to measure growth in clam (*Donax*) species from different climate areas, Herrmann *et al.* (2009b) found that by carrying out a relatively short-time mark-recapture experiment they obtained a good growth estimate for temperate bivalves, but they recommended that both methods should be used to determine growth in tropical species. The decided advantage of GIA is accuracy, allowing daily growth rate measurements on a much smaller number of individuals than for LFD analyses. GIA can also be used on scattered populations where it is difficult to sample sufficient individuals for clear cohort detection. On the other hand, the LFD method allows

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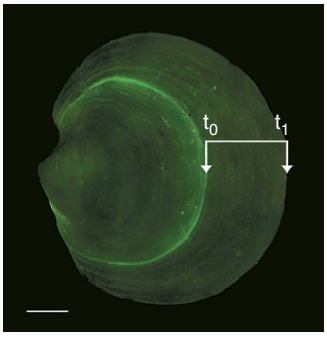


Figure 6.6 Photograph of the right valve of a calcein-marked *Loripes lacteus* shell. The position of the growing edge at the time of calcein administration is given by t_0; the position of the growing edge 3 months later is given by t_1. Thus, t_0 to t_1 represents shell growth (increase in height). Scale bar: 1 mm. From van der Geest *et al.* (2011). Reproduced with permission of Elsevier.

detection of seasonal growth and causes much less disturbance than applying GIA, with its much shorter sampling intervals. Herrmann *et al.* (2009b) found that GIA was more time-saving compared to the LFD method, achieving results after only 6 weeks of sampling time and 3 months of laboratory time, compared to 25 months of sampling time for LFD analysis, with costs twice that of GIA.

Annual growth rings

Bivalve age can be determined from growth checks or rings on the external shell, or from growth lines in shell or ligament cross sections. Such rings or lines are usually produced annually during the winter period of suspended shell growth (Figures 6.7 and 2.4a). Mark-recapture studies are used to confirm that growth lines are formed annually. Confirmation that the rings are annual is essential before they can be used to construct growth curves. There are several difficulties associated with using rings on the shell surface to age bivalves. Firstly, while the method is successful for most scallop and clam species it is of little use in mussels and oysters because the rings are either absent or difficult to discern. Annual marks can often be difficult to distinguish from spawning or disturbance lines on the shell, caused as a result of storms, dredging, handling, transplanting or predation attempts (reviewed in Richardson 2001). In some scallop species winter rings are coincident with the spawning season, but in other species winter and spawning rings are separate, although one type is usually more conspicuous than the other (Orensanz *et al.* 1991). Age determination is often unreliable in species that do not mark a clear ring during the first year of life, and also in older bivalves where growth rings near the border of the shell are so close together that they are difficult or impossible to tell apart.

The use of growth lines in shell sections is an alternative ageing method for species where external growth rings are absent or difficult to see. The shell valves are sectioned whole, or embedded in epoxy resin and then sectioned along the axis of maximum growth (Figure 6.8). Other parts of the shell, for example the umbo, hinge plate, pallial line scar or posterior adductor muscle scar, may also be used. The cut surfaces are ground, polished and etched with a decalcifying agent and then either viewed directly, or more commonly the etched shell surface is flooded with acetone and a thin sheet of acetate (~3 mm thick) is firmly applied to the surface. After about 30 min the acetate peel is removed, mounted on a glass slide and viewed under the microscope to determine the number of growth lines in the middle prismatic and inner nacreous shell layers (Richardson 2001; Masu *et al.* 2008). An alternative is fluorescence spectroscopy, which does not require any chemical treatment

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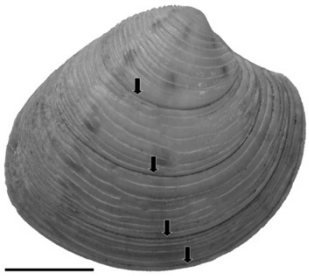


Figure 6.7 External growth rings (arrows) visible in the shell of the striped venus clam *Chamelea gallina*. Scale bar = 1 cm.
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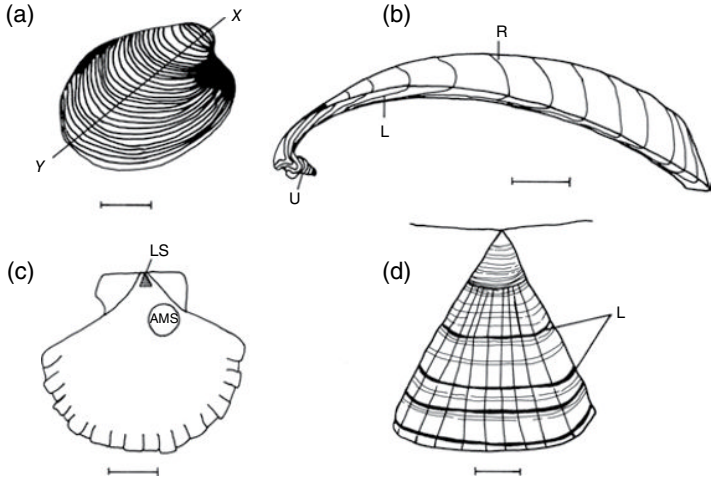


Figure 6.8 Diagrammatic representation of (a) the shell of the clam *Arctica islandica* with prominent external growth rings, X–Y line of cross section. (b) Radial shell section taken along the X–Y axis to reveal the internal growth lines (L) in the outer shell layers and umbone (U); R, surface growth rings. (c) Inner surface of the *Pecten maximus* scallop shell to show the position of the ligament scar (LS) and adductor muscle scar (AMS). (d) Growth lines (L) on the inner surface of the ligament scar. Scale bar = 1 cm.
From Richardson (2001). Reprinted with permission of R.N. Gibson, Series Editor *Oceanography and Marine Biology: An Annual Review*.

of the polished shell section and yields results comparable to the acetate peel method (Wanamaker *et al.* 2009). A less common method is by thin sectioning, and although it is more labour-intensive thin sections tend to produce better results than acetate peels (Cerrato 1980; Fiori & Morsán 2004). In long-lived species such as the ocean quahog, *Arctica islandica* (see later), where migrogrowth bands can number greater than 400 per year, backscattered electron imaging of polished shell sections is used to clearly discriminate between growth increments (Karney *et al.* 2011).

Nacreous lines are formed annually, while fine microgrowth bands within the prismatic layer have a tidal periodicity (Abades *et al.* 2000). The width and definition of these microgrowth bands is influenced by such factors as season, temperature at time of emmersion, tidal amplitude and tidal cycle, and reproductive stage (Richardson 2001; Kanazawa & Sato 2008). Tidal periodicity of microgrowth increments has also been observed in the subtidal species, *Ruditapes philippinarum*, and could be explained by a behavioural adaptation of valve closure

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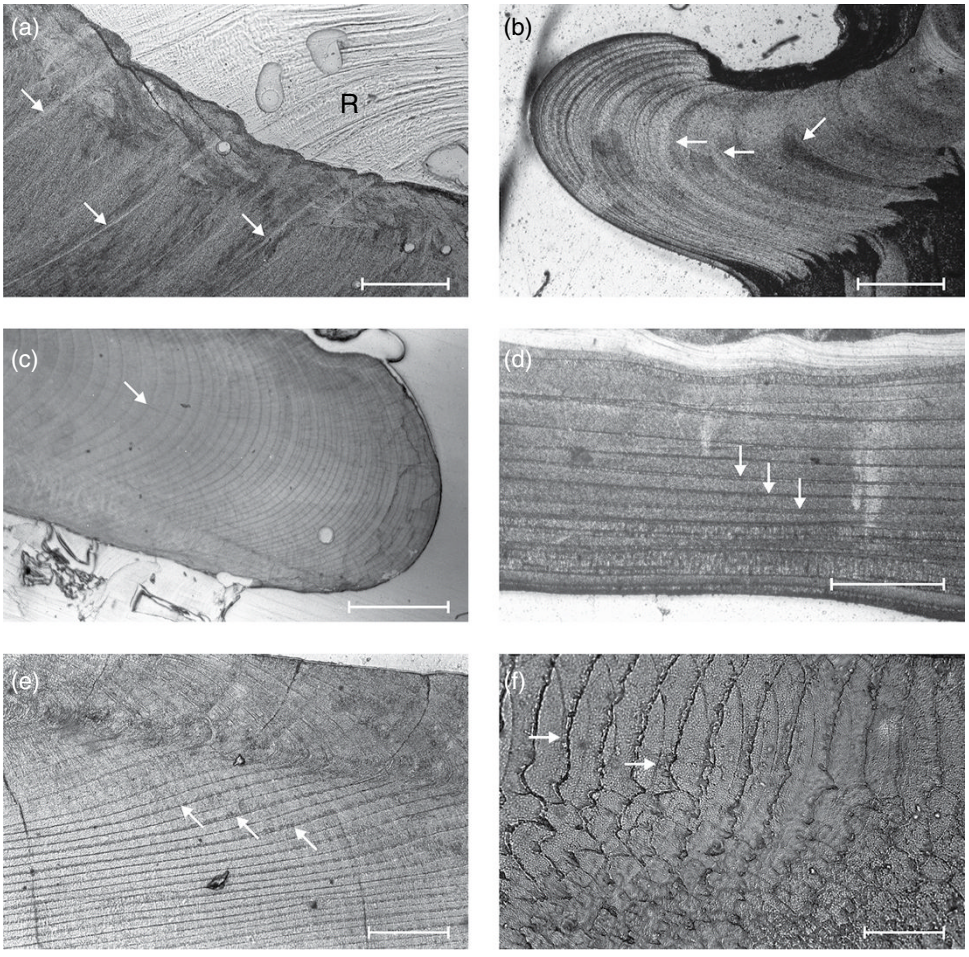


Figure 6.9 Acetate peel replicas of shell sections of (a) the ligament scar in the oyster *Ostrea edulis*. R, resilium (enlargement of the ligament); arrows indicate positions of annual growth lines. (b), (c) Umbones of the mussel *Modiolus modiolus* and the cockle *Glycymeris glycymeris*, respectively, showing annual growth lines (arrows). (d) Inner nacreous layer of *M. modiolus* and annual growth lines (arrows). (e) Strongly defined growth bands (arrows) alternating with weakly defined bands in the crossed lamellar layer of the cockle *Cerastoderma edule*. (f) Weakly defined endogenous bands in the prismatic layer of the clam *Ruditapes philippinarum*. Scale bar = 100 μm, except (d) and (e) where scale bars = 0.5 mm. Photomicrographs courtesy of C. Richardson, Bangor University, Wales, United Kingdom.

at low tide to protect the clam from low salinities and/or to synchronise with food availability (Poulain *et al.* 2011). The annual lines are thin, translucent and darkly pigmented and first appear at the end of the winter. Between each winter line is a wider area that represents the spring–summer growth increment (Figure 6.9). Counts should be made at or near the umbonal region of the shell, as this is the only region of the nacreous layer that contains a relatively complete record of growth (Lutz 1976). Abrasion of the umbo or poor definition of the first winter ring can result in an underestimate of age, such as in large, old oysters (Richardson *et al.* 1993a). However, the method is generally reliable, but requires more time and expertise than the external shell ring method of ageing. The method is particularly useful in habitats such as offshore platforms or water intake pipes where conventional methods involving repeated sampling of the population are difficult or impracticable (Richardson *et al.* 1990).

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Additional methods of measuring absolute growth include optical techniques such as laser diffraction, photographic techniques and the uptake and decay of radionuclides, often referred to as radioisotopes, from seawater into shells. The nuclei of such chemicals are unstable and spontaneously change or decay into a more stable nucleus of another element. Once in the shell and isolated from its seawater source the radionuclide atoms undergo radioactive decay and the number of atoms remaining is a function of the time since the deposition of the shell. In addition, the seasonal variation in the ratio of the oxygen stable isotopes ^{18}O and ^{16}O in shell calcite may be used to validate the annual formation of growth rings (Chute *et al.* 2012 and references). The ratio is determined primarily by thermodynamic equilibrium between the two isotopes in calcite and in seawater at the time of shell deposition. Calcite that has been deposited at equilibrium with seawater has a higher $^{18}O/^{16}O$ ratio (or $\delta^{18}O$ when expressed relative to a standard) than that of seawater. In addition, the difference between $\delta^{18}O_{calcite}$ and $\delta^{18}O_{seawater}$ increases as temperature decreases (see later). Ratios $\delta^{18}O/\delta^{13}C$ and $\delta^{14}C$ in shell carbonate have been used to verify the annual formation of rings (Brey & Mackensen 1997; Lomovasky *et al.* 2007; Vadopalas *et al.* 2011).

The bivalve shell as a marine archive

The amount of ^{18}O incorporated into the shell is temperature-dependent and therefore $\delta^{18}O$ values from sequential calcite samples taken along the direction of growth from the umbo to the shell margin produce a record of relative water temperature over the life of the bivalve during the time it was depositing shell material (Chute *et al.* 2012). To date, high-resolution records of $\delta^{18}O$ from fossil and recent bivalves have been used to reconstruct sea surface temperatures (Kennedy *et al.* 1999; Goodwin *et al.* 2001; Schöne *et al.* 2002; Strom *et al.* 2004; Henry & Cerrato 2007; Black *et al.* 2009; Lartaud *et al.* 2010). Where records of measured sea surface temperatures (SSTs) are available to compare directly with $\delta^{18}O$ records, shells record within 1–2°C of the actual temperature (Richardson 2001). Stable oxygen and carbon isotope records from annual growth increments have also been used to reconstruct changes in surface water salinity, nutrient concentrations, water depth, seasonal phytoplankton blooms, upwelling currents, melt-water inflow and climatic influences, for example El Niño (Richardson 2001 and references; Tada *et al.* 2006; Harding *et al.* 2010). Elemental ratios, for example Mg/Ca or Sr/Ca in shell carbonate, have also been used as temperature proxies (Richardson *et al.* 2004; Freitas *et al.* 2005), although Freitas *et al.* (2006, 2008) caution against the use of the Mg/Ca ratio as a temperature proxy because of the substantial intra- and interspecies variation in Mg incorporation into shell calcite (see also Dick *et al.* 2007).

An exciting development in recent years is the realisation of the enormous potential of the North Atlantic clam, *A. islandica*, as a high-resolution marine archive (see Wanamaker *et al.* 2009 for an overview). The species exhibits the essential criteria for use in a shell-based chronology: synchronised growth, annual banding, longevity (up to 3–4 centuries) and growth throughout life (Butler *et al.* 2009 and references). Because of its longevity the clam is a potential model organism in ageing research (reviewed by Ridgway & Richardson 2011). Various programmes are used to cross-match the growth-increment series between shells (Scourse *et al.* 2006; Black *et al.* 2009). Several authors have cross-dated live caught *A. islandica* to fossil shells to produce master shell-growth chronologies of varying lengths, the longest being 1350 years (Wanamaker *et al.* 2012). The geochemical signatures ($\delta^{18}O$, $\delta^{13}C$ and $\delta^{14}C$) from the shell material and shell-growth chronologies can be used to reconstruct ocean circulation, hydrographic changes and ecosystem dynamics. As there is no ontogenetic trend in carbon or oxygen isotopes, geochemical records can be used from young and old shell portions to reconstruct past

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oceanic environmental conditions (Schöne *et al.* 2005). In the North Sea shell growth records were used to infer changes in zooplankton cycles and productivity, which were partly related to the North Atlantic oscillation (NAO), a climatic phenomenon caused by differences in atmospheric pressure at sea level between the Icelandic low and Azores high (Witbaard *et al.* 2003). Clam species, for example the geoduck *Panopea abrupta* from the Pacific (Strom *et al.* 2004), the northern quahog *M. mercenaria* from the western North Atlantic (Ridgway *et al.* 2011) and the Antarctic soft shell clam *Laternula elliptica* (Dick *et al.* 2007) can also serve as reliable climate proxies, thereby extending the geographic range in which ocean climates can be reconstructed. A relatively recent development is the use of multiple chronologies in climate reconstructions. For example, Black *et al.* (2009) developed SST reconstructions in the northeast Pacific by combining tree-ring and geoduck clam growth-increment chronologies. Six geoduck chronologies from sites along the Washington–British Columbia coasts were compared and combined with tree-ring chronologies from California to Alaska. Both proxy types closely tracked SST, and when used in combination produced a more robust SST reconstruction than tree-ring or geoduck chronologies could provide alone (Figure 6.10).

Allometric growth

Bivalves exhibit progressive changes in the relative proportions of the shell with increasing body size. These changes are the result of differential growth vectors operating at different points around the mantle edge (Seed 1980). The mantle plays a key role in shell secretion (Chapter 2). The relationship between any two size variables can be described by the allometric equation

$$y = ax^b$$

where y is one growth variable, for example shell length, x is the other variable, for example shell height, and a and b are coefficients. The exponent b is the growth coefficient and represents the relative growth rate of the two variables, while a is the value of y when x is unity. In logarithmic form the equation becomes

$$\log y = \log a + b \log x$$

The slope b and intercept a of the transformed data are estimated by regression analysis. If x and y have the same units of measurement, a value of unity for b indicates that the relative growth of the two variables is identical (isometric) and that geometrical similarity is maintained with increasing size. Values of b greater than unity indicate that y is increasing relatively faster than x (positive allometry), while values of b less than unity indicate the reverse (negative allometry). Different criteria for isometry and allometry apply if the dimensions of the two variables x and y differ (Seed 1980). However, b is not necessarily constant and it may change either continuously or abruptly at specific breakpoints (points of discontinuity in slope b); thus the allometric model quite often fails to describe allometric growth successfully (see Katsanevakis *et al.* 2007).

Allometric changes in the shell are generally gradual and are associated with the maintenance of physiologically favourable surface area-to-volume ratios rather than with changing environmental conditions. For example, in the mussel *M. edulis* differential growth among various shell parameters (height, length, width and weight) is reflected in a gradual change in shell shape, and larger (older) mussels have relatively heavier and more elongate shells in which width frequently exceeds shell height (Seed 1968). This may be particularly beneficial to mussels living in densely crowded conditions as a wedge-shaped profile effectively elevates the posterior current flow among

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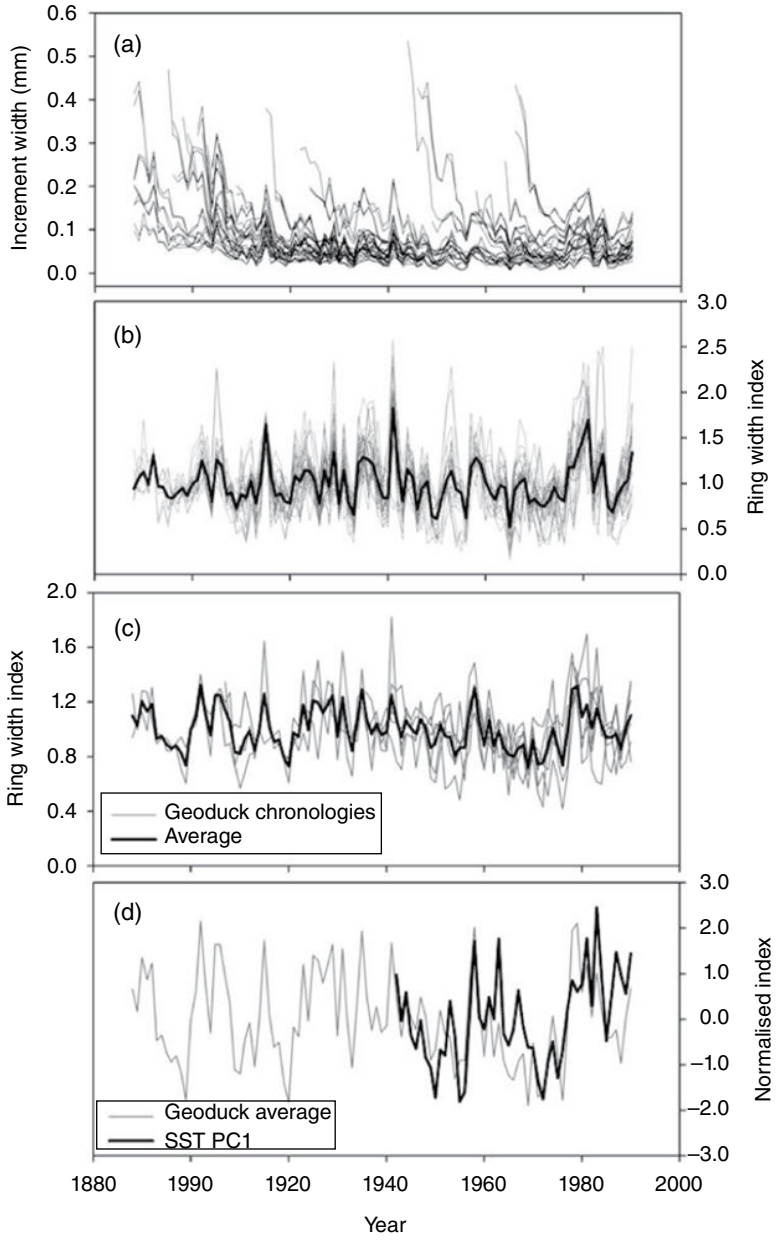


Figure 6.10 (a) Measurement time series for the geoduck clam (*Panopea abrupta*) Tree Nob chronology, British Columbia, Canada. (b) Master chronology (heavy line) and individual measurement time series after detrending with negative exponential functions. (c) Six master geoduck chronologies from the same region, and their average. (d) The average of the six geoduck chronologies and the leading principal component for regional sea surface temperatures (SSTPC1); with one exception, correlations between individual chronologies and SSTPC1 were significant ($p < 0.05$ to $p < 0.001$). From Black *et al.* (2009). Reproduced with permission of Elsevier.

conspecifics (Yonge & Campbell 1968). A variety of environmental factors such as latitude, shore level, wave impact, currents, water depth, type of bottom, type of sediment (Seed 1980; Gaspar *et al.* 2002 and references) and, more recently, disease (Caill-Milly *et al.* 2012) are believed to influence shell proportions in bivalves. The allometric

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equation is also employed in physiological investigations, and for obtaining estimates of seasonal variation in growth or productivity.

One disadvantage of the use of bivariate data in allometric studies is that only two variables are compared at any given time, so that this type of analysis deals only with changing proportions, but not changing shape of shell. Size-independent shape characterisation can now be rapidly and accurately assessed by a method known as Fourier analysis. Shell outlines are captured by video imagery and the Fourier series mathematically compartmentalises the shape outline into a series of standard sinusoidal components, which when summated converge to the empirical shape of the outline. The method, which is capable of discerning rather subtle differences among very similar, nearly oval shapes, has been used on mussel (Krapivka *et al.* 2007), scallop (Kenchington & Full 1994) and clam (Costa *et al.* 2008) shells. However, in recent years geometric morphometric techniques are becoming the standard approach in shell shape analysis (Valladares *et al.* 2010). These techniques are based on 2D and 3D discrete (landmarks) or continuous (outlines) matrix data, thereby allowing the partitioning of shape and size components of morphological change. It is not possible to obtain such partitioning using inter-landmark distances because of their one-dimensionality. In addition, geometric morphometric techniques also preserve the main geometric properties of the biological specimens generating a visual output of the morphometric change after applying thin-plate splines, a mapping function for coordinate transformation (Bookstein 1991). Serb *et al.* (2011) used landmark-based geometric morphometrics to quantify shell shape in seven scallop species that display a range of behavioural habits (Figure 6.11). They found that all species had a distinct shell shape with the exception of the two long-distance swimmers, whose shells were indistinguishable. These two species also displayed reduced morphological variance relative to the other five species. Both these findings demonstrated that convergent evolution of shell shape occurs in scallops, and suggested that selection for shell shape and behaviour may be important in the diversification of the group.

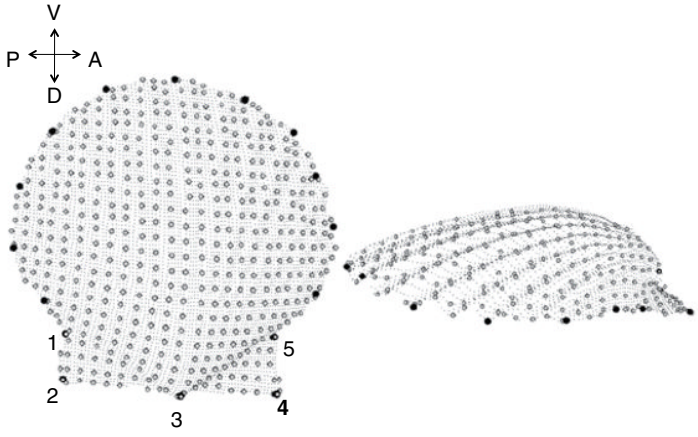


Figure 6.11 Three-dimensional surface scan of the left valve of a representative scallop, with the positions of 506 landmarks and semi-landmarks indicated. Fixed landmarks are shown as numbered, open circles (landmark 1, ventro-posterior auricle; 2, dorso-posterior auricle; 3, umbo; 4, dorso-anterior auricle; 5, ventro-anterior auricle), semi-landmarks along the ventral edge of the valve are shown as closed circles and surface semi-landmarks on the scallop valve are shown as grey circles. Dorso-ventral and antero-posterior axes are provided.
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Scope for growth and dynamic energy budget models

An alternative to direct measurements of growth is a method based on the physiological energetics of the test individuals. Physiological energetics is concerned with the study of energy balance within individuals, not only in terms of the acquisition and expenditure of energy, but also with the efficiency with which it is converted from one form to another (Bayne & Newell 1983).

The most common way to assess energy balance in an individual is to measure the various components of the balanced energy equation:

$$C = P + R + F + U$$

where C is total consumption of food energy, F is faecal energy loss, U is the loss of the end products of nitrogen metabolism, R is the respiratory energy expenditure and P is the energy available for shell, soft tissue and gametes. The absorbed ration (A) is the actual amount of material digested and is represented by $C-F$. The efficiency with which the ingested ration (C) is absorbed, A/C, is called the absorption efficiency.

By rearranging the energy balance equation we get

$$P = A - (R + E)$$

where $E=F+U$. Production is, therefore, the difference between energy gains from the absorbed ration (A) and metabolic losses through respiration and excretion ($R+E$). An organism can only allocate energy to growth or reproduction if the energy gain from A exceeds total metabolic losses. This energy, surplus to metabolic demands, is referred to as 'scope for growth' (SFG; Figure 6.12; Warren & Davis 1967). For routine monitoring, the energy balance equation may be further simplified to

$$P = A - R$$

SFG can be measured either in the field using a mobile laboratory or in the laboratory under standardised conditions (see Widdows & Staff 2006 for details). Thus, the effect of

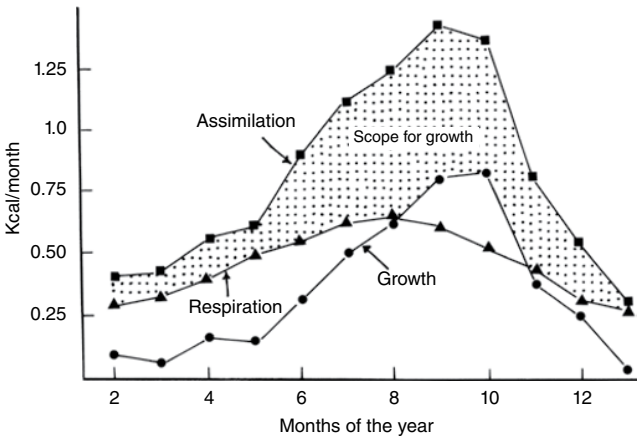


Figure 6.12 Seasonal variations in energies of growth, respiration and assimilation in a 40 g intertidal oyster *Crassostrea virginica* over a 12-month period. The stippled area represents scope for growth. Adapted from Dame (1972) and Bayne and Newell (1983). Reprinted with permission from Springer and Elsevier.

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parameters such as temperature, salinity, pollutants, food type and ration on SFG can be quantified. The energy consumed or ingested is calculated as follows: C = [maximum clearance rate: $l\,g^{-1}\,h^{-1}$] × [mg POM l^{-1}] × [23 J mg^{-1} POM], where the energy content of particulate organic matter (POM) or algal food is 23 J mg^{-1} ash-free POM. Absorption efficiency (AE) is measured by comparing the proportion of the organic matter in the food and faeces of animals kept in individual experimental chambers (Conover 1966). The energy absorbed (A) is then estimated from (C) × (AE). Oxygen consumption (R) is measured using an oxygen electrode to track the decline in oxygen partial pressure for individual animals maintained in closed-glass respirometers held in a temperature-controlled water bath. The energy respired is expressed as R = (μmol O_2 $g^{-1}\,h^{-1}$) × 0.456, where the heat equivalent of oxygen uptake is 0.456 J μmol^{-1} O_2. All physiological rates are converted to mass-specific rates for a standard individual of 1 g dry weight using the appropriate weight exponent b; in mussels b has a value of 0.67 (Widdows & Staff 2006). As all measured physiological responses have been converted into energy equivalents J $g^{-1}\,h^{-1}$, SFG (P) is expressed in these terms. Generally there is good agreement between growth rates estimated from energy budgets and growth rates measured by more direct methods (references in Bayne 1998). The methods that are used for estimating production in individual animals can equally well be applied to whole populations, or even to communities. Data on populations are normally derived by integrating field measurements on population density, age structure and production with laboratory estimates of C, A, R and E of different-sized individuals. The results are generally expressed in terms of energy flux per unit area of habitat, kJ m^{-2} year^{-1}. Production, expressed as a percentage of consumption, along with P_g and P_s (gonad and somatic growth, respectively) values for a variety of bivalve species are given in Table 6.1. Values for production vary from a low of 1–2% in the scallop *Chlamys islandica* to over 20% for the two oyster species *Crassostrea gigas* and *Crassostrea virginica*, and the scallop *Patinopecten yessoensis*. Such variation reflects interspecific growth differences (somatic and reproductive) and differences in recruitment, immigration, mortality and emigration. The variation in P_g and P_s estimates between species largely reflects differences in age structure. This can also be seen when individuals of the same species are analysed from a single location. For example, in Killary Harbour, a fjord on the Irish west coast, production of cultured mussels exceeded production of the wild population on the shore by an order of magnitude (Rodhouse *et al.* 1985). However, the wild population, which is dominated by large, older mussels (40–60 mm shell length) with a high reproductive output, makes a major contribution to total gamete production in the inlet.

Table 6.1 Energy budget parameters for a variety of bivalve populations.

Species	C	P_s	P_g	P
Aulacomya ater	27 499	3.4	5.8	9.2
Chlamys islandica	55 711	1.0	0.4	1.4
Choromytilus meridionalis	831 890	1.6	4.8	6.4
Crassostrea gigas (mature)	10 140	0.4	20.7	21.1
Crassostrea virginica	8 811	17.7	3.4	21.1
Mercenaria mercenaria	5 400	5.6	4.7	10.3
Mytilus edulis	–	8.9	4.8	13.7
Ostrea edulis	134–468	6.4	5.5	11.9
Patinopecten yessoensis	–	22.7	4.3	27.0
Mean		7.5	6.0	13.5

Adapted from Griffiths and Griffiths (1987); based on data in Griffiths and King (1979) and Stuart (1982). Reproduced with permission of Elsevier.

Dashes indicate that no measurement was made. The enormous variation in C mainly reflects differences in population density (<10–10 000 individuals m^{-2}).

P_s (somatic growth), P_g (reproductive output) and production (P) are each expressed as a percentage of consumption (C; kJ m^{-2} year^{-1}).

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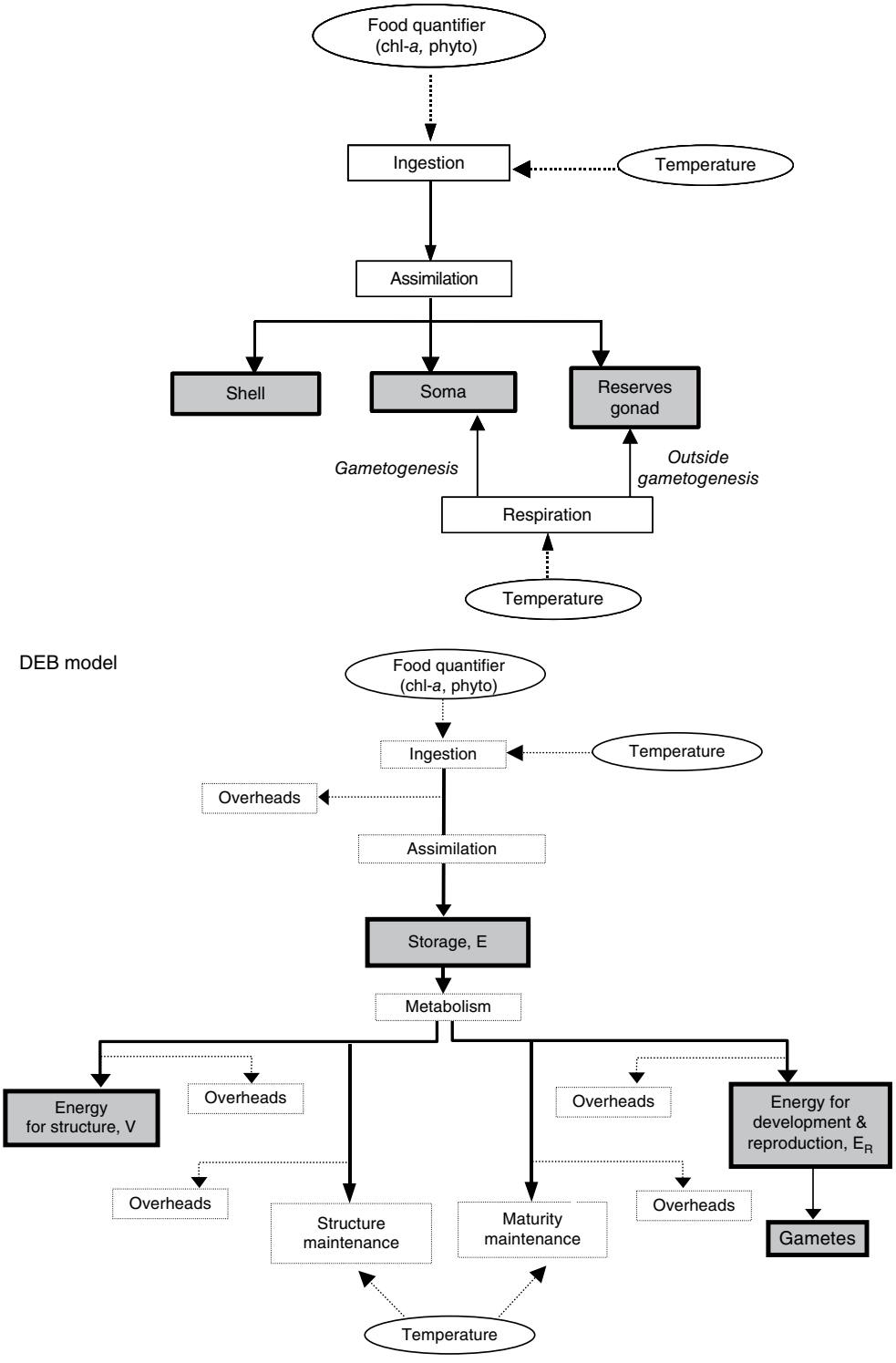


Figure 6.13 Conceptual diagrams of the scope for growth (SFG) and dynamic energy budget (DEB) models for the Pacific oyster *Crassostrea gigas*. Forcing variables are shown in the ellipsis while state variables are in the grey boxes.
From Barillé *et al.* (2011). Reproduced with permission of Elsevier.

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The dynamic energy budget (DEB) bioenergetics model is another model that has received considerable attention in recent years. A very comprehensive account of the major differences between the SFG and DEB models are provided by Filgueira *et al.* (2011), and these are summarised as follows. The main difference is that in the SFG model maintenance costs are subtracted from the assimilated products before they are allocated to reserves and somatic tissue, while in the DEB model assimilated energy is first stored in reserves, which in turn are utilised to fuel other metabolic processes. Thus, reserves reflect the feeding history of the organisms and consequently the structural growth dynamics in a DEB model become different from the SFG model, particularly in situations with temporal fluctuations in energy supply. Another important assumption of the DEB model is the κ-rule, which implies that a fixed proportion κ of the available energy is allocated to somatic maintenance and growth, with priority for maintenance, and the remaining $1-\kappa$ is allocated to maturation and reproduction/maturity maintenance for juveniles and adults, respectively. So, SFG models are forced by environmental conditions such as temperature, food concentration and quality, without taking into account the internal state of the organism, while DEB models describe the rates of energy absorption and utilisation as a function of the organism and the environment (see Duarte *et al.* 2010 and references). Although the conceptual basis is different in the two models, each has been used successfully to simulate growth in a variety of bivalve species (Figure 6.13; see Barillé *et al.* 2011; Filgueira *et al.* 2011 and Larsen *et al.* 2014 for references). Recently, models have been increasingly used to simulate physiological responses of cultured bivalves reared under varying conditions of food quantity and quality (Pouvreau *et al.* 2006; Bourlès *et al.* 2009; Rosland *et al.* 2009; Rico-Villa *et al.* 2010; Alunno-Bruscia *et al.* 2011; Bernard *et al.* 2011; Handå *et al.* 2011; Thomas *et al.* 2011; Wijsman & Smaal 2011; Duarte *et al.* 2012), temperature (Bourlès *et al.* 2009; Rico-Villa *et al.* 2010; Bernard *et al.* 2011; Thomas *et al.* 2011), turbidity (Barillé *et al.* 2011) and water depth (Ren & Schiel 2008). DEB models have also been used to estimate the carrying capacity of coastal ecosystems for aquaculture (Ren & Ross 2001; Duarte *et al.* 2003, 2008). Information from these studies can then be used to evaluate the suitability of coastal ecosystems for bivalve culture.

Factors affecting growth

There are many factors that influence growth in bivalves. Of these, food supply is considered to be the most important as, without this, sustained growth is impossible (Seed & Suchanek 1992). But food supply to bivalves is influenced by factors such as temperature, aerial exposure, water depth and population density. Some modulators of growth such as temperature and salinity also interact, often synergistically. It is for these reasons that it is usually very difficult to quantify the precise influence of a single environmental factor on growth in natural populations of bivalves. In addition, endogenous influences that are inherent to the organism, for example genotype and physiological status, interact in a complex way with environmental factors. Although the bulk of published information deals with the effect of environmental factors on absolute growth, where possible their effect on SFG will also be dealt with in the following sections.

Environmental modulators

Food

In the wild, bivalves derive nutrients from a variety of POM, including phytoplankton, re-suspended benthic microalgae, detritus from a mixture of sources, bacteria and microheterotrophs (Pernet *et al.* 2012; details in Chapter 4). Of these, phytoplankton is the primary

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food source, as growth of somatic and reproductive tissue follows the annual cycle of phytoplankton biomass (Kang *et al.* 2006). But phytoplankton quality, as opposed to overall biomass, is often more important as bivalves preferentially select phytoplankton for ingestion based on size, morphology and nutritional content (Chapter 4). For example, in the Thau lagoon, South France, periods of high growth rates in bivalves are mainly fueled by diatoms, a highly nutritious component of seasonal phytoplankton blooms. An earlier report by Beukema and Cadée (1991) also found that in the Wadden Sea the clam, *Macoma balthica*, grew fastest in years of high diatom abundance. Increased nutrient enrichment to coastal waters worldwide increases microalgal production, thereby increasing food quantity and quality for primary consumers such as bivalves, and, in turn, increasing bivalve secondary production in terms of shell and soft tissue growth. But increased algal production can have negative effects, for example low oxygen concentrations, habitat loss or degradation, which can result in reduced survival and lower recruitment rates in bivalves (reviewed in Carmichael *et al.* 2012). There is growing evidence, however, to show that growth may in fact be phytoplankton-limited owing to the enormous filtration capacity of bivalves. Consequently, in order to meet their energy requirements bivalve populations must exploit non-phytoplanktonic carbon in the form of re-suspended sediment, a complex mixture of benthic microflora, microalgae, fine organic detritus as well as quantities of inorganic material. Results from both laboratory and field experiments have shown that in appropriate concentrations sediment suspensions enhance growth. In the mussel, *M. edulis*, an increase in growth has been reported at low or moderate seston concentrations of 1–25 mg l^{-1} with a general decrease at high seston concentrations of 40–50 mg l^{-1} (see Fréchette & Grant 1991 for references). Similar results have been obtained from SFG experiments. When SFG in mussels (*Aulacomya ater*) fed on kelp detritus was compared with SFG in mussels fed on algae (Griffiths & King 1979; Stuart 1982), absorption of algal cells differed markedly from absorption of detritus. Moreover, mussels continued to maintain positive and increasing SFG at high ration levels of detrital material (Figure 6.14). This is even more obvious when classes of different-sized mussel are

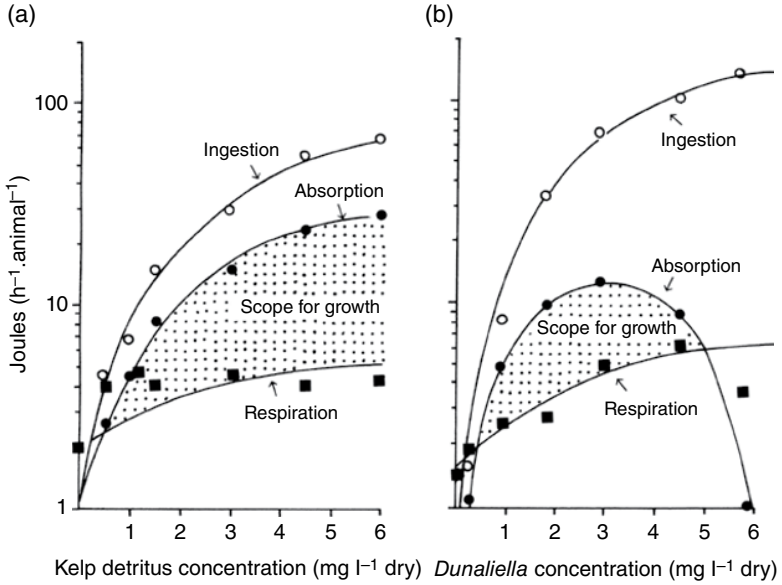


Figure 6.14 Ingested ration, absorbed ration, respiration rate and resultant scope for growth in the mussel *Aulacomya ater* (50 mm shell length) as a function of increasing concentration of (a) *Dunaliella primolecta* and (b) kelp detritus.
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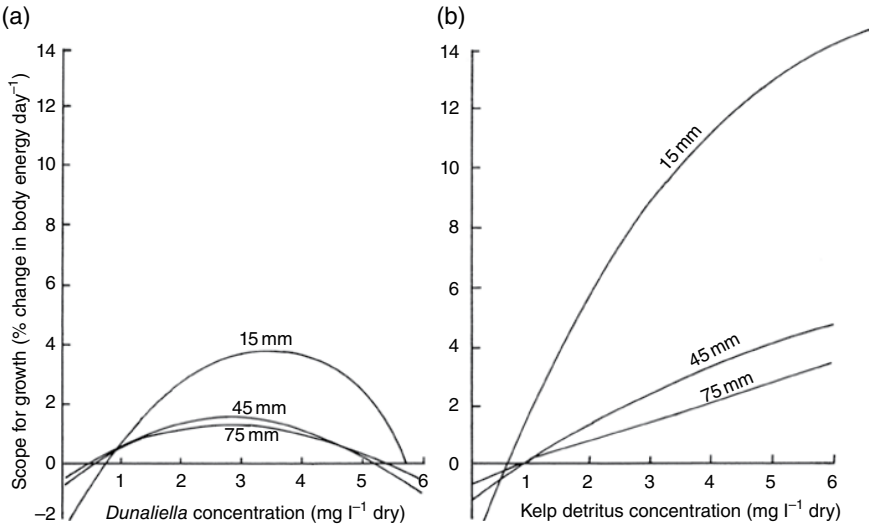


Figure 6.15 Scope for growth in relation to food concentration in *Aulacomya ater* of differing shell lengths when fed on (a) *Dunaliella primolecta* and (b) kelp detritus.
From Griffiths and Griffiths (1987) based on data in Griffiths and King (1979) and Stuart (1982). Reproduced with permission of Elsevier.

compared (Figure 6.15). On both diets small mussels exhibit faster relative growth rates largely because they are metabolically more efficient, consistently filtering larger volumes per unit of oxygen consumed than larger mussels (Griffiths & Griffiths 1987). Response to variation in the concentration of suspended particles is also species-specific. In the scallop *Placopecten magellanicus*, SFG at low particle concentrations equalled or exceeded SFG in the clam *Mya arenaria*, but the opposite was true at high concentrations. The latter species appears to be better suited to cope with higher concentrations of seston, especially if it consists of relatively poor-quality particles (MacDonald *et al.* 1998).

The availability of re-suspended material (plankton and organic detritus) as a source of food to bivalves is dependent on several factors. This source of food is more readily available to bivalves in suspended culture than to benthic-dwelling bivalves. The potential for re-suspension of organic matter is greater over mud than over sand. But growth in bivalves is in fact poorer over mud than over sand. This is due to excessive turbidity over muddy bottoms. The seston becomes diluted with inorganic particles, resulting in a decrease in seston quality and also in progressive clogging of the bivalve filtering mechanism. Other factors such as the hydrography of an area and weather patterns also affect re-suspension. In addition, the response to re-suspension is species-dependent. Growth enhancement by low additions (<5–10 mg l^{-1}) were reported for the mussel *M. edulis* and the surf clam *Spisula subtruncata*, but not for the hard clam *M. mercenaria* (references in Bricelj & Shumway 1991). Because re-suspended material plays an important role in bivalve diet, Smaal and van Stralen (1990) have suggested that primary production data based on carbon turnover rather than chlorophyll values should be used in evaluating the feeding capacity of sites for bivalve culture (see Chapters 4 and 9 for additional information on feeding and food).

Tidal level and wave action

Bivalves only feed when they are submerged; the longer a species is out of water, the less time it has to feed. Therefore, it is to be expected, that animals in the high intertidal zone exhibit markedly reduced growth rates compared to animals that are permanently covered by the tide. This is well illustrated for the oyster species *Tiostrea lutaria*, introduced into the

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United Kingdom from New Zealand in 1963. Growth of intertidal and subtidal oysters was similar over the first 3 years of life but after that time growth of the intertidal population slowed appreciably (Richardson *et al.* 1993b) (Figure 6.16). Similar findings have been reported for the commercially important clam, *M. arenaria*, where the time to reach the legal size of 50.8 mm shell length is 4–5 years in the lower mid tide region, whereas clams near the upper mid tide take 7–8 years to reach legal size (Figure 6.17; Beal 2006). Bivalves

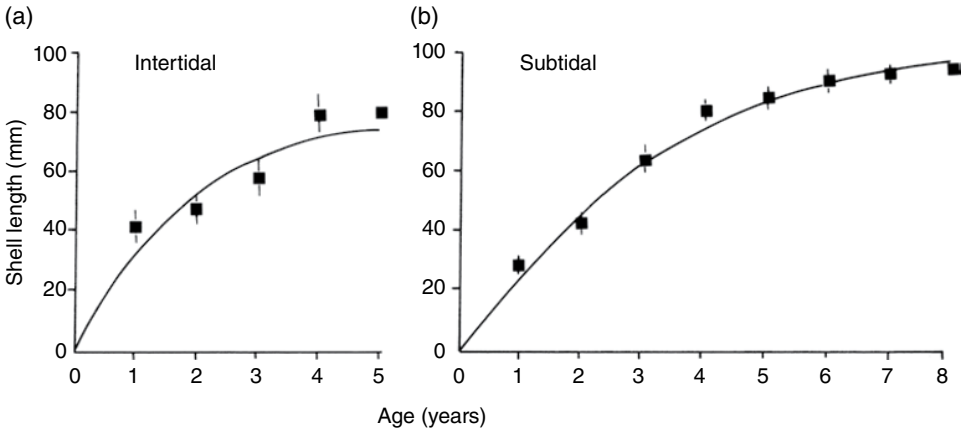


Figure 6.16 Growth rates of (a) intertidal and (b) subtidal populations of *Tiostrea lutaria* at Tal-y-foel, Menai Strait, North Wales. Values are means (± SD). Curves were fitted using the von Bertalanffy equation: intertidal, $L_t = 79.89\ (1 - \exp(-0.597t))$; subtidal, $L_t = 108.48\ (1 - \exp(-0.299t))$, where L_t is shell length at time t. From Richardson *et al.* (1993b). Reproduced with permission of the National Shellfisheries Association, United States.

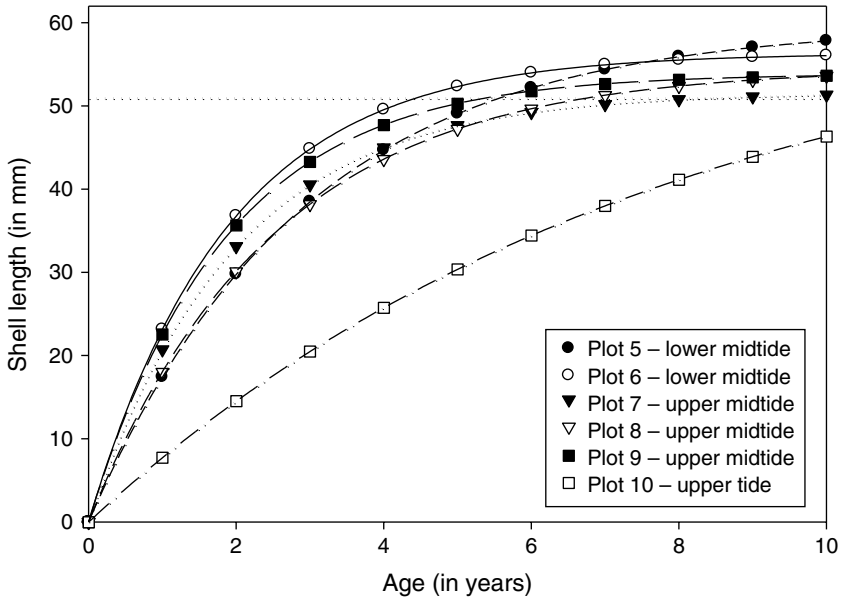


Figure 6.17 Von Bertalanffy growth curves of wild clams along a tidal gradient at Carrying Place Cove, Eastport, Maine, United States from 22 April 1998 to 20 April 1999 (Beal 2006). The dotted line parallel to the *x*-axis is the minimum legal size of softshell clams (*Mya arenaria*) in Maine (50.8 mm, or 2 inch shell length).
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do, however, retain the potential for growth, even when they have been unable to exploit it due to unfavourable growing conditions. Seed (1968) found that when old slow-growing mussels were transferred downshore they grew rapidly.

Species differ in their ability to tolerate aerial exposure. For example, van Erkom Schurink and Griffiths (1993) found that for four mussel species *Mytilus galloprovincialis* and *Perna perna* continue to grow at about 80% of the submerged rate even at 50% exposure level, compared to 66% for *Choromytilus meridionalis* and only 54% for *A. ater*. But for most bivalve species a figure of around 50% exposure represents the point of zero growth, when the energy required for metabolism during aerial exposure exceeds that available during the feeding period (Seed & Suchanek 1992). In reality this figure is an overestimate as other factors, such as tolerance to extremes of temperature and desiccation, may become limiting well before SFG declines to zero (van Erkom Schurink & Griffiths 1993).

The effect of strong wave action on bivalve growth is often site- or species-specific, and the reasons for this are presently unclear. In some regions, for example western Europe, Chile and northern Russia, mussels are smaller on shores with strong wave action (Seed 1969; Alvarado & Castilla 1996; Sukhotin *et al.* 2006), while in South Africa, *P. perna* grows almost twice as fast on exposed as on sheltered shores, probably due to greater food availability at sites with greater water flow (McQuaid & Lindsay 2000, 2007). The same finding was reported for *M. galloprovincialis* in South Africa, except that growth declined at sites experiencing extreme wave action (Steffani & Branch 2003; Hammond & Griffiths 2004).

Temperature

Water temperature varies with latitude and there has been a general consensus in the literature that bivalves from low latitudes grow more rapidly at ambient temperature than do conspecifics from higher latitudes. Chauvaud *et al.* (2012) have recently examined this in more detail by analysing variation in growth patterns of the scallop *Pecten maximus* along a latitudinal gradient using three main parameters: maximum annual growth rate, daily growth rate and length of the growing season. The maximum observed age was 6–7 years for the southern populations, and 7–10 years for the northern populations. A temperature–size gradient was clearly identifiable within the species distribution area, with a significant positive correlation for the first five classes. Lower annual growth characterised shells from the northern sites, but this growth differential decreased with age so that size differences between the populations were no longer significant after six winters. However, scallops from lower latitudes continued to grow, thus achieving a higher maximum size than those from lower latitudes. They also observed that the lower annual growth rates in northern populations were not due to lower daily growth values, but were a consequence of the fewer number of days available each year to achieve this growth in the north compared to the south, a finding that highlights the importance of using growth rate as well as growth duration when exploring growth patterns of bivalves. Interestingly, daily growth rate of other scallop species are significantly and positively related to temperature. Even in the Antarctic scallop *Adamussium colbeki*, growth rates do not differ from growth rates of other scallop species living in temperate or warm waters (Figure 6.18; Heilmayer *et al.* 2005 and references).

Food supply, while predominantly light/nutrient-limited, is also temperature-dependent. In temperate seas winter minimum temperatures and food availability are generally coincident, and growth in many species of bivalves seem to reflect changes in food availability rather than temperature. For example, growth rate in mussels on a submerged platform off the Californian coast was dependent on variations in phytoplankton concentrations, and temperature was virtually eliminated as an important growth regulator over the temperature range 10–18°C experienced by these mussels (Page & Hubbard 1987). This is because bivalves (and other aquatic invertebrates) are able to adjust the components of energy balance

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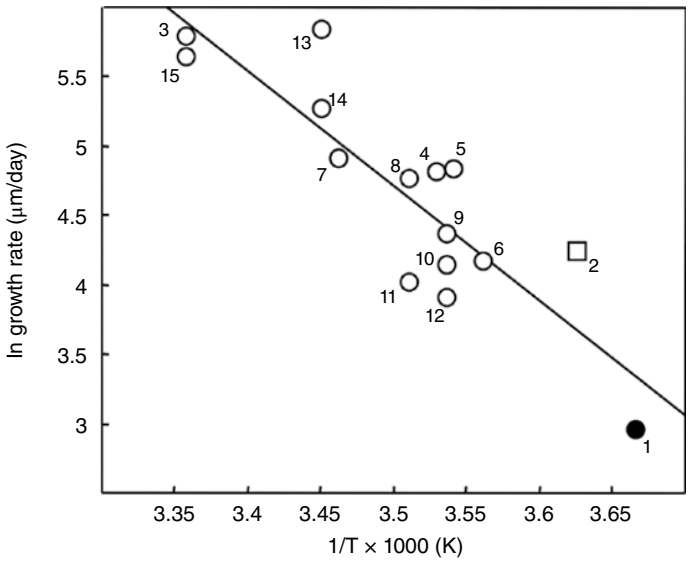


Figure 6.18 Mean daily shell growth (µm/day) for six scallop species from different latitudes. Shown are values measured at the main growth periods in juvenile/premature scallops: 1–2, *Adamussium colbecki*; 3, *Argopecten circularis*; 4–5, *Chlamys islandica*; 6, *Mizuhcopecten yessoensis*; 7–12, *Placopecten magellanicus*; 13–14, *Pecten maximus* (13 1-year-old, 14 2-year-olds); 15, *Pecten vogdesi*. Arrhenius plot, with fitted least-squares regression line (only populations 3–15): ln shell growth (µm/day) = 33.65–8.268 1000/T (N = 13, r^2 = 0.693, p < 0.001).
From Heilmayer *et al.* (2005). Reproduced with permission of Springer Science and Business Media.

(compensatory acclimation) in order to maintain positive SFG over a range of environmental temperatures. Widdows (1978) has shown that SFG in *M. edulis* was virtually independent of temperature over the range 5–20°C. However, at 25°C, which is close to the lethal temperature for this species, SFG declined sharply. A similar result, albeit using higher temperatures, was reported for *R. philippinarum* (Sobral & Widdows 1997). These findings reflect a breakdown in the mechanism for metabolic compensation, resulting in an increase in metabolic costs but a decline in filtration rate. Seawater temperatures are predicted to increase by 0.2°C per decade (IPCC 2007; also see Chapter 3). Therefore, it is important that we understand the relationship between growth patterns and temperature because global climate change will be a thermal challenge to most ectotherms (Chauvaud *et al.* 2012).

Salinity

Bivalves respond to changes in external salinity by closing the shell valves and by adjusting the intracellular concentrations of ions, amino acids and other small molecules to maintain a relatively constant cell volume. Initially rates of feeding and respiration are depressed but gradually recover as osmotic equilibration is reached. The period required for complete acclimation depends on the extent of the initial salinity change (Almada–Villela 1984).

Bivalves grow well in brackish estuaries but this is probably related to increased food levels in this habitat rather than to reduced salinity *per se* (Seed & Suchanek 1992). Mussels are able to adjust growth at changing salinities. To illustrate, in laboratory experiments when mussels were exposed to salinities between 10 and 30 psu, they showed a reduced growth rate with decreasing salinity (Riisgård *et al.* 2012). Mussels that were initially exposed to 10 psu exhibited fast growth when subsequently exposed to 30 psu, and this trend was reversed when 30 psu mussels were exposed to 10 psu (Figure 6.19). However, in the northern Baltic Sea, where

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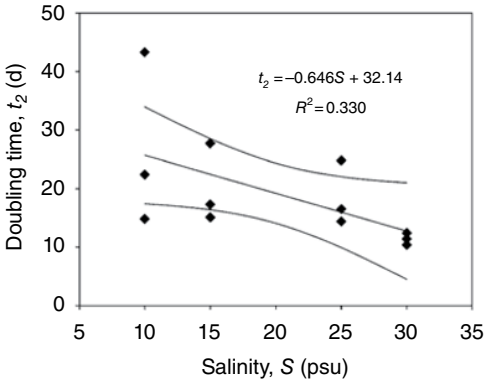


Figure 6.19 Doubling time of body dry weight for the mussel *Mytilus edulis*, grown at different salinities during 11–22 days on *Rhodomonas salina* cells. Regression line, its equation and the 95% confidence interval are indicated.
From Riisgård *et al.* (2012). Reproduced with permission of H.U. Riisgård and Scientific Research Publishing.

salinity ranges from 6.5 to 3.0 psu, mussels are at the limit of their salinity tolerance and consequently show a marked decline in size with decreasing salinity (Westerbom *et al.* 2002; Riisgård *et al*. 2014). The slow growth of mussels in the Baltic Sea has been attributed to the increased excretion of ammonia and metabolism of free amino acids in this hypo-osmotic environment, which probably represents a continuous energy loss (Tedengren & Kautsky 1986; Chapter 7). Some bivalves cope very well with steep, permanent changes in habitat salinity. For example, when adult clams (*M. balthica*) were transplanted into habitats with strongly reduced salinities (~50% of the original habitat salinity) there was no reduction in growth rate. Only clams that were transplanted from the central Baltic Sea (6–7 psu) to the Gulf of Bothnia (0.5–3.0 psu), further north, revealed reduced growth rates compared to the control group (Jansen *et al.* 2009). As a result of climate change water temperatures are predicted to increase and salinity to decrease in the Baltic Sea over the coming decades (Lehmann *et al.* 2011). This predicted scenario of warming and desalination could impose severe stress on bivalve populations if evolutionary adaptation does not happen at a similar rate (Hiebenthal *et al.* 2012).

Stock density

Most of the information on density as a modulator of growth has come from studies on bivalves grown in suspended culture. Indeed, carrying capacity is often defined as the stocking density at which production is maximised without negatively affecting growth rates (Duarte *et al.* 2003). When scallops (*P. magellanicus*) were held at different stocking densities (15–90 individuals per net; initial shell height 10–15 mm) individuals held at lower density had faster growth rates, greater individual whole weights, higher individual meat weights and increased production compared to those held at higher stocking densities (Table 6.2). Similar results have been observed for other species of scallop (Rheault & Rice 1996; Román *et al.* 1999; Maguire & Burnell 2001; Bacher *et al.* 2003), for cultivated clam species (Broom 1982; Beal & Kraus 2002; but see Jimenez *et al.* 2009; Powers 2009), for rope-grown mussels (Rodhouse *et al.* 1984; Alunno-Bruscia *et al.* 2000; Cubillo *et al.* 2012 and references) and for field and hatchery-cultured oysters (Holliday *et al.* 1993; Rheault & Rice 1996; Honkoop & Bayne 2002). One explanation is that a greater stocking density reduces food availability per individual. An alternative hypothesis is that growth is reduced at high densities because of the reduction in space; this leads to increased physical contact between individuals, with more

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Table 6.2 Average daily growth rates (mm day^{-1}) of scallops *Placopecten magellanicus* at different stocking densities for three different sampling intervals: I = August 1989–January 1990 (151 days); II = January–May 1990 (105 days); III = May–August 1990 (108 days), and for the whole year August 1989–August 1990.

Density	I	II	III	Year
15	0.158	0.054	0.100	0.111
30	0.154	0.044	0.084	0.101
45	0.148	0.050	0.060	0.094
60	0.141	0.051	0.047	0.087
75	0.140	0.042	0.051	0.085
90	0.115	0.055	0.070	0.084

Adapted from Parsons and Dadswell (1992). Reprinted with permission from Elsevier.

frequent irritation and retraction of the mantle, or valve closure, resulting in less feeding (Côté *et al.* 1993). To test these hypotheses Côté *et al.* (1994) grew *P. magellanicus* juveniles in nets at different densities in two series: one in which density was increased by adding living scallops, and a second in which density was increased by adding 'dummy' (non-living) scallops. The dummies occupied space but did not compete for food resources. For the first series, increasing the density of the scallops from 25 to 250 individuals per net caused a marked decrease in growth. In contrast, no significant decrease occurred in the 'dummy' series. This clearly demonstrates that at least for scallops food depletion is the major factor causing decreased growth at high densities. Few studies take population size structure, within the groups assayed, into account although this could be a strong determinant of the outcome of stocking experiments (discussed by Fréchette *et al.* 2005).

Wild populations also show a negative relationship between growth rate and density. Seed (1969) found that *M. edulis* spat recruited into a population of 1-year-old mussels grew at less than half the rate than those recruited onto an adjacent bare rock surface. Density also significantly reduced growth rate in the clam *Spisula solidissima*, and the effect was apparent in clams from 3 to 17 years of age (Weinberg 1998). The effect of density on growth appears to be species-specific. For example, in mussels, growth in *C. meridionalis* is more sensitive to density than is the case for *A. ater* or *M. galloprovincialis*. This appears to correlate with natural packing densities in the wild: *C. meridionalis* usually occurs as a monolayer, while both *A. ater* and *M. galloprovincialis* form dense multilayered beds (van Erkom Schurink & Griffiths 1993).

Water flow

Suspension-feeding bivalves depend on sufficient water movement to bring in new supplies of suspended food and carry away waste, while deposit feeders require low water flow to allow accumulation of deposited seston as a food source (Dame 2012). Generally speaking, reduced growth rates are observed for suspension feeding bivalves in areas of low current speed and high population densities. This is attributed to a reduction in seston supply and consequent food limitation (Wildish & Kristmanson 1985), and is one of the main reasons why bivalves held in suspended culture grow much more rapidly than those held under comparable conditions on the shore. Excessively high current speeds, however, reduce growth by inhibiting feeding activity. The underlying mechanism of growth inhibition is believed to be a build-up of a pressure differential between inhalant and exhalant apertures that interferes with feeding (Wildish & Kristmanson 1988).

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The effect of water flow on growth in juvenile seed clams (*M. mercenaria*) was examined using two manipulated water flow regimes: ambient (0–22 cm s^{-1}) and low (0–11 cm s^{-1}). After 12 months 69% of clams were of harvestable size in the ambient flow, compared with 42% in the low flow, and no relationship between planting density and flow regime was evident, indicating that food supply was not limiting and that the increase in growth was a physiological response to water flow increase (Powers 2009). The potential applicability of these results to clam culture operations remains to be tested.

In another experiment, this time under culture conditions in the wild, Claereboudt *et al.* (1994a) placed juvenile scallops (*P. magellanicus*) glued to panels either inside or on the outside of pearl nets, and placed these at two sites where environmental conditions were similar except for a twofold difference in the current velocity (mean velocity <0.9 m and >1.6 m s^{-1}, respectively). For scallops on the outside of the pearl nets daily specific growth rates of the shell were similar at the two sites, but the mass of shell and flesh scaled to shell height was greater under weaker current conditions. At the strong current site tissue masses were greater for scallops inside than for those outside the net, probably because the net reduced the frequency of velocities that inhibit feeding. Pearl nets decreased water flow by 46–61%. In contrast, at the slower current site, tissue masses were less inside the pearl net, probably because flow was slowed to the extent that seston depletion was limiting growth. These results highlight the importance of adapting culture methods to hydrodynamic conditions at bivalve growing sites in order to optimise growth.

Additional studies on the influence of flow velocity on growth include works by Cahalan *et al.* (1989), Grizzle *et al.* (1992), Wildish and Saulinier (1992), Lesser *et al.* (1994), Lenihan *et al.* (1996) and Sobral and Widdows (2000).

Pollutants

Bivalves, in particular mussels, have been widely used to assess the effects of specific toxicants on mortality, shell and tissue growth and SFG. In addition a variety of biosassays using bivalve embryos and larvae have been developed to monitor the toxic effect of marine pollutants on growth and development, both in the laboratory (His *et al.* 1999) and in the field (Geffard *et al.* 2002). The most common toxicity test is the 96 h LC50, which determines the concentration of toxicant that results in a 50% lethal response over a period of 96 h exposure. But LC50 estimates may also be determined after 24, 48 or 72 h exposure depending on the toxin and/or species being tested (see Ramakritinan *et al.* 2012). The water concentrations of various toxicants that produce a 50% reduction in shell growth rate in juvenile mussels are presented in Table 6.3. In general, tissue growth is more sensitive to toxicants than shell growth. In addition, the effect on growth depends on the type of pollutant being tested. For example, pearl oysters (*Pinctada imbricata*) showed significant reduced total growth (shell plus soft tissue) when exposed for a period of 2 months to high concentrations (270 µg l^{-1}) of either lead or zinc, but this high concentration of lead completely halted shell growth. However, exposure to aliphatic hydrocarbons had no effect on total growth (Gifford *et al.* 2006). Toxins can also accumulate in the sediment. Culbertson *et al.* (2008) have studied the effect of exposure of the ribbed mussel *Guekensia demissa* to residual petroleum from a major oil spill in 1969, which still remains buried in the sediments of this species habitat. Examination of short-term exposure was done by transplanting mussels from Great Sippewissett Marsh, a control site, into Wild Harbor, the site of the oil spill, both situated on Cape Cod, Massachusetts, in the United States. They also examined the effects of long-term exposure with transplantation of mussels from Wild Harbor into Great Sippewissett. Both the short- and long-term exposure transplants exhibited slower growth rates, shorter mean shell lengths, lower condition indices and decreased filtration rates.

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Table 6.3 Water concentration of various toxicants that induce a 50% reduction in shell growth rate of juvenile *Mytilus edulis*.

Toxicant	Water concentration ($\mu g l^{-1}$)
Copper (Cu)	4
Cadmium (Cd)	100
Mercury (Hg)	<1
Zinc (Zn)	60
Lead (Pb)	>200
Nickel (Ni)	>200
Tributyltin (TBT)	<1
Oil	1500

Data from Strømgren (1982, 1986) and Strømgren and Bonard (1987) and adapted from Widdows and Donkin (1992). Reprinted with permission from Elsevier.

Although the larval bioassay is routinely used to test the toxicity of chemicals there is increasing evidence to show that early larval stages of bivalves are not necessarily more sensitive than adults to a wide range of contaminants (Widdows and Donkin 1992). Beaumont *et al.* (1987) found that EC50 (median effective concentration) values for shell growth in response to Cu were about 30 times lower for adult mussels (water concentration of $5 \mu g l^{-1}$) than those reported for larvae ($150 \mu g l^{-1}$) (Strømgren 1986), or juveniles (see Table 6.3). Generally, adults tend to be about 4–10 times more sensitive than larvae to a range of pollutants such as Cu, hydrocarbons, tributyltin (TBT) and sewage sludge. The reduced sensitivity of larvae has been ascribed to a combination of their reliance on energy reserves, rather than direct feeding, and the absence of a developed nervous system, an important site of toxic action (Widdows & Donkin 1992).

Toxicants often exhibit synergistic effects on growth. For example, when oyster spat (*Saccostrea commercialis*) were exposed to both copper and TBT they suffered a greater reduction in growth than spat exposed to only one toxin (Nell & Chvojka 1992). Similar results were reported when juvenile *Argopecten ventricosus* scallops were exposed to a cadmium–chromium–lead combination (Sobrino-Figueroa *et al.* 2007).

SFG is one of the most sensitive measures of pollution-induced stress, and has proved to be a powerful and cost-effective method of assessing environmental pollution (Widdows *et al.* 2002). Using this approach in the mussel *M. edulis*, Widdows *et al.* (1997) found significant negative correlations between SFG and tissue concentrations of petroleum hydrocarbons, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexanes (HCHs), but no association between SFG and tissue levels of metals (Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn). Similar studies have been carried out on *M. edulis* (Widdows & Johnson 1988; Widdows *et al.* 1995; Shuhong *et al.* 2005; Booth *et al.* 2007; Mubiana & Blust 2007) and other mussel (Wang *et al.* 2005; Tsangaris *et al.* 2007) and clam species (Sobral & Fernandes 2004; Burt *et al.* 2007).

Other factors

Another factor that influences growth is concentration of seawater CO_2. When larval hard clams (*M. mercenaria*) and scallops (*Argopecten irradians*) were reared under CO_2 conditions representative of the pre-industrial era (250 ppm), they exhibited significantly faster growth compared to individuals exposed to modern-day CO_2 levels (390 ppm) (Talmage & Gobler 2011 and references therein). Increased acidification of seawater, due to CO_2, leads to

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decreased calcification in bivalves, as well as in other marine species with calcified parts (Michaelidis *et al.* 2005). Decreased pH also reduces growth in bivalves (Timmins-Schiffman *et al.* 2013). Predicted increases in atmospheric CO_2, as well as temperature (IPCC 2007), in the future are likely to have negative consequences for coastal bivalve populations.

Storms also can affect growth. Juvenile clams *M. mercenaria* maintained under simulated storm conditions showed up to a 40% reduction in shell growth compared to clams under gentle wave conditions (Turner & Miller 1991). The decrease in shell growth was attributed to a reduction in filtration due to high concentrations of re-suspended sediment, coupled with energy costs of increased pseudofaeces production.

Other factors that have been shown to decrease bivalve growth are reduced dissolved oxygen concentrations (Norkko *et al.* 2005; Wang *et al.* 2011), toxic algal blooms (Chauvaud *et al.* 1998; see Chapter 12), disease and parasites (see Chapter 11), chlorinated water (Thompson *et al.* 2000), manual handling of bivalves in land-based culture (Jakob & Wang 1994), predation (Nakaoka 2000), epibionts (Thieltges 2005), fouling (Claereboudt *et al.* 1994b) and intra- (Fréchette *et al.* 2005) and interspecific competition (van Gils *et al.* 2012).

Endogenous growth modulators

Genotype

That variation for growth in bivalves has a genetic basis is without question. This is based on the observation that highly variable rates of growth are routinely observed in bivalve larvae reared under identical, controlled laboratory conditions. While considerable progress has been made in understanding the role of exogeneous factors in growth modulation (see previous section), it has been more difficult to understand the role that endogenous mechanisms play in the process (Pace *et al.* 2006), although allozyme analysis, breeding studies and chromosome manipulations have gone some way in helping to elucidate the mechanistic basis of genotype-dependent growth.

Greater heterozygosity, calculated for individual animals as the mean number of allozyme loci that are polymorphic, is positively correlated with growth rate in many wild populations of juvenile and adult bivalves (see Britten 1996 for review). But the magnitude of the correlation appears to depend on factors such as age, reproductive state, environmental conditions, background genetic effects, and the number and type of loci analysed (Saavedra & Guerra 1996; Myrand *et al.* 2002 and references). It is also important to note that where a heterozygosity/growth correlation has been observed heterozygosity typically explains only a small percentage (2–4%) of the variation in size among individuals, although a higher figure of 27% has been reported by Bayne and Hawkins (1997). There are conflicting views regarding the genetic interpretation of the heterozygosity/growth correlation. One view is that allozymes are merely neutral indicators of selection acting against deleterious recessive mutations at linked loci with fitness effects (associative overdominance hypothesis). Another view is that selection acts directly on allozyme genotypes and the heterozygosity/growth correlation is a result of the functional superiority of allozyme heterozygotes (the overdominance hypothesis) (see David *et al.* 1997; Launey & Hedgecock 2001). However, to date it has not been possible to demonstrate any direct connection between an electrophoretic locus and bivalve growth.

A more direct approach to the study of growth heterosis is to use established inbred lines that can be crossed to produce larvae with reproducibly different growth phenotypes. Bayne *et al.* (1999) compared various physiological parameters (clearance rate, absorption efficiency, rates of oxygen consumption and ammonia–nitrogen excretion) in a selected

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fast-growing line of adult oysters (*S. commercialis*) with a control (unselected) line. Fast growth was associated with faster rates of feeding, reduced metabolic rates and lower metabolic costs of growth. The reduced costs of growth were due in part to increased efficiencies of protein turnover in fast growers (Bayne 2004). Control oysters, however, deposited more lipid than the selected oysters, though the unit costs of lipid deposition did not differ between selected and control oysters (Table 6.4). Similar results have been reported for fast- versus slow-growing larval families of the oyster *C. gigas* (Pace *et al.* 2006). Results from both studies indicate a wide plasticity of physiological rates and efficiencies and show how, through selection, interactions between physiological traits can serve to enhance growth (Bayne 2000). Turning now to possible genetic causes of heterosis, one study reported that the wild founders of inbred lines of *C. gigas* carried a minimum of 8–14 highly deleterious recessive mutations. This evidence for a high genetic load strongly supports the associative overdominance explanation of heterosis (Launey & Hedgecock 2001). Genetic linkage maps have been constructed for *C. gigas* (Hubert & Hedgecock 2004) and other species (Yuan *et al.* 2010 and references), and several quantitative trait loci (QTLs)[1] for size-related traits have been mapped in *C. gigas* (Hedgecock *et al.* 2007; Guo *et al.* 2012), as well as in the scallops *Chlamys farreri* (Zhan *et al.* 2009) and *A. irradians* (Li *et al.* 2012) (see Chapter 10). Quantitative trait loci (QTLs) may ultimately be used in marker-assisted selection (MAS) programmes (Chapter 10). To identify the genes that establish growth differences in bivalve larvae, Meyer and Manahan (2010) used another approach by comparing expression patterns in contrasting phenotypes (slow and fast growth) that were experimentally produced by genetic crosses of *C. gigas*. They identified 34 candidate genes, half of which were ribosomal proteins associated with protein synthesis, in addition to other well-characterised genes involved in protein metabolism, energy metabolism and regulation of feeding, thus supporting the general conclusion that genes involved in protein metabolism and feeding regulation are key regulators of growth. It is clear from these studies that the oyster *C. gigas* has played a pivotal role in helping us understand both the physiological and genetic bases of growth heterosis.

Chromosome set manipulation can produce phenotypic changes that are commercially important in bivalve aquaculture. For example, triploids (3*n*), which have been produced in many bivalves, invariably grow significantly faster than diploids (reviewed by Guo *et al.* 2009). Conversely, when chromosome numbers were analysed in fast-, medium- and

Table 6.4 Percent allocations of the metabolisable energy intake to maintenance, growth and storage in oysters (*Saccostrea glomerata*) selected for fast growth and in wild (control) oysters.

Allocations to	Selected oysters	Control oysters
Maintenance*	26.2 ± 5.6	42.8 ± 8.4
Growth in dry flesh*	23.9 ± 17.6	10.0 ± 8.0
Growth in protein†	61.0 ± 25.2	21.6 ± 9.7
Lipid storage†	28.2 ± 8.4	102 ± 30.4
Carbohydrate content†	−16.5 ± 19.0	−52.3 ± 13.2

From Bayne (2000)‡ in Bayne (2004). Reproduced with permission of Elsevier.
*Values for maintenance and dry flesh growth are calculated relative to dietary organic matter.
†Biochemical components are calculated relative to the appropriate component of the diet.
‡Sample size was eight oysters in each category; values are means ± SD. The ration was a mixture of three algal species dosed at approximately 2% of dry tissue weight per day.

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slow-growing clams (*R. philippinarum*) with a diploid number of 38, aneuploid cells of $2n = 34$, 35, 36 or 37 were observed in all groups, but slow-growers showed significantly higher levels of aneuploidy (~60%) than fast-growers (~20%) (de Sousa *et al.* 2011). Similar findings have been reported from studies on *C. gigas* (Leitão *et al.* 2001 and references). The genetic or physiological basis of this phenomenon is not yet understood.

Size

In bivalves growth is rapid in the first year(s) of life but progressively slows down with increasing age (see Figure 6.5). For animals the surface area available for oxygen diffusion limits general metabolism. Metabolism is proportional to a constant power of the body weight as described earlier by the allometric equation

$$y = ax^b$$

In this case y is the metabolic rate (measured as oxygen consumption), x is the body size, b is the exponent and a denotes the metabolic rate of an animal of unit weight. While the value of a varies according to factors such as temperature, b approximates 0.75 (Hawkins & Bayne 1992). From this it is clear that as size increases the metabolic rate decreases. This has been verified for clams (*Marcia opima*) with higher weight-specific oxygen consumption values observed in smaller than in larger individuals (Suja 2007). In addition, ingestion rate also declines with increasing size, but at a faster rate than metabolic rate. Both these factors contribute to a decline in growth efficiency (growth per unit of absorbed ration) with size. There is also evidence in mussels that metabolic efficiency *per se* decreases with increasing size (Figure 6.20). But it is probably the increasing proportional allocation of available energy into gamete production that ultimately puts the brake on growth (Griffiths & Griffiths 1987; Hawkins & Bayne 1992). Because of various exogenous and endogenous influences the proportional allocation of energy to somatic growth or reproduction varies widely between species, and even between populations of the same species (see Figures 5.6 and 5.7, Chapter 5).

Growth factors

While there is a significant amount of information on the role that hormones play in bivalve reproduction, there have been few publications on their role in bivalve growth. Growth factors are widespread, locally acting, signalling polypeptides that are involved in the regulation of cell proliferation and differentiation during the development of organs and tissues in embryos and adults (Lelong *et al.* 2000). The majority of growth factors were initially identified in vertebrates. To investigate the possibility that known growth factors may have counterparts in molluscs, studies on the effects of some of these factors on tissue cells were carried out. Various growth factors have now been identified in bivalves, such as molluscan growth and differentiation factor (mGDF), which may play a central role in the biological processes that allow larvae to become competent to metamorphose (Lelong *et al.* 2000). Insulin-related peptides have also been identified and appear to stimulate protein synthesis in mantle-edge cells in *M. edulis* (Kellner-Cousin *et al.* 1994), *C. gigas* (Gricourt *et al.* 2003) and both mantle-edge and digestive gland cells in *P. maximus* (Giard *et al.* 1998). In *C. gigas* the growth-promoting effect was mediated by specific receptors initially characterised and expressed in mantle and mantle-edge cells. Hamano *et al.* (2005) conducted cDNA cloning and expression analysis of the oyster (*C. gigas*) insulin-related peptide gene. The gene showed seasonal variation in expression with the period of highest expression at the onset of body growth and gamete development.

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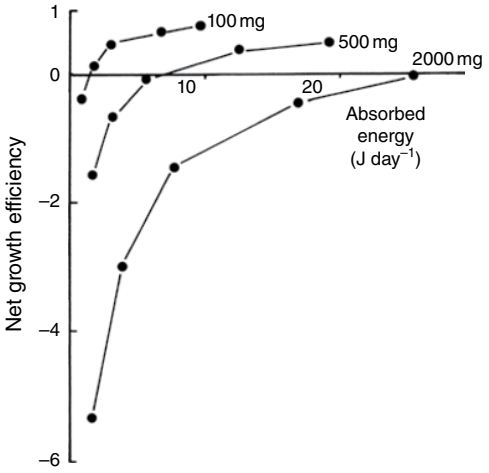


Figure 6.20 Net growth efficiency (net energy balance/net energy absorbed) in relation to absorbed energy and body size (mg total dry tissue) in the mussel *Mytilus edulis*.
Adapted from Hawkins and Bayne (1992) based on data in Thompson and Bayne (1974). Reproduced with permission of Elsevier.

Note

1 Stretches of DNA containing or linked to the genes that underlie a quantitative trait such as growth.

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7 Circulation, respiration, excretion and osmoregulation

Circulation

A detailed description of the heart and associated haemolymph ('blood') vessels is presented in Chapter 2. Briefly, haemolymph flows from the gills into the heart, which contracts to drive the fluid into a single vessel, the anterior aorta, which divides into many arteries. The most important of these are the pallial arteries, which supply the mantle, and the visceral arteries (gastrointestinal, hepatic and terminal) that supply the stomach, intestine, shell muscles and foot with haemolymph (see Figure 2.15, Chapter 2). The arteries break up into a network of vessels in all tissues and these then join to form veins that empty into extensive spaces called sinuses. The haemolymph system is therefore an open circulatory system, with haemolymph in the sinuses bathing the tissues directly. In addition to having a respiratory function these sinuses also serve as a fluid skeleton giving temporary rigidity to various parts of the body, for example labial palps, foot and mantle edges (Morton 1967). From the sinuses the haemolymph flows to the kidneys for purification. From there a branch enters the gill circulation for oxygenation and returns to the heart via the kidney. In some bivalves oxygenated haemolymph from the gills returns directly to the heart. In all bivalves the mantle has an extensive system of haemolymph sinuses, and thus may function as the primary respiratory site.

The haemolymph plays an important role in gas exchange, osmoregulation, nutrient distribution, elimination of wastes and internal defence (see later; also Chapter 11). The haemolymph contains cells called haemocytes that float in a colourless plasma. Most bivalves lack circulating respiratory pigments, although haemocyanin is found in some protobranchs, and haemoglobin has been reported in several heterodont families (Giribet 2008).

Haemolymph plasma and haemocytes

The osmotic concentration of haemolymph is determined chiefly by inorganic ions (Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, SO_4^{2-}) and is equal to, or marginally greater than, the osmotic concentration of seawater (Bayne *et al.* 1976a). The metabolic costs of maintaining a steep

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plasma–seawater osmotic gradient would be prohibitive because of the vast surface areas exposed to the external environment. However, the K^+ concentration of bivalve plasma is greater than seawater by a factor of 1–2 (Burton 1983), which probably reflects the normal intracellular–extracellular K^+ gradient. Haemolymph plasma also contains numerous dissolved organic molecules (Table 7.1), and concentrations fluctuate depending on food ration, gametogenesis and short-term osmoregulation (see later).

Haemocytes are not confined to the haemolymph system but move freely out of the sinuses (haemolymph spaces) into surrounding connective tissue, mantle cavity and gut lumen. Haemocytes represent the most important internal defence mechanism in marine bivalves (Chapter 11). Haemocytes also have a digestive function, ingesting particles of nutritional value that are too large to enter the cells of the digestive gland (Chapter 4). Several distinct categories of haemocytes have been described using a combination of morphology, cytochemistry and function, as well as flow cytometry, density gradient centrifugation and monoclonal antibodies that react with haemocyte surface proteins (Aladaileh *et al.* 2007). These authors identified three major types of haemocytes in the Sydney rock oyster *Saccostrea glomerata*. Haemoblast-like cells, which comprised about 15% of the total haemocyte population, were the smallest ($4.0 \pm 0.4\,\mu m$ diameter) cells observed and had a spherical shape with a high nucleus/cytoplasm ratio. Hyalinocytes, representing about 46% of all haemocytes, were large cells ($7.0 \pm 1.0\,\mu m$) with a low nucleus/cytoplasm ratio and few cytoplasmic granules. Four discrete sub-populations of hyalinocytes were identified. The third major cell type comprised granulocytes, large cells ($9.3 \pm 0.3\,\mu m$) with cytoplasm containing many granules and comprising 38% of the haemocyte population. Five sub-populations of granulocytes were identified based on the types of granules in the cytoplasm. Other, albeit similar, haemocyte classifications have been reported in mussels (Carballal *et al.* 1997), scallops (Zhang *et al.* 2005), clams (Cima *et al.* 2000; Allam *et al.* 2002) and oysters (Xue *et al.* 2000; Hégaret *et al.* 2003; Donaghy *et al.* 2009). Granulocytes contain high levels of intracellular enzymes that enable them to kill pathogens after phagocytosis. This cell type also plays a prominent role in encapsulation, a process used by molluscs to sequester pathogens that are too big to be phagocytosed (see Chapter 11). Hyalinocytes play a lesser role than granulocytes in phagocytosis but have been shown to play a central role in haemocyte aggregation processes associated with wound healing. Unlike granulocytes and hyalinocytes, haemoblast-like cells do not contribute to defensive responses like phagocytosis or encapsulation, and they lack the common intracellular enzyme systems associated with host defence, suggesting that they may act as stem cells for the other two cell types (Aladaileh *et al.* 2007).

Table 7.1 Organic constituents in the haemolymph of the mussel *Mytilus edulis* and the scallop *Placopecten magellanicus*.

	Concentration (mg 100 ml^{-1})	
	M. edulis	***P. magellanicus***
Protein	115–282	153 ± 28
Non-protein nitrogen	3.5–23.4	No data
Ammonia	0.31–1.79	0.24 ± 0.12
Sugars	9.8–35.7	No data
Total carbohydrate	No data	5.2 ± 1.2
Lipid	20.4–84.3	13.7 ± 2.4

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Values for *M. edulis* were over an annual cycle (Bayne 1973a), while those for *P. magellanicus* (means and standard deviations) were collected after a single sampling event (Thompson 1977).

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Heart rate

Several methods have been used to study heart rate in molluscs. Investigators have drilled windows in the valves of bivalves to view the heart directly, a method that was later modified by covering the windows with glass or cellophane. Subsequently, an electrode of platinum, silver or stainless steel inserted through the shell into the pericardial cavity around the heart was used (references in Bayne *et al.* 1976a). This method, which has been modified in recent years (Nicholson 2002; Braby & Somero 2006), creates minimal disturbance to the animal and heart rate can be monitored from a distance. Depledge and Andersen (1990) developed a non-invasive method for continuously monitoring heart rate, which involved cementing transducers to arthropod and bivalve shells. Later, Haefner *et al.* (1996) used a non-invasive ultrasound technique to monitor heart rate in the mussel *Mytilus edulis*. This method involved positioning an ultrasound probe, parallel to the long axis of a submerged mussel. Dual video monitors provided a rectilinear image scan of the heart as well as its frequency of movement. A recent technique used infrared sensors that were glued externally to the left shell valve close to the heart position. Signals obtained from the heart were amplified and filtered by using a special amplifier card and then detected by means of a portable oscilloscope (Fluke™125) connected to a laptop computer equipped with the Fluke View 3.0 software (Sarà & de Pirro 2011; see also Burnett *et al.* 2013). These non-invasive methods are reliable, not just for physiological investigations, but can also enhance behavioural analysis.

Heart beat rate (HBR) has been used successfully in bivalves as a proxy for whole-animal stress in response to toxins, temperature, salinity and predation (references in Braby & Somero 2006). HBR increases linearly with ambient temperature (Figure 7.1). The upper thermal limit varies for each species but is coincident with the temperature at which other physiological processes are disrupted. For example, in *M. edulis* this temperature is 25–27°C, whereas for *Perna perna* it is as high as 32°C (Bayne *et al.* 1976a). Salinity also affects heart

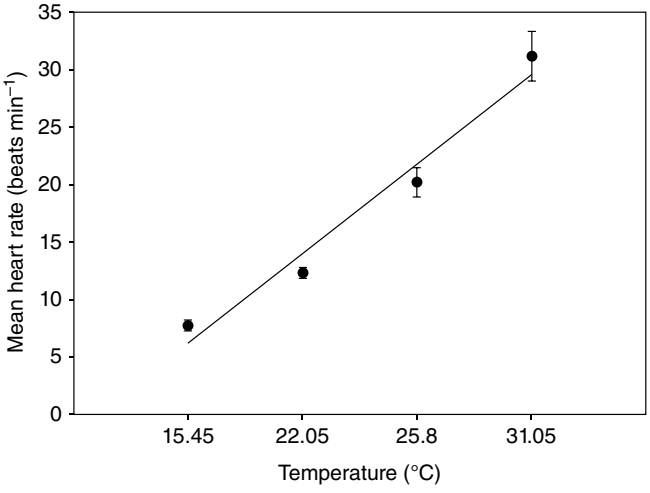


Figure 7.1 Relationship between mean (±SE, $n = 5–7$ individuals) heart rate and temperature in immersed *Perna viridis*. Line represents regression equation where $y = 7.812 + 3.072x$; $r = 0.99$; $P < 0.0001$. The upper tolerance limits for cardiac activity were between 30 and 35°C. When seawater temperature was allowed to return to ambient conditions prior to thermal manipulation cardiac activity returned to initial rates, indicating that elevated heart rate shown by mussels exposed to the higher temperatures were transient.
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activity by interfering with the osmotic balance of heart tissue. Changes in HBR of *Brachidontes pharaonis* (an invasive species) and *Mytilaster minimus* (a native Mediterranean species), in response to changes in salinity values from brackish (20 psu) to extreme hyper-saline (75 psu) water, were investigated both *in situ* and in the laboratory (Sarà & de Pirro 2011). The two mussel species displayed different responses to varying salinity, with clear differences in HBR observed between groups investigated at different salinities and between groups originating from different environments. The native species, adapted to marginal salinity changes as found in the Mediterranean Sea, showed signs of stress at salinities slightly above 37 psu (in the range 37–45 psu). On the other hand, the invasive species displayed clear signs of stress only at salinities over 45 psu and exhibited heart activity even under hyper-saline conditions, indicating that the invasive species has the potential to invade most of the transitional environments across the entire Mediterranean basin. The effects of various contaminants on cardiac activity are well documented. For example, exposure to heavy metals causes a marked decline in cardiac activity (bradycardia) in mussels. This could be due to the direct effects of metals on nervous control of the mussel heart, rather than to valve closure and consequent hypoxia in response to elevated metal concentrations (Rajkumar 2009). The heart rate of *M. edulis* increases significantly when it is in the presence of effluent from the dogwhelk *Nucella lapillus*, probably due to an olfactory-mediated perception of predation threat (Rovero *et al.* 1999). When attacked through a drilled hole mussel heart rate increases significantly, but when attacked by penetration between the valves heart rate decreases throughout the attack, supporting the hypothesis that dogwhelks that penetrate between the valves induce muscular paralysis by injection of toxins. Other factors such as body size, food, oxygen availability and aerial exposure affect heart rate. As these same factors also affect oxygen consumption they will be discussed in more detail in the following section.

Respiration

The gill filaments are essentially hollow tubes within which the haemolymph circulates. Haemolymph flows from the kidney to the gill via the afferent gill vein, which gives off to each filament a vessel that descends one side and ascends the other. The ascending vessels join to form the efferent gill vein that returns to the kidney, or goes directly to the heart (Figure 2.15, Chapter 2). As water passes through the gills, oxygen from the water diffuses into the haemolymph. However, the efficiency with which this occurs is low, usually in the range of 1–13%, although this figure can rise up to 30% or more by an increase in the heart rate (Bayne *et al.* 1976b).

Several methods for measuring oxygen consumption rate ($\dot{V}O_2$), a proxy for metabolic rate (MR), have been described (Peck *et al.* 2002; Goulletquer *et al.* 2004; Jansen *et al.* 2009; Brown *et al.* 2010). One method used to measure ($\dot{V}O_2$) in the ocean quahog *Arctica islandica* is that described by Begum *et al.* (2009). Animals were maintained for 3 days without food and then allowed to accommodate to the respiration chambers overnight. The chambers were Perspex tubes that allowed adjustment of chamber volume (100–600 ml) depending on animal size. Three respiration chambers, each with one animal, and a control chamber without an animal were used for simultaneous measurement in each experiment. Temperature was kept constant throughout the experiment. Oxygen microoptodes connected to a MICROX TX3 array (PreSens, Neuweiler, Germany) were used to record at regular intervals the oxygen content of water flowing into and out of the respiration chambers. After each measurement the animal was carefully removed from the chamber and

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oxygen consumption was recorded for another 3 h to determine the microbial oxygen demand in the respective chamber. Soft tissues of each individual were dried to obtain dry mass (DM), and then combusted to calculate ash-free dry mass (AFDM = DM − ash). Individual mass-specific respiration rates (MSR, μmol O^2 h^{-1} $gAFDM^{-1}$) were calculated according to

$$\mathrm{MSR} = \frac{\dot{V}\mathrm{O}_2}{\mathrm{AFDM}}$$

To gain a full understanding of oxygen consumption in a given species, measurements should be made at different times of the year and include animals of different sizes, and at different reproductive stages. Values for weight-specific oxygen consumption of a variety of bivalves are presented in Table 7.2. For some of these species values for aerial O_2 consumption (see later) are also included.

Table 7.2 Weight-specific oxygen consumption rates ($\dot{V}O_2$) for a variety of bivalves.

	O_2 uptake (O_2 ml h^{-1} g^{-1})			
Species	**Temperature (°C)**	**Water**	**Air**	**References**
Mytilus edulis	10	0.37	0.017	Vahl (1973)
	15	0.38*	–	Bayne (1973b)
	–1–4.8	0.12–0.28	–	Loo (1992)
Mytilus galloprovincialis	25	0.33	0.044	Widdows *et al.* (1979)
Mytilus californianus	13	0.23	0.17	Widdows *et al.* (1979)
Cerastoderma glaucum	15	0.15	0.012	Boyden (1972)
Cerastoderma edule	15	0.20	0.13	Boyden (1972)
Crassostrea gigas	Ambient†	0.51	–	Bernard & Noakes (1990)
Crassostrea virginica	10	0.171	–	Dame (1972)
	20	0.372	–	
	30	0.423	–	
Ostrea edulis	5	0.364	–	Rodhouse (1978)
	15	0.962	–	
	25	2.655	–	
O. edulis larvae	–	3.0–6.0	–	Holland & Spencer (1973)
Mya truncata	Ambient†	0.63	–	Bernard & Noakes (1990)
Argopecten irradians	10.5	0.43	–	Bricelj *et al.* (1987)
Placopecten magellanicus	10	0.24	–	Shumway *et al.* (1988)
Patinopecten yessoensis	9	0.29	–	Fuji & Hashizume (1974)
Chlamys islandica	8	0.20	–	Vahl (1978)
Chlamys hasata	Ambient†	0.38	–	Bernard & Noakes (1990)
Chlamys varia	10	0.34	–	Shafee (1982)

Data from Bayne and Newell (1983), Bernard and Noakes (1990) and Bricelj and Shumway (1991).
–, indicates no data.
**Mytilus edulis* can acclimate its routine oxygen consumption between 10 and 20°C.
†experiments carried out in a flow-through system at near-ambient summer temperature, Vancouver Island, British Columbia, Canada.

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Factors affecting oxygen consumption

Size

The relationship between body size (usually expressed as weight) and the rate of oxygen consumption ($\dot{V}O_2$) is generally described by the allometric equation $y = ax^b$ where y is $\dot{V}O_2$, x is the body weight, and a, the intercept, and b, the slope, are fitted parameters. The intercept a is a measure of $\dot{V}O_2$ of an individual of unit weight (or length), and its value changes depending on environmental conditions and on the species being tested. The slope b is a measure of the rate of increase in $\dot{V}O_2$ with size. Bayne and Newell (1983) reported that the value of b in 11 different bivalve species ranged between 0.44 and 1.09, with a mean value of 0.728 ± 0.130. This value is very similar to the mean value of 0.76 reported by Bricelj and Shumway (1991) for 8 species of scallop. Table 7.3 presents data on the relationship between

Table 7.3 Parameters a and b of the allometric equation $y = ax^b$ relating oxygen consumption ($\dot{V}O_2$ ml O_2 h^{-1}) and tissue weight (g) in a variety of bivalve species.

Species	Size range	Temperature (°C)	*a*	*b*
Argopecten irradians irradians	0.47–2.99	17.4	0.931	0.725
	0.84–2.86	10.5	0.368	0.733
	0.87–4.37	1.5	0.065	0.986
Placopecten magellanicus				
10 m	1.8–42.0	10–12	0.339	0.78
31 m	0.5–25.0	5.5–7.2	0.234	0.79
Chlamys islandica				
Immature	0.02–0.9	5.7*	0.145	0.486
Mature	0.5–5.0		0.251	0.567
Mature	0.4–6.0		0.242	0.759
Patinopecten yessoensis	0.5–15.0	22.4	0.579	0.817
		14.8	0.398	0.777
		5.8	0.181	0.862
Crassostrea virginica	–	10	0.171	0.734
		20	0.372	0.710
		30	0.423	0.603
Ostrea edulis	0.1–2.7	10	0.281	1.079
		15	0.392	0.940
		20	0.756	0.825
		25	1.072	0.886
		30	1.635	0.726
Crassostrea gigas	0.1–3.0	5	0.422	0.777
		10	0.448	0.823
		15	0.868	0.775
		20	0.860	0.945
		25	1.288	0.865
Modiolus demissus	–	14	0.260	0.690
		22	0.629	0.798
Mytilus edulis				
Winter	–	–	0.549	0.744
Summer			0.339	0.702
Mytilus californianus				
Fed	–	–	0.540	0.650
Starved			0.230	0.650

Data from Bayne and Newell (1983), Bricelj and Shumway (1991). Bougrier *et al.* (1995) and Haure *et al.* (1998).
In scallops, weights are dry tissue weights.
*value applies to all three size classes.
–, indicates no data.

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body size (g) and oxygen consumption for some of these species. However, as $\dot{V}O_2$ has been measured under a range of environmental conditions it is difficult to examine the effect of size *per se* on $\dot{V}O_2$ from the data in this table (but see Table 7.2. for comparisons of $\dot{V}O_2$ in larvae versus adults of the oyster *Ostrea edulis*). For example, $\dot{V}O_2$ has been measured in three weight classes of *Argopecten irradians irradians* but each of these measurements has been taken at three different temperatures, making it impossible to separate the individual effects of size and temperature on $\dot{V}O_2$. A more recent study looking at $\dot{V}O_2$ in three different size groups of clams (13–23, 28–35 and 41–46 mm), but using a constant temperature, found that small individuals had a higher $\dot{V}O_2$ than larger ones (Suja 2007). Indirect evidence that $\dot{V}O_2$ is influenced by body size comes from MR studies where MR has been shown to decrease with increasing body size (see Chapter 6).

Food

In the absence of particulate food, $\dot{V}O_2$ declines to a steady-state condition, called the 'standard (basal) rate', which is typical of an animal with shell valves open but showing minimal feeding activity. With a return to feeding there is a marked increase in $\dot{V}O_2$ to the 'active rate'. There can be as much as a fivefold difference between these two rates (Figure 7.2). Between active and standard rates there are a variety of 'routine rates' that are dependent on variations in filtration or ventilation rates, which in turn are dependent on factors such as animal size, ration, season and gametogenesis (Bayne *et al.* 1976b; MacDonald *et al.* 2006 and references). The relationship between $\dot{V}O_2$ and food supply is complex, involving not only energy expenditure associated with the mechanical process of filtration, but also the physiological costs of digestion and excretion. In *Mytilus californianus* Bayne *et al.* (1976c) have estimated that the mechanical cost of feeding is 18% of ingested ration, while the physiological cost is about 6%. For *M. edulis* the equivalent values were 24% and 4%, respectively (Bayne & Scullard 1977a). However, in *M. chilensis*, while the physiological costs of feeding were similar to those observed for *M. californianus* and *M. edulis*, the mechanical costs were about 5%, reflecting the tendency of this species to reduce its filtration rate in response to increased ration (Navarro & Winter 1982). But in a more recent study an even lower value of ~1% of mechanical costs relative to total metabolic expenditure has been reported for *M. edulis* (Riisgård & Larsen 2001).

Temperature

Temperature is one of the major factors influencing $\dot{V}O_2$ and MR in marine bivalves (Table 7.2). Changes in temperature elicit two types of responses. If the animal is subject to a sudden change in temperature it responds by a change in $\dot{V}O_2$, which may initially result in overshoot, but which is followed by a period of stabilisation that takes place over a period of minutes or hours. If the temperature change persists over days or weeks there is a gradual adjustment in $\dot{V}O_2$ to a level comparable with that preceding the temperature change (Bayne & Newell 1983). However, these responses are by no means universal among bivalves (see later).

Acute temperature response

The effect of change in temperature on $\dot{V}O_2$ is often expressed in terms of temperature coefficient or Q_{10} values:

$$Q_{10} = \left(\frac{V_2}{V_1}\right)^{10/(t_2 - t_1)}$$

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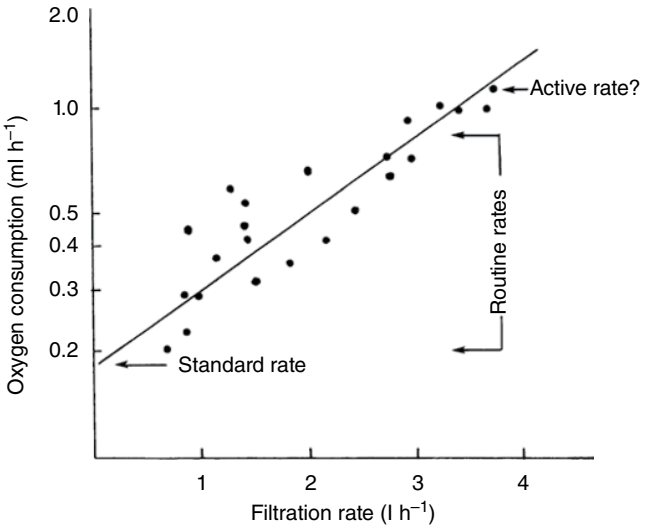


Figure 7.2 The relationship between rate of oxygen consumption and filtration rate in the mussel *Mytilus californianus* (1 g dry flesh weight at 13°C). The metabolic cost of filtration increases logarithmically with filtration rate. The standard metabolic rate is estimated by extrapolation of the regression line to a point where filtration activity is zero (0.19 ml h^{-1}). However, it is not possible to identify active metabolic rate from plots such as this.
From Griffiths and Griffiths (1987). Reproduced with permission of C.L. Griffiths.

where V_1 and V_2 are the rates of oxygen consumption at temperatures t_1 and t_2, respectively. The Q_{10} provides an index of the dependence of the physiological rate on temperature (Bayne *et al.* 1976b). In bivalves Q_{10} values range from 1.0 (temperature-independent) to more than 2.5, which represents over a doubling of rate with each 10°C increase in temperature. The values obtained are dependent on thermal history of the animal, body size, activity and reproductive condition.

Temperature effects on $\dot{V}O_2$ are usually depicted in the form of rate/temperature or R/T curves. One such curve is illustrated in Figure 7.3. Here the acute responses of standard, routine and active rates of $\dot{V}O_2$ in the mussel *M. edulis* (1 g dry flesh weight, sexually mature and acclimated to 15°C) are illustrated over the temperature range 5–25°C (Widdows 1973). Between 10 and 20°C the Q_{10} values for standard, routine and active rates were 1.6, 2.4 and 1.9, respectively. Lower values (1.0–1.2) for the standard rate of oxygen consumption have been reported in young, sexually immature *M. edulis* (Newell & Pye 1970), and in *M. californianus* after spawning (Bayne *et al.* 1976d). The fact that standard, as opposed to routine, $\dot{V}O_2$ is relatively independent of temperature allows *M. edulis* resources to be conserved during stress periods, such as those of high temperature and low food availability experienced during aerial exposure in summer (Table 7.2). Routine rate is temperature-dependent, but this is less crucial to the animal as increased ventilation is usually accompanied by increased filtration. However, in some species, for example the scallop *Chlamys varia*, both standard and routine rates are temperature-dependent (Shafee 1982), which means that the species experiences a metabolic deficit during periods of aerial exposure in summer (see later). Unlike *Mytilus* this species is limited to low-shore levels, where environmental temperatures are more uniform.

The degree of separation (Figure 7.3) of routine and standard rates at any given temperature is taken as an estimate of scope for routine activity, for example feeding (Newell 1979). For *Mytilus* this tends to be greatest at the upper end of the normal thermal range, but declines at both the upper and lower limits of temperature tolerance.

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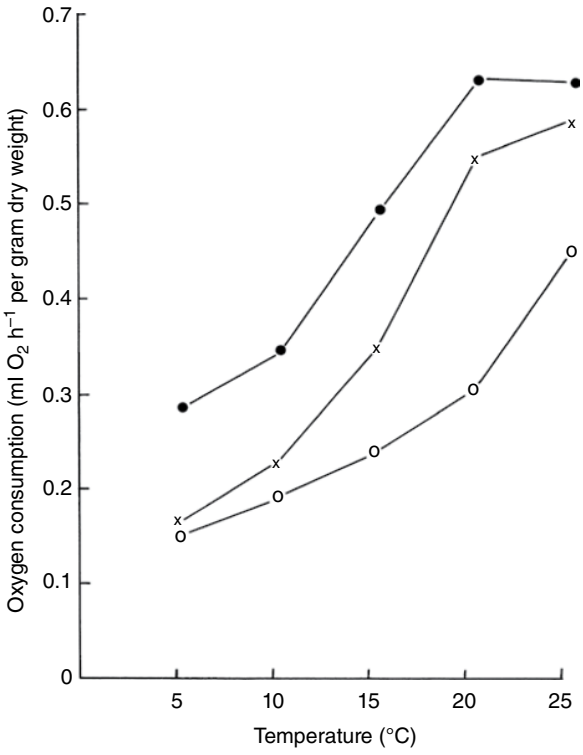


Figure 7.3 The effects of temperature on the standard (○), routine (×) and active (●) rates of oxygen consumption of the mussel *Mytilus edulis* acclimated to 15°C.
From Bayne *et al.* (1976b). Reproduced with permission of Cambridge University Press.

Acclimation to temperature change

Bivalves experience quite different temperature regimes depending on the season, and in the case of intertidal species can also experience daily fluctuations in temperature associated with the tidal cycle. Some bivalves respond to temperature changes lasting days or weeks by adjusting $\dot{V}O_2$ to a level comparable with that which occurred before the temperature changed. This process of adjustment is called acclimation and has been demonstrated in many bivalve species to date. In *M. edulis* collected from 10°C waters and acclimated to 5 and 15°C, Widdows and Bayne (1971) recorded an initial 50% decrease in $\dot{V}O_2$ at 5°C and a doubling at 15°C. However, rates subsequently declined in the warm-adapted group and increased in the cold-adapted group so that after 14 days both groups had approximately the same $\dot{V}O_2$ as the control mussels (Figure 7.4). In *Mytilus* compensation is achieved by shifting the rate–temperature curve to the right following warm acclimation. This means that the animal tolerates higher temperatures but still maintains $\dot{V}O_2$ levels relatively constant. This acclimation process ensures that respiration and also feeding and excretion are maintained at approximately constant levels, but this is only possible over a temperature range that is species-dependent. In oysters, for example *O. edulis* (Newell *et al.* 1977) and *Crassostrea virginica* (Shumway & Koehn 1982), there is no evidence of metabolic adjustment following warm acclimation. When the oyster *O. edulis* was acclimated to temperatures ranging between 5 and 25°C for 70 days $\dot{V}O_2$ was positively correlated with temperature (Figure 7.5). The increase in energy expenditure with increasing temperature was offset by increased filtration rate, thus illustrating that this species uses a different compensatory strategy than *M. edulis*.

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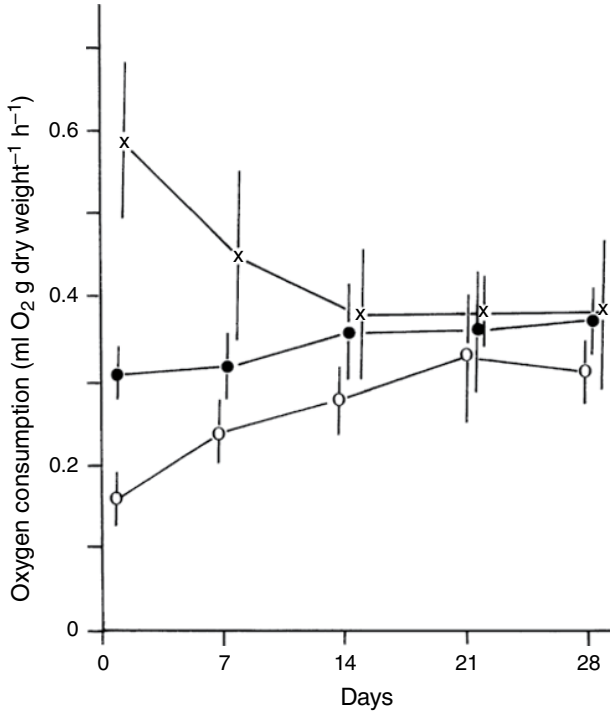


Figure 7.4 Oxygen consumption in the mussel *Mytilus edulis* acclimated to 5°C (○) and 15°C (×) compared with mussels at 10°C (●; ambient temperature in November).
From Widdows and Bayne (1971). Reproduced with permission of Cambridge University Press.

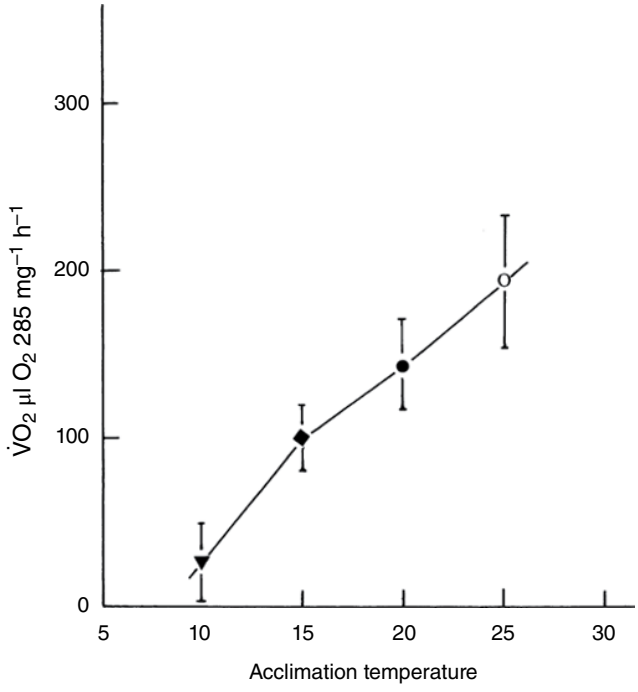


Figure 7.5 Routine rates of oxygen consumption in the oyster *Ostrea edulis* following acclimation to temperatures of 10°C (▼), 15°C (♦), 20°C (●) and 25°C (○) for up to 70 days.
From Newell *et al.* (1977). Reproduced with permission of Springer Science and Business Media.

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Temperature tolerances

Critical temperatures (T_c) for marine invertebrate and fish species are characterised by a switch from aerobic to anaerobic metabolism, caused by a mismatch of O_2 demand and O_2 supply. Extended exposure to temperatures above high T_c or below low T_c finally leads to death of the animal unless thermal acclimation, that is a shift of T_c values, occurs. Tolerance windows in marine species, estimated by lethal and T_c limits, are related to climate regime. Ectotherm species in Antarctic and tropical regions have narrow thermal tolerance windows compared to temperate species, which show broad thermal tolerance windows that reflect the higher variation in their environmental temperatures. In Antarctic waters temperature variation is about 1–2°C, similar to the 1–3°C in tropical waters, but very different from the 10–15°C reported for temperate waters (references in Peck *et al.* 2010). This relationship between climate variation and thermal tolerance windows has been termed the climate variability hypothesis. To test this hypothesis Compton *et al.* (2007) compared the upper and lower lethal thermal tolerance limits of numerous bivalve species from a tropical (Roebuck Bay, northwest Australia) and a temperate location (Wadden Sea, northwest Europe), and found that species from the tropical site had higher upper and lower lethal thermal limits than species from the temperate site (Figure 7.6). However, Wadden Sea species were able to survive freezing temperatures and this resulted in thermal tolerance windows that were on average 7°C higher than for Roebuck Bay species. In addition, when they reviewed previous studies on a global scale, they found that upper lethal thermal limits of tropical species were closer to maximum habitat temperatures than the upper lethal thermal limits of temperate species, suggesting that temperate species are better adapted to temperature variation, findings that are consistent with the climate variability hypothesis.

Adaptation and acclimation to low temperatures

In the past two decades there has been considerable interest in how polar ectotherms survive in what is regarded as an extremely harsh environment. In the Antarctic benthic invertebrates exhibit slow growth, low production-to-biomass ratios, prolonged longevity and low activity (Heilmayer & Brey 2003). It has been postulated that their MRs should be elevated to compensate for the physiological constraints imposed by living at temperatures near or below 0°C. This has been referred to as 'metabolic cold adaptation' (see Peck 2002 for review). However, in the past two decades data have shown that a low standard or routine MR is the norm for polar ectotherms, including bivalves (Peck & Conway 2000 and references therein; Heilmayer & Brey 2003). When $\dot{V}O_2$ was measured at 0 ± 0.2°C in two Antarctic bivalves, *Laternula elliptica* and *Cyclocardia astartoides*, values were 46.3 µl O_2 g dry tissue mass^{-1} h^{-1} in *C. astartoides*, and 239 µl O_2 g dry tissue mass^{-1} h^{-1} in *L. elliptica*. These values are low compared to species from temperate and tropical locations (Peck & Conway 2000; Figure 7.7). Although there was a fivefold difference between the two values, both were within the range reported for other polar bivalves. Moreover, when the data were compared with those for bivalves from other latitudes they found that such variability is characteristic of the group as a whole and not just of polar latitudes (Figure 7.7). Reduced metabolism may be an important strategy of energy conservation for bivalves in the Antarctic, where food may be in short supply for up to 9 months of the year (Ahn & Shim 1998).

There are few data on the upper temperature limits of polar bivalves. A value of +4.5 and +6°C has been reported for *Limopsis marionensis* (Pörtner *et al.* 1999) and *L. elliptica* (Pörtner *et al.* 2006), respectively, which is probably representative of other polar bivalves, but is two to five times less than for temperate or tropical bivalves (Peck & Conway 2000). Using acute upper lethal temperatures as a metric for ability to acclimate, Peck *et al.* (2010) found that five out of six polar species tested, including the infaunal bivalve *Yoldia eightsi*, failed to acclimate over 60 days to temperatures only 3.5°C above the annual average, and

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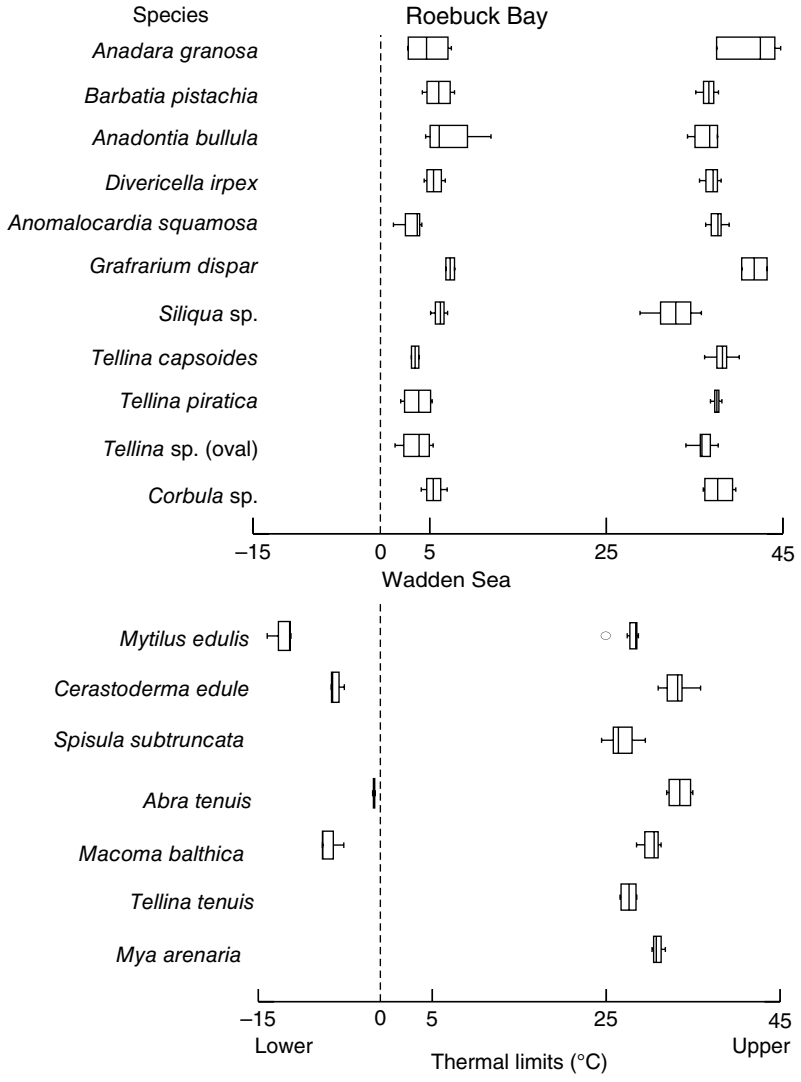


Figure 7.6 Upper and lower lethal thermal limits of bivalve species from Roebuck Bay (northwest Australia) and the Wadden Sea (northwest Europe). The boxplot of each species represents the range of temperature treatment basin, day and season estimates. The stippled line represents the 0°C value. A boxplot displays the median, the upper and lower quartile. The values lying outside of the box are shown as whiskers, and a large outlier is indicated by a circle.
From Compton *et al.* (2007). Reproduced with permission of Elsevier.

1–2°C above the current summer maximum. Poor acclimation capacity has also been reported for *L. elliptica* (Morley *et al.* 2012). Polar ectotherms may need periods in excess of months before acclimination has been achieved. Interestingly, doubling the oxygen content of ambient seawater raised the upper temperature limits of the Antarctic marine bivalve *L. elliptica* by ~2.5°C, thus providing further evidence that oxygen limitation through reduced aerobic capacity (see earlier) is the limiting factor in acclimation to increased temperatures (Pörtner *et al.* 2006). However, Peck *et al.* (2009, 2014) take a wider view and argue that the capacity to acclimate depends on the *rate* of temperature change and that a range of mechanisms will limit survival and resistance to temperature change, particularly

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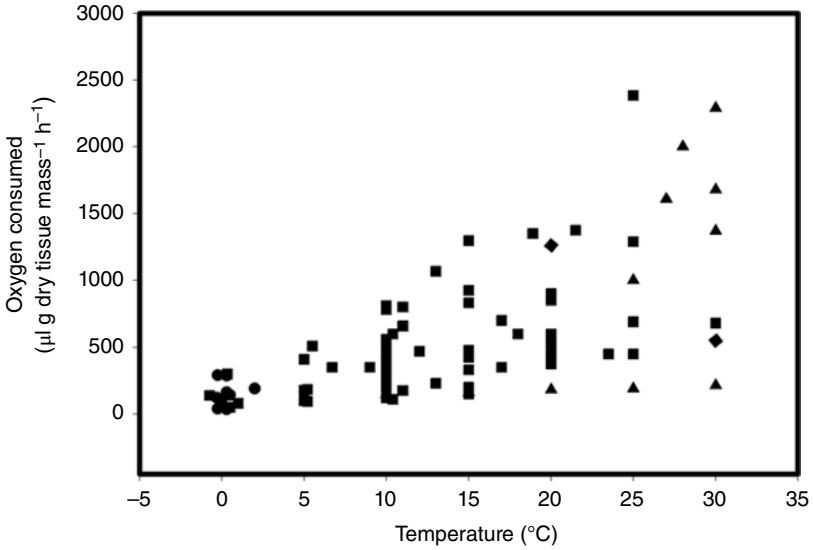


Figure 7.7 Oxygen consumption values (µl O_2 g dry tissue mass^{-1} h^{-1}) for bivalve molluscs from different latitudes. Data presented are resting routine or standard rates for species at their normal temperatures. Where data were available for winter and summer rates for species inhabiting a wide temperature range, more than one value is included. ●, polar species; ■, temperate species; ▲, tropical species.
See Peck and Conway (2000). Reproduced with permission of the Geological Society, London.

in the long term. These mechanisms include other physiological tolerances such as altered immune responses, changes in reproductive capacity, energy trade-offs associated with metabolism at higher temperatures and ecological interactions such as larval dispersal and recruitment (see Peck *et al.* 2010 for more details).

Thermal acclimation involves not just compensatory adjustments in $\dot{V}O_2$ and differential utilisation of metabolic pathways, for example shift from aerobic to anaerobic metabolism, but also changes in enzyme activities, changes in membrane composition and up-regulation of stress protein genes.

Salinity

Many bivalves are euryhaline, that is they can tolerate an extremely wide range of salinity in their natural environment. The salinity range tolerated is, however, species-dependent. For example, *M. edulis* is found from fully marine conditions to salinities as low as 4–5 psu, while the lowest salinities tolerated by *M. galloprovincialis* and *M. californianus* are 12 and 17 psu, respectively (references in Bayne *et al.* 1976b). *C. virginica* appears to have a higher tolerance of salinity fluctuation than other oyster species (Berquist *et al.* 2006), with self-sustaining populations occurring in salinities between 5 and 35 psu (Buroker 1983; Wilson *et al.* 2005).

When the respiration rates of mussels from three different salinities (5–6, 15 and 30 psu) were measured in the field they were found to be similar (Remane & Schlieper 1971). However, if mussels are exposed to a sudden change in salinity the immediate response is to close the shell valves. The mussel *Guekensia demissa* may remain shut for up to 7–10 days, depending on the extent of the salinity change (Pierce 1971). During this time the valves open periodically for short periods of time and eventually ventilation is resumed, or else death occurs. During this initial period of 'shock', respiration rate is depressed. However,

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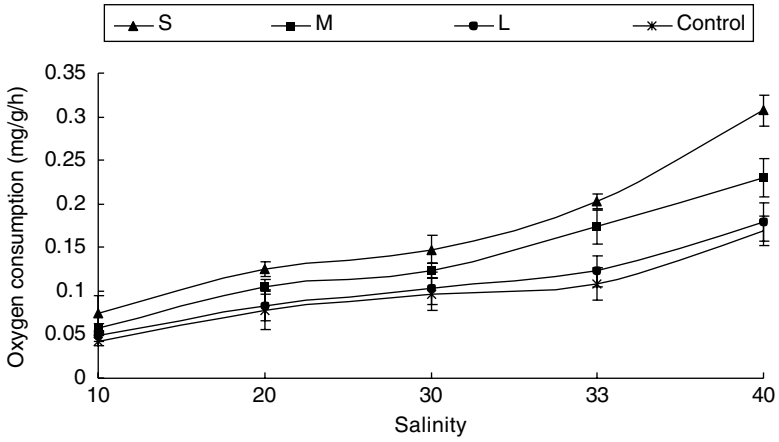


Figure 7.8 Oxygen consumption rates of *Scapharca broughtonii* spat at different salinity values. Small (S) spat (▲) had a shell length of 11.8 ± 2.1 mm, medium (M) spat (■) were 21.8 ± 1.1 mm and large (L) spat (●) were 30.1 ± 3.0 mm. Adults (71.2 ± 2.7 mm) were used as a control. From Kang *et al.* (2008). Reproduced with permission of John Wiley & Sons.

should the new salinity regime persist there is gradual acclimation, which may take as long as 4–7 weeks, depending on the magnitude of the salinity change, the ambient temperature and the size of the animal (Bayne & Newell 1983). The length of the acclimation period far exceeds the 2 weeks normally required for temperature acclimation, probably a reflection of the time needed for intracellular osmotic regulation (see osmoregulation later). However, a higher acclimation capacity for salinity than for temperature, both for chronic and acute conditions, has been reported in the mussel *P. perna* (Resgalla *et al.* 2007). Oxygen consumption rates at different salinities may also be related to shell size. For example, in the ark shell *Scapharca broughtonii*, $\dot{V}O_2$ increased with increasing salinity (10–40 psu), but rates were higher in small versus larger spat, and the lowest rates were observed for adults (Kang *et al.* 2008; Figure 7.8).

There is not much information on the combined effects of salinity and temperature on $\dot{V}O_2$. Shumway and Koehn (1982) measured the acclimated and acute rates of $\dot{V}O_2$ in *C. virginica* at three temperatures (10, 20 and 30°C) and three salinities (7, 14 and 28 psu) giving nine temperature–salinity regimes. They found that as acclimation salinity decreased the effect of exposure temperature became more pronounced, and the effect of exposure salinity decreased. As acclimation temperature increased the effect of exposure salinity decreased and the effect of exposure temperature increased (Table 7.4). They also demonstrated that oysters regulated $\dot{V}O_2$ when exposed to declining oxygen tension at all of the temperature–salinity combinations tested, but in general the degree of regulation decreased with increasing temperature or decreasing salinity. Oysters are thus able to utilise available oxygen over a wide range of temperature–salinity–oxygen combinations without resorting to anaerobiosis.

Oxygen availability

Bivalves frequently experience periods of hypoxia, that is dissolved oxygen (DO) concentrations below 2 mg l^{-1}. For example, low oxygen concentration is a normal occurrence for species that live deep within soft sediments, or for intertidal species during shell closure on the low tide, or in crowded tide pools. Reduced O_2 concentration can also be due to environmental conditions such as stagnation or pollution of the water column. Over the past

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Table 7.4 Oxygen consumption ($\dot{V}O_2$, ml O_2 $0.4\,g^{-1}\,h^{-1}$) in the oyster *Crassostrea virginica* acclimated to nine salinity–temperature combinations, and exposed to the same nine combinations.

	Experimental conditions								
	28 psu			**14 psu**			**7 psu**		
Acclimation conditions	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**	**10°C**	**20°C**	**30°C**
28 psu									
10	*0.0531*	0.1211	0.2174	0.0951	0.1713	0.4122	0.1484	0.3603	0.5068
20	0.0335	*0.0962*	0.2196	0.1748	0.2100	0.5193	0.1590	0.2505	0.4248
30	0.1210	0.0594	*0.1783*	0.1122	0.3965	0.6742	0.0408	0.2344	0.5264
14 psu									
10	0.0809	0.2005	0.2916	*0.0933*	0.2298	0.4710	0.1072	0.2489	0.4349
20	0.0542	0.2166	0.4007	0.0892	*0.1845*	0.4538	0.0693	0.2740	0.4901
30	0.0652	0.2114	0.2351	0.0472	0.3171	*0.6216*	0.0351	0.3512	0.5532
7 psu									
10	0.0582	0.2849	0.4500	0.1040	0.2925	0.4849	*0.1371*	0.2752	0.5191
20	0.1224	0.2491	0.4907	0.1352	0.2542	0.4671	0.1114	*0.2464*	0.4624
30	0.0867	0.2296	0.5243	0.0402	0.2003	0.4351	0.0704	0.3001	*0.5031*

From Shumway and Koehn (1982). Reproduced with permission of Inter-Research, Germany.
Values in italics on the diagonal are acclimated rates. Each value is the mean of six determinations. Mean standard deviation for all determinations is 0.029.

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few decades there has been an exponential increase (>5.5% year^{-1}) in the number of coastal sites reporting hypoxic conditions. This growth is expected to increase in the coming decades due to the combined effects of the continued spread of coastal eutrophication and global warming (Vaquer-Sunyer & Duarte 2008).

In some species, for example the mussel *G. demissa*, the rate of oxygen consumption is directly dependent on the ambient O_2 concentration, which therefore declines as the O_2 concentration surrounding the gill surface is reduced. Such species are called 'oxyconformers'. In the face of declining O_2 concentration other species are able to maintain a constant rate of oxygen consumption. Such species, termed 'oxyregulators', do this by increasing ventilation rate, or by increasing the efficiency with which oxygen is removed from the water, or a combination of these two strategies (Bayne & Newell 1983). For example, the clam *Arctica islandica* more than doubles the volume of water pumped per unit time as O_2 concentration (pO_2) falls from 160 to 60 mm Hg (Taylor & Brand 1975); in *M. edulis* extraction efficiency can more than treble as pO_2 falls from 160 to 20 mm Hg (Bayne 1971). Oysters (*Crassostrea* and *Ostrea* spp.) are also strong regulators (Shumway 1982 and references therein). The distinction between conformers and regulators is, however, more apparent than real. Species that regulate only maintain a constant rate of oxygen consumption down to some minimum 'critical' oxygen tension that is species-dependent. Individuals within a species may also show different responses to hypoxia, depending on body size (Wang & Widdows 1991; Hole *et al.* 1995), temperature (Hicks & McMahon 2005; Jansen *et al.* 2009) or food availability (Morley *et al.* 2007). Some species, for example *A. islandica* survive hypoxic conditions by depressing their MR and using anaerobic fermentative pathways to produce adenosine triphosphate (ATP) (Stead & Thompson 2003; Strahl *et al.* 2011). As a result, acidic end products of glycolysis, for example acetate, alanine, opines, propionate and succinate, accumulate causing a decrease in haemolymph pH and an increase in CO_2 concentration due to fermentation of glycogen during anaerobiosis. In addition, increases in the expression levels and enzymatic activities of a number of glycolytic enzymes are also observed in bivalves during anaerobiosis (Kawabe *et al.* 2010, Sussarellu *et al.* 2010, 2012, Strahl *et al.* 2011). Hypoxia-tolerant species, for example the oyster *Crassostrea gigas*, have optimised fermentation pathways to increase ATP yield and produce less acidic alternative end products (Michaelidis *et al.* 2005a). Other species that burrow use behaviour, for example siphon extension or a reduction in burial depth, to increase oxygen supply, although these may result in an increased predation risk (Long *et al.* 2008 and references therein).

Thresholds of hypoxia for benthic organisms range from 0.28 to 4 mg O_2 l^{-1} although the majority of reports refer to a value of 2 mg O_2 l^{-1} or lower. Vaquer-Sunyer and Duarte (2008) carried out a broad comparative analysis across a range of contrasting marine benthic organisms, including bivalves, and showed that hypoxia thresholds vary greatly across organisms, and that the conventional definition of 2 mg O_2 l^{-1} to designate waters as hypoxic was below the empirical sublethal and lethal O_2 thresholds for half of the 206 species tested. Median lethal concentration ($LC_{50} \pm SE$) for all organisms tested was 1.60 ± 0.12 mg O_2 l^{-1} and the coefficient of variation across experiments was 78%, indicative of considerable variability in these thresholds across taxa. Ninety percent of the experiments showed LC_{50} values below 4.59 mg O_2 l^{-1}. Bivalves had a value[1] of 1.312 ± 0.43 mg O_2 l^{-1}, which was lower than the value for fish species (1.54 ± 0.07) and lower also than the value for crustaceans (2.45 ± 0.14). They found that median lethal O_2 thresholds differed significantly with the extent of mobility of the organisms tested, that is motile groups such as fish and crustaceans had higher threshold values than more sedentary groups. The median sublethal concentration ($SLC_{50} \pm SE$) was 2.24 ± 0.21 mg O_2 l^{-1}, and the coefficient of variation was 76% across experiments. Once again fish (4.41 ± 0.39) and crustaceans (3.21 ± 0.28) showed the highest median sublethal O_2 thresholds, with the bivalve value, albeit based on

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few experiments, considerably lower (0.75 ± 0.18). It is clear from this study that the species tested experience substantial mortality and sublethal responses to hypoxia at oxygen thresholds more than twice the 2 mg O_2 l^{-1} threshold generally used in the literature.

Aerial exposure

Many bivalves are capable of utilising oxygen in air during aerial exposure (Table 7.2). Their ability to do this depends on behavioural responses, such as intermittent air gaping. For example, when the mussel *G. demissa* is emersed it opens its valves slightly (~2 mm), which is sufficient to allow direct access by air to a large surface area of water trapped in the mantle cavity (Lent 1968). In contrast, during emersion the cockle *Cerastoderma edule* expels some water from the mantle cavity by rapid valve movement, and replaces it with a bubble of air, keeping the valves 50–100% agape. The cockle maintains a level of $\dot{V}O_2$ that is about 65% that of the aquatic rate (Boyden 1972), and it does not accumulate anaerobic end products during exposure. However, in the closely related, but mainly subtidal species *C. glaucum*, which keeps the shell valves tightly closed during exposure, this figure is only 8%, and anaerobic end products accumulate. On a return to aerobic respiration there is a transient, above normal increase in $\dot{V}O_2$ for the oxidation of anaerobic end products, and possibly also re-oxygenation of the haemolymph. In the mussel *M. edulis*, tolerance to aerial exposure varies along the intertidal–subtidal gradient with the average survival rate of intertidal mussels in sustained aerial exposure 50% higher than that of subtidal mussels (Altieri 2006). Reciprocal transplant of mussels between the intertidal and subtidal revealed that higher tolerance to aerial exposure is an inducible response that is gained or lost depending on whether the mussels had acclimated to intertidal or subtidal conditions, respectively, prior to experiments. In addition to behaviours related to respiratory requirements, some species may gape periodically during aerial exposure as a means of preventing lethal body temperatures (Helmuth 1999). In all species, however, exposure to air probably results in metabolic stress through the inability to feed, a limit to gas exchange and desiccation, all of which act to reduce scope for growth (Bayne *et al.* 1976a; see also Chapter 6).

Other factors

Shumway *et al.* (1988) showed that seasonal changes in $\dot{V}O_2$ in the scallop *Placopecten magellanicus* were clearly related to changes in the gametogenic cycle, with highest rates of exhibited during the summer months when the gonads ripen, and the lowest rates during the winter months. Similar results have been reported for other bivalves (references in Bayne *et al.* 1976b; Sukhotin 1992; Ekaratne & Davenport 1993; Rueda & Smaal 2004). While these changes in $\dot{V}O_2$ mirror environmental temperatures, Shumway *et al.* (1988) suggest that seasonal changes in food availability and reproductive stage have a greater effect on $\dot{V}O_2$ than temperature *per se*. Another factor that affects $\dot{V}O_2$ is a high level of CO_2 in the haemolymph (hypercapnia). In the mussel *Mytilus galloprovincialis*, this leads to a reduction in haemolymph pH. To limit the degree of acidosis mussels increased bicarbonate levels through shell dissolution, which was accompanied by a significant reduction in $\dot{V}O_2$, indicating a lowering of MR (Michaelidis *et al.* 2005b). A close correlation was evident between the reduction in extracellular pH and the reduction in MR during hypercapnia. Such effects are relevant in the context of future scenarios of progressive accumulation of anthropogenic CO_2 in marine surface waters. Other factors that affect $\dot{V}O_2$ in bivalves are suspended sediment (turbidity) or pollutants in the water column. Grant and Thorpe (1991) have found that in the clam *Mya arenaria*, long-term exposures to sediment concentrations of 100–200 mg l^{-1} for 35 days caused a significant decrease in $\dot{V}O_2$ but an increase in

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ammonia excretion compared to controls. Reducing ventilation rate is an effective strategy for coping with intermittent turbidity in the wild, but this can lead to starvation if exposure is long-term. Pollutants such as copper, cadmium and zinc significantly depress respiration rate in mussels (Akberali *et al.* 1984, 1985; Cheung & Cheung 1995) and oysters (Chen 1994). Finally, the predatory oyster drill *Urosalpinx cinerea* uses the $\dot{V}O_2$ of its prey species *C. virginica* and *G. demissa* to mediate its choice of a particular individual within a group of prey (Blake 1960).

Excretion and osmoregulation

The kidneys and pericardial glands are the major excretory organs (see Chapter 2 for details), although excretory products are possibly also lost across the general body surface and especially across the gills (Bayne *et al.* 1976a). Waste accumulates in certain cells of the pericardial glands and this is periodically discharged into the pericardial cavity, from where it is eliminated via the kidneys. Other cells of the pericardial glands are probably involved in filtering the haemolymph, the first stage of urine formation. The filtrate then flows through the renopericardial canals to the glandular part of the kidneys, where the process of reabsorption occurs. The end result is urine that has a high concentration of ammonia, and smaller amounts of amino nitrogen, urea and uric acid nitrogen (Table 7.5). By analysing the water in which animals have been held for several hours these compounds can be identified. With this method one should be aware that some of the measured nitrogen might come from bacterial contamination, from faeces voided during the experiment or from 'leakage' rather than true excretion (Bayne *et al.* 1976a; Griffiths & Griffiths 1987). This can be avoided by using high-performance liquid chromatography (HPLC), which directly measures the net

Table 7.5 Nitrogenous compounds in the urine of some bivalves.

	Excreted components as percentage of total measured nitrogen (N)				
Species	**NH_4-N**	**Urea-N**	**Amino-N**	**Uric acid-N**	**References**
Crassostrea virginica	68	8	21	3	Hammen (1968)
Modiolus demissus	62–75	0	25–38	–	Lum and Hammen (1964)
Mercenaria mercenaria	67	0	29	4	Hammen (1968)
Mya arenaria	94	6	–	–	Allen and Garrett (1971)
Mytilus californianus	100	–	0	–	Bayne and Scullard, (1977b)
Mytilus galloprovincialis					
Summer	37–58	–	42–63	–	Bayne and Scullard (1977b)
Autumn	100	–	0	–	Bayne and Scullard (1977b)
Mytilus edulis					
Summer	71	–	29	–	Bayne and Scullard (1977b)
Winter	97–100	–	0–3	–	Bayne and Scullard (1977b)

Data from Griffiths and Griffiths (1987). Reproduced with permission of C.L. Griffiths.
–, signifies that the component was not measured.

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fluxes of metabolites during a single passage of natural seawater over the bivalve gill (Hawkins & Bayne 1992). It is clear from Table 7.5 that the proportions of excreted ammonia to amino acid nitrogen differ considerably between species, and with season. The quantities of amino acids that are lost amount to 60–70% of the total measured excreted nitrogen in some species. This represents a loss in energy, which for *M. edulis* is about 11% of routine MR, rising to much higher values during stress (Bayne 1973a). It is not clear whether the amino acids are lost through leakage or through active excretion (Bayne *et al.* 1976a).

Nitrogen excretion rates are extremely variable in bivalves; average values for a range of species varied from 9.6 to 94.7 μg ammonia nitrogen $day^{-1}\,g^{-1}$ fresh weight (references in Bayne *et al.* 1976a). Excretion rates are influenced by factors such as size, temperature, salinity, season, origin and contaminants. Several studies have investigated the relationship among these by means of the O/N ratio, the atomic ratio of oxygen consumed to ammonia excreted $[(\text{mg } O_2) \div 16] \div [(\text{mg } NH_4) \div 14]$, which provides an index of the balance in an animal's tissues between the rates of catabolism of protein, lipid and carbohydrate substrates. A low value of ~10 indicates considerable protein catabolism, for example during starvation or low food rations, while higher values signify that greater proportions of lipid or carbohydrate are being metabolised (Bayne & Newell 1983). It now appears that much of the reported variability in excretion rates can be attributed to feeding history and the gametogenic stage of the animals being analysed. For example, Bayne and Scullard (1977b) have shown that in *M. edulis*, nitrogen excretion peaks prior to spawning followed by a progressive decline to minimum rates in autumn and early winter (Figure 7.9). Just before spawning glycogen reserves are at a minimum and mussels must catabolise protein to offset nutritional stress. Similar findings have been reported for rope-grown mussels (*M. galloprovincialis*) in northwest Spain (Babarro *et al.* 2000). The utilisation of protein reserves under stress

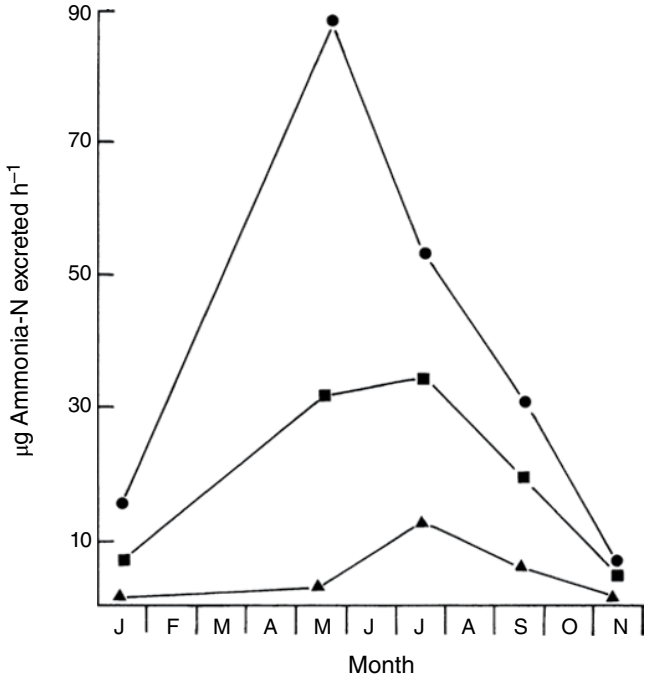


Figure 7.9 Rates of excretion of ammonia (NH_4–N) by the mussel *Mytilus edulis* of 0.2 g (▲), 1.0 g (■) and 2.0 g (●) dry flesh weight during different months of the year.
From Bayne and Scullard (1977b). Reproduced with permission of Cambridge University Press.

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conditions has been further substantiated by the results of Bayne and Thompson (1970) for starved *M. edulis* in the laboratory, and protein was utilised more rapidly at higher (16°C) than at lower temperatures (6°C). In the wild oxygen consumption tends to decline during starvation and this results in a marked reduction in the O/N ratio. These changes are most pronounced in smaller individuals, probably because glycogen reserves are depleted more rapidly, and also because they have higher weight-specific MRs than larger individuals. In the autumn, when glycogen reserves are high, but food is at a premium, ammonia excretion rates are low, which indicates that glycogen is preferentially being used to meet metabolic requirements (Figure 7.9). Salinity also affects excretion rate, with increased amounts of ammonia and amino acids being excreted when animals are initially exposed to reduced salinities. However, excretion rates return to normal after a period of time, the duration of which depends on the extent of the salinity decrease (Allen & Garrett 1971). Origin of the test species may also influence ammonia excretion. For example, when mussel seed from two origins (rocky shore and collector ropes) were cultivated on a raft in northwest Spain the rocky shore mussels showed significantly higher ammonia excretion rates than the collector rope mussels over the experimental period (7 months), which probably reflects the slow adaptive process of osmotic adjustment in these mussels to the littoral habitat (Babarro *et al.* 2000). Factors such as pollutants (Radlvc *et al.* 2007) and pesticides (Führer *et al.* 2012), which induce shell closure and a switch from aerobic to anaerobic metabolism, alter various physiological parameters, including ammonia excretion.

Bivalves exhibit wide variation in salinity tolerance. Euryhaline species, for example the mussel *M. edulis*, tolerate a wide range of salinities (5–35 psu), whereas stenohaline species, for example most scallops, are confined to a much narrower range. Despite such differences, all bivalves are osmoconformers possessing little if any capability for osmotic regulation of their extracellular fluid (haemolymph). Therefore, the cells bear the burden of volume regulation by adjusting the concentrations of intracellular free amino acids (FAAs) and other small organic molecules. Bivalves are thus able to maintain their cells isoosmotic with their extracellular fluids while at the same time regulating their volume. The capacity for volume regulation is, however, species-dependent. Euryhaline species such as the mussels *M. edulis* and *Geukensia demissa* exhibit near-perfect volume regulation in comparison to the stenohaline species *Modiolus modiolus* (Gainey 1994; Sadok *et al.* 1997). Generally, a bivalve's first response to fluctuations in salinity is shell closure. As a result the tissues are isolated from osmotic changes in the external medium. This is a short-term response, however, and should salinity change persist the animal may be compelled to regulate cell volume by its demand for oxygen and food (Bayne *et al.* 1976a). Alternatively, it may open, begin pumping and then die due to its inability to regulate cell volume.

The amino acids alanine, arginine and aspartic and the glutamic acids, glycine, taurine and betaine are particularly important in the process of cell volume regulation (Table 7.6 and Shumway *et al.* 1977). The quaternary base, glycine–betaine also plays a role in osmoregulation (de Vooys & Geenevasen 2002; Kube *et al.* 2006). Amino acids are generally separated and quantified from lyophilised bivalve tissue using HPLC (see Kube *et al.* 2006). Hawkins and Hilbish (1992) have shown that the principle source of FAAs is from breakdown of whole-body protein, and contributions from dietary sources, *de novo* synthesis and direct uptake of dissolved amino acids are negligible. With an increase in salinity there is a rapid increase in the concentration of FAAs and a corresponding decrease with reduced salinity. The decrease is effected by elevated excretion of FAAs and ammonia. Bayne (1975) recorded rates of amino nitrogen loss in *M. edulis* of $0.42\,\mu g\,h^{-1}\,g^{-1}$ dry weight at 32.5 psu, which increased to $2.95\,\mu g\,h^{-1}\,g^{-1}$ dry weight within 3 h of transfer to water of 14.5 psu. This high excretion rate of amino nitrogen gradually declined over the next 20 days. Such losses represent a major component cost of cell volume regulation, and Hawkins and Hilbish (1992)

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Table 7.6 The concentration of amino acids in the adductor muscle of *Mytilus edulis* in 100 and 50% seawater.

	Concentration (μmol per g wet weight)	
Amino acid	**100% seawater**	**50% seawater**
Alanine	18.40	13.0
Arginine	11.94	8.38
Aspartic acid	9.77	1.73
Glutamic acid	9.79	10.61
Glycine	61.87	18.40
Histidine	1.80	1.22
Isoleucine	0.31	0.39
Leucine	0.42	0.60
Lysine	2.81	1.71
Phenylalanine	0.51	0.28
Proline	6.29	2.80
Serine	5.99	3.05
Threonine	4.37	1.93
Tyrosine	0.77	0.48
Valine	0.94	0.50
Taurine	58.09	44.51
Betaine	62.99	40.66
Water content	69.80	80.20
Osmotic pressure (mOsm/kg H_2O) due to		
Amino acids	216.54	90.19
Taurine	92.47	61.66
Betaine	100.27	56.23
Osmotic pressure of the medium (mOsm)	1180	573

Adapted from Bayne *et al.* (1976a). Reproduced with permission of Cambridge University Press.

have suggested that this may help to explain stress and even mortality resulting from small but frequent fluctuations in salinity. Species, or even populations within a species, may differ in the size and content of their FAA pool. In the brackish waters of the southern Baltic Sea, the main pool of FAA in the clam *Macoma balthica* is composed of alanine, glutamate, arginine, glycine and ornithine, which represent approximately 80% of the FAA total (Sokolowski *et al.* 2003). The composition differs substantially to that found in clams from full saline environments, which can be attributed to the lower salinity in the southern Baltic. Moreover, some amino acids play a more predominant role than others, depending on salinity. For example, in the Baltic, glycine seems to play a most important role in regulating intracellular osmolarity in *M. balthica*, a function performed primarily by taurine in Atlantic and North Sea populations (Sokolowski *et al.* 2003). Another study that examined differences in the concentration of FAA in populations of *M. balthica* and *Mytilus* spp. exposed to high versus low intra-annual salinity fluctuations along their European distribution found that seasonal FAA variations were more pronounced in *M. balthica*, and in both species from different locations within the Baltic, highest FAA concentrations were found in autumn and winter, and low FAA concentrations were measured in summer (Kube *et al.* 2007). Seasonal patterns were less pronounced in both taxa at locations with constant salinity conditions. In contrast to Baltic Sea populations, Atlantic and Mediterranean bivalves showed high FAA concentrations in summer and low values in winter, regardless of seasonal salinity fluctuations. The authors concluded that seasonal patterns of the main FAA pool components (alanine, glycine and taurine) within and between populations should be considered in the

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context of seasonal variations of other environmental factors, besides salinity, and physiological state (glycogen content, reproductive stage) of the test species. FAA profiles have also been used as a convenient indicator of stress in contaminated environments (see Babarro *et al.* 2006 and references therein), although its use has inherent risks of misinterpretation in view of the large temporal fluctuations in FAA profiles seen earlier. Recently, biologists have been using a vast repertoire of biosensors that allow testing of mRNA expression (transcriptome analysis) in response to environmental stressors such as temperature, salinity, anoxia and contaminants (reviewed in Li *et al.* 2013). For example, when the euryhaline oyster *C. gigas* was exposed to a range of salinities (5–40 psu) for 7 days, FAA metabolism key enzyme genes were differentially expressed in low salinity–adapted individuals compared with controls, confirming their important role for oyster low-salinity adaptation (Meng *et al.* 2013).

Note

1 This value is lower than that calculated by Vaquer-Sunyer & Duarte (2008) because gastropod data were included in their estimate for bivalves.

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8 Fisheries and management of natural populations

Introduction

Between 1950 and 1970 world marine and inland capture fisheries production increased on average by as much as 6% per year, trebling from 18 million tonnes in 1950 to 56 million tonnes in 1969 (SOFIA 2000). By the 1970s and 1980s the rate of increase had fallen to 2% per year, and by the 1990s and the following decade it had dropped to zero (SOFIA 2006, 2012). It would appear that most of the world's fishing areas have reached their maximum potential, with the majority of stocks now being fully exploited. In contrast, since 1990 inland and marine aquaculture production grew at an annual rate of 10%, and by 2012 production had more than trebled (SOFIA 2000, 2006, 2012).

Although bivalves contribute a small percentage (~2%) to global capture fishery landings their generally high unit price compensates for the smaller landed weight when compared with the combined categories of fish, crustaceans and other molluscs (Tables 8.1 and 8.2). The economic importance of bivalves therefore calls for efficient approaches to the conservation and management of wild populations. Much of the methodology developed for finfish stock assessment and management has also been applied to bivalve molluscs, although much is inappropriate. Unlike fish these animals are sedentary, or almost so, and this has important implications for their population biology, conservation and management.

The first part of this chapter deals briefly with the main elements of bivalve population dynamics: abundance, mortality, growth, reproduction and recruitment. Strategies for the development and management of bivalve fisheries are also discussed. The focus thereafter is on commercial fisheries of selected species, described under quantity and landed value from the main fishing regions, types of fishing methods utilized and practices for handling, processing and marketing. In each case management practices and constraints are also explored.

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Table 8.1 Global landings from fisheries (live weight, metric tonnes) of four bivalve groups for 2009–2011.

Group	2009	2010	2011
Mussels	99601	86682	91459
Oysters	132969	157881	203778
Scallops	496640	810081	527036
Clams	743424	591086	492307
Total	1472634	1645730	1314580

Data from FAO (2013).

Table 8.2 The monetary value (US$ per metric tonne) of marine bivalves in comparison to various categories of fish in 2010.

	$/mt
Mussels	410
Oysters	845
Scallops	1800
Clams	970
Mean	1066
Salmon	3000
Flatfish	2450
Tuna	1740
Cod	1200
Tilapia	1020
Herring	320
Mean	1622

Data from FAO (2010).

Population dynamics

What is a stock?

It is almost impossible to get universal agreement on what constitutes 'a stock'. Some define it as a production or management unit, where differences within the group or exchanges with other groups are disregarded. Others regard a stock as a genetically discrete population, or a mixture of populations with limited genetic exchange (Cobb & Caddy 1989; see also Chapter 10). One definition that does not demand reproductive isolation or genetic discontinuity, and which approaches the functional concept of a stock that a fisheries manager must deal with, is 'a group of individuals that sustains itself over time, and that responds in a similar way to environmental changes within a discrete geographic area' (Campbell & Mohn 1983). For sedentary organisms the 'geographic area' can be as large as a sea, major offshore bank or estuary, or as small as a single bivalve bed. Cobb and Caddy (1989) feel that Campbell and Mohn's definition is a reasonable working definition, and suggest that unless stocks are severely overexploited it may not be vitally important to know the extent of gene flow from adjacent populations. If characteristics such as growth rate and size at maturity are sufficiently different to allow populations from different geographic areas to be clearly discriminated, then this allows the corresponding stocks to be managed separately, whether or not they are genetically distinct. See Carvahlo and Hauser (1995), Grant *et al.* (1999), Secor (2005) and Waldman (2005) for discussions of the stock concept in fisheries and conservation.

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Distribution and abundance

Assessment of abundance and distribution, two closely related parameters, is a necessary prerequisite for stock assessment. A feature of most sedentary invertebrate populations, for example bivalves, is the non-uniform distribution of individuals, due to variable patterns of recruitment, predation, competition for space and natural and anthropogenic disturbances. These processes are better understood for benthic organisms, for example mussels, living on hard surfaces, but they also apply to species living in soft sediments, for example clams (Underwood & Chapman 2005). The most direct method to measure absolute abundance is to count all individuals in a given area, but this is possible for very few organisms. Therefore, absolute abundance is most often estimated by counting the numbers in small samples taken from the total stock, using sampling surveys. Alternatively, relative abundance – the number of individuals in one area relative to the number of individuals in another area, or in the same area at a different time – can be measured (King 2007). The most commonly used index of relative abundance in fisheries is catch per unit effort (CPUE; see later). Other methods such as mark-recapture techniques and fishing success methods that are routinely used in fish stock assessment provide information on abundance but are essentially uninformative on spatial patterns although they are useful in the assessment of movements, migrations and growth rate.

Within the selected stock area it is important that sampling is random, that is that each potential sample unit has an equal chance of being included in the sample. A range of sizes of sample units (quadrats) should first be tested to determine the appropriate size to use for the species being sampled. The number of sample units to collect depends on the level of precision required. There is usually a trade-off between the need for a high level of precision and the cost of taking a large number of samples (see King 2007 for details). Which sample units are chosen is usually determined using a table of random numbers or a string of random numbers generated by a computer or calculator. In addition, a mandatory component of sampling surveys is that sampling units are replicated both spatially and temporally (see Underwood & Chapman 2005).

Methods for surveying the distribution and abundance of bivalve stocks involve direct methods such as the use of quadrats, benthic corers, dredging and trawling gear, underwater photography, TV and model-based approaches or indirect methods such as CPUE. A brief account of these methods follows. Those wishing for more detailed information on sampling methodology may consult the many excellent texts available, for example Sutherland (2006), King (2007) and Eleftheriou and McIntyre (2013).

Quadrats

Quadrats are used to measure abundance of one or several species in an area. On rocky shores, for example, a square metal or rigid plastic frame is laid on the substratum, and the animals within the frame are counted, weighed or estimated in terms of percentage cover of the surface. Good estimates of percentage cover can be made subjectively using subdivisions of the quadrat as a guide. This may be done *in situ* or, alternatively, all organisms within the frame may be photographed (see later), or scraped off the substrate for subsequent analysis. Quadrat size will vary depending on the abundance and size of animals, as well as the topology of the rock surface. For mussels it is best to use a quadrat size of $1.0\,m^2$ for estimating abundance on sheltered rocky shores, but on exposed shores where mussels are smaller, a $0.50\,m^2$ quadrat is best. Sampling stations should be spaced out from high to low water mark and several samples (4–5) should be taken at each tidal height, spanning the full range of microhabitats, for example rock pool, crevice and rocks exposed to or sheltered from sun or wind. At each station quadrat locations can be assigned by using random

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distances on either side of the transect line. For repeat sampling the positions of sample points can be marked with paint, chisel marks or drill holes; on soft substrates (see later) posts driven deeply into the sediment may be used. For small-scale underwater studies quadrat-based individual counting and cover estimates can sometimes be useful. However, patchy distribution, which is a characteristic of sublittoral scallop and clam species, reduces the likelihood of a randomly placed quadrat finding the species. This problem may be overcome by increasing the number of replicates per station, and by using larger quadrats that can be assembled *in situ*. Quadrats-based counts are impractical in large-scale studies where estimates on the size of whole populations, fishing grounds or stocks are needed. Pech *et al.* (2004) compared three different methods to estimate abundance using 40 quadrats randomly selected along a 5 m transect on a rocky shore in Maine, in the United States. Data from high-resolution digital photography of quadrats and digital image analysis (DIA) were compared with visual scan and photo quadrat (PQ) conventional methods. The results from the three techniques were similar, but were produced in a much reduced time with DIA, potentially lowering the cost of benthic monitoring programmes.

An alternative to making many replicated counts in quadrats is to use abundance scales, semi-quantitative estimates of percentage cover or density, which fall into five to seven broad categories from 'super abundant' through to 'common' to 'rare' (SACFOR scale). Estimates are made in an area covering a few square metres around a station. Such estimates are particularly useful for the rapid assessment of abundance of a particular species along a stretch of coast, a headland or an island, where shore topography can make the use of quadrats a daunting, if not an impossible, task.

Benthic corers, suction samplers and grabs

The corer is a quick, easy and effective sampling tool for estimating abundance and distribution of burrowing bivalves. For intertidal areas or in very shallow water, it can be a tube or a pipe of rigid material that is manually pushed into the substrate to a depth greater than the burrowing depth of the species being sampled. The dimensions of the corer are determined by the size of the animals being sampled, their expected abundance and the maximum depth at which the animals are found. If the substrate is firm enough the core is simply lifted up, but in sandy areas a core retainer in the form of a rubber bung or cap is slid across the bottom by underwater divers to prevent material falling out as the corer is lifted up. A recently developed device, the pedal corer, specially designed for sampling in shallow water areas that are accessible on foot, uses a pedal lever to close the lower part of the sampling chamber (Parada 2008). Sampling can be done at different levels by inserting a series of horizontal plates introduced through slots in the core tube.

This sampling method was employed by the Washington Department of Fish and Wildlife to determine the density and size of the Manila clam *Tapes* (*Ruditapes*) *philippinarum* before and after gravelling, a process in which gravel and crushed oyster shell are mixed and used as habitats to improve recruitment, growth and survival in clams (Thompson 1995). A randomised block design of nine plots (3 controls, 3 gravel and 3 gravel + shell) was used and core samples were randomly selected in relation to a central line running the length of each plot. The sampling device was a piece of PVC pipe (10 cm diameter and 20 cm long) that was inserted into the substrate to a depth of approximately 15 cm. The core was removed and placed in a 1 mm mesh bag, and later sieved through a series of screens to a minimum diameter of 1 mm. Clams were measured, counted and weighed for recruitment, survival and biomass data. Recruitment was higher on the control plots, while survival and biomass were enhanced by gravelling. Recruitment improved on gravel, and gravel and shell, as the plots aged and accumulated a layer of fine sediment and organic debris.

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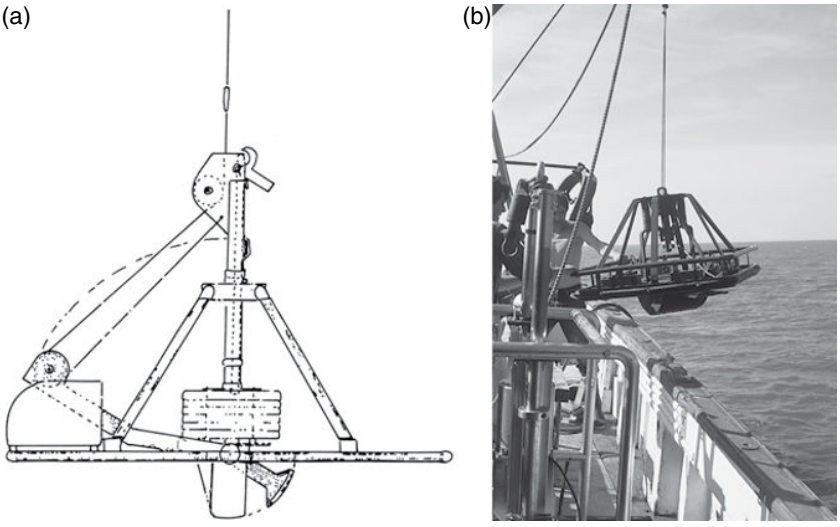


Figure 8.1 Reineck box sampler. (a) The rectangular coring tube is closed by a knife edge actuated by pulling on the lever on the left. An attachment can be fitted to show the inclination and compass orientation of the core.
Adapted from Holme and McIntyre (1984). Reproduced with permission from John Wiley & Sons.
(b) The corer being lifted back on board.
Photograph by John Costelloe, Aquafact International Services Ltd., Galway, Ireland. Reproduced with permission.

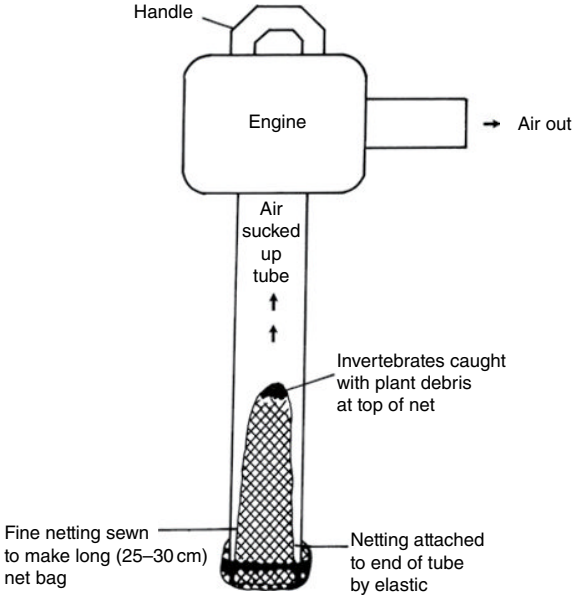


Figure 8.2 A small lightweight suction machine for sampling invertebrates.
Adapted from Sutherland (1996). Reproduced with permission of Cambridge University Press.

Bivalves in deep water are usually sampled from a boat using a mechanical corer that is longer and larger than manually operated corers. The one illustrated in Figure 8.1 is a rectangular box corer supported in a pipe frame that samples an area of 20×30 cm to a depth of 45 cm. The advantage of box corers is that they provide deep and relatively undisturbed

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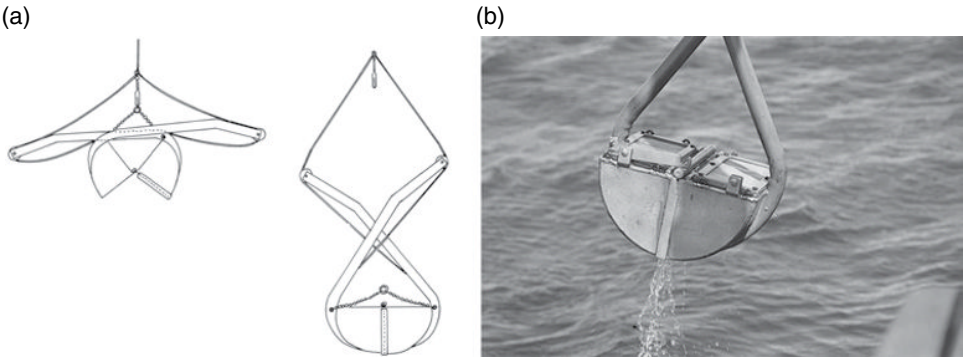


Figure 8.3 (a) The van Veen grab, open and closed.
Adapted from Holme and McIntyre (1984). Reproduced with permission from John Wiley & Sons.
(b) The grab being lifted back on board.
Photograph by John Costelloe, Aquafact International Services Ltd., Galway, Ireland. Reproduced with permission.

samples from a variety of sediments, but a large vessel and calm weather are essential for the safe deployment of this big and heavy piece of equipment.

Suction samplers employ a coring tube, which is forced into the substrate and held for a set period of time to draw up sediment and associated fauna into some form of self-sieving collector (Figure 8.2). Most suction samplers are mechanically operated, using either pumped water or compressed air to suck up samples. Samplers are designed to take account of different water depths, faunal and sediment types. Grabs are lowered vertically from a stationary ship, and usually sample a surface area of 0.1–0.5 m^2, depending on the type of grab. The van Veen grab (Figure 8.3) or the Day grab, a modified Smith–McIntyre grab, are the most common types used in sampling macrofauna. Details on a whole range of corers, suction samplers and grabs, and an evaluation of their general performance and efficiency in capturing macrofauna can be found in Eleftheriou and Moore (2005).

Dredges

The dredge is a type of fishing gear that is towed behind a boat fishing for bivalves, for example oysters or scallops. It has a heavy metal frame that is attached to a steel mesh bag and the leading edge of the dredge has a heavy chain, a sharp-edged blade or a toothed bar that scrapes or digs the animals from the substrate (Figure 8.4). The dredge is hauled in and put out by hand-operated or powered hydraulic winches. Alternatively, a suction pump is attached to the top of the dredge bag, through which the catch is continuously pumped aboard the fishing vessel, thus eliminating the need to haul the dredge on board. However, dredges are environmentally destructive because they disrupt the substrate and associated fauna, and can also damage the shells of the bivalves left behind (Gaspar *et al.* 1994; Piersma *et al.* 2001; Hauton *et al.* 2003; van Gils *et al.* 2006; Beukers-Stewart & Beukers-Stewart 2009; Leitão *et al.* 2009; Boulcott & Howell 2011; Vasconcelos *et al.* 2011; Narvarte *et al.* 2012).

Abundance surveys are performed by towing a dredge over a measured area for a set time and taking samples at a predetermined number of stations (tows) at set intervals. It is assumed that the number of bivalves caught per tow directly assesses local density. However, efficiencies of dredges can vary with values between 46 and 95% reported for clam dredges fishing for surfclams (Weinberg *et al.* 2005), down to values as low as 4–7% for some scallop dredges (Orensanz *et al.* 2006) and oyster dredges (Banta *et al.* 2003). Dredge efficiency

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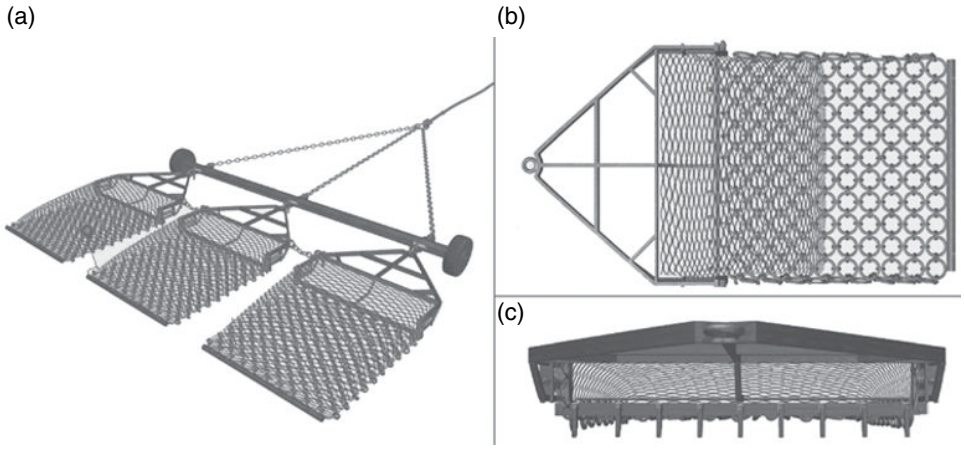


Figure 8.4 The Newhaven dredge commonly used in UK commercial scallop fisheries. (a) View of three such dredges and tow bar; the usual number used by scallop boats is 4–8 per side. (b) Steel rings (70–80 mm diameter) of the collecting bag (top elevation). (c) The spring-loaded, nine-toothed bar (side elevation). Boulcott and Howell (2011). Reproduced with permission of Elsevier.

is affected by the nature of the bottom, operation conditions, dredge design, gear leaping, gear saturation, skill of the operator, and size and behaviour of the species being sampled. A dredge can be calibrated by comparing dredged samples with those taken by Scuba divers from marked quadrats. An estimate of efficiency is needed to convert catch-per-tow figures into actual abundance. Catches are numbered and either the whole sample or a subsample is measured to get an estimate of the size distribution of the catch at each station.

The most commonly used index of relative abundance in fisheries is CPUE. It may be recorded as the number or weight of bivalves caught per metre of dredge width per hour of towing. If CPUE is twice as large in area A than in area B the inference is that there are twice as many individuals in area A. Provided that the same type of fishing method is used and the distribution of bivalves in the two areas is similar, CPUE can provide a good index of abundance in relative terms. The relationship of CPUE to stock size (N) is linear

$$\text{CPUE} = qN$$

where q is the catchability coefficient (see later). However, in commercial bivalve operations CPUE has severe limitations as an abundance indicator. Because bivalves are sedentary they do not mix after each fishing operation. The spatial structure of a bivalve stock is persistent and fishermen do not fish at random over the fishing ground, but tend to fish a bed until the density drops to some threshold level before they move on to another bed. Because of this sequential pattern of patch depletion CPUE is rarely a good index of abundance in bivalve populations. The method is adequate only when dense patches of bivalves are small relative to the length of the tow, that is when they are invisible to the fishermen, and when the boundaries of the fishing bed are well defined and data are collected at the appropriate spatial scale (Orensanz *et al.* 2006).

Underwater visual methods

One advantage of working underwater is that the diver, with little effort, can get a preliminary qualitative overview of the area to be sampled, thus leading to precise quantification at a later stage. Underwater visual methods are generally restricted to epibenthic bivalves and

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have been mainly used in surveys of scallop species. Recordings are made by divers or by a towed underwater video camera. The diver swims along a fixed rope or chain, laid on the substrate, which is marked at 1–5 m intervals (see Munro 2005 for information on different types of transects). Numbers of individuals lying within a fixed distance (1–2 m in temperate waters; greater in clear tropical waters) of the transect line are recorded. The diver's swimming speed must be slow and constant to ensure optimum accuracy. To illustrate, Centoducati *et al.* (2007) carried out an underwater visual census of the endangered fan mussel *Pinna nobilis* in the Gulf of Taranto, Italy. Six macroareas were classified on the basis of parameters such as depth, substrate, flora, anthropogenic pressure and landmarks, and within each area ten microareas (~1 ha) were randomly selected, giving a total surveyed area 60 ha wide. The geographical coordinates for each microarea were identified with Global Positioning System (GPS) and marked with buoys, and each area was surveyed in both longitudinal and latitudinal directions in corridors 2–5 m apart by a team of 4–6 divers and the support of a rubber dinghy. The diver was towed with a depressor wing at a speed of less than $<2\,km\,h^{-1}$ and when a pinnid was sighted the wing was released and the operator in the dinghy surveyed the GPS coordinates so that the exact same location could be subsequently revisited, while a second diver recorded various biometric and ecological data on a plastic slate. All field data were processed, stored in a database and linked to the study area by means of geographic information system (GIS) technology. Density of clams was low (0.1–0.7 individuals per hectare), but indicated a tentative recovery of the population despite illegal fishing methods, and the degraded and heavily polluted environment it inhabits. Density surface modelling with line transect sampling via SCUBA diving is another abundance method, as illustrated in a study on a population of the same species, *Pinna nobilis* (Katsanevakis & Thessalou-Legaki 2009).

Alternatively, a diver may be carried on a manta board towed behind a boat, or on an underwater vehicle towed at a set speed behind a boat. If the diver needs to manually operate the vehicle then a tape recorder is used to record data. Bivalve densities can also be assessed by video camera, either hand-held by a diver on an underwater vehicle, or towed on a sledge or dredge. Ragnarsson and Thórarinsdóttir (2002) estimated abundance of the ocean clam *Arctica islandica*, with underwater photography and a hydraulic dredge at 15 m depth in a fjord in northwest Iceland. Estimates based on counts of siphons from the photographs were four times higher ($53\,m^{-2}$) than from the analysis of the dredge catches ($14\,m^{-2}$), almost certainly due to the low efficiency (21%) of the dredge. Stock assessments performed with underwater photographic techniques may provide more accurate quantitative estimates of abundance in this species than dredges, although surveys conducted during winter may greatly underestimate abundance because a large proportion of individuals may be buried deep in the sediment.

Factors that increase biomass

Growth

Growth in bivalves is usually described in terms of an increase in some dimension of the shell valves. Direct measurements of growth rates can be made by marking individual shells and measuring them at fixed intervals. Alternatively, a time series of size–frequency histograms can be used to follow the position of individual modes over time; from this growth rates of individual cohorts can be estimated. Growth rate can also be determined from growth checks or rings on the external shell, or from growth lines in shell or ligament cross sections. The mean size of individuals for each year class is then calculated and a direct plot of size versus age gives a growth curve for the population. It should be pointed out that these

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methods are not equivalent in that shell marking provides data on individual growth rates, while growth rings and size–frequency histograms provide estimates of population growth rate (details in Chapter 6).

The life expectancy of bivalves varies enormously depending on the species. This is well illustrated using scallops (Orensanz *et al.* 1991 and references therein), which fall into two main groups:

- Long-lived species that live in temperate waters; large-sized species (>100 mm) usually live more than 12 years, while medium-sized species (60–100 mm) live less than 10 years. Examples of the former are *Patinopecten caurinus* (>25 years), *Pecten maximus* (~22 years) and *Placopecten magellanicus* (>12 years). Examples of the latter are *Aequipecten* (*Chlamys*) *opercularis* (6–9 years) and *Chlamys varia* (4 years).
- Short-lived species that live in tropical seas grow to more than 100 mm but seldom live for more than 3 years, for example species of *Amusium* or warm–temperate species like *Argopecten irradians* and *Argopecten gibbus*, which do not reach sizes above 80 mm, and rarely live beyond 2 years. Species in this group have a shorter larval life, a faster growth rate and higher natural mortality than long-lived species.

Reproduction and recruitment

Of most interest to fishery biologists/managers are the size/age at sexual maturity and the timing of spawning and recruitment. The term 'recruitment' needs to be carefully defined as it has different meanings in different contexts. To a fisheries biologist the term refers to entrance of individuals to the exploited population.

From a commercial point of view it is useful to be able to predict when maximum recruitment can be expected to take place. However, forecasting the timing in a particular year can be difficult because peaks of reproductive activity in a population do not always correlate with subsequent recruitment in that area. This is because a variety of factors may affect the prerecruit, such as hydrography of the area, weather conditions, larval abnormalities, and pre- and postsettlement mortality through predation and inadequate settlement surfaces. In addition to the unpredictability of recruitment time in a particular area there is often an enormous variability in the strength of recruitment from one year to the next. This is not always correlated with stock size as one might intuitively expect (see Haddon 2011). Indeed, even if local stock numbers are very low recruitment can be high because of larval influx from other populations. Year class strength variability most often reflects inter-annual variation in reproductive effort and success, the survival of pelagic larvae or the survival of spat.

While the size of whole populations, or subpopulations, is largely determined by environmental and hydrographic factors that influence spawning and the fate of pelagic larvae, once larvae are ready to settle it would seem that density-dependent processes are major influences on settlement, growth and mortality at the smaller scale of single grounds or beds. Details on the effects of density on these processes are provided in Chapters 5 (settlement), 6 (growth) and 3 and 11 (mortality).

Factors that decrease biomass

Mortality

Mortality in bivalves is caused by physical factors such as extremes of temperature and salinity, and biological factors such as predation, disease and fouling, plus interactions between these factors (see Chapters 3 and 11). For commercial species fishing is an

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additional source of mortality. The larval stages of bivalves are especially vulnerable to predation and consequently suffer extremely high mortality rates. With increasing size natural mortality rates decrease, but not in a constant manner because of seasonal influences and random catastrophic events such as oxygen depletion, algal blooms and disease. But as animals increase in size they become increasingly susceptible to fishing mortality.

The loss of individuals in a population can be estimated in terms of the percentage of individuals that survive (survival rate) over a particular time period or the percentage that die (mortality rate). The total mortality rate is referred to as Z, and is the sum of the instantaneous rate of fishing mortality (F) caused by the fishing operation, and the instantaneous rate of natural mortality (M), which includes deaths due to all other factors (King 2007).

Total mortality (Z)

An indirect method to estimate mortality entails plotting the natural logarithms of the numbers of individuals surviving by age as a catch curve. In an exploited species, assuming a constant rate of mortality, numbers surviving tend to decline exponentially with time or age. This is expressed as follows:

$$\ln(N_t) = \ln(N_0) - Z_t$$

N_t is the number surviving at time t, N_0 is the initial number of individuals at time zero and Z_t is the total mortality rate at time t. When the natural logarithm of N_t is plotted over successive years the result is a straight line referred to as a catch curve. An estimate of Z is given by the slope of the line of best fit through these data. The utility of this type of analysis assumes that the age composition of the sample truly represents the age composition of the stock, and that recruitment and total mortality rates are constant for each age group. If recruitment and mortality rates are constant across year classes (Table 8.3a) then a large sample of several year classes may be used to construct a catch curve. Figure 8.5 is a catch curve constructed using the data in Table 8.3a. Total mortality Z is 0.92 per year and using the equation (King 2007)

$$\text{Mortality}(\%) = 100(1 - \exp(-Z))$$

this works out at 60%. If recruitment is variable from year to year, which is usually the situation, then a single cohort is followed diagonally from upper left to lower right over 3 years to provide an estimate of Z (Table 8.3b), which gives 2000 individuals at age 1 in the first year, 800 at age 2 in the second year and 320 at age 3 in the third year. Plotting

Table 8.3 Hypothetical age – composition data over 3 consecutive years under conditions of (a) constant recruitment and (b) variable recruitment; a constant annual mortality rate of 60% has been applied (see text).

	(a) Constant recruitment				**(b) Variable recruitment**		
	Age 1	**Age 2**	**Age 3**		**Age 1**	**Age 2**	**Age 3**
Year 1	1000	400	160	Year 1	2000	2200	100
Year 2	1000	400	160	Year 2	1000	800	880
Year 3	1000	400	160	Year 3	1500	400	320

From King (1995). Reproduced with permission of John Wiley & Sons.

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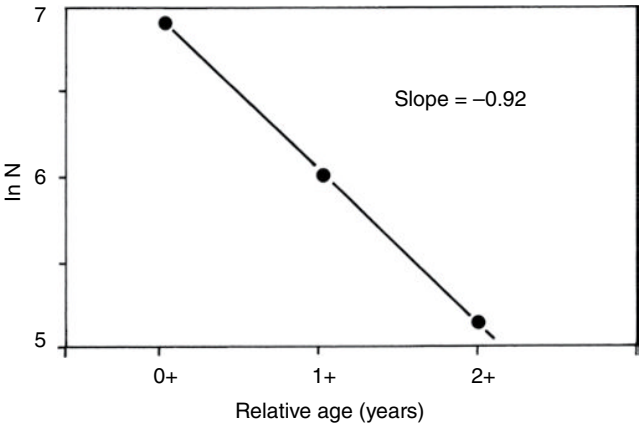


Figure 8.5 A catch curve, natural logarithms of the number of individuals against age for the data in Table 8.3a. The absolute value of the slope, and therefore total mortality, is 0.92 per year, which is equivalent to 60% (see text).
King (1995). Reproduced with permission of John Wiley & Sons.

the natural log of these numbers against age provides a catch curve (not illustrated), which has the same slope as in Figure 8.5, and therefore the same estimated mortality rate of 60%.

In bivalves that show no annual growth rings on the shell, such as many oyster and mussel species, catch curves from length frequency distributions can be constructed to give estimates of *Z*. Once growth data for the species are available a length frequency distribution can be converted to an age frequency distribution. Details of how this is done are covered by King (2007).

CPUE data – commonly used as an index of abundance – can also be used to provide an estimate of *Z*. The natural logarithm of CPUE values for a particular cohort can be plotted over several years as a catch curve. This requires both age composition data and CPUE data. If the former are not available CPUE data may be used on their own, provided that recruitment occurs during well-defined periods. The decrease in total CPUE over the period between one recruitment and the next gives an estimate of the average total mortality for all age groups combined. The assumptions here are that all age groups, including the newly recruited group, are equally vulnerable to the fishing gear, and that mortality is constant (King 2007).

Mark-recapture data – normally used in growth studies – have been used to estimate survival and mortality in fish and crustacean species. However, mortality during the actual tagging operation, tag-induced death after tagging, loss of tags and possible emigration, in the case of scallops, from the survey area, are just some of the reasons why the technique has not been useful for bivalves, or indeed for fish and crustaceans.

Natural mortality (M)

Natural mortality (*M*) may be estimated by following change in abundance of a cohort (or year class) or by analysis of dead shells. The former involves taking intertidal or subtidal quadrat samples (see earlier and Figure 6.2), and following the decay in the numbers of a particular cohort or age class over a set period of time. This is the most direct method for estimating natural mortality but is really only suitable for small-scale studies.

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Analysis of dead shells is only useful in species groups such as scallops and clams with well-defined annual growth lines on the shell. The use of dead shells in estimating *M* rests on the assumption that empty shells held together by the ligament – sometimes referred to as 'cluckers' or 'clocks' – are a result of natural mortality alone, fished bivalves being either exported from the fishing grounds or their shells separated at shucking. The ratio of cluckers to living animals of a particular cohort can give an estimate of natural mortality for that cohort, provided that mean clucker life (the time elapsed between death of the bivalve and decomposition of the ligament) is known. This ranges from 28 days for *Chlamys tehuelcha* (Orensanz 1986) to 1 year or more for *P. magellanicus* (Dickie 1955). It is important to have an accurate estimate of mean clucker life for individual species because an underestimate will inflate natural mortality rates (Orensanz *et al.* 1991). In addition, bivalve damaged by fishing gear that is subsequently eaten by predators will also lead to inflated natural mortality rates, while the opposite bias will be seen if a proportion of cluckers are separated by fishing gear (Caddy 1989).

Another method for estimating *M* is to use the ratio of live shells to cluckers of a given cohort, where all have the same last growth ring marked on the shell (Caddy 1989). The number of cluckers counted is taken to be an estimate of the total number of natural deaths that have occurred since the formation of the last growth ring. However, the life of a clucker could often be shorter than the time elapsed since the formation of the last ring, in which case both cluckers and separated valves that have this growth ring should be counted. This will, however, give an overestimate of *M* as some of the separated valves could be due to fishing gear!

Jonasson *et al.* (2006) have examined fishing (see later) and natural mortality in the scallop *Chlamys islandica* in western Iceland. In Iceland yields declined drastically from 1993 to 2003 and today there is no longer a fishery for this species in Icelandic waters. Natural mortality (*M*) was based on the occurrence of cluckers in survey tows:

$$a = 1 - e^{-(C/t)(1/L)365}$$

where *a* is the annual ratio of natural death, *C* the number of cluckers, *t* the average time in days required for the shells of the cluckers to separate (211 days) and *L* the number of live scallops in the sample. The exponent is equal to the instantaneous mortality rate. The number of cluckers was adjusted for the number of scallops that disarticulate during the tow by multiplying the number of cluckers in the sample by 1.211 (Naidu 1988). During the 10-year survey period *M* increased after the year 2000 from 0.1 in 2001 to 0.4 in 2003, probably due to disease and scarce food availability; part of the *M* estimate could also be dredge-induced.

Fishing mortality (F)

Fishing mortality (*F*) may be estimated directly by obtaining density estimates before and after harvesting, or by swept-area methods. Both rely on having a reliable estimate of fishing gear performance.

In swept-area methods annual fishing mortality for a particular age group is calculated by using the formula

$$F = fe\left(\frac{a}{A}\right)$$

where *f* is the annual total of effort units exerted, *e* is the efficiency of the fishing gear, *a* is the average area swept by a unit of fishing effort and *A* is the total area of the fishing ground. The assumption of the formula is that units of fishing effort, for example hours dredged per

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day, are distributed at random within the total fishing area A. However, because of the patchy nature of bivalve distribution, fishermen do not fish at random over the fishing ground, but tend to fish a bed until the density drops to some threshold level before moving on to another bed. Thus, fishing effort tends to match the non-homogeneous distribution of the stock, thereby resulting in a mosaic of fishing mortalities. Serious underestimates of F will result unless stock and effort contagion are taken into account (Caddy 1975).

Indirect methods for estimating F can be obtained by subtracting the natural from the total mortality rate ($F=Z-M$), or if M is known, by cohort analysis; or calculated from total removals (C) over mean stock numbers (N) derived from surveys, that is $F=C/N$. Alternatively, F may be calculated using non-model methods such as the Beverton and Holt length-based estimate (^{BH}F), or the equilibrium fishing mortality estimate (^{Y}F). See Quinn and Deriso (1999) for details and Jonasson *et al.* (2006) for the use of both methods in estimating fishing mortality in Icelandic scallops.

Fisheries assessment and management

The economic importance of bivalve fisheries calls for efficient methods of management to ensure sustainable production over time. Current practices are mostly based on methods that have been developed for finfish stock assessment and management. Some useful references are Jennings *et al.* (2001), Hilborn and Walters (2003), Cooper (2006), King (2007), Haddon (2011) and Ley (2011).

Stock assessment

Before management measures can be implemented an assessment of the stock(s) in question should be undertaken. Stock assessments are designed to answer a number of questions that help managers make the best decisions to ensure sustainable fisheries (NOAA 2012a):

- What is the current status of the stock, for example is it experiencing overfishing or is it overfished?
- How much catch is sustainable while maintaining a healthy stock?
- If a stock is depleted what steps are needed to rebuild it to healthy abundance levels?

Stock assessments require data on catch, relative abundance and the life history of the species, and this information is obtained from both fishery-dependent and fishery-independent data. The former comprise catch data or landing records, along with measures of fishing effort. These data are most often collected by commercial fishing operations. This is a cheap and comprehensive way of collecting data, provided that samples are reasonably large and representative of the population. Subsequently, the information is made available to stock assessment scientists and managers. Fishery-independent data are generally obtained from state agency-funded research surveys. Survey vessels use standardized sampling methods to collect data the same way each year, thus providing a relative index of abundance over time (NOAA 2012a). Surveys may focus on a single species, multiple species or a specific age range or cohort. The survey data provide an index of abundance, and can also provide a variety of biological data, for example size and age structure of the stock, sexual maturity and reproduction, natural mortality and geographical boundaries of the stock. Catch, abundance and biological data feed into mathematical models that represent the factors causing changes in harvested stocks. The model outputs indicate the measures needed for managers to make informed decisions on how best to regulate the fishery (NOAA 2012b). The National

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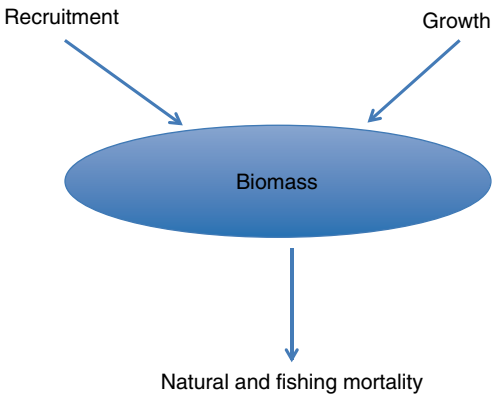


Figure 8.6 Stock biomass is increased by recruitment and growth, and is reduced by natural and fishing mortality.

Oceanic and Atmospheric Administration (NOAA) website http://nft.nefsc.noaa.gov/index.html also provides a suite of biological modelling software programs that can be used in fisheries stock assessments.

Commonly used models are surplus production models and age-structured models. Of these, surplus production models, sometimes called surplus yield models or biomass dynamic models, are the least complex, only requiring a time series of catch and effort data. Such models are based on the assumption that a stock produces a surplus abundance, or biomass, that can be harvested. Factors that increase stock biomass are recruitment and growth, that is production, while natural and fishing mortality decrease biomass (Figure 8.6). If production is greater than mortality, biomass will increase, and if biomass is over and above that needed to replace losses, this surplus production can be harvested without adverse effects on the stock (King 2007). The point at which the rate of surplus production is maximal is referred to as the maximum sustainable yield (MSY), that is the largest average catch that can be continuously taken from a stock without impacting on its long-term productivity. Surplus production models seek to ascertain the level of effort that is needed to produce the MSY for a stock. However, these models do not include processes that affect biomass, such as growth and mortality. Consequently, models are now available that examine the effects of these two opposing processes on the biomass of a single cohort or year class.

Age-structured models can be classified into two groups: virtual population analysis (VPA) and statistical catch-at-age analysis. VPA models use catch-at-age data to calculate fishing mortality and stock numbers in a single age group or cohort by basically working backwards year by year.

The number of individuals surviving from one year (N_t) to the next (N_{t+1}) is given by

$$N_{t+1} = N_t \exp\left(-(F+M)\right)$$

The number of individuals dying is therefore $N_t\,(1-\exp[-Z])$. The catch (C_t) is the proportion due to fishing, and can be estimated from the following catch equation:

$$C_t = \left(\frac{F_t}{Z}\right) N_t \left(1-\exp(-(F+M))\right)$$

The estimation of stock abundance (N_t) is calculated by rearranging this equation. Once this is estimated stock abundance in the previous year (N_{t-1}) can be estimated

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retrospectively if the catch for the previous year is known, and assuming that M is a constant. Details are provided by Jennings *et al.* (2001) and King (2007).

Statistical catch-at-age models, unlike VPA models, are almost always forward-projecting models, and are based on the age or length classes of a fished population. These models use the proportional catch-at-age to predict the relative abundance of each age class. These calculated relative abundances are then used to estimate future abundances of the stock and harvest regulations are set based on the predicted future abundances. Some missing catch-at-age data are allowed, unlike VPA, and some implementations allow the catch data to be separated into landings and discards (ICES 2012).

In addition to the information provided by VPA and statistical catch-at-age analyses (population size, F and M estimates) yield-per-recruit (Y/R) models track growth and hence total biomass, the objective being to select fishing mortality rates that maximize the yield (biomass) per recruit. If fishing levels are set too high, too many individuals will be taken before they have a chance to grow (growth overfishing). On the other hand, if levels are set too low individuals will be large when captured but total biomass will be low (King 2007). Beverton and Holt (1957) probably provided the best-known Y/R model. This predicts the total yield to be obtained from a cohort over its entire lifespan, as a function of the year class abundance, age at recruitment, growth and mortality. The model assumes that the total yield in any one year from all age classes is the same as that from a single cohort over its whole lifespan. The yield (catch in weight) of a single year class from age at first capture (t_c) to some maximum age (t_{max}) is an integral of the fishing mortality (F), the number of bivalve present (N) and their mean weight (W), which is

$$Y = \int_{t_c}^{t_{max}} F_t N_t W_t dt$$

Although this model only addresses the problem of growth overfishing, data can be tied to some index of mean reproductive potential per recruit, for example mean gonad weight per scallop recruit, and trade-offs between gains in yield and reproductive losses can be evaluated for different harvesting regimes (Orensanz *et al.* 2006). See Hart (2001) on the limitations of Y/R models in sedentary organisms such as bivalves.

Recruitment should also be integrated into Y/R models to avoid recruitment overfishing, which is when fishing pressure is too heavy to allow a population to replace itself. By plotting recruitment against spawning stock over a number of years, the level of recruitment needed to maintain the population in equilibrium, despite fishing mortality, can be estimated; the higher the mortality, the higher the recruitment needs to be to maintain this status (King 2007). Because of their high fecundity, bivalve fisheries are rarely considered to be at risk from recruitment overfishing. However, results from a 24-year survey (1978–2001) showed that recruitment in the hard clam *Mercenaria mercenaria* fell more than 70% in the fishery in North Carolina, United States (Peterson 2002). Over the same period landings initially increased fivefold, but subsequently fell by over 50%, clearly illustrating how unsustainable fishing mortality can lead to recruitment overfishing in this species.

Fishery management

Controls

Ideally a fishery should be managed from the beginning but in the majority of cases management measures are only applied when there is already evidence for overexploitation (overfishing) of the resource. Historically, the goal of fishery management has been to

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maximise yield, either in weight or value, and to maintain a particular stock level to provide a buffer against poor recruitment years, or to maintain a minimum spawning stock (King 1995). More recently, fisheries policies have been extended to address economic, social and environmental objectives (King 2007). In addition, fishery scientists are adopting a more precautionary approach to management due to uncertainties associated with alternative management options (see Sethi 2010 for review on risk management for fisheries).

Optimising yield can be achieved by regulating the size or age at which bivalves can be harvested, and/or controlling fishing mortality. The former involves implementing regulations governing gear selectivity, minimum meat weights or meat counts, while the latter involves controlling effort or catch quotas.

Size limits are usually regulated through selectivity of the fishing gear. In dredges, for example (Figure 8.4), there are two potential routes for escaping individuals, the inter- and intra-ring spacing, the latter being larger and thus the most likely exit. Retention characteristics of dredges are regulated by stipulating minimum ring size and also through spacing of teeth in front of the dredge (see Table 8.5). However, this does not guarantee that small individuals escape entrapment, as exit from the dredge is often blocked by large animals and debris. It may be necessary to cull the catch on board, or land the catch unsorted.

In addition to regulating gear selectivity a minimum legal landing size is usually applied. If the aim is to protect breeding individuals it should be taken into account that reproduction is often age- rather than size-dependent. Where environmental conditions are less than optimal for a species, individuals may grow more slowly and mature at a smaller size, and a lower minimum legal size would be justified in these areas. Meat counts (number of meats permitted per unit weight) may be used when shell size is not a good predictor of muscle weight (see Naidu & Robert 2006, and section on scallop fisheries later).

Yield is optimised by controlling fishing mortality by means of effort limitation and catch controls. Catch controls aim to control fishing mortality by limiting the weight of catch that fishers can take (Jennings *et al.* 2001). These include total allowable catch (TAC), which is a catch limit set for a particular fishery generally for a year or a fishing season, and quota shares, which are dedicated portions of the TAC allocated to individuals, that can be bought, sold and leased. How this works in the European Union (EU) is that TACs and quotas are agreed by the member states, after consultation with advisory groups of independent scientists and stakeholders. Effort is controlled by setting limits on the size, type and number of fishing gear per boat, the size of fishing boats and/or power of engines, entry to the fishery, number of fishing hours, fishing days and by employing temporal and spatial closures.

In recent years marine-protected areas (MPAs) have been seen as a promising tool for fisheries management (Claudet *et al.* 2008). However, MPAs need to be evaluated and compared to viable alternative fisheries management measures, and used as one element in a broader package of measures (Hilborn *et al.* 2004). For example, closing approximately 8% of the North Sea to protect groundfish species diversity increased fishing impact on benthic invertebrates. But closure, specifically to protect benthic invertebrates, reduced fishing mortality by just 2–4%, but when closure was combined with appropriate reductions in TAC, approximately 17% reductions in fishing mortality were achieved (Greenstreet *et al.* 2009). The beneficial effects of MPAs in scallop fishery management in the Isle of Man are described later.

Regulations must be enforced for the management of a fishery to be effective. This should be done through public education (public meetings, radio and TV, press articles and poster displays) rather than by coercion. While prosecution should be regarded as a measure of last resort, regulations must be seen to be enforced. Penalties must be significant to the offender and appropriate to the offence. Enforcement staff must be trained in, for example, public relations, fishery management, evidence collecting and court procedures. Economic but

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effective ways of enforcing regulations should be explored. For example, to enforce a legal minimum size it may be more economic, and just as effective, to inspect the bivalves at point of sale rather than at point of capture (King 1995). A regulation making it illegal to sell rather than catch undersized bivalve would be easier to enforce.

More information on management measures, and enhancement procedures such as habitat improvement, restocking, reseeding and predator control for fisheries can be found in the sections dealing with specific bivalve fisheries later in this chapter.

Types of management

Management and enforcement are generally more effective if managers and fishers work together (Jennings *et al.* 2001). Three types of management – co-management, community management and ecosystem-based management – will be described although these pertain, almost exclusively, to management of commercial fish species.

Co-management means that fishers and government authorities share responsibility for the management of the fishery. Although their roles can vary the most common situation is where government authorities and fishers consult, advise and cooperate fully. To illustrate, Castilla and Defeo (2001) examined three Latin American fisheries in which co-management and field experimentation were used on different temporal and spatial scales. One of these was the yellow clam (*Mesodesma mactroides*) fishery in Uruguay, where clams are picked by hand and shovel along 22 km of beach. Due to decreasing catches the fishery was closed as a management experiment between 1987 and 1989 to investigate the effect of fishing on clam demography. Coastal marine authorities, scientists and a well-defined group of local fishers participated in the experiment with the latter group also involved in enforcing regulations (Defeo 1996). The fishery was reopened in 1989 with additional management strategies imposed on the fishers but, unfortunately, the fishery collapsed after 1993 (details in Castilla & Defeo 2001). However, the authors concluded, based on the three case studies, that co-management constitutes an effective institutional arrangement by which fishers, scientists and managers interact to improve the quality of the regulatory process and may serve to sustain Latin American shellfisheries over time. Another example of co-management is the hard clam (*M. mercenaria*) fishery in Puget Sound, Canada, where the Swinomish Indian Tribal Community (SITC) utilizes many beaches for commercial, recreational, ceremonial and subsistence clam harvests. The fishery is currently managed by Washington State Parks and SITC, and both carry out annual surveys to determine clam biomass, and establish annual TAC (details in Barber *et al.* 2012).

Community-based management is most common in tropical regions where coastal communities are dependent on seafood as a protein food source. Such communities are spread along coastlines and islands, and typically consist of large numbers of fishers who use many different methods to make small individual catches of a great variety of species (King 2007). There are many examples of community-based fishery management of fish, but few describing such management in molluscs. One example is the fishery managed by the Seri indigenous community, which stands out among other fishing communities in the Gulf of California, Mexico, for their ability to govern and conserve their fishing resources without collapsing the social-ecological system on which they depend (Basurto & Ostrom 2009). The Seri fish for the pen shell (*Atrina tuberculosa*), a sessile byssally attached bivalve that commands high market prices and is harvested by divers using rudimentary breathing apparatus. The fishery is solely self-governed by the Seri who have historic property rights in a region they have inhabited for thousands of years, as well as multi-species fishing concessions, both granted by the Mexican government in 1975. The cultural and biological factors that help the community to control access,

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avoid overexploitation and ensure long-term sustainability of their fishing resources are documented by Basurto (2006, 2008) and Basurto and Coleman (2010).

In the last decade there has been increasing interest in community-based approaches to fisheries management in developed regions, for example the northwest Atlantic coasts of Canada and the United States (Graham *et al.* 2006). But fisheries in these areas are already highly regulated by local and national governmental authorities, and therefore a community-based approach to fishery management will differ substantially from the community-based approach in the example described earlier.

The Ecosystem Approach to Fisheries (EAF) is a risk-based management planning process that covers the principles of sustainable development including the human and social elements of sustainability, not just the ecological and environmental components (http://www.fao.org/fishery/eaf-net/topic/166237/en). It represents a union, therefore, of ecosystem management and fisheries management. The approach has been adopted by the FAO Committee on Fisheries (COFI) in 1995 as the appropriate and practical way to fully implement the Code of Conduct for Responsible Fisheries. The key principles addressed by the EAF are as follows:

- Fisheries should be managed to limit their impact on the ecosystem.
- Ecological relationships between species should be maintained.
- Management measures should be compatible across the distribution of the resource.
- Precaution in decision-making and action is needed because the knowledge of ecosystems is incomplete.
- Governance should ensure both human and ecosystem well-being and equity (FAO 2005).

The steps needed to implement EAF successfully are quite similar to those described for the implementation of Ecosystem Approach to Aquaculture (EAA) described in Chapter 9. While a number of pilot-scale commercial initiatives have adopted an ecosystem approach to bivalve culture (Chapter 9), there are no reports to date on the implementation of EAF in bivalve fisheries, although there are several ongoing projects on fish, such as the EAF-Nansen Project (http://www.eaf-nansen.org/nansen/en/), and the Bay of Bengal Large Marine Ecosystem Project (BOBLME; http://www.boblme.org/). For further details on EAF see FAO (2003, 2005), Garcia and Cochrane (2005), Plagányi (2007), Carocci *et al.* (2009) and De Young *et al.* (2008).

The following sections deal with fisheries for key bivalves within the four major groups in terms of global landings, principal fisheries, fishing methods, treatment and processing, management methods, and, where appropriate, restoration initiatives.

Scallop fisheries

Although there are about 400 species most of the commercial harvest comes from just two of these: the yesso scallop, *Patinopecten yessoensis*, and the sea scallop, *P. magellanicus*. Global landings from scallop fisheries have remained relatively stable averaging about 700 000 t annually in the 10 years between 2002 and 2011 (http://www.fao.org/fishery/statistics/software/fishstatj/en) Annual landings for *P. magellanicus*, mostly from the United States, increased by a small amount from 186 000 t in 2002 to 220 000 t in 2011, while landings of *P. yessoensis* mainly from Japan remained relatively stable at approximately 300 000 t over this period. In 2011, 74% of total global landings of scallops were from aquaculture operations, and this is probably an irreversible trend as a larger number of countries are moving increasingly towards semi- or total-cultivation methods, not just for scallops but for the other bivalve groups as well (see Table 9.1).

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This section first focuses on fishing and processing methods for scallops, then on the king scallop, *P. maximus*, and the queen scallop, *A. opercularis*, fisheries in Europe, followed by the *P. magellanicus* fishery on the east coast of North America. Although *P. yessoensis* accounted for about 57% of total global landings of scallops in 2011, the fishery is not a wild fishery in the generally accepted sense in that it depends on semi-cultivation methods. For this reason the species is not considered in this section but will be dealt with in Chapter 9. As far as possible the same approach will be taken in the sections on oyster, mussel and clam fisheries.

Fishing methods

The main methods for catching scallops are by dredging and diving, the former being the one most commonly used. Other methods include trawling, used in calico (*A. gibbus*) and saucer scallop (*Amusium balloti*) fisheries in southeastern United States and Queensland, Australia (Campbell *et al.* 2010), respectively; and hand devices, for example hand rakes, hand tongs, dip nets and scoops, in the bay scallop (*A. irradians*) fishery on the east coast of the United States (Blake & Shumway 2006).

Dredges

The type of dredge that is used varies depending on whether the sea bottom consists of mud, sand, pebble, rocks or boulders. In the offshore fishery in Canada and the United States, the New Bedford scallop dredge, which has undergone little change since it was first introduced, is used to capture *P. magellanicus*. The dredge consists of a heavy metal frame, about 3.5 m wide, attached to a bag made from steel rings (10–12 cm diameter) with interconnecting chains as reinforcement. On smooth terrain the bag may last several trips, but on rough ground it may not even last one trip (Naidu & Robert 2006). In shallow inshore waters vessels may use as many as 13 small dredges (~1 m wide) individually shackled to a single tow bar. The efficiency of this system is only 5 or 12% for rocky and smooth bottoms, respectively (Dickie 1955); the value for the New Bedford dredge is somewhat higher at 15–20% (Caddy 1989). In the British Isles (English Channel and Irish Sea), the standard dredge for *P. maximus* is a 0.75 m wide spring-toothed Newhaven dredge, with nine teeth and a collecting bag, the underside of which is constructed from steel rings (>70 mm internal diameter) while the back of the bag is made from rope netting of a similar diameter. If the dredge becomes caught, the whole tooth bar folds backwards on a spring, thus allowing the dredge to pass over the obstacle, and regain its original position once free (Figure 8.4). Most UK boats pull 4–8 Newhaven dredges per side, that is 8–16 per boat; a few larger vessels pull 20 dredges per side (Brand 2006). In a depletion experiment where an area of the seabed was fished repeatedly and run concurrently with a diver survey of the dredge tracks on the same fishing ground, Beukers-Stewart *et al.* (2001) found dredge efficiency to range between 24 and 30%, consistently lower than dredge efficiency estimates from the diver surveys (38–41%).

Two components contribute to overall dredge efficiency (e): efficiency of capture (E) and gear (mesh) selectivity (s); $e = E \times s$, where E is the number of scallops entering the dredge divided by the number in the dredge path, and s is the number of scallops caught divided by the number entering the dredge. One way of measuring s is by releasing marked scallops of known size from fragile plastic bags that burst as the dredge starts to move. On sea bottoms with abundant epifauna, dredge selectivity can be reduced to zero when the mesh becomes clogged with shells and debris. Efficiency of capture can be measured by comparing catches with densities photographed in front of the dredge (Caddy 1989). Capture efficiency

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tends to increase with increasing shell size; smaller scallops are able to swim away from the approaching dredge, or swim out of the dredge if captured. Many factors (some already mentioned) affect overall gear efficiency (*e*): nature of the seabed, speed of tow, weather conditions, time of day (gear less visible to scallops at night), swimming ability and endurance of scallops (see also Currie & Parry 1999 and Table 14.2 in Orensanz *et al.* 2006 for additional information on dredge efficiency).

Diving

Divers are limited to working in shallow waters (<30 m depth) because of safety and economic considerations. Usually teams of two to three divers operate from a small boat with an outboard engine. Each diver systematically covers lucrative spots within a given area, the choice invariably based on a prior working knowledge of the fishing ground. The scallops are picked up off the bottom and placed in a net bag that is attached to a plastic drum filled with water, making it only partially buoyant. As the bag fills up the diver adds more air to compensate for the increasing weight of the bag. When the bag is full the diver fills the drum with air and the drum rises to the surface for collection by the boatman (Hardy 2006). The diver only picks scallops of market size, leaving smaller individuals and all other epibenthic fauna undisturbed. This contrasts with the damaging effects of dredges already mentioned. Some regions have actually banned the use of dredges in favour of commercial diving. In the San José Gulf, Argentina, for example, dredges were banned in 1970 when the nearby San Matías Gulf fishery for *C. tehuelcha* collapsed. Since 1975 the fishery has only been opened to commercial divers (details in Ciocco *et al.* 2006).

Treatment and processing

Sorting of the catch usually takes place on board. Scallops are size-graded, packed into weight lots and are transported either directly to the consumer or to a processing plant. In the case of *C. islandica* the catch is machine-washed and sorted before it is landed in 300–500 kg lots in tanks or bags for machine shucking and processing at landing ports the next day (Strand & Parsons 2006). The meats are size- and quality-graded, then packed and quick-frozen. If both meat and gonad are required these are manually removed from the shell – a costly and labour-intensive process. The main market for this species is the United States. In New Zealand legal-sized 100 mm scallops (*Pecten novaezelandiae*) are sorted from the catch, packed in wooden boxes or sacks, and under-sized scallops and shells are returned to sea. Scallops are transported on a daily basis to processing factories, where they are shucked, after which the meats (muscle plus gonad) are usually chilled, frozen in layer packs or free flow form, or further processed by adding breadcrumbs or batter (Marsden & Bull 2006). About 50% supplies the home market and the rest is exported mainly to France, Australia and the United States. In the case of the *A. gibbus* fishery on the coasts of Florida and the Gulf of Mexico, the unsorted catch is relayed to a processing plant, where a shaker removes broken and dead shell, sand and unwanted species. The scallops are steamed to remove the meat and other tissues from the shell. A system of rollers separates out the adductor muscles, which are then rapidly cooled and packaged (Blake & Shumway 2006). The adductor muscle, the only part consumed by Americans, is rapidly cooled and can reach the consumer within 24–48 h without being frozen.

In all of the fisheries mentioned so far processing of the catch takes place on land, but in the case of *P. magellanicus*, by far the most important commercial species on a global scale, shucking takes place at sea and the scallop meats are washed, bagged and stowed on ice for the duration of the fishing trip (DuPaul *et al.* 1993).

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European scallop fisheries

The major contributors to the European scallop fishery are *P. maximus* and *A. opercularis.* Species of minor importance are *Mimachlamys varia* and *Pecten jacobeus* in the Mediterranean Sea.

Pecten maximus *and* Aequipecten opercularis

Commercial fishing for *P. maximus* began in the 1930s and is based on stocks in the waters on the Atlantic coasts of western Europe (Figure 8.7). Total landings (2002–2011) for *P. maximus* are shown in Table 8.4, together with the contributions made to the total catch by individual countries. From 2002 to 2011 landings increased two- to threefold for Belgium, Ireland and the United Kingdom, remained relatively stable for France and Norway, and decreased considerably for Spain and the Netherlands. Some aquaculture production of *P. maximus* is under way, mainly in Scotland and Ireland, but in 2011 this represented less than 1% of the landings of wild scallops.

Table 8.5 provides information on current fishing methods and fishery regulations in the European *P. maximus* fishery. In Scotland the fishery began in the Clyde area in the 1930s but has since expanded around the coast of mainland Scotland and its islands (Figure 8.7). Annual landings in the period 1983–1993 were approximately 4000–5000 t, but subsequently rose to approximately 9000 t, a figure that remained relatively stable up to 2011. Some areas such as the Irish Sea have shown progressive increases in annual landings, while some areas, for example northeast and northwest Scotland, are characterised by occasional rapid increases or declines, some of which are associated with fishery closures due to toxic algal blooms, but others with strong year classes (Dobby *et al.* 2012). The Isle of Man, not an EU member, has the smallest fishery in Europe with annual landings in or around 1500 t since 2002. The government of the island plays a leading role in the management of the fishery, with very strict regulations, some of which have been adopted by the UK government for the north Irish Sea, for example closed season and legal minimum landing size (MLS) (Brand 2006). To secure the future of the fishery six closed or restricted areas have been created since 1989, a large number for such a small island. These areas, generally referred to as MPAs, are often re-seeded with juvenile scallops as part of the closure process. Studies on scallops at Bradda, on the southwest coast, the longest established MPA, have shown that densities of scallops above the MLS (110 mm shell length) increased by a factor of approximately 10 over the period 1989–2006 in the closed versus the fished area (Figure 8.8; Beukers-Stewart & Beukers-Stewart 2009), and exploitable (adductor muscle and gonad) and reproductive biomass increased by a factor of 11 and 12.5, respectively, between 1989 and 2003 (Beukers-Stewart *et al.* 2005). This is significant in terms of fishery management because the build-up of high densities of large individuals enhances local reproductive potential, leading to the export of larvae to surrounding fishing grounds. In addition, juveniles in the closed area had higher survival and growth rates in the closed area, likely in response to reduced fishing disturbance (Beukers-Stewart *et al.* 2005). The local fishing industry in the Isle of Man is strongly supportive of these closures and is actively involved in research and stock enhancement programmes. Similar findings have been reported for the Isle of Arran MPA on the west coast of Scotland (Howarth *et al.* 2011).

The main fisheries in England and Wales are concentrated in the English Channel and Irish Sea. The fishery has developed strongly since the late 1980s due mainly to increased exploitation of offshore fishing grounds by large (28–35 m) beam trawlers towing 14–20 Newhaven dredges per side (Brand 2006). Annual landings, which exclude those from Scotland and the Isle of Man, are approximately 21 000 t, with most of this coming from the

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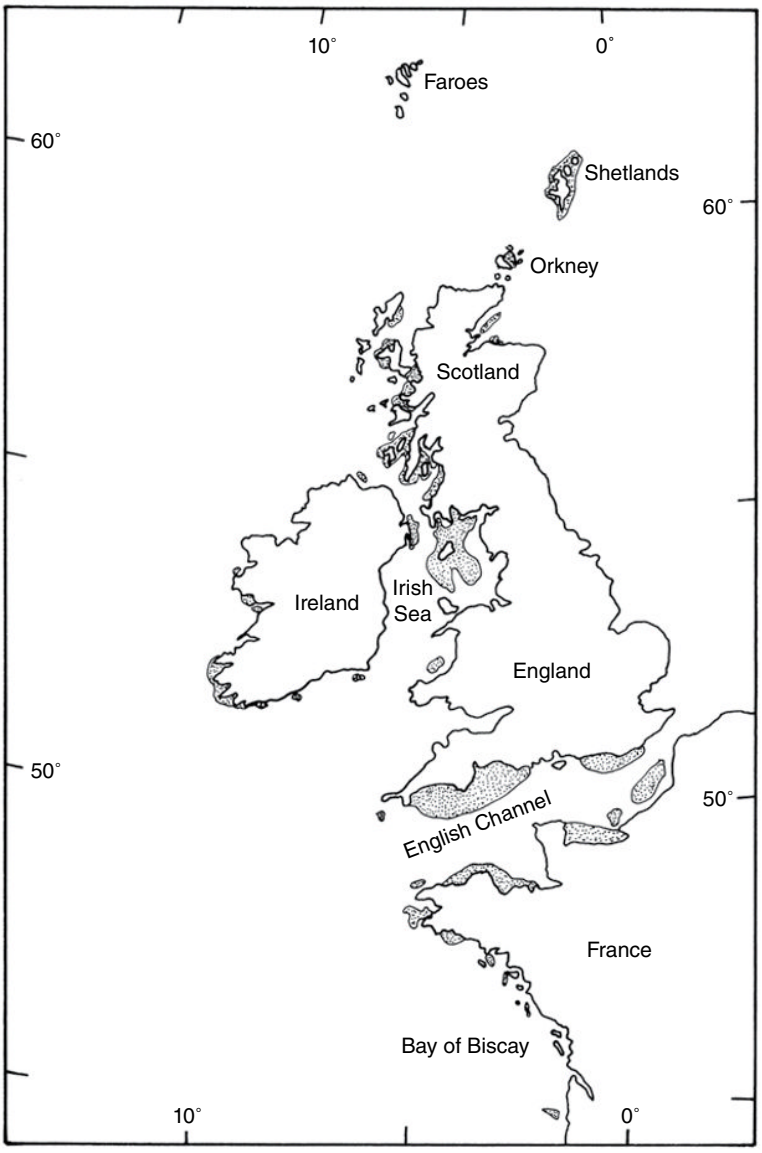


Figure 8.7 Distribution of the major fishing areas (stippled) for the scallop *Pecten maximus* in western Europe.
Adapted from Mason (1983). Reproduced with permission of John Wiley & Sons.

English Channel. There are no catch limits on UK scallop fisheries, and licence number restrictions are widely regarded as ineffective (Beukers-Stewart & Beukers-Stewart 2009). Restrictive licensing was introduced in 1999 for vessels over 10 m long. Under this scheme licences were granted to any vessel that had caught more than 1 t of scallops in any year between 1 January 1994 and 31 May 1998. This low entry qualification means that more than 450 vessels greater than 10 m are currently entitled to fish for scallops (Brand 2006).

In Ireland the largest fishery is off the southeast coast in the Celtic and Irish Seas, while smaller inshore fisheries are in sheltered bays on the south and west coasts. Since the 1990s the offshore fishery has expanded into the English Channel and southwards to the west coast

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Table 8.4 Annual landings (live weight, metric tonnes) of the scallop *Pecten maximus* in Europe.

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Belgium	432	521	530	485	534	634	674	886	1037	898
France	20109	18992	23335	26952	26876	26631	24104	25081	26888	27820
Ireland	1140	1719	1715	1196	694	901	1117	2641	1975	2266
Spain	223	176	151	135	105	226	150	124	150	60
United Kingdom	10611	11528	22225	19147	19873	22451	25027	29597	32612	31919
The Netherlands	473	536	612	702	781	757	781	754	315	0
Norway	575	504	679	681	799	866	899	748	748	739
Area total	33563	33976	49247	49298	49662	52466	52752	59831	63725	63702

UK landings include Scotland, Wales, the Channel Islands and the Isle of Man. All landings in these tables exclude landings from aquaculture production.

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Table 8.5 Annual landings for 2011 (live weight, metric tonnes), together with information on fishing gear, fishing vessels and current regulations in the scallop *Pecten maximus* fishery in Europe.

Fishery/ reference	Fishing gear	Fishing fleet	Current regulations
Scotland ~8000t The Prohibition of Fishing for Scallops (Scotland) Order 2003; Dobby *et al.* (2012)	Spring-loaded toothed dredge (maximum 0.76m wide)	75 vessels, 10–30m length	Minimum legal landing size (MLS) of 100 mm length (larger dimension than shell height), but 110 mm in Irish Sea area; no limits on TACs or quotas; maximum between 8 and 14 dredges per boat side depending on the distance of fishing from the coast; French dredges prohibited; restrictions on licensing vessels >15m length to reduce effort; curfew from 21:00 to 06:00; closed season from 1 October to 31 March; in some areas there are seasonal and temporary closures. One no-take zone (NTZ) on west coast
Isle of Man 1534t Brand (2006), Murray *et al.* (2011)	Spring-loaded toothed dredge (maximum 0.76m wide)	~25 vessels, 9–18m	MLS of 110 mm length; maximum of 7 dredges per side permitted within the 3–12 mile limit; French dredges prohibited; fishing not permitted after 20:00h or before 6:00h local time within the 3–12 mile limit; all vessels must carry satellite-tracking devices; recreational fishermen are prohibited from taking >18 scallops in any 1 day; closed season (1 June–31 October); six small closed/restricted areas to protect inshore stocks
England, Wales and Northern Ireland ~22000t Brand (2006); The Scallop Fishing (England) Order 2012; The Scallop Fishing (Wales) (No.2) Order 2010; AFBI (2012)	Spring-loaded toothed dredge (<0.85m wide) and beam trawl	>400 vessels, >10m	MLS of 110mm in the northern Irish Sea, eastern English Channel and Welsh waters, and 100mm length elsewhere; no limits on TACs or quotas; maximum of 8 (7 in Wales, 6 in Northern Ireland) dredges per side of boat from 0 to 12 miles offshore; French dredges prohibited; restrictive licensing for vessels >10m; curfew 19:00–07:00 but 21:00–05:00 in Northern Ireland; closed season (1 May–31 October, 1 June–31 October, or 1 July–30 September depending on the region); a number of closed areas on west coast of Wales and southwest coast of England to protect habitats
Republic of Ireland 2266t Tully *et al.* 2006	Spring-loaded toothed dredge (maximum 0.80m wide) and beam trawl	Boats up to 36m length; number not available	MLS of 100mm length but 110mm in the Irish Sea; up to 36 dredges per boat allowed; effort restrictions on vessels >10m; licence to fish based on track record; no closed season except in the Irish Sea (1 June–31 October)
France (St Brieuc Bay) 27820t Brand (2006), Binet (2010)	French dredge with diving plane and fixed teeth, heavy Breton dredge, and spring-toothed dredges; dimensions vary depending on fishing ground	In St Brieuc Bay more than 250 boats; mean length 10.6m	MLS of 102mm length for all waters except the eastern English Channel, where MLS is 110mm; maximum of 2 dredges per boat with a maximum width of 2m; max vessel length of 13m; local complex series of regulations including licensing, effort, catch and gear restrictions; in St Brieuc Bay the closed season is May–October; only 2 days fishing allowed per week and only 45–60min per day; no spatial closures within St Brieuc Bay, but different quotas are set for inshore and offshore grounds; several fishing areas are enhanced with hatchery-produced spat and fished in rotation

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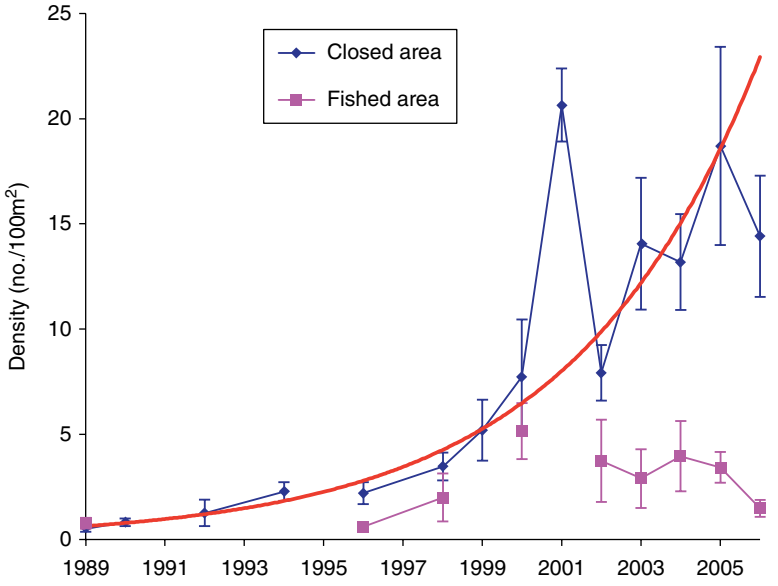


Figure 8.8 *Pecten maximus* density (mean number/$m^2 \pm$ SE) estimated by dive surveys (1989–2006) in the Bradda closed and fished areas off the southwest coast of the Isle of Man. Beukers-Stewart and Beukers-Stewart (2009). Reproduced with permission of B. Beukers-Stewart.

of France (Tully *et al.* 2006). Offshore stocks are fished by large 20–36 m long vessels, which may each tow as many as 34 spring-loaded dredges. From 2002 there was a gradual decline in fishing effort due to various economic constraints, for example physical condition of the vessels, rising fuel costs and declining market prices for scallops. In addition, a days-at-sea regime was imposed on the Irish fleet by the European Commission (Council regulation 1415/2004) in 2005, which, when imposed by Irish legislation, limited the activity of the vessels. However, despite these limitations, overall landings for Ireland have remained in or around 2000 t per year.

Two scallop fisheries, St Brieuc Bay and the Bay of Seine on the English Channel coast, are the mainstay of the French scallop industry. The following description focuses on the St Brieuc fishery, the largest scallop beds in France spanning 150 000 ha. Annual landings peaked at 12 500 t in the mid 1970s, but subsequently decreased to a figure of approximately 7500 t in 2008, with a concomitant decrease in the number of licences from 460 to 240 (Binet 2010). In the 1990s, in an attempt to rebuild the fishery, licence holders were not authorised to transfer their licences or any of their catch/hour quotas to a person of their choice. Moreover, to stop the growth in fishing capacity, vessels were limited to 13 m length and 250 horsepower. Scientific support also helped to build trust between researchers and fishers in order to develop management measures compatible with the biological needs of the stock. Consequently, this fishery is currently regarded as one of the best-managed fisheries in France (for details see Binet 2010).

Fishing countries and their individual contributions to the total catch of the queen scallop *A. opercularis* (2002–2011) are presented in Table 8.6. Annual landings have remained relatively stable at 15 000 t between 2002 and 2009, but increased twofold in 2010 and threefold in 2011, mainly due to increased UK landings. Currently, queenie landings are 57% of those for king scallops (Tables 8.4 and 8.6). The major fishing areas are the northern Irish Sea, western Scotland, the western English Channel and eastern Faroe Islands (Figure 8.7). Queenies are fished using both toothed and toothless dredges as well as bottom trawls. Due

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Table 8.6 Annual landings (live weight, metric tonnes) of the scallop *Aequipecten opercularis* in the European fishery.

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Faeroe Island	2366	3659	4055	4512	5237	4970	2851	3115	4771	4216
France	4260	4275	2978	4058	4614	5905	3282	1241	3937	2528
Isle of Man	1655	1426	859	1133	448	2028	858	1473	2817	4529
United Kingdom	10774	7317	5094	3856	5056	5661	4659	6843	13082	25096
Area total	19055	16677	12986	13559	15355	18564	11650	12672	24607	36369

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to differences in their swimming behaviour, trawls tend to be used during the summer and autumn months when water temperatures are high and scallops are active swimmers, while dredge fishing tends to occur during the cooler water temperature months when queenies are unable to swim up from the seabed (Jenkins *et al.* 2003).

In the United Kingdom the main fishing area is off the southwest coast of Scotland, but to date there are few regulations in place for management on the fishery. The EU minimum size is 40 mm shell height but it is generally uneconomic to process queens less than 55 mm shell height. There are no closed seasons for queenies or restrictions on fishing time or catches (Beukers-Stewart & Beukers-Stewart 2009). In the Faroe Island fishery, only one dredge vessel is currently allowed to participate in the fishery, and fishing is only permitted in two areas. Therefore, it is likely that only a small proportion of the population is being fished. There are seasonal closures in the East from April through August and to the North from August through March, as well as spawning closures but there is no minimum size limit although scallops less than 55 mm are typically not processed (Hoydal *et al.* 2010). The Bay of Brest supports the main French queenie fishery, and here there are limits on the number of fishing days, the size of the vessel, licences and individual catch quotas (Townsend *et al.* 2008).

In the Isle of Man substantial management measures for the queen scallop fishery are in place. These include a complete closure from April through May in Isle of Man territorial waters, and a dredge closure in effect from June through August (DEFA 2010). Vessels larger than 212 kW are not allowed to enter the fishery, there is a curfew for fishing from dusk to dawn (18:00–06:00) and a weekend ban. Catches information must be recorded, there is a minimum catch size of 50 mm shell height, as well as a mandatory satellite vessel monitoring system, and a recommended TAC limit is set based on the current status of the population (DEFA 2010; Andrews *et al.* 2011; Read 2011). A queen scallop conservation zone, covering more than 50% of territorial waters, has been created and all forms of dredging are permanently banned in this zone. During the 2010/11 and 2011/12 fishing seasons, catches of queenies were well over the recommended catch limit, but at the time this was allowed by managers. However, it was later determined that these high catches (see Table 8.6) were not sustainable (Murray *et al.* 2011; Murray and Kaiser 2012). In 2011 the Isle of Man trawl fishery, but not the dredge fishery, received Marine Stewardship Council eco certification as a sustainable and well-managed fishery (www.msc.com). However, to keep their certification, they must ensure that the catches are sustainably controlled in subsequent years (Andrews *et al.* 2011; Andrews and Brand 2012).

Northwest Atlantic fishery

This fishery is based on the sea scallop *P. magellanicus*, which is by far the most important species in wild fisheries, accounting for 53% of the total annual global scallop catch in 2011 (Tables 8.1 and 8.7). The species is found only in the Northwest Atlantic from Cape Hatteras to Labrador and inhabits depths of 10–100 m. Sea scallops are large, commonly reaching sizes of 100–150 mm and frequently larger (Naidu & Robert 2006). All landings come from just two countries, Canada (20–25%) and the United States (75–80%), and while the combined catch has remained remarkably stable from 2002 to 2011, there has been a decrease in Canadian landings and a concomitant increase in landings from the United States over that period (Table 8.7). Consumers in North America prefer shucked scallop meats as opposed to scallops in the shell, and as scallops cannot maintain tight shell closure for prolonged periods and die soon after their removal from the sea, they are usually shucked at sea and the meats are stored on ice until the vessel returns to port. Hence, national annual landings for sea scallops are given as weight of meats in metric tonnes rather than live

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Table 8.7 Annual landings (live weight, metric tonnes) of the scallop *Placopecten magellanicus* from Canada and the United States.

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Canada	93 971	92 520	81 260	53 684	61 406	64 681	67 620	62 590	59 792	59 293
United States	186 336	200 279	243 424	214 245	222 948	220 751	202 265	218 064	215 270	221 750
Area total	280 307	292 799	324 684	267 929	284 354	285 432	269 885	280 654	275 062	281 043

weight as in FAO statistics (see Table 8.7); for *P. magellanicus* meat weight works out at approximately 8–10% of live weight. Until 2002 the Canadian offshore fleet fished primarily for fresh scallops but since then with the introduction of freezer trawlers, individually quick-frozen (IQF) scallops began to be landed. In recent years these vessels have accounted for nearly 60% of the landings.

The main fishing grounds for sea scallops are the mid-Atlantic (from Virginia to Long Island, New York), the Bay of Fundy and waters off Nova Scotia and Newfoundland (Figure 8.9). There is also a small, primarily inshore fishery for sea scallops in the Gulf of Maine. The offshore fishery is concentrated on Georges Bank situated in both US and Canadian territorial waters, about 200 km southwest of Cape Sable on the southern tip of Nova Scotia. The New Bedford scallop dredge is used to fish for scallops, and has already been described earlier. For inshore fishing small wooden boats, about 20 m long, are used, while larger steel vessels, up to 46 m long, operate in offshore waters. The latter are technically well equipped



Figure 8.9 Map showing scallop (*Placopecten magellanicus*) and oyster (*Crassostrea virginica*) fishery regions in Canada and the United States.

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and are capable of fishing on a 24 h, year-round basis (Naidu and Robert 2006). Offshore vessels typically employ two dredges, each varying in width from 4 to 6 m.

Georges Bank produces about 40% of total sea scallop landings in North America and represents the world's largest single scallop resource. Attention will mainly focus on management of this fishery, but, where appropriate, information will be provided for the other fisheries mentioned earlier. The fishery is independently reviewed each year by the Canadian Science Advisory Secretariat (CSAS) of the Department of Fisheries (DFO), and by the New England Fishery Management Council of NOAA's National Marine Fishery Service in the United States. The International Court of Justice (ICJ) established an international boundary line in 1984, which awarded the northeast portion of Georges Bank to Canada. About 80% of sea scallop landings in Canada comes from this portion (DFO 2011), and within it there are two management zones, *A* and *B*, with *A* being more productive than *B*, which is regarded as a marginal scallop habitat (Figure 8.10; Jonsen *et al.* 2009). Management of the two zones includes TACs and meat counts of 33 and 50 meats per 500 g for *A* and *B*, respectively. Meat count regulations are imposed in order to protect young scallops. There is limited entry to the fishery, mandatory logs for vessels of 19.8 m and over, and regulations governing trip catch limits and trip duration (not exceeding 12 consecutive 24 h periods), electronic

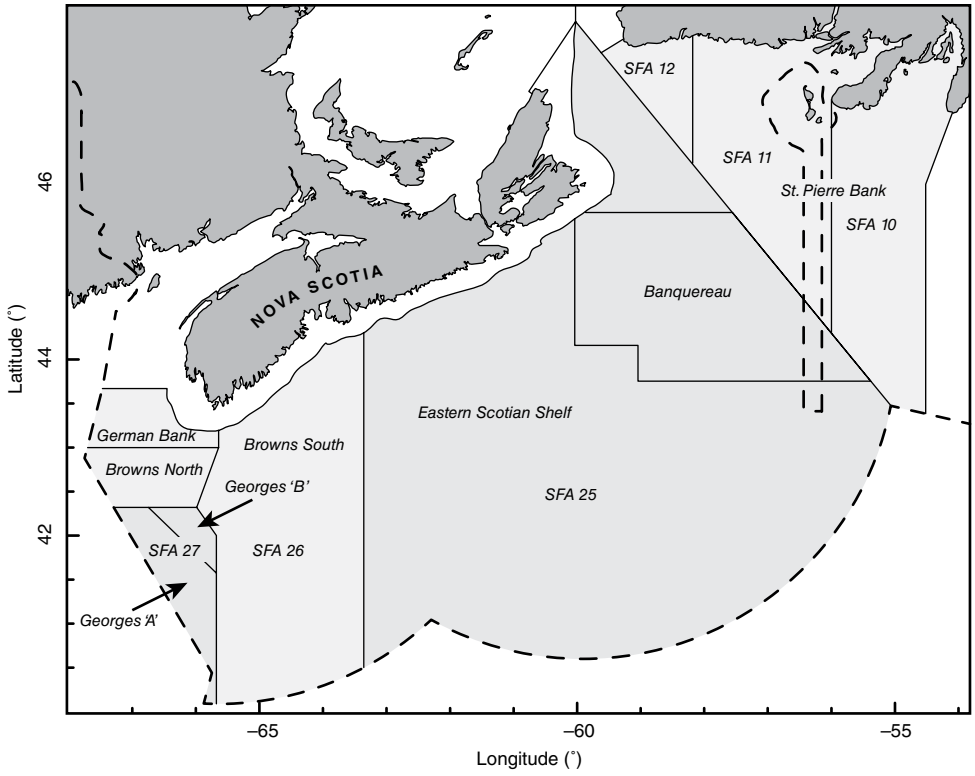


Figure 8.10 Offshore scallop (*Placopecten magellanicus*) fishing areas (SFA) in the eastern part of Canada and the United States. The areas are SFA 10, 11, 12 near St. Pierre Bank and Newfoundland (upper right of figure). SFA 25 comprises Banquereau Bank, and Eastern Scotian Shelf. SFA 26 is composed of Browns Bank and SFA 27 is composed of Georges Bank *A* and *B*. The 200-nautical-mile limit is shown from the coast line. Within the Canadian area, the boundaries of each fishing area are outlined. Eastern Canada Sea Scallop (offshore) Figure 1. www.dfo-mpo.gc.ca/fm-gp/sustainable-durable/fisheries-peches/scallop-petoncle-eng.htm. Reproduced with the permission of Fisheries and Oceans Canada.

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vessel monitoring, dockside monitoring of all landings and industry-managed closures to protect juvenile scallops and increase yields (DFO 2011). There are two specific area closures for approximately 7 weeks from early February to the end of March to protect spawning cod, and for the month of June to protect yellowtail flounder (DFO 2011). Enforcement of conservation measures include at-sea inspections, frequent flyovers by aircraft, at-sea independent observers, a vessel monitoring system and full independent verification of all catch landed (DFO 2011). The condition of the eastern Canadian offshore fishery, which includes scallop fishing areas (SFAs) 10–12, 25 and 26 as well as Georges Bank (see Figure 8.10) is regarded as healthy, with current biomass levels above their long-term medians, and annual fishing quotas in line with scientific advice to maintain the future health of the population (DFO 2011; but see Table 8.7). On 25 March 2010, the fishery received Marine Stewardship Council (MSC) eco certification, the first scallop fishery in North America to receive this certification.

Inshore Canadian stocks are generally not subjected to a minimum legal size or meat count regulations. However, where regulations are in force, for example the Bay of Fundy, the fishery is managed using limited entry, gear type and size dimensions, seasonal closures, a minimum shell height of 105 mm and a meat count from 33/500 g to 72/500 g, depending on location and time of year. Quotas were introduced in 1997. The Full Bay Fleet operates under an Individual Transferable Quota (ITQ) system, while the Mid and Upper Bay fleets fish with competitive quotas (DFO 2013).

In the United States the main scallop fisheries are Georges Bank and the mid Atlantic (Figure 8.9). Sea scallops are now the most valuable fishery in the United States, worth about $580 million in 2011 (Rowe and Smith 2012). On Georges Bank, biomass index remained low from 1982 through 1994 due to overfishing, but subsequently increased markedly, and has remained at a high, roughly stable level since 2000. This recovery was due to the introduction of a suite of new management measures in 1994. The fishery changed from open to limited access whereby vessels that had a history of fishing for scallops were given permits, but no new permits were issued. Each permitted vessel was given a fixed number of days to fish. Meat count regulations were replaced with gear restrictions that gradually increased ring size from 76 to 102 mm in 2004. A minimum shell height of 4 in. (101.6 mm) was imposed, but has since been reduced to 3.5 in.(89 mm). Three large areas on or near Georges Bank were closed indefinitely to help rebuild groundfish and scallop stocks, and between 1994 and 2005 biomass increased about fivefold compared to adjacent open fished areas (Hart 2012). In 1999 fishery managers created the first controlled access to one of the closed areas and during a 5-month opening fishers harvested $36 million in landings, representing about one-third of US landings for that year, demonstrating how quickly scallops could recover during closures (Frady 2007). However, since 1999, despite several short-term openings of the three closed areas, Georges Bank landings have only recovered to historic levels. It is likely that effort controls, such as days-at-sea limits and ring size increases, have played a more important role in restoring the Georges Bank fishery than have closed areas.

The mid-Atlantic fishery (from Virginia to Long Island, New York; Figure 8.9) is currently more lucrative than Georges Bank, being responsible for 50% more landings than the latter. From 1979 to 1998 the survey biomass index in the fishery was low but increased about tenfold between 1998 and 2003. This was due to strong recruitment combined with conservation measures such as reduced fishing mortality, increased dredge ring requirements, and the implementation of rotational closures of two areas, with further closures in 2004 and 2007 that allowed many scallops to grow to larger sizes (Hart 2006). The US sea scallop fishery from Maine to North Carolina was certified to MSC standard in 2013. For more details on US management measures see Electronic Code of Federal Regulations (e-CFR) at www.ecfr.gov.

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Other scallop fisheries of commercial importance are the Patagonian scallop *Zygochlamys patagonica*, off the coast of Argentina with landings of 48 000 t in 2011; the Peruvian scallop *Argopecten purpuratus*, off northern Peru (93 050 t); and the calico scallop *A. gibbus* off southeast United States and in the Gulf of Mexico (18 392 t).

Interestingly, there are increasing efforts to culture *P. magellanicus* using the technology that has so successfully been employed by Japan and China for other scallop species (Chapter 9). So far, experimental culture of *P. magellanicus* has been tested at a number of locations in the northeast Atlantic, for example Québec (Milke *et al.* 2004), Nova Scotia (Pilditch *et al.* 2001) and Maine (Parsons *et al.* 2002), but the industry is currently largely constrained by the availability and affordability of high-quality spat.

Oyster fisheries

The history of oyster production in both Europe and North America is well documented. In the nineteenth century flat oyster (*Ostrea edulis*) production in Europe was booming, but from the end of the century there was a gradual decline in production in the British Isles, most likely due to overfishing and an increase in pollution. To reverse the trend non-native *Crassostrea virginica* were imported for relaying, carrying with them slipper limpets and predators such as oyster drills, which decimated much of the remaining native oyster beds. Much the same scenario occurred in France but was exacerbated by disease, which affected not just the indigenous flat oyster stocks but also the Portuguese oyster *Crassostrea angulata*, introduced into France in the 1860s (Buestel *et al.* 2009). Flat oyster production fell from 28 000 t in 1960 to 2000 t in 2000, and is currently even lower at approximately 1000 t per annum. Concomitant with this decline has been the spectacular increase in the aquaculture production of *C. gigas* in France (95 000 t in 2011). A similar situation is occurring in other European countries.

A corresponding decline in oyster production also occurred in the *C. virginica* fishery on the east coast of the United States. Annual landings fell from 200 000 t in the 1950s to 104 000 and down to 44 000 t in the 1990s, and today they are about 76 000 t (Figure 8.11; note that data in this figure are meat weights). The initial decline, which started in the late 1880s, was primarily due to a fall in demand for oysters because consumers became aware that oysters could contain pathogens, three economic depressions, and biological and physical damage to oysters and their beds through predation, siltation and harvesting by dredges (MacKenzie 2007). In addition, the diseases MSX and Dermo (see Chapter 11) have had a devastating effect on stocks from Chesapeake Bay southwards to Florida. For those interested in the history of the fishery the fascinating and well-illustrated account by MacKenzie (1996) is highly recommended. Kirby (2004) also reviews the historical expansion and collapse of other oyster fisheries in North America and Australia.

Total global landings from wild oyster stocks for the years 2009–2011 are presented in Table 8.1. This table does not include oyster landings from aquaculture, which presently account for 95% of all oyster production (Table 9.1). Out of 200 oyster species there are only three (*C. virginica*, *C. gigas* and *O. edulis*) that are fished in commercial quantities, although the category 'cupped oysters nei' probably includes other species of *Crassostrea* (Table 8.8). In the 1990s more than 80% of annual landings were from *C. virginica* and about 8% from *C. gigas*. However, for *C. gigas* this increased in the 2000s from 6% in 2003 to 30% in 2011 (Table 8.8), which was primarily due to the increasing contribution of Korea to global landings of this species.

In the following section the *C. virginica* fishery in the United States is described in some detail. While oyster numbers continue to fall the fishery still remains a wild fishery, although

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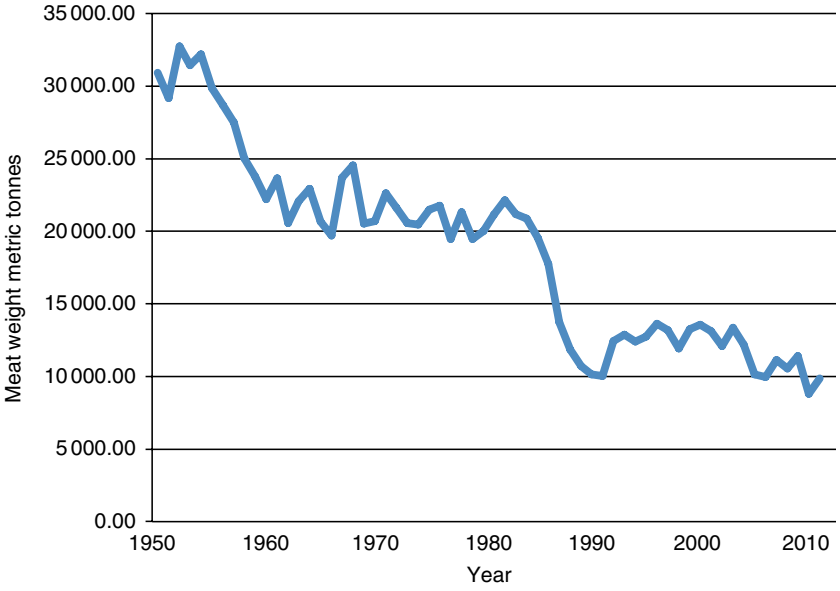


Figure 8.11 The fall in annual US landings (meat weight in metric tonnes) of the eastern oyster *Crassostrea virginica* between 1950 and 2011.
Data from the National Marine Fishery Service (NMFS), NOAA, USA.

the situation may change with the development of hatchery-produced, disease-resistant strains for relaying (see Chapter 10). For information on the *C. virginica* fishery in Mexico see the Instituto Nacional de la Pesca (INP) website http://www.inp.gob.ec/, and http:// www.dfo-mpo.gc.ca for information on the Canadian fishery.

The *Crassostrea virginica* fishery in the United States

The distribution of *C. virginica* extends from the St Lawrence River in Canada to the Gulf of Mexico, the Caribbean and may even extend onto the coasts of Brazil and Argentina. There have been numerous introductions of the species into the Pacific coast of North America but none of these plantings has survived, with the exception of a few small, but not commercially viable, populations in British Columbia (Carlton & Mann 1996) and Pearl Harbour, Hawaii (Coles *et al.* 1999).

The fishery (Figure 8.9) is centred on Cape Cod (Connecticut), Chesapeake Bay (Maryland and Virginia), North Carolina, Florida, Louisiana and Texas, which altogether contribute about 60% to annual landings of *C. virginica* in North America (Table 8.9). Mexico, on the Gulf side, also lands substantial quantities, contributing about 38% to the annual global catch of this species. Canada, on the other hand, contributes only about 2%. The species grows and matures very quickly, reaching 100–115 mm in length during the first 2 years of life, although in some areas such as Maryland this can take up to 5 years.

The main fishing methods for harvesting subtidal oysters are tongs, dredges and diving. In some areas such as the southern states, intertidal oyster reefs are harvested by hand. Hand tongers comprise two long flexible wooden poles, about 5–5.5 m long, joined like scissors towards one end. Metal basket rakes are attached at this end, and periodically the contents of the baskets are lifted and spilled out onto a culling board on deck for sorting. Market-size oysters (>3 in. or >76 mm shell length) are separated from undersized oysters, shell and debris, which are returned to the sea to allow further growth in the case of the oysters, or to

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Table 8.8 Global landings (live weight, metric tonnes) of the main oyster species fished in commercial quantities.

Species	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Crassostrea gigas	9048	21546	27226	28620	39398	34607	31554	26748	30452	35935
Crassostrea virginica	159168	165108	112789	125097	91430	109590	90948	96073	115925	120795
Ostrea edulis	1523	2369	2926	1915	2297	2894	2152	1822	1467	1138
Cupped oysters nei*	3475	2831	3140	4081	3799	4029	5199	6193	9867	45687††
Others†	3066	3003	2600	2731	2760	2623	2615	2156	208	255
Total	176280	194857	148681	162444	139684	153743	132468	132992	157919	203810

†includes the Chilean flat oyster *Ostrea chilensis*, the mangrove oyster *C. rhizophorae* and the slipper cupped oyster *C. iredalei*.
††this value is more than fivefold higher than the value for 2010.
More than 88% of this figure is from Mexico; the reason for the huge increase in one year is unknown, but it cannot be due to inclusion of cultured species as annual production of cultured oysters in Mexico was only 4000 t in 2011.
nei*, not elsewhere indicated (countries concerned did not state the specific name of the oysters landed).

Table 8.9 Global landings (live weight, tonnes) of the eastern oyster *Crassostrea virginica*.

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Canada	2410	1470	4255	3245	1651	2428	1685	2100	1934	1877
United States	109188	118308	63493	81958	46877	61214	48791	57115	67327	76037
Mexico	47570	45330	45041	39894	42902	45948	40472	36858	46664	42881
Total	159168	165108	112789	125097	91430	109590	90948	96073	115925	120795

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serve as cultch for settling larvae. The deeper the water, the more difficult it is to use hand tongs with two rigid arms, so at depths greater than 7 m heavy metal 'patent tongs', operated by a winch system to close the tongs, are used. In dredge fishing, small dredges called hand scrapes are used from small boats. Alternatively, larger heavier dredges similar to those used in scallop fisheries are towed, usually in twos, on alternate sides of a boat, and the contents are winched up and brought onto the deck for culling. In waters where clumps of oysters may be widely separated from each other, diving is a very efficient method for collecting oysters, particularly large ones that cannot be reached by tongers or dredgers, either because they are too shallow or too deep (Kennedy 1989).

The oysters are bagged on board and brought to the packing house on shore for grading. Unlike the situation in other oyster fisheries, most oysters in the United States are sold as shucked meats rather than as shellstock. When sold as shellstock the oysters are graded and packed in sacks, bags, baskets or boxes. Fresh shucked meats are packed in containers (half-pint to a gallon) or IQF for coating with breadcrumbs or batter, block-frozen for stews and soups, or canned. For example, Kellum Seafood, a large family-run company in Virginia, sells their oysters live in the shell, fresh-shucked or quick-frozen (breaded and unbreaded). Their shucked oysters are designated according to size range: small (>500 meats/gallon; US gallon = 3.785 l), standard (301–500), select (211–300), extra select (160–210) and largest count (<160).

Between 2002 and 2011 annual landings in the United States fell from 12 102 t of meats to 9846 t (http://www.st.nmfs.noaa.gov/commercial-fisheries/commercial-landings/annual-landings/index). The Louisiana fishery is currently the most successful with 50% of total landings in the United States coming from this state, followed by Texas (18%), Florida (14%), Chesapeake Bay (12%), North Carolina (4%) and Connecticut (<1%). Management regulations for the Louisiana fishery are now described, keeping in mind that each state has its own specific control methods. Efforts to restore the Chesapeake fishery, once one of the world's largest oyster producers (Kennedy & Breisch 1983), is then considered.

The Louisiana oyster fishery

The Louisiana Department of Wildlife and Fisheries (LDWF; http://www.wlf.louisiana.gov/) manages the public (state) beds, which comprise almost 1.7 million acres. Management of public beds includes setting oyster seasons, monitoring harvest levels, and cultch planting (reef building) projects. These beds serve as a source of seed oysters for transplant to private leases. The harvest season on public beds runs from the first Wednesday following Labor Day (first Monday in September) to 30 April of the following year, although in recent years the season may have been closed or delayed until the end of October if biological concerns or enforcement problems were encountered. An individual may lease part of the bottom of any public bed by applying to the LDWF and paying a fee. A private lease expires after 15 years and may be renewed for a further 15 years. Leased beds are clearly demarcated from public beds and prominently marked with name and number of the lessee. On these beds fishing is permitted at any time of the year. However, on both public and leased beds harvesting oysters is prohibited from 30 min after sunset to 30 min before sunrise. Public beds were once the backbone of the Louisiana fishery but since 2009 more than 70% of all oysters landed have come from private leases.

Oysters may be taken from public grounds by dredgers, scrapers and tongs. Dredges and scrapers should be no wider than 6 ft (183 cm) and the teeth should be no longer than 5 in. (127 mm). No more than seven dredges are allowed per vessel, with the exception of private lessees who are permitted to use any fishing implement, provided it does not impair or destroy the water bottom. Oysters taken from public beds must be at least 3 in. (76 mm) in

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length, but this restriction does not apply to private leases. On public beds undersized or dead oysters must be immediately returned to the substrate from which they were taken. The exceptions are lawfully fished seed oysters and leases on private beds. No more than 25 sacks (1 sack = 1.5 bushels; 120–180 oysters depending on size) per day per vessel can be taken on public beds, while the harvest amount from private leases is unlimited. All individuals harvesting oysters must be residents of Louisiana and have a commercial fisherman's licence, a fresh products licence if selling directly to consumers, a commercial gear licence and a vessel licence.

All harvested oysters are tagged, whether in containers or sacks. Unshucked oysters must be tagged with the harvester's name, address, licence number, area harvested and the harvest date. Containers of shucked oysters must be dated and have the name and address of the original processor, shucker-packer or repacker, and the Louisiana certification number. All tags are kept for 90 days and there is zero tolerance for untagged oyster containers or sacks. To control the growth of harmful bacteria, for example *Vibrio vulnificus* (Chapter 12), containers of shucked oysters should be stored at 0–3.5°C, ideally packed in ice, and live oysters should be stored at 4.5–7.0°C. For more details see http://www.seagrantfish.lsu.edu/resources/factsheets/index.htm.

Part of the LDWF management plan is the placement of the substrate called cultch, which provides free-swimming oyster larvae with a firm attachment site on which to settle and grow. The practice began in the 1930s when the state used fossil clam shell as cultch material on the seed grounds. After shell dredging was stopped in the1990s, the state looked to other possible sources and materials for cultch. Today, cultch deposition is carried out on public seed grounds using materials such as oyster shell, limestone and crushed concrete (LDWF 2011; Figure 8.12). In November 2011 the LDWF announced the closure of seed-oyster harvest in public areas east of the Mississippi River, a region that contributes about 30% to all oyster landings in the state. The reason for the closure was that these areas held small amounts of seed-oyster stock because a significant portion of the available stock had been harvested during the 2011/2012 oyster season. Additionally, sampling of seed-oyster loads on commercial vessels had shown that excessive amounts of cultch material were being removed during seed harvest, an activity that threatens the long-term sustainability of the reefs.



Figure 8.12 Men use a high-pressure hose from a barge to blow oyster cultch into the water. A person on the crew boat beside the barge first checks the bottom, using a cane pole, to make sure that the barge is over an existing oyster bed.
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In April 2010 an explosion on the British Petroleum *Deepwater Horizon* oil rig off the coast of Louisiana uncorked an underwater gusher, spewing more than 200 million gallons of oil into the Gulf of Mexico. This is considered as the largest accidental marine oil spill in the history of the petroleum industry to date. The well was finally sealed 3 months after the accident, although there are indications that it continues to leak. As a consequence there has been extensive damage to marine and wildlife habitats along the Louisiana coast; about 125 miles of the 400 miles of coast have been polluted by the oil spill. In many areas 60–80% of oysters were wiped out, not by the oil but by the massive infusion of freshwater diverted from the Mississippi River into wetlands in an effort to keep oil from the coast. As a result, oysters were killed en masse by the reduced salinity (Brand 2011). Three months after the spill a large area of 84 000 square miles, approximately 35% of the Gulf of Mexico, was closed to fishing, and although some of this is now open, additional closure areas are required when tar mats are reported in their vicinity (http://www.wlf.louisiana.gov/). In 2011 British Petroleum agreed to fund up to $1 billion for eight projects in phases I and II of an early restoration programme to accelerate efforts to restore natural resources damaged as a result of the oil spill. Funding for two projects in phase I has been allocated to Louisiana, one of which is the Louisiana Oyster Cultch Project costing more than $15 million. The project involves the placement of oyster cultch onto approximately 850 acres of public oyster seed grounds throughout coastal Louisiana, and the construction of an oyster hatchery facility that would produce supplemental larvae and seed to an existing oyster hatchery (see details in NOAA 2012c). The latter is the Louisiana Sea Grant's hatchery on Grand Isle, which is the largest oyster hatchery along the Gulf coast of the United States. To date, the hatchery has focused on triploid production, breeding oysters for disease resistance and developing off-bottom cultivation techniques, for example longline systems, to protect oysters from predation, fouling and disasters such as hurricanes. The two facilities will collaborate to supply 50% of its larval production to the state for setting in public beds (http://www.laseagrant.org/2013/hatchery-20-years/). Phase II projects, initiated in 2012, will help restore nesting habitats for beach-nesting birds and sea turtles.

In the Gulf region there are high mortalities (>50%) of market-sized oysters from Dermo disease. However, Gulf oyster populations still persist and support a viable fishery because of a balance between oyster recruitment and mortality. While an increase in salinity causes a rise in Dermo prevalence and intensity (Chapter 11), recruitment is also enhanced during periods of high salinity (Soniat *et al.* 2012).

The Chesapeake Bay oyster restoration programme

At the end of the nineteenth century, the *C. virginica* fishery in Maryland and Virginia was the largest in the world with combined landings at 60% of US landings. At that time annual landings in Maryland and Virginia were 20 and 11 million bushels, respectively, with most of these from the Chesapeake Bay (Figure 8.9; Kennedy *et al.* 2011). Over the next century landings declined precipitously, not just in Chesapeake Bay but also in other states on the US coast (Figure 8.11). Today, populations in Chesapeake Bay are at less than 1% of historic levels. During this decline numerous restoration efforts were made but with limited success. These were initially implemented to maintain the fishery using conventional fishery management measures. However, in the 1990s efforts began to focus more and more on restoration of the oyster habitat, more specifically on the reef substrate on which oysters like to settle and grow. These reefs, often referred to as bars, are primarily built by *C. virginica* through successive reproduction and settlement of larvae onto an existing reef structure. Oyster spat produce a crystallizing cement of calcium carbonate that allows them to bond together and expand the reefs spatially in three-dimensional space (Piazza *et al.* 2005).

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The species has been dubbed an 'ecosystem engineer' because its reefs provide many benefits to coastal and estuarine systems including food and habitat for fish, crabs and birds; water quality improvement; and shoreline stabilization and coastal defence (Grabowski and Peterson 2007).

The rapid decline of the Chesapeake Bay fishery was accompanied by the loss of the reef habitat. In the Maryland waters of the Bay it is estimated that 90% or more of historic reef acreage has been lost to direct damage from overharvesting and sedimentation of remnant reefs. Globally, 85% of oyster reefs have been lost, making oyster reefs one of the most severely impacted marine ecosystem on the planet (Beck *et al.* 2009). In Chesapeake Bay the first reef-restructuring programme started in 1990, and the majority of restoration activities since then have been conducted by two state agencies, the Maryland Department of Natural Resources (MDDNR) and the Virginia Marine Resources Commission (VMRC).

The most fundamental step for successful reef restoration is to clearly specify the goals of the project. The next step is site selection. Acoustic seabed-surveying systems are used to identify oyster habitat and planting locations that will maximize the survival of spat-on-shell. Preferred sites are hard, geologically stable terraces, of generally uniform depth, of moderate to high surface irregularity, with sand and/or oyster shell as the base. Such sites are typically located on historic oyster bottom (http://chesapeakebay.noaa.gov/oysters/oyster-restoration). In 2013 an online mapping tool that displays a range of information relevant to oyster restoration, from historic reef boundaries and maps of the seafloor to the rate of oyster disease, death and spatfall, was released by NOAA. Other considerations for site selection include nature of the bottom for shell planting, recruitment history, water quality, salinity, food, current velocity and threats such as predation and disease (see Brumbaugh *et al.* 2006). Following site selection, a number of interrelated restoration activities are employed, which fall into two categories: adult stock enhancement, which includes wild seed transplanting and hatchery seed planting; and habitat enhancement, which comprises the addition of substrate, bar (reef) cleaning and bagless dredging (see Table 8.10 for details).

In Chesapeake Bay, 1037 bars were targeted for restoration or monitoring between 1990 and 2007 (Figure 8.13). Restoration activities took place at 378 bars in Maryland and 216 in Virginia, while monitoring occurred at 453 and 437 bars in Maryland and Virginia, respectively, with 43% of bars both monitored and restored (details in ORET 2009; Kennedy *et al.* 2011). In total, 10 500 and 2124 acres have been restored in Maryland and Virginia, respectively. Restoration activities in Virginia covered a shorter period (1999–2007) than in Maryland. Substrate addition and wild seed transplantation were the most used activities, but after 1997 hatchery seed planting began to dominate, and bar cleaning only started in 2003 in Maryland. The cost of restoration activities has been estimated at $17 million. To evaluate the success or failure of these efforts, records of restoration and monitoring activities over the 17-year period were analysed by a team of individuals from funding agencies and academic institutions (ORET 2009; Kennedy *et al.* 2011). The key findings of the team were that limited monitoring efforts, a lack of replicated post-restoration sampling and the effects of harvest on some restored bars hindered evaluations of the effectiveness of restoration activities. In order to evaluate these effectively experimental designs should include replication, quantitative sampling and robust sample sizes, supplemented by pre- and post-restoration monitoring. In addition, future restoration activities should have clearly articulated objectives, and be coordinated among agencies and across bars, which should also be off limits to fishing (see Kennedy *et al.* 2011 for details).

In the wake of the ORET study a new restoration master plan for Chesapeake Bay was prepared by the US Army Corp of Engineers (USACE), in close partnership with the MDDNR and VMRC. The plan (USACE 2012) aims to carry out large-scale, concentrated oyster restoration throughout the Chesapeake Bay and its tributaries. Past restoration efforts

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Table 8.10 The six major activities employed in the period 1990–2007 to restore populations of the oyster *Crassostrea virginica* in Chesapeake Bay.

Substrate addition	Oyster shell is transported in large quantities by barge to new locations for natural spat settlement, or as a base for hatchery-reared animals. The suitability of other substrates, for example construction rubble, precast concrete structures, decking and limestone marl, is being investigated.
Wild seed transplanting	Large-scale shell plantings are made in areas that receive consistent natural spat settlement, but where disease pressure may be prohibitive for extended survival. Wild seed on planted shell is harvested in the first spring following planting and relocated to areas with low natural recruitment but also low disease pressure for the grow-out phase.
Hatchery seed planting	Small oysters or spat set on shell are often washed off a specially built boat over suitable planting sites in the Bay.
Bar cleaning	The site-based practice is intended to remove older, infected oysters from an oyster bar prior to rehabilitation in order to maximize the survival rates of the newly planted oyster spat by reducing their exposure to the highly infectious parasite *Perkinsus marinus*. Bar cleaning is typically accomplished by repeated dredging under power using a commercial dredge on a planned restoration site and the area surrounding it. Any collected shell is returned to the reef and some of the oysters are retained as hatchery broodstock.
Bagless dredging	Bagless dredging uses a harvesting dredge modified by removing or opening the catch bag. The gear is dragged across bars, stirring up shell and sediment. Shell resettles while sediment is dispersed into the water column and advected from the reef. Bagless dredging is thought to be less disruptive than bar cleaning with power dredges.
Monitoring	Monitoring at sites not undergoing restoration activities provide important reference points (or controls) for comparison with sites targeted for restoration. Data collected at restoration and reference reefs include measurements of oyster abundances, growth rate, disease prevalence and intensity, ecological observations and water quality measurements collected at or near oyster reefs.

Adapted from ORET (2009). Reproduced with permission of Maryland Sea Grant, College Park, Maryland, United States.

had been scattered throughout the Bay and were on too small a scale to make a system-wide impact. To illustrate, restoration efforts in the past have only addressed 2 and 11% of historic acreage in Virginia and Maryland, respectively. The plan now is to focus on 63 tributaries for restoration based on current physical and biological conditions; all are already designated as permanent sanctuaries, and therefore closed to fishing. Twenty-four have been selected as high-priority tributaries on the basis that they have the highest potential to develop self-sustaining oyster populations. The remaining tributaries (Tier 2) have physical or biological constraints that either restrict the scale or predict long-term sustainability of the project. For Tier I tributaries, the plan is to restore 17 400–35 000 acres, which represents about 20–40% of the historic acreage, at an overall cost of $1.8–6.5 billion. The long-term plan is to have 20 of Tier 1 tributaries restored by 2025. For updates on oyster restoration programmes in Chesapeake Bay see http://www.dnr.state.md.us/fisheries/oysters/eco_resto/Implementation_update_2-25-14.pdf.

Other ongoing oyster restoration projects, albeit on a smaller scale than those in Chesapeake Bay, are under way in the Gulf States, Georgia, North and South Carolina, and Delaware. Information on these can be found on the NOAA website http://www.noaa.gov/.

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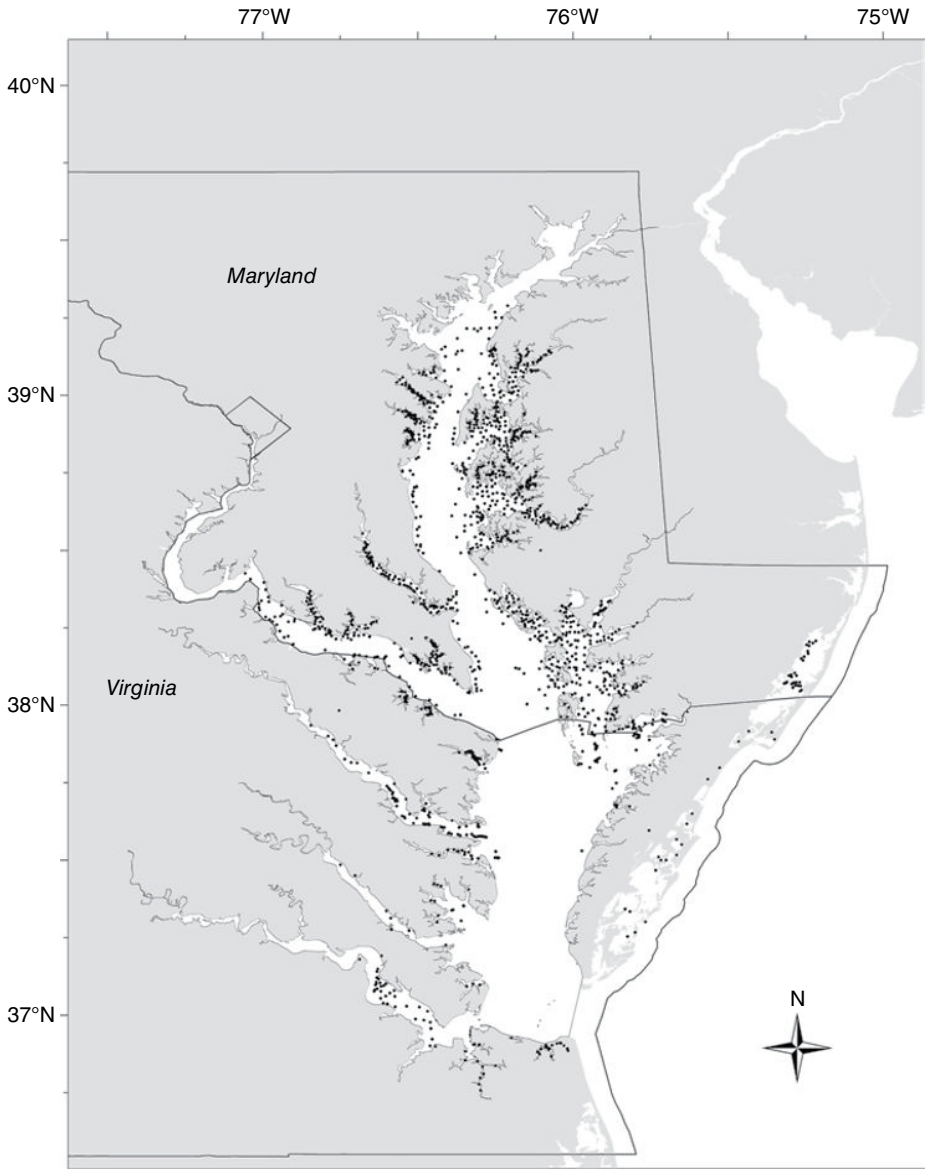


Figure 8.13 Spatial coverage of oyster (*Crassostrea virginica*) bars ($N = 1037$) with restoration or monitoring activities in Chesapeake Bay and coastal sites in Maryland and Virginia from 1990 to 2007. Kennedy *et al.* (2011). Reproduced with permission of the *Journal of Shellfish Research*.

Mussel fisheries

There are hundreds of species of mussel but only about a dozen or so are fished commercially and most of these are in the genus *Mytilus*. Annual global landings from fisheries for the years 2009 to 2011 are presented in Table 8.1a. Three species, the blue mussel *Mytilus edulis*, the Mediterranean mussel *Mytilus galloprovincialis* and the cholga mussel *Aulacomya ater*, are the main contributors to global mussel fisheries, contributing 82, 3 and 15%, respectively, in 2011 (Table 8.11). One striking observation is the downward trend

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Table 8.11 Global landings (live weight, tonnes) for the three most important contributing species to mussel fisheries.

Species	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Mytilus edulis	142433	108635	123841	100460	80615	73711	56316	54903	50278	59066 (41%)
Mytilus galloprovincialis	51452	51044	41264	12638	9485	2367	803	7330	1407	1879 (4%)
Aulacomya ater	19433	13596	11646	10684	8391	12054	12949	11979	12664	10735 (55%)
Total	213318	173275	176751	123782	98491	88132	70068	74212	64349	71680 (34%)

The figure in parentheses in the last column represents landings in 2011 as a percentage of landings in 2002.

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in landings for all three species between 2002 and 2011, in line with the global trend from capture fisheries over to aquaculture. In 2011 most landings (58%) for *M. edulis* came from Denmark, followed by the United States (18%), the United Kingdom (16%) and France (7%). Almost all landings of *M. galloprovincialis* were from Turkey (96%), while Peru (85%) and Chile (14%) were the two countries responsible for virtually all landings of *A. ater*.

As the vast majority of annual landings (95%) come from aquaculture production (see Chapter 9) it is almost impossible to locate a natural fishery, that is one that is not subject to human intervention at some stage of the mussel life cycle. For example, in Germany and the Netherlands mussel seed is harvested from natural habitats and relayed onto controlled beds for on-growing to market size. Other than collection and relaying there is no other human input. However, landings for these two countries have been treated as aquaculture rather than capture production in FishStatJ statistics since the 1950s. The Danish fishery, described in the following section, is closest to being a natural fishery as almost 100% of the mussels are wild-grown, and consequently Danish landing data in FishStatJ appears under capture production.

The *Mytilus edulis* fishery in Denmark

In the 1950s and 1960s between 10 000 and 20 000 t of mussels were landed annually in Denmark, but over the period 1970–2003 annual landing gradually rose from 20 000 t to between 86 000 and 136 000 t. However, from 2003 onwards landings started to decline, and currently they stand at about 30 000 t per year. The majority of annual landings in 2011 (75%) came from Limfjorden in the north of the Jylland peninsula (Figure 8.14). Other areas such as the Kattegat/Lille Baelt, and Isefjorden on Sjaelland are of minor importance, contributing about 17 and 7%, respectively. In 2008 the mussel fishery in the Danish Waddenzee was closed to ensure that there would be more than 35 000 t available for mussel-eating birds, for example eider ducks, herring gulls and oystercatchers (Laursen *et al.* 2010).

Limfjorden is Denmark's largest estuary (1526 km^2), with average water depths of 5.5 m. It is situated in the north of the Jylland peninsula and is connected by a shallow channel to the North Sea on the west coast and to the Kattegat on the east coast. Limfjorden's catchment area is 7528 km^2, of which 62% is arable land (Dinesen *et al.* 2011). Nutrient loading from the land, in the form of nitrogen and phosphorous, increased sixfold over the past 100 years, peaking in the 1980s, and while this caused a decline in the finfish fishery there was a concomitant increase in mussel landings. In recent years, however, nutrient loading has decreased, aided by implementation, over large areas of the fjord, of the EU Water Framework Directive (WFD; 2000/60/EC), and other EU directives such as the Habitat Directive (HD; 92/43/EEC) and the Birds Directive (BD; 2009/147/EC). The BD requires the establishment of Special Protected Areas (SPAs) for birds, while the HD requires Special Areas of Conservation (SACs) to be designated for species other than birds, and for habitats. Together, SPAs and SACs make up the Natura 2000 network of protected areas (http://en.wikipedia.org). With the continuing decrease in nutrient loading in Limfjorden there is concern for the future of the fishery, not helped by increasing conflicts between mussel fishers and mussel farmers (Dinesen *et al.* 2011). This is a real concern since 18 mussel farms were in production in Limfjorden in 2010 (Ahsan and Roth 2010).

The Danish AgriFish Agency (http://agrifish.dk/), an agency under the Ministry of Food, Agriculture and Fisheries, is responsible for regulating and inspecting the fishery. DTU-Aqua, the National Institute of Aquatic Resources at the Danish Technical University, provides consulting services to the Danish AgriFish Agency. DTU-Aqua (http://www.aqua.dtu.dk/) estimates the abundance of mussels in the entire Limfjorden from annual samples collected at 250–300 stations in waters deeper than 3 m, and these data are used to set

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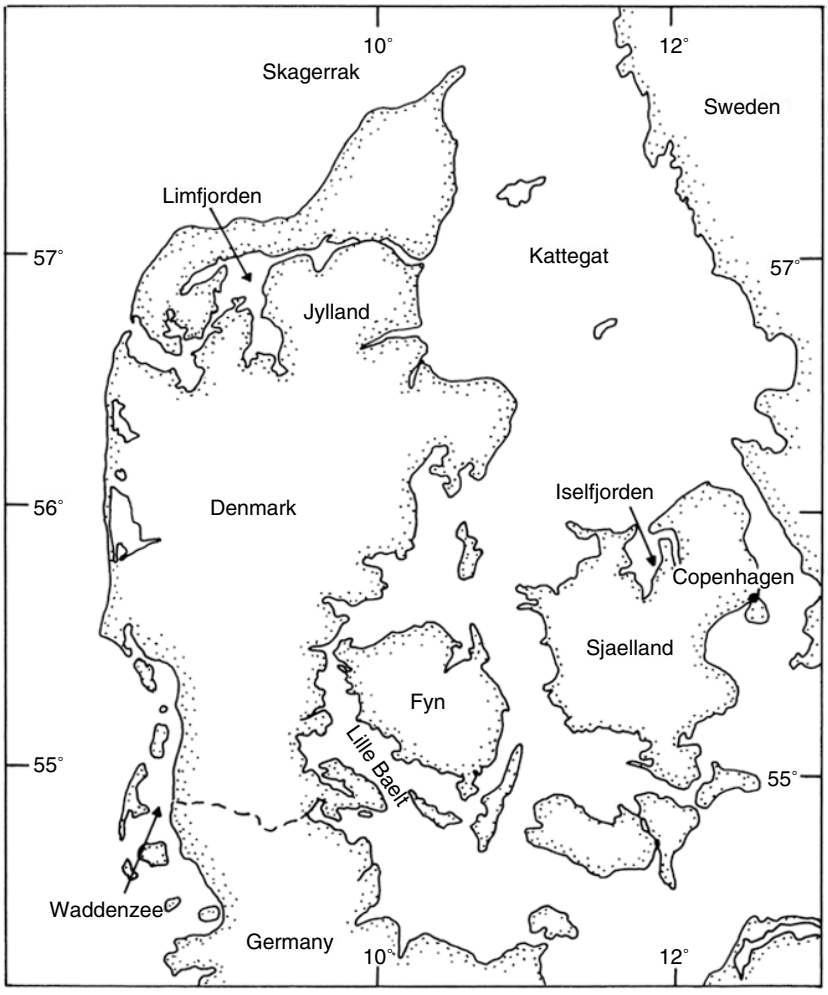


Figure 8.14 Map of Denmark showing the main production areas of the largest mussel (*Mytilus edulis*) fishery in Europe: Limfjorden on the Jylland peninsula, the Kattegat, Lille Baelt and Isefjorden on Sjaelland.

weekly fishing quotas, and identify areas where there is concern about stock status. Fishing plans are drawn up for those areas by the Central Fishermen's Association, made up of representatives from local Fishermen's Associations around the fjord. A fishing plan for a particular area may contain measures to reduce fishing pressure or recommend complete closure of the fishery. The local environmental protection agency monitors stocks in shallow waters and intertidal areas. The overall biomass is currently estimated at around 800 000 t (Andrews & Brand 2011), many orders of magnitude larger than annual landings from the fjord. DTU-Aqua also conducts impact assessments of mussel fisheries in nature conservation areas (Natura 2000 sites). A recent report by DTU-Aqua indicates that currently there is no effect of the fishery on fish-eating bird abundances. Another body, the Association of the Mussel Industry (www.muslingeerhvervet.dk), was set up by mussel fishermen and the processing industry. Their main tasks are to oversee the relaying of undersized mussels (see later) and administer the industry's programme for algal surveillance. Much of the information in the following section has been kindly provided by Grete Dinesen at DTU-Aqua.

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Limfjorden is divided into 42 production areas and specific regulations may differ between these areas. No fishing is permitted in any of these areas from the middle of June until the end of August. Fishing licences, issued by the Danish AgriFish Agency on the first Monday in September, are just for 1 year, and for a specific vessel and fishing area. In Limfjorden there is a maximum of 51 licences issued and currently there are 39 licensed vessels fishing for mussels. Vessels with a license to fish in Limfjorden for a specific year cannot fish elsewhere for that or the subsequent year. Each vessel is equipped with one or two 50 kg lightweight dredges, with a maximum length and width of 1.8 and 1.5 m, respectively. Vessels cannot fish in waters shallower than 4 m, and in Natura 2000 sites there are further depth restrictions (see later). Landings from each vessel are limited to a quota of 45 t per week, but all fishers voluntarily apply a lower limit. The maximum gross catch per day is 30 t, which includes by-catch (see later), the weight of which is estimated from net landings of mussels. Mechanical processing (rinsing, sorting, etc.) on board is not permitted, and food safety regulations must be adhered to. Fishing in a particular production area must begin with an empty vessel and all mussels must be unloaded first before fishing in a new production area. Unloading must be pre-notified to authorities exactly 1 h prior to unloading, and the gross weight of catch must be preregistered. There are additional restrictions on the landing and handling of the mussels on land. More than 90% of all landings are exported ready-shelled, cooked and frozen, or as whole live mussels to markets all over Europe. A small quantity is sold live on the home market.

The minimum landing size is 4.5 cm shell length but seed mussels are always dredged up along with adults. In the past these were discarded during the sorting process on land. This represented a significant loss to the industry because about one-third of gross landings are generally undersized mussels. Nowadays these small mussels are sorted out and put back on selected areas within Limfjorden, which are subsequently closed to fishing to allow the mussels to grow. In addition, transplantation often protects these mussels from oxygen depletion events, which occur on an annual basis in Limfjorden (P.S. Kristensen, personal communication 2013; see also Dolmer *et al*. 2012). Undersized mussels must not exceed 10% of the catch, or 30% if the vessel is involved in a national relaying programme. There is extensive use of closed areas resulting in only 5% of Limfjorden being fished in any 1 year. Reasons for closure can be because of too few/small mussels, or because of food hazards, for example toxic algal blooms or bacterial contamination.

There has been concern in the industry about the effects of removing by-catch such as boulders and pebbles from the seabed when dredging for mussels. These substrata are now returned to the sea and records must be kept of their capture (Andrews & Brand 2011). Vessels are allowed a by-catch maximum weight of 1% oysters and 49% clams. The clam by-catch is registered, and the weight is subtracted from the maximum catch of 45 t allowed per vessel per week. To reduce habitat impacts a new design of dredge is being considered (Frandsen *et al*. 2014), and there are indications that this will be adopted with enthusiasm by the industry.

Several Natura 2000 areas are located within Limfjorden, and currently two of these, Lovns Broad and Løgstør Broad, are partly open to restricted fishing. Fishing is permitted subject to an Environmental Impact Assessment (EIS) that must be carried out annually and issued along with the general licences. For 2013–2014 maximum permitted combined landings from these two broads are 25 000 t, and maximum weight of clam by-catch is 10%. Other precautions/restrictions stipulate that locating mussels on the seabed must be carried out by underwater TV surveys prior to fishing; during fishing vessel location must be registered every 10 s; fishing is limited to specific areas of these broads and to depths greater than 5–6 m to protect eelgrass meadows, other habitat types and Annex 4 species (see Habitat Directive); and the number of fishing vessels within each broad must not exceed 15 at any one time.

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As seen earlier, management of the fishery is supported by a comprehensive monitoring, control and surveillance system and there is good cooperation and communication between scientists, management and the fishery. This system is transparent and all documentation is in the public domain. The fishery receives no direct subsidies and there are no incentives that contribute to unsustainable fishing or ecosystem degradation (http://www.msc.org). Consequently, in 2010 the Limfjorden fishery was the first mussel fishery in the world to be Marine Stewardship Council (MSC)-certified for sustainability, and a surveillance report in 2013 recommended that MSC certification should continue (Andrews *et al*. 2013). In 2012 several other, more minor, mussel fisheries in eastern Jutland were certified, as well as the oyster (*O. edulis*) fishery and cultured mussel production in Limfjorden.

Clam fisheries

There are thousands of species of clams but only about two dozen are fished in commercial quantities. Global annual landings for clams for 2002–2011 ranged between 500 000 and 900 000 t (Table 8.12). This represents 20–40% of total global clam landings, the remainder being from aquaculture production (see Chapter 9). Seven species contributed 78% to clam fishery landings in 2011. Of this figure, the surfclam *Spisula solidissima* contributed 20.6%, with almost all of this (>99%) coming from the United States. The ocean quahog *A. islandica* contributed 24.2%, with, once again, the majority (>95%) coming from the United States. The blood cockle *Anadara granosa*, contributed 9%, with 80% of this landed in Indonesia. The Manila clam *Ruditapes philippinarum* contributed 8.4%, with most of this from Japan (70%). Smaller amounts were contributed by the striped venus *Chamelea gallina* (6.5%), Stimpson's surfclam *Mactromeris* (*Spisula*) *polynyma* (4.7%) and the taca clam *Protothaca thaca* (4.1%), with most landings coming from Turkey (>85%), Canada (100%) and Chile (100%), respectively. It should be pointed out that a significant proportion of landings, 14–27% between 2006 and 2011, are categorized as 'nei' (not elsewhere indicated), which means that the countries concerned did not state the specific name of the clams landed. Over the 10-year period 2002–2011 there was a decrease in total clam landings of 40%, primarily due to a large decrease in category 'nei' clam landings, as well as more moderate decreases in *S. solidissima*, *A. islandica* and *A. granosa* landings (Table 8.12).

The following section describes the wild fishery for the surfclam *S. solidissima* on the Atlantic coast of North America. This species and the ocean quahog *A. islandica* are fished in the same geographic region so many of the fisheries management measures pertain to both species.

The *Spisula solidissima* fishery

The species is large, with a maximum shell length of 20–25 cm. Individuals 15–20 years are common in many areas with some as old as 30 years (Weinberg 1999). The species is distributed along the western North Atlantic Ocean from the southern Gulf of St. Lawrence to Cape Hatteras, North Carolina. Major concentrations are on Georges Bank (GBK), southern New England (SNE), off Long Island (LI), southern New Jersey (NJ), the Delmarva Peninsula (DMV) and southern Virginia (SVA) (Figure 8.15). The species is harvested from the subtidal zone to depths of about 60 m, although abundance declines sharply beyond depths of 40 m. Despite encouraging results from earlier trials (Goldberg 1989) there is no aquaculture production of *S. solidissima*.

The fishery started in the 1870s when clams were initially fished for bait for the Atlantic cod fishery. In the 1930s a food fishery developed and expanded rapidly over the next four

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Table 8.12 Global landings (live weight, metric tonnes) for the seven most important species contributing to clam fisheries.

Species	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Spisula solidissima	176322	169003	150379	143949	145770	152101	139095	123123	99453	101593
Arctica islandica	162212	171124	162406	116186	119635	134403	136183	130634	132233	118941
Anadara granosa	73512	50756	67665	59592	54623	66195	50179	58304	41960	44309
Ruditapes philippinarum	49252	50518	49081	48708	42543	44462	60136	53997	40228	41590
Chamelea gallina	14548	23920	21904	17038	54001	52191	41027	28783	29973	32232
Mactromeris polynyma	19960	27339	24038	19286	21922	18823	20014	25594	23764	23061
Protothaca thaca	5360	8345	18914	14113	13868	9400	15725	15739	21591	20359
Species in category 'nei'	317932	403996	302129	229515	303746	301431	300729	307250	201884	110222
Total	819098	905001	796516	648387	756108	779006	763088	743424	591086	492307

See text for main contributing countries for each species.

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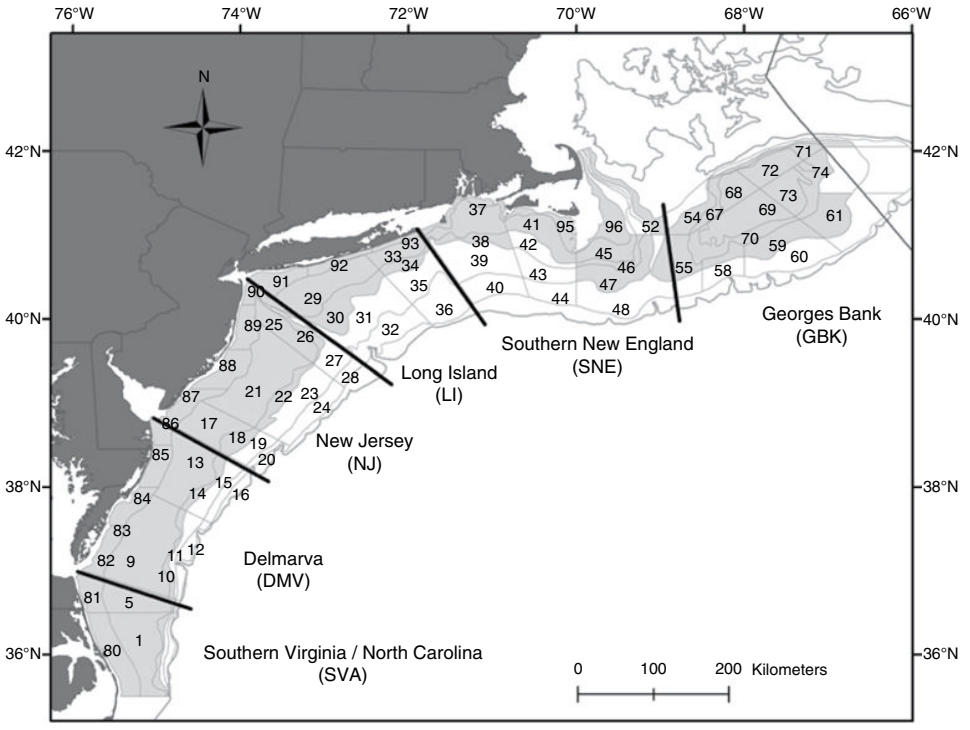


Figure 8.15 Assessment regions for the surfclam *Spisula solidissima* in the US Exclusive Economic Zone (EEZ; waters 3–200 miles offshore). Northeast Fisheries Science Center (NEFSC) shellfish strata with potential surfclam habitat are shown in grey, and are identified by stratum numbers. NEFSC (2013b).

decades due to increased fishing effort, improved harvesting, shucking and processing methods, and exploitation of new areas. Landings peaked in 1974 at 180 000 t, but by 1980 this figure had halved due to a combination of overfishing and widespread hypoxic water conditions off the coast of New Jersey during the summer of 1976. It was about this time that a fishery for the ocean quahog *A. islandica* developed in the offshore waters of New Jersey and Maryland. To conserve the surfclam fishery stringent regulations were implemented in 1977 (see later), and from 1980 onwards annual landings started to increase, reaching a peak of 182 000 t in 1992. From then until 2009 annual landings remained relatively stable at approximately 150 000 t, but in recent years landings have dropped to about 100 000 t, with those for the ocean quahog also showing a similar decrease (Table 8.12). Most surfclam landings in 2011 were from NJ (64%), with smaller amounts from GBK (13%), SNE (13%), DMV (8%) and LI (2%). Since 2003 there have been no landings from SVA (NEFSC 2013a).

Surfclams are recruited to the fishery at 10–12 cm shell height, at 5 years of age or older. The clams are harvested by hydraulic dredges that use high pressure water jets at the 1.52 m cutting edge of the dredge to loosen the clams from the substrate. The dredge knife then lifts the clams into the rear portion of the dredge. The bars on the dredge are spaced several inches apart so as not to collect anything but surfclams. Therefore, there is limited by-catch with virtually no impact on protected species. The fishing vessels are large (50–150 gross registered tonnes) and typically operate two dredges, one off each side of the boat.

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Since 1977 the fishery in the US Exclusive Economic Zone (EEZ; waters 3–200 miles [5–320 km] offshore) is managed under the Surfclam–Ocean Quahog Fishery Management Plan (FMP) of the Mid-Atlantic Fishery Management Council. Very restrictive measures were implemented under this plan in an attempt to rebuild and conserve surfclam stocks. These included annual and quarterly catch quotas, a moratorium on vessel entry into the fishery, mandatory logbook reporting for vessels and processing plants, fishing time limits and closed areas in order to protect small clams. A few years later a minimum size limit of 140 mm shell length and a target discard rate no larger than 30% of the landed portion of the catch were also implemented. The minimum size limit was later reduced to 4.75 in. (121 mm) shell length. The dredge allows clams smaller than 80–90 mm shell length to escape. These measures, along with good recruitment, have been responsible for the recovery of the fishery (see earlier). Because the harvesting capacity of the fishing fleet is considerably greater than that necessary to catch the annual quota (applied to the entire US EEZ area), an Individual Transfer Quota (ITQ) system was adopted in 1990. This system allocates individual fishing quotas to fishermen or vessel owners that can be sold or leased. In1991 the number of vessels fell from 128 to 75 as quota shares were bought, sold and combined on fewer vessels; currently only 25 vessels are active in the fishery. Catch quotas are specified for a 3-year period based on the most recent Northeast Fisheries Science Center (NEFSC) stock assessment report (see later), as well as from data supplied by harvesters and processors.

Harvested clams are loaded into cages that weigh almost 2 t when full. A tag must be fixed to each cage and must not be removed until the cage is emptied by the processor, who retains the tag for 60 days beyond the end of the calendar year. The cages are offloaded by crane and transported to the shucking and processing plants. Shucking is allowed at sea provided that an observer carried on board the vessel can measure accurately the total amount of surfclams harvested in the shell prior to shucking. The average meat yield of market-sized (~130 mm shell length) clams usually exceeds 100 g. All of the visceral mass is used to provide chopped flesh (fresh, frozen or canned) for chowder, clam cakes or stuffed clams. The mantle and foot are sliced into thin strips, breaded, fried and packaged dry. There is no market for live or whole clams.

Certain areas are closed to fishing, usually because of adverse environmental conditions or because of the presence of undersized clams. For example, an area located in Georges Bank was closed in 1990 due to paralytic shellfish poisoning. After 22 years the fishery reopened in 2012 when testing showed that toxins were consistently below harmful levels in the preceding few years. This is good news for surfclam fishermen and may relieve fishing pressure on the more southerly areas of the fishery, which have experienced a decline in landings over the past two decades (NOAA 2012d). For updates on surfclam fishery regulations see the website of the Electronic Code of Federal Regulations (e-CFR) (http://www.ecfr.gov).

Every 3 years the NEFSC carries out an assessment of the total fishery. The survey area is divided into six regions, each of which is subdivided into a number of sampling strata, 96 in all (Figure 8.15). The results of the latest assessment, carried out in 2011, showed that the estimated biomass for the entire fishery was 106 000 t of meats (95% confidence interval of 802–1401), overlapping with the biomass target of 972 000 t meats, but entirely above the biomass threshold (1/2 biomass target) of 486 000 t meats for 2011 (Figure 8.16; NEFSC 2013b). Biomass has dropped by 2–10% per year between 2002 and 2011, primarily due to poor recruitment (Figure 8.17) and slow growth rates associated with warm water conditions probably due to global warming (see Chapter 3). Estimated annual fishing mortality during 2011 for the entire fishery was $F = 0.027$ (95% CI 0.016–0.045), which is well below the overfishing threshold, based on an assumed natural mortality rate $M = 0.15$ (NEFSC 2013b).

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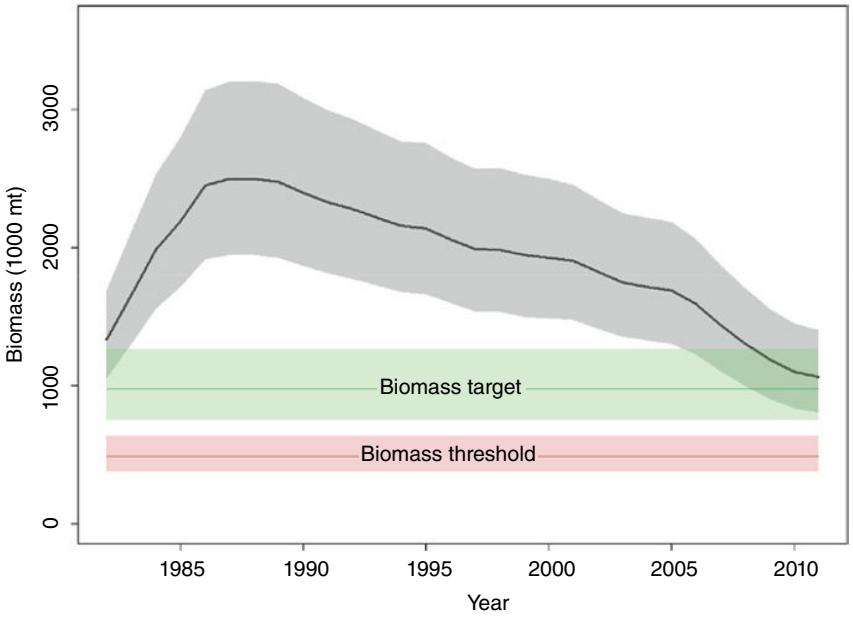


Figure 8.16 Surfclam *Spisula solidissima*, whole stock biomass status with approximately 95% confidence intervals on the estimates and biomass target and biomass threshold reference points. NEFSC (2013b).

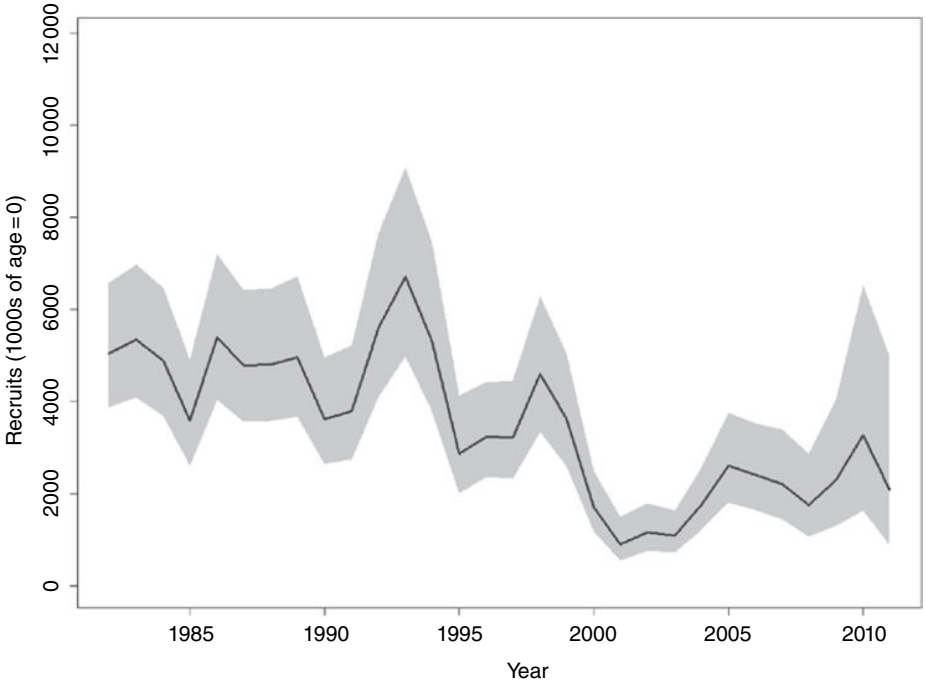


Figure 8.17 Surfclam *Spisula solidissima*, whole stock recruitment estimates with approximately 95% confidence intervals.
NEFSC (2013b).

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9 Bivalve Culture

Introduction

Aquaculture dates as far back as 500 BC when Fan Lei, a Chinese politician, wrote the *Classic of Fish Culture*, which describes the rearing of carp in fishponds as a commercial venture. Bivalve culture has almost as long a history. About 350 BC Aristotle mentions the cultivation of oysters in Greece, and Pliny describes commercial holding ponds for oysters near Naples, Italy, around 100 BC. However, farming of oysters, as we know it today, did not begin until 1624 in Hiroshima Bay, Japan (Fujiya 1970). Mussel culture is said to have started when a resourceful Irish sailor, shipwrecked off the Atlantic coast of France in 1235, used poles and netting on tidal mudflats to trap birds. He quickly discovered that the poles became covered with mussels and that the mussels grew better and were of a superior quality to mussels living on the bottom. This apparently was the start of the bouchot method of culture, still practised today in France and parts of Asia. However, it is only in the last four decades that the full potential of the mussel as a culture organism has been realised. Simple methods of clam culture have been practised for several centuries in China (Pillay 1993) and today China is the largest global producer of the Manila clam, *Ruditapes philippinarum* (see later). Culture of scallops started in Japan about 1965 when a fisherman used an onion bag to collect seed (newly settled larvae) in the open sea (Ito, 1991). This novel method of seed collection formed the basis of what was later to become a thriving scallop culture industry in Japan.

Towards the end of the nineteenth century reliable techniques for culturing bivalves were developed. This allowed a rapid growth in production that was greatly augmented about a century later by the development of hatchery techniques for seed production. Overexploitation of wild stocks, deteriorating water quality and debilitating disease have compelled the industry to actively manage the resource and turn to restocking and culture in deep water using juveniles originating in the hatchery.

An examination of the FAO (Food and Agriculture Organization) Global Aquaculture Production data for 1950–2011 shows that the number of bivalve species being cultured is increasing annually, and that more and more underdeveloped countries are becoming

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Table 9.1 Annual yields (live weight, metric tonnes) from aquaculture production (2001, 2010 and 2011) and capture fisheries (2011).

	Aquaculture				Fisheries
	Global production			China	Global
	2001	2010	2011	2011	2011
Mussels	877 884	1 711 118	1 639 973 (95%)	707 401 (43%)	91 459
Oysters	3 787 804	4 548 784	4 409 415 (95%)	3 756 628 (87%)	203 778
Scallops	1 102 345	1 696 145	1 489 284 (74%)	1 306 124 (83%)	527 036
Clams	2 809 555	4 910 753	4 917 563 (91%)	4 388 269 (98%)	492 307
Total	8 577 588	12 866 800	12 456 235 (90%)	10 158 422 (82%)	1 314 580

Data from FAO (© FAO 2013) FishStatJ Global Aquaculture Production database 1950–2011. Figures in parentheses in aquaculture 2011 column represent production as a percentage of the combined annual yields from aquaculture and fisheries. Figures in parentheses in China 2011 column are the percentage contributions by China to global aquaculture.

involved. Factors that have contributed to the continuing growth in bivalve culture include the acknowledged need to achieve greater self-reliance in food production, especially by developing countries; the recognition that it is an efficient way to produce animal protein and that it improves the income and nutrition of rural populations; improved technology transfer in terms of hatchery and nursery techniques and the availability of better transportation by road and air.

In 2011 almost 12.5 million metric tonnes (t) of cultured bivalves were landed, which represents about 90% of the combined yield from fisheries and culture, in contrast to the situation for finfish where only 15% of the yield is cultured (FAO 2010). In 2011, 95% of all oysters landed were from culture operations whereas the corresponding figures for mussels, scallops and clams were 95, 74 and 91%, respectively (Table 9.1). China is by far the biggest contributor, producing over 10 million of the total 12.5 million tonnes cultured in 2011. An examination of the data in Table 9.1 shows that over a 10-year period, harvest almost doubled in the case of mussels and clams, while increases were more moderate for oyster and scallop production. But when the data for 2010 and 2011 are compared there was a small decrease in production in 2011 for mussels, oysters and scallops, but a slight increase for clams (Table 9.1).

This chapter will first deal with some basic aspects of bivalve culture and will focus then on a number of key species for detailed treatment. Topics such as choosing a species for culture, selecting culture sites, and designing and constructing hatcheries and nurseries will not be included, but interested readers should consult Helm *et al.* (2004) and Sarkis and Lovatelli (2007) for details.

Fundamentals of bivalve culture

In bivalve culture the supply of seed, also known as spat, is a critical element. Seed may be produced by wild or cultured stocks in the field, or alternatively from broodstock maintained in a hatchery. In the former case reproduction is left to nature, whereas in the latter situation it is controlled, albeit to varying degrees. Generally speaking, mussel culture relies on wild-caught seed while scallop, clam and oyster culture use either wild or hatchery-produced seed, depending on the species being cultured (see later).

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Wild seed collection

The basic requirements for successful seed collection in the wild are information on the reproductive cycle of the population and knowledge of the spawning, larval development and settlement process of the species concerned. With this kind of information it is possible to forecast when and where to collect spat.

Thin sections of gonad or gonad squashes are examined to assess seasonal changes in the reproductive tissue of bivalves (Chapter 5). To take account of asynchronous development a sample size of about 25 females per month is recommended for squashes, and at least 25 individuals per month for gonad sections (Quayle & Newkirk 1989). In scallops, where the gonad is a discrete structure, gamete development is often assessed through gross visual examination of gonad size, colour and shape. In oysters condition factor is sometimes used as an indirect way of assessing reproductive stage.

Temperature and food supply are the main exogenous factors influencing the reproductive cycle in bivalves (see Chapter 5). Temperature is also the main environmental trigger for spawning in temperate water species. In the tropics, where water temperatures are relatively stable, changing salinity, rather than temperature, may be the chief spawning stimulus. Endogenous factors, for example, neurohormones, and neurotransmitters, also play an important role in gametogenesis and spawning, and interact in a complex fashion with exogenous agents (see Chapter 5). As spawning triggers are species- and site-specific it is important that careful records of spatfall, temperature, salinity, lunar phase, tidal fluctuations and weather conditions are kept in order to establish relationships with spawning activity (Quayle & Newkirk 1989).

After the gametes are released into the water column fertilisation occurs and the fertilised egg rapidly divides and goes through the trochophore and veliger larval stages. As soon as the veliger reaches 250–300 μm shell length it is ready to settle and metamorphose. The length of time that larvae are planktonic depends on temperature, salinity and food supply. Generally speaking, the larval period for species in temperate waters is between 15 and 30 days, but is less than 15 days for tropical species. The spat settle on a wide variety of substrates and attach themselves using either byssus threads (mussels, clams and scallops) or cement (oysters). Attachment by byssus and cement persists throughout the life of mussels and oysters, respectively. In scallops and clams the byssus is lost some time after settlement, although there are some species where it persists into adult life.

Accurate spatfall forecasting is a prerequisite for successful seed collection. This is particularly important when collection involves setting out poles, ropes or cultch, which require prior conditioning in seawater before spat settle. If collectors are put out too early they may become covered with fouling organisms and hinder spat settlement. Alternatively, late placement of collectors, or not placing them at the proper place and depth, can result in low setting success. At the best of times forecasting is a difficult task but knowledge of the life cycle of the bivalve and of the local hydrographic conditions will help to improve accuracy. In temperate waters forecasting is based primarily on plankton sampling but in tropical regions forecasting is often not necessary because of the continuous nature of settlement. Until relatively recently, light microscopy and SEM were the only techniques used to identify bivalve larvae. But these methods are only effective for those larval stages and species that can be distinguished morphologically. Molecular methods (antibody and DNA markers), although not as accessible as microscopy, have been very successful in identifying larvae to species regardless of developmental stage (see Chapter 5).

A wide variety of physical and chemical factors influence settlement in the wild (see Chapter 5). Generally speaking, larvae settle on clean, silt-free, irregular surfaces that can range all the way from filamentous algae through stone, wood, dead shell and shells of their

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own species. Therefore, culture operations use many different materials to collect wild seed. For on-bottom oyster culture the preferred material is an empty oyster shell (cultch), chiefly because it is generally available, cheap and attractive to the larvae. However, a myriad of other materials can also be used to good effect, such as rope, bamboo, rubber tyres, roof tiles, wood laths, tree branches and coconut shell. Mussel seed for off-bottom culture is collected on poles or stakes set into the seabed, or on ropes or mesh stockings suspended from rafts or longlines. For on-bottom culture the seed is dredged from natural beds and transplanted to culture plots (see Chapter 8). Newly settled clam spat are collected on oyster cultch or brush fences, or, because the byssal attachment phase is short, spat are more often sieved from the substrate using a series of graded sieves. This method is labour-intensive and also requires expertise in clam identification. Scallop spat are typically collected on net bags filled with monofilament gillnetting that are attached to lines suspended from a raft or longline. In Japan bundles of live kelp seaweed can be used in addition to net bags. In scallop species where the attachment phase is short the spat are removed from collectors to pearl nets or lantern nets.

Hatchery production of seed and juveniles

Hatchery production of seed began in response to declining stocks and the consequent seed shortage in the 1960s. Hatcheries guarantee the supply and maintenance of broodstock, provide the means to control maturation and spawning and supply food for the early vulnerable stages in the life cycle. In addition, breeding techniques can be used to produce seed for desired characteristics such as fast growth or disease resistance. However, the costs involved in setting up and running hatcheries are high and at present very little of the seed used in culture operations actually comes from hatcheries, although future improvements in technology should make it possible to produce seed at competitive prices.

The basic techniques for the production of seed in hatcheries are very similar for all bivalves, although each hatchery will invariably modify these depending on local conditions and the species being cultured. The procedures include the holding, conditioning and spawning of broodstock, the rearing of larvae and juveniles and the production of large quantities of microalgae as food. The latter will be dealt with first because of the crucial role it plays in the production of hatchery seed.

Culture of microalgae

In most hatcheries the incoming seawater is filtered and sterilised to remove contaminants. But in doing so potential food is also removed, thus making it necessary to produce microalgae cultures for the larvae, spat and breeding stock in the hatchery. Algal requirements of the various stages depend on whether the hatchery is mass-producing larvae for remote setting (see later) or growing millions of seed up to planting size. In either scenario juveniles consume the largest volumes of cultured algae (Figure 9.1). The species chosen for culture chiefly depends on their nutritional value and ease of culture. Cell size, cell wall structure and chemical composition of the species are also important. The most widely used species for bivalve culture are the diatoms: *Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana* and *Skeletonema costatum*, and the flagellates *Tetraselmis suecica*, *Isochrysis galbana*, *Isochrysis* (T-ISO) and *Pavlova lutherii* (Helm *et al.* 2004). Algae are grown either indoors in intensive culture under artificial light or outdoors in tanks using natural light. Essentially, in the former, a pure culture of the species is grown in a well-aerated enriched medium under temperature and lighting conditions that will ensure rapid growth. To avoid contamination the media, containers and tubing are

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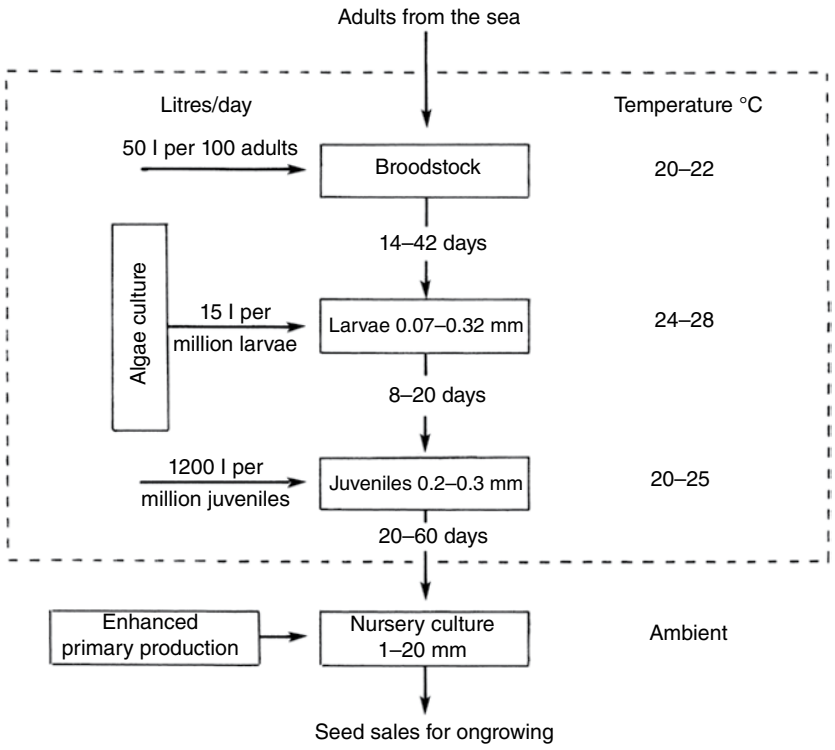


Figure 9.1 A summary flow diagram of the various aspects of hatchery production showing the temperature range and the daily food requirement per unit number of animals at each stage. From Utting and Spencer (1991). Reprinted with permission from the Office of Public Sector Information (OPSI), UK.

sterilised and frequent examination of cultures under the microscope are carried out to check their purity. Stock cultures (≤250 ml) are maintained in specialized enriched medium under controlled temperature and lighting conditions (Figure 9.2). A small volume (e.g. 20 ml) of algae from these is aseptically transferred into starter cultures (250 ml to 4 l volume) and grown quickly for 7–14 days at higher temperature and light intensity with a supply of CO_2-enriched air. Later, a small volume is used to for a new starter culture, and the remainder to initiate an intermediate-scale culture (4–20 l volume). The latter may be used as food for larvae or to start a large-scale culture (80–500 l volume) maintained in tall, narrow, polyethylene or fibreglass cylinders (Figure 9.3). A haemocytometer or Coulter Counter is used to measure algal cell density at the different stages in the culture process. Typical concentrations of cells in mass cultures are between 1 and 5 million cells ml^{-1}. For further information on algal culture methods see Lavens and Sorgeloos (1996) and Helm *et al.* (2004).

While the culture methods described earlier provide food for larvae and small juveniles, outdoor tanks (60–450 m^3 capacity) are ideal for producing algae for larger juveniles and maintaining broodstock. The tanks are provided with the necessary nutrients (nitrogen, phosphorus and silica) to induce a mixed diatom and flagellate population at higher densities than normally found in the sea. It is possible to induce monospecific blooms but over time these become contaminated with other microorganisms. Although the composition of multispecies blooms varies depending on season and environmental condition, such blooms are nutritionally very valuable for larger bivalve individuals (Helm *et al.* 2004).

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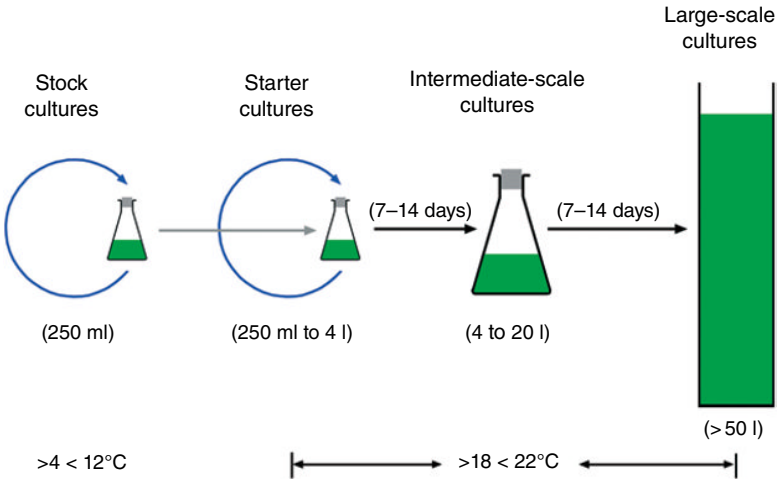


Figure 9.2 Steps in the production of algae. Stock cultures (250 ml or less) remain in isolation under light and climate control (low temperature) and are only used to inoculate starter cultures when necessary. They are not aerated nor is carbon dioxide added. Starter cultures (250 ml to 4 l in volume) are grown quickly for 7–14 days at higher temperatures and light intensity with a supply of carbon dioxide-enriched air. When ready, a small portion of the volume is used for a new starter culture and the main portion is used to begin an intermediate-scale culture. Intermediate-scale cultures (usually of between 4 and 20 l in volume) may be used as food for larvae or to start a large-scale culture. Large-scale cultures are generally of a minimum of 50 l and are frequently much greater in volume.
From Helm *et al.* (2004). Reprinted with permission from the Food and Agriculture Organization of the United Nations (FAO).



Figure 9.3 Large, transparent plastic tubes used in the culture of microalgae.
Photo courtesy of Kevin O Kelly, Lissadell Shellfish Co. Ltd., Sligo, Ireland.

In the last two decades there has been increasing interest in screening new algal species for culture, and testing single or mixtures of algae on larval growth and mortality (Ponis *et al.* 2006a). Biochemical composition is a major factor determining the nutritive quality of microalgae, and it is now well established that there is a link between the presence of poly-unsaturated fatty acid (PUFA) content, specifically eicosapentaenoic (20:5n-3, EPA) and

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docosahexaenoic (22:6n-3, DHA), and high nutritional value (Martínez-Fernández *et al.* 2006). EPA and DHA are important in maintaining the functional integrity of biological membranes, while EPA and another PUFA, arachidonic acid (20:4n-6, AA), are precursors of several hormones. Therefore, it is important to take into account the overall balance between EPA, DHA and AA in conjunction with the PUFA level to avoid nutritional deficiency (Pernet & Tremblay 2004). Sterols, for example, cholesterol, are also an essential requirement for bivalve health. As bivalves have a limited capacity to synthesise these, they must be provided in the diet although it must be kept in mind that phytosterols, similar to PUFA content, vary between microalgal species (Soudant *et al.* 2000; Tremblay *et al.* 2007). The whole area of larval nutrition is complex in that the nutritional value of a specific microalgae can depend on the bivalve consuming it and the particular growth stage of the bivalve concerned. Also unialgal diets produce good growth and survival in some species, but for others a mixture of two or more species is required (see Ponis *et al.* 2006b; Ben Kheder *et al.* 2010; Marshall *et al.* 2010, for review). There is also evidence that the PUFA composition of a species can change, depending on environmental conditions. For example, *Thalassiosira pseudonana* cultured under high light was a superior diet for oyster larvae than the same species grown under low light (Thompson *et al.* 1996).

Microalgae culture is an expensive process, representing on average 30% of hatchery operating costs (Coutteau & Sorgeloos 1992). Therefore, there is considerable interest in the development of suitable artificial diets to replace or supplement microalgae. One such replacement is concentrated algal pastes, which are produced by concentrating algal cells from mass cultures and preserving the resultant paste through refrigeration, freezing or drying (Ponis *et al.* 2008). Results from feeding trials on live and concentrated algal species showed that *Tetraselmis suecica* was the best suited as a preserved feed because dried and live *T. suecica* produced similar growth rates when fed to larval clams, *R. philippinarum*. Also, cold-stored *T. suecica* produced growth rates very similar to that of fresh *T. suecica* when fed in combination with live feed to larval oysters, *Crassostrea gigas* (see Marshall *et al.* 2010 and references). One major advantage of *T. suecica* is that it can be grown in fermentors using organic carbon instead of light as an energy source; and the technique costs 30% of what it costs to produce algae autotrophically. Another promising supplement is microcapsules containing a mixture of animal proteins. Southgate *et al.* (1992) have shown that when these were fed to oyster larvae (*Saccostrea commercialis*) they were able to support a growth rate that was in excess of 80% that of algae-fed larvae. Similar results were reported for mussel spat fed MySpat, a microencapsulated fine powder of vegetal protein, fish oil lipids and carbohydrate (Nevejan *et al.* 2007). A major advantage of microcapsules is that they are made when required and, unlike microalgae, require no additional maintenance. However, possible problems include settling, clumping and bacterial degradation of the particles, leaching of nutrients and low digestibility of the cell wall material (Lavens & Sorgeloos 1996). High larval growth rate can also be achieved when lipid spheres are used as a supplement to live feed (Hendriks *et al.* 2003). Another algal substitute that has been tried with good results is the use of yeast-based diets. Coutteau *et al.* (1994) found that when baker's yeast mixed with algae (50:50 on dry weight basis) was fed to clam seed (*Mercenaria mercenaria*) the growth rate was similar to algal-fed controls. The high lipid content of the yeast formulation probably compensated for the deficiency in calories of the algae/yeast mix. Cornmeal and cornstarch also serve as good complements to live microalgal diets (Pérez–Comacho *et al.* 1998). Adding certain species of bacteria and picoplankton, or dissolved organic material (fatty acids, amino acids), to the culture water may also be beneficial, but to date there has been little research on their potential benefits to larvae (see Marshall *et al.* 2010). Despite their benefits, none of the supplements described earlier can fully replace live microalgae diets.

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Broodstock conditioning, spawning and fertilisation

Broodstock conditioning is essential in order to produce larvae for culture. The process extends the production season, which is especially relevant for hatcheries based in marginal climates or for exotic species that may not reproduce naturally in the wild, or where small-sized seed may not be as cold hardy as that of native species (Helm *et al.* 2004). Broodstock are normally picked for the hatchery on the basis of desirable traits such as fast growth, disease resistance, meat weight and shell appearance. Genetic improvements can potentially be achieved through selective breeding, crossbreeding, hybridisation or chromosome manipulation (Chapter 10).

The methods for broodstock conditioning are basically similar for all bivalves. Broodstock are usually kept in a quiet area of the hatchery where disturbance is minimal. The stock are maintained in flow-through systems in trays at ambient temperature or, if out-of-season reproduction is required, are conditioned in indoor tanks with heated seawater and added algal food (Figure 9.4). A water flow of >25 ml min^{-1} $adult^{-1}$ is recommended with no more than 5 kg live weight biomass of stock in a tank of 120–150 l volume (Helm *et al.* 2004). The water is not filtered as it contains a diversity of food organisms that are beneficial to broodstock. Salinity and temperature regimes are

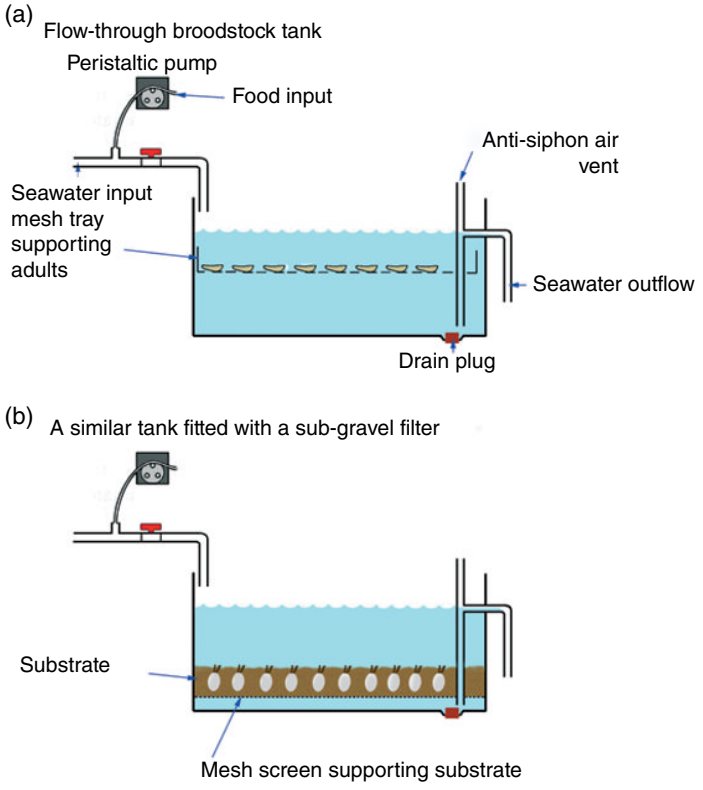


Figure 9.4 Diagrammatic representation of (a) a flow-through broodstock tank in which adults are suspended off the bottom in a mesh tray with large apertures in the base so as not to retain faeces and detritus; (b) a similar tank fitted for subgravel filtration. Systems of type (a) are suitable for most species that do not require a substrate. Clams and some scallop species often condition better in tanks of type (b). From Helm *et al.* (2004). Reprinted with permission from the Food and Agriculture Organization of the United Nations (FAO).

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species dependent, but most cultured bivalves become sexually mature at salinities >25 psu and at temperatures between 16 and 24°C (Chávez-Villalba *et al.* 2002; Ojea *et al.* 2008; Matias *et al.* 2009). Cultured microalgal species are the principal food used for conditioning although natural phytoplankton bloomed in outdoor tanks or algal pastes may also be used. The recommended food ration is based on the dry weight of the adults, usually 2–4% of their mean dry meat weight at the start of the conditioning period in dry weight of algae fed per day (for details see Helm *et al.* 2004). It is important that attention is paid to the fatty acid profile of the broodstock diet since it has been shown that it affects gonad composition and hatching rate and quality of larvae (Pronker *et al.* 2008; Nevejan *et al.* 2008; Rivara & Patricio 2010). The length of the conditioning period depends on the species being conditioned (Table 9.2), the initial condition of the broodstock, the stage in gametogenesis when the bivalves begin conditioning and hatchery-related factors such as temperature, diet and ration.

The easiest way to assess broodstock maturity is to examine a small piece of gonad from a few individuals under the microscope. Alternatively, a hole can be drilled in the shell and a piece of gonad extracted using a syringe, a method that does not involve sacrificing broodstock. Ripe oysters can be maintained without spawning for several months; in the case of

Table 9.2 A summary of information relevant to the conditioning and egg (or larval) production for a number of commonly cultured bivalves.

Group/species	Sex type	Conditioning period (days*)	Temperature (°C)	Fecundity (10^6)	D-larva size (μm)
Oysters					
C. gigas	O-D	28–42	20–24	50+	70–75
C. virginica	O-D	28–42	20–22	50+	60–65
C. rhizophorae	O-D	21–35	20–22	7–12	55–60
O. edulis	L-A	28–56	18–22	1–3	170–190
T. lutaria	L-A	28–56	18–20	0.02–0.05	450–490
Clams					
R. philippinarum	O-D	28–42	20–22	5–12	90–100
M. mercenaria	O-D	28–42	20–22	10–20	90–100
Scallops					
P. yessoensis	O-D	14–21	7–8	20–80	100–115
P. magellanicus	O-D	28–42	12–15	20–80	80–90
P. maximus	O-M	35–56	10–15	20–80	90–100
A. gibbus	O-M	14–28	20–22	4–7	90–100
A. irradians	O-M	21–35	20–22	4–7	90–100
Mussels					
M. edulis	O-D	28–35	12–16	5–12	90–100

Adapted from Helm *et al.* (2004). Reprinted with permission from the Food and Agriculture Organization of the United Nations (FAO).
A key to the meaning of the symbols under sex type is given at the bottom of the table. Conditioning times are for adults brought back to the hatchery early in the season (* time in days will vary considerably according to the reproductive stage of the adults when brought to the hatchery). Fecundity values are a guide only and will vary according to the size of adult spawned, its condition and other factors. The average shell lengths of fully developed, early-stage D-larvae (2–3 days after fertilization) are also given for comparative purposes.
Key to sex-type: O, oviparous (gametes shed into the water); L, larviparous (adults brood larvae which are then shed into the water); D, dioecious (sexes are separate); M, monoecious (hermaphroditic, both sexes in the same animal); A, alternate sexuality (sex switches in the same animal after each spawning).

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clams this holding period can be as long as 8 months (Castagna & Manzi 1989). However, holding broodstock for lengthy periods invariably entails extra costs.

It is recommended to have an equal-sex ratio and about 50 pairs of spawners in order to ensure a good supply of eggs and to keep inbreeding to a minimum (see Chapter 10). A number of methods are used to induce spawning. Thermal cycling is the most widely used method for oviparous species. Individuals are placed in a black-bottomed tray in about 10 cm of cool water (12–15°C) together with a small amount of algae to stimulate feeding. After 30–40 min the water is drained and replaced by water at a higher temperature (25–28°C) with once again a small amount of algae. This cool/warm cycle is repeated until spawning occurs. If no spawning has taken place within a 2–3 h period the animals undergo a further week of conditioning. Stripped eggs or sperm from opened individuals may be added as a spawning stimulant. For dioecious species, males invariably spawn first and these should be removed from the tray and kept out of water until a sufficient number of eggs have been collected from females, the reason being that sperm ages faster than eggs and fertilisation rate may be reduced if this procedure is not followed. Hermaphroditic species, for example, many scallop species, liberate their sperm first, followed by the eggs, thus minimising the chances of self-fertilisation. Oysters species, for example, *Ostrea* and *Tiostrea*, spawn during the conditioning process and brood the larvae within the mantle cavity for 6–20 days depending on the species. Released larvae or larvae removed before completion of the brooding period are reared using standard methods of larval culture (see later). If spawning does not occur, gonad tissue may be dissected from a number of males and females. The tissue is blended with seawater for 5–10 s and the eggs are collected on a screen, washed, and a sample is then examined under the microscope to check for fertilisation. Although this method produces fewer larvae than from a natural spawn, it is a common way of obtaining gametes from *Crassostrea* species.

The fertilisation protocol is fairly similar among bivalves. Before the addition of sperm, egg suspensions are filtered (90 µm aperture) to remove faecal pellets. The sperm to egg ratio for optimal fertilisation varies widely between species. For example, in oysters (*Crassostrea* sp.) for optimum fertilisation success, and to avoid polyspermy – the penetration of the egg by more than one sperm – the recommended sperm concentration is 500–5000 per egg (references in Thompson *et al.* 1996). Polyspermy results in abnormal embryonic development. In the cockle, *Clinocardium nuttalii*, the recommended ratio is 10 000:1 (Liu *et al.* 2008). After fertilisation the eggs are then washed and sieved at 25 µm to remove excess sperm. Fertilisation rates are generally high (>90%) and can be estimated using a light microscope.

Sperm cryopreservation has received considerable attention since the 1970s, primarily focusing on *Crassostrea* and *Pinctada* oyster species (Acosta-Salmón *et al.* 2007; Adams *et al.* 2008; Yang *et al.* 2013). The technique provides reliable supplies of sperm without seasonal limitations and costly hatchery maintenance of adult males, and provides a safe repository for improved lines with desirable traits (Dong *et al.* 2005). Cryopreserved sperm can also be used for shipment to other locations, or to cross populations that breed asynchronously. Fertilisation success is about 70–90% of the rate for natural sperm, but larval yields are often as low as 20% (Yankson & Moyse 1991). There are a number of reports on the successful cryopreservation of oocytes, embryos and larvae of oysters (Paniagua-Chavez & Tiersch 2001; Liu & Li 2008; Adams *et al.* 2011), mussels (Wang *et al.* 2011) and clams (Choi *et al.* 2008). Cryopreservation of gametes and larvae has yet to be applied on a commercial scale by the aquaculture industry, but before this happens there is a real need for the standardisation of protocols across laboratories (Dong *et al.* 2005, 2007).

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Table 9.3 Summary data of typical embryo densities (thousands per l), initial D-larva size (shell length, μm), densities of D-larvae (thousands per ml) and culture conditions in terms of suitable temperature (±2 °C) and salinity (±5 psu) for the culture of embryos and early larvae of a number of bivalves.

Group/species	Embryo density (thousands per litre)	D-larva size (μm)	D-larva density (thousands per litre)	Temperature (°C)	Salinity (psu)
Oysters					
C. gigas	15–20	75	10–20	25	28
C. virginica	15–20	65	10–20	25	28
C. rhizophorae	15–20	60	10–20	25	35
O. edulis	N/A	175	5–10	22	30
Clams					
R. philippinarum	20–40	95	10–20	25	30
M. mercenaria	15–25	95	10–20	25	28
M. arenaria	15–25	95	10–20	19	30
Scallops					
P. yessoensis	*	105	1–2	15	30
P. magellanicus	*	90	1–2	15	30
P. maximus	*	95	1–2	14	30
P. ziczac	10–15	95	2–5	25	32
A. gibbus	10–15	95	5–10	24	30
A. irradians	10–15	95	5–10	23	30
Mussels					
M. edulis	15–25	95	10–20	16	30

From Helm *et al.* (2004). Reprinted with permission from the Food and Agriculture Organization of the United Nations (FAO).
N/A: not applicable: embryo development takes place within the mantle cavity in *Ostrea edulis*.
*Embryo densities in coldwater scallops are calculated as embryos per unit area of the base of tanks rather than per unit volume; maximum density should not exceed 1000 fertilized eggs/embryos per cm^2.

Larval rearing

The fertilised eggs are moved to flat-bottomed cylindrical tanks (2–5 l capacity) with high-quality, filtered (1–2 μm particle size), well-aerated seawater (18–24°C, cooler for cold water species), and are initially held at densities of 10 000–40 000 l^{-1} depending on the species (Table 9.3). In *C. virginica* fertilised eggs at 24°C reach the trochophore stage within 12 h of fertilisation, and the shelled D-larval stage within 24 h (Figure 9.5). A recovery of 35–85% perfectly formed D-larvae from the initial number of embryos is usual in large hatcheries (Helm *et al.* 2004). There is no need for food during the first 24 h as the early larval stages do not feed. Larvae are held in static water systems and therefore need regular water changes, initially at 24–48 h post-fertilisation and about thrice weekly after that. When the tanks are being emptied the cultures are passed through a series of sieves to size-grade the larvae. In some hatcheries only the best-growing larvae are kept, but in others several size classes are segregated for rearing with only the slowest growers being discarded. At regular intervals samples of larvae are counted, staged and checked for disease under the microscope.

Shelled larvae are held at varying densities depending on the species (Table 9.3) and are fed once or twice daily at concentrations of 30–100 algal cells per μl depending on the size of the larvae and the microalgal species being consumed. For example, daily consumption rates for *Ostrea edulis* larvae fed on *Isochrysis galbana* vary from 20 000 cells ml^{-1}, when the larvae are first liberated at 180 μm shell length, to 60 000 cells ml^{-1} as they approach

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Figure 9.5 Mussel (*Mytilus edulis*) larvae. Upper left: D-larva; upper right: D-larva at 28 days; lower left: D-larva at 35 days; lower right: different sizes of D-larvae.
Photos courtesy of H. Gurney-Smith, Biological, Earth and Environmental Sciences, University College Cork, Ireland.

metamorphosis at 250–300 µm (Walne 1974). The time it takes for the larvae to reach settlement stage depends on initial D-larval size, temperature and food quality. Generally, this stage is reached 14–21 days after fertilisation.

Setting

Cultch is the term used for the substrate provided for settling larva. The most popular cultch that hatcheries use is mollusc shells. For oyster setting the best cultch is clean dry oyster shells in plastic mesh bags that have been presoaked in filtered seawater for 12 h so that the shell surfaces have a bacterial film to attract settling oysters. A few examples of other oyster cultch are grooved plastic PVC tubes, plastic cones, lime-coated strips of wood veneer and rubber tyre chips. Some hatcheries use cultchless techniques for setting oysters that are to be grown off the bottom in trays. For example, the larvae may be allowed to settle on a flexible plastic sheet from which they can be easily removed soon after setting, or alternatively, finely crushed shell chips small enough to accommodate only one to two larvae at most can be used. Sometimes the chemicals epinephrine, norepinephrine or γ-aminobutyric acid (GABA) are used to induce oyster larvae to settle and metamorphose without the provision of a settlement surface (see García-Lavandeira *et al.* 2005 for references). Scallop larvae settle on fibreglass panels or sieves suspended in the setting tanks and the spat can be easily removed at a later stage. They also settle on presoaked (see earlier) cultch such as monofilament, shell, pebbles, polypropylene line, artificial turf, jute and sisal; maximum settlement occurs on filamentous material and kinran, an artificial material made in Japan. Postsettlement survival rates in scallops are 15–30% from the initial number of pediveligers to 2 mm shell length, much lower than the 50–70% reported for oysters (Helm *et al.* 2004). Clam larvae generally attach by a byssal thread on the bottom or to the sides of the rearing vessel, not needing any special substrate for setting.

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Some oyster hatcheries do not allow the larvae to settle but filter them on screens and place them in a piece of nylon mesh to form a bundle which is kept damp and placed in a cooler maintained at 5–10°C. The larvae can be maintained like this for about 5 days without ill effects. A bundle of 5 cm diameter can contain as many as two million mature oyster larvae. These may be kept in the hatchery for subsequent setting or they may be transported to another location for setting. This technique has made it possible for oyster farmers working in locations remote from a hatchery to receive and set viable larvae. This process – called remote setting – has revolutionised Pacific oyster production on the west coast of North America and is worth about $40–50 million annually. It has the potential to restore the Virginia oyster (*Crassostrea virginica*) industry in the United States, which has been decimated by disease over the past five decades. Its success depends on producing very large numbers of larvae at a very low cost. Theoretically, the remote setting concept could also be applied to other bivalve species, but so far it has been used only for those that attach firmly to cultch. For more detailed information on remote setting procedures see Helm *et al.* (2004) and Congrove *et al.* (2009).

Nursery culture of spat

If newly settled spat are placed out directly in the natural environment for on-growing they can suffer high mortality from smothering, predation and competition. Therefore, maintaining the spat in a nursery system until they have reached a large enough size to withstand these factors often makes economic sense. Generally speaking, spat are held in the nursery until they have reached a few centimetres in size.

Many hatcheries initially maintain the newly settled spat in their larval rearing containers until they have reached about 1 mm shell length. The water is changed daily and the spat are fed large amounts of algae, starting at 2.5×10^6 cells ml^{-1} day^{-1} but increasing significantly as the spat grow (Figure 9.1). As this is a costly process, the spat are transferred to a number of different rearing systems, the type depending on the bivalve in question and the hatchery's production system and location.

Nursery systems are either land-based (indoors or outdoors) or field-based. One type of land-based system uses raceways – long, shallow tanks or troughs made of fibreglass or concrete – that are continuously supplied with pumped seawater. The spat are layered on the bottom with water to barely cover them, or in deeper raceways they may be held on tiers of plastic mesh or tiers of shallow trays. Raceways can be either indoor or outdoor nursery systems. Alternatively, the spat may be held in tiers of rectangular, shallow fibreglass trays. A more common nursery system is the upflow or upweller unit that consists of screen-bottomed cylinders of spat held inside a larger container; seawater flows upward through the cylinders partially fluidising the seed mass, and discharges through a side exit (Figure 9.6). The water is recirculated by means of an electrically driven pump or airlift. If upwellers are used indoors there can be significant costs in heating the seawater (22–25°C) and providing the microalgae, although the latter can be supplemented with naturally occurring algae if the incoming seawater is only coarsely filtered (Utting & Spencer 1991). With an indoor system, however, growth is rapid and the total time required for bringing post-set spat to sizes large enough for flow-through nursery systems is minimised (Castagna & Manzi 1989). Spat held in raceway or upflow systems show similar rates of growth and survival. However, upflow systems allow much higher stocking densities and are also easier to maintain than raceways. Periodically, spat are graded using hand-held screens, and biomass and survival rates of each size grade are estimated (details in Helm *et al.* 2004).

Instead of land-based nurseries, spat containers may be mounted on rafts or barges moored in protected areas in the subtidal or low intertidal zones, or to saltwater ponds or

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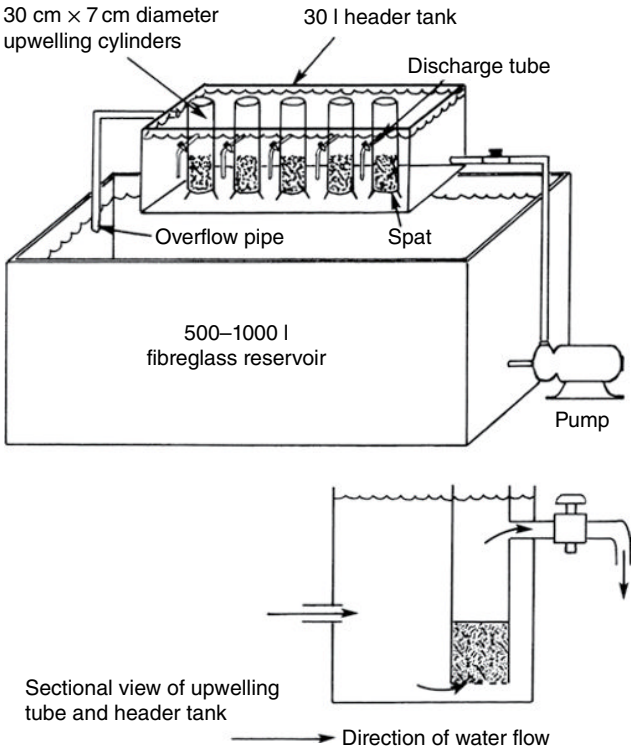


Figure 9.6 Diagram of an upwelling tank system showing the reservoir from which water is pumped to a header tank by an overflow pipe through which excess water is discharged back to the reservoir. The holding tank contains a number of tall, narrow cylinders with mesh bases in which the spat are held. Holes are drilled in the holding tank below water level to take flexible tubes that interconnect with the cylinders. Thus, there is a head difference between the water level in the holding tank and the water level that can be maintained within the cylinders. Water flows through the mesh bases of the cylinders, up through the bed of spat and then back to the reservoir through the flexible tubes. Flow rate can be changed to alter the extent to which the spat are lifted by the flow.
From Utting and Spencer (1991). Reprinted with permission from the Office of Public Sector Information (OPSI), UK.

lagoons. Primary production in ponds or lagoons can be enhanced through application of fertiliser to encourage natural algal blooms, thus allowing more control of food supply than in sea-based systems (Helm *et al.* 2004).

Whatever system is used, the spat must be cleaned routinely of fouling organisms and silt, and thinned to reduce competition. Fouling organisms such as sponges, bryozoans, ascidians, barnacles, mussels and algae can settle and grow on the shell of spat, and will smother the spat eventually if they are not removed. Fouling is more of a problem in field-based than in land-based nursery systems; intertidal systems have fewer problems than suspended systems. Various removal methods include high-pressure water hoses, air-drying in the sun or submergence in a bath of fresh water, brine or dilute copper sulphate. Knowledge of the breeding cycle, settlement time and vertical distribution of the fouling organisms can help in deciding when and where to place spat (and larger individuals), especially for suspended culture. Predation can also be a significant problem in land-based systems as the young stages are particularly vulnerable to major predators such as crabs, boring gastropods and starfish (Chapter 3). Disease can also be a major problem in larval and nursery systems (see

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later). By the time the spat have reached 2–3 cm they are less vulnerable to predators and are ready for the grow-out phase. It is virtually impossible to give an estimate as to how long it takes to reach this size. This is because so many different factors affect growth rate, for example, type and location of the nursery system, water temperature, quantity and quality of food, density of rearing, fouling, predators, pests, pollutants and disease.

Disease in the hatchery

The artificial, high density and sometimes stressful conditions under which hatchery larvae and juveniles are reared is an environment conducive to the spread of bacterial disease. The most important bacterial pathogens in hatchery cultures belong to the genus *Vibrio*, the causative agents of larval vibriosis, a disease that affects a wide range of bivalve species (see Beaz-Hidalgo *et al.* 2010 for review). Symptoms of infection include abnormal swimming behaviour, reduced feeding and general inactivity due to proliferation of the bacteria through the soft issues. The spread of vibriosis is rapid; in experimental infections signs of the disease appear 4–5 h after exposure and death begins at 8 h, and complete mortality of the cultures occurs by 18 h (Sindermann & Lightner 1988). Because the spread of infection is so rapid, antibiotics are often ineffective, may cause larvae to stop feeding and can lead to the development of resistant strains. An alternative to antibiotic treatment is the recent use of probiotics, beneficial bacteria that override pathogens by producing inhibitory substances or preventing pathogenic colonisation in the host (see Prado *et al.* 2010 for review, and Lim *et al.* 2011; Karim *et al.* 2013). Outbreaks of vibriosis often coincide with high concentrations of *Vibrio* in the water supply to the hatchery, but continue well after field concentrations have declined. The bacteria may also enter larval cultures through the algal food supply or vertically through the broodstock. Once the disease has been diagnosed all equipment, containers and water must be sterilised and infected broodstock and larval cultures should be destroyed. Preventative measures include good hatchery hygiene; reduction of stress factors such as overcrowding, high temperatures and insufficient food and routine water monitoring to provide advance warning of problems (Sindermann & Lightner 1988).

Another disease that affects only hatchery-reared oysters, *Crassostrea virginica*, is *Roseovarius* oyster disease or ROD (formerly known as juvenile oyster disease), caused by a novel bacterium *Roseovarius crassostreae* (Maloy *et al.* 2007 and references). The first signs are growth inhibition and mantle lesions that appear about 1 week before the onset of mortality. Severe mantle retraction occurs, followed by conchiolin deposits around the shrunken tissue and mantle lesions. Bacteria are present in both lesions and shell deposits. Other tissues develop lesions, and additional deposits between the shell and mantle sometimes cause the adductor muscle to detach from the shell. This in turn causes the soft tissue to fall out of the shell. The disease outbreak lasts between 4 and 6 weeks. Highest mortality (60–90%) occurs in juveniles averaging 5–20 mm shell height; those larger than 25 mm suffer much lower mortality (0–30%) but a similar disease incidence (95%) to smaller juveniles. One of the most effective ways to avoid ROD is to transfer oysters into the field as early in the growing season as possible so that they will have reached the 25 mm size refuge before ROD appears. In the hatchery reducing density and increasing water flow through upwelling systems can reduce mortality.

Serious diseases such as bonamiasis, Dermo, brown ring disease (BRD) and MSX (multinucleate sphere X; Chapter 11) do not tend to be a problem in spat or juvenile nursery systems. This is mainly because small bivalves filter less water and thus encounter fewer infective stages than older individuals.

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Grow-out

The grow-out phase brings the small bivalve up to market size and is therefore the longest phase in the culture process. The choice of site is of paramount importance not just for good growth but also for management and economic reasons.

Oysters are either on-grown in trays or in nylon rearing nets in suspended culture, or in mesh bags or trays placed on trestles in the low intertidal area. They may also be grown directly on the bottom within protected crab-proof plots or on unprotected plots if the oysters are large enough to resist crab attack (Spencer 1990). Scallops are mainly grown in suspended culture in pearl nets, lantern nets or plastic trays. Clams are on-grown in the ground in parks or plots in the intertidal area of the shore.

During grow-out the culture set-ups are regularly cleaned of fouling organisms and silt, samples are taken for measurement of growth rate (see Chapter 6), densities are regulated to optimise growth and the animals are checked for signs of disease and predation. As the animals mature, samples are taken to assess reproductive condition (see Chapter 5).

Rather than present a general account of the techniques used to on-grow bivalves, five key species have been chosen for a detailed account: *Mytilus galloprovincialis*, the Mediterranean mussel, in Spain, culture of the Pacific oyster, *Crassostrea gigas* in France, the yesso scallop, *Mizuhopecten* (*Patinopecten*) *yessoensis* in Japan, the zhikong scallop, *Chlamys farreri* in China and the Manila clam, *R. philippinarum* in Europe.

Mussel culture

Just under 1.7 million metric tonnes (t) of mussels were cultured in 2011 and about 43% of this figure was produced in China (Tables 9.1 and 9.4). FishStatJ Global Aquaculture Production data for China does not specify individual mussel species, but it is largely *Mytilus galloprovincialis*, with small amounts of *M. coruscus*, *Musculus senhousei* and *Perna viridis* that are cultured, although we have no information on annual yields for these species (Tseng 1993; Guo *et al.* 1999). Global production in 2011 was highest for *M. galloprovincialis* (almost 400 000 t), with Spain the largest producer (55%), followed by Korea, Italy and Greece (Table 9.4). The next largest production was for *M. chilensis* (~290 000 t) in Chile, followed by *M. edulis* (170 000 t), with France the main producer (>36%), followed by the Netherlands, Canada, Ireland and Germany. Thailand was the largest producer of the green mussel, *Perna viridis*, in 2009 and 2010 (>167 000 t), but in 2011 this figure had fallen to 85 000 t, while production of *P. canaliculus* in New Zealand increased over the same period to >100 000 t. It is likely that *M. edulis* data also include *M. galloprovincialis* in the case of the United Kingdom and Ireland, and *M. trossulus* in the case of Canada.

Culture of *Mytilus galloprovincialis* in Spain

The following references have been sources of information for some of the culture methods: Caceres–Martinez and Figueras (1997); Goulletquer and Heral (1997); Figueiras *et al.* (2002); FAO (©2005–2012a).

In 2011 Spain produced nearly 210 000 t of *Mytilus galloprovincialis*, making it the second largest producer of cultured mussels in the world (Table 9.4; see also Table 9.1). The industry is mainly based in the rías of Galicia, northwestern Spain (Figure 9.7). Rías are deep sunken river valleys up to 25 km in length, 2–25 km wide and 40–60 m deep. Their mean surface water temperature ranges from 10 to 20°C, salinity is about 34 psu and tidal range averages 3–4 m. Upwelling of cold nutrient-rich waters and run-off from the

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Table 9.4 Yields (live weight, metric tonnes) 2009–2011 from the main producers of cultured mussels (FAO FishStatJ 1950–2011).

Country		Species	2009	2010	2011
China	R, L	Sea mussels*	637 373	702 157	707 401
Spain	R	*M. galloprovincialis*	198 531	189 089	208 583
Thailand	P	*P. viridis*	193 626	166 927	84 665
Chile	R, L	*M. chilensis*	166 952	221 522	288 583
New Zealand	R, L	*P. canaliculus*	89 850	95 168	101 311
Korea	L	*M. galloprovincialis*	55 035	54 440	70 416
Italy†	L, HP	*M. galloprovincialis*	76 800	64 256	64 300E
France	B, L	*M. edulis*	61 620	61 800E	61 800E
		M. galloprovincialis	15 204	15 000E	15 000E
The Netherlands	OB	*M. edulis*	45 618	56 227	32 418
Ireland	OB, R, L	*M. edulis*	26 802	22 234	22 671
UK	R, L	*M. edulis*	28 000E	30 212	6 996
Greece	R, L	*M. galloprovincialis*	22 383	17 064	20 000E
Canada	R, L	*M. edulis*	23 912E	24 484	25 509
Philippines	P, HP	*P. viridis*	19 936	20 877	22 443
Germany	OB	*M. edulis*	3 600	4 905	20 830

Countries with values <2500 t year^{-1} are excluded.
E, estimated value. Method of culture: R, raft; L, longline; HP, hanging park; B, bouchot; P, bamboo pole; OB, on bottom.
*Species not specified, but largely *Mytilus galloprovincialis* with small amounts of *M. coruscus*, *Musculus senhousei* and *Perna viridis* (Tseng 1993; Guo *et al.* 1999). Values from Canada may include *M. trossulus*, while those from the United Kingdom and Ireland may include *M. galloprovincialis*.
†Mediterranean and Black Sea data.

surrounding hills support a high mean primary production of ~1.4 g C m^{-2} day^{-1} (Figueiras *et al.* 2002). Protected from the full force of the Atlantic Ocean by islands at their mouth, these sheltered, nutrient-rich rías provide the perfect environment for suspended mussel culture.

The first culture started at the beginning of the twentieth century at Tarragona and Barcelona in northeastern Spain. Initially poles, similar to French bouchots (see later), were used but these were soon replaced by floating structures. These consisted of a central flotation chamber that supported a square wooden framework (raft) from which ropes were hung. In 1946 this system was introduced to Galicia and production increased rapidly from 22 460 t in 1956 to the present-day figure of almost 267 000 t. About 70% of production comes from the Ría de Arosa (Figure 9.7).

Today the rafts consist of a framework of eucalyptus wood (~500 m^2) supported by cylindrical floats made of wood or steel covered with fibreglass or polyester, or filled with expanded polyester (Figure 9.8). A raft has a maximum of 500 hanging ropes, each a maximum of 12 m long. The ropes are ~3 cm thick and are made of nylon, polyethylene or esparto grass. Rafts are secured by mooring chains and a heavy concrete anchor and are spaced at a distance of 80–100 m from each other and located together in groups called parks. Currently, there are over 3340 mussel rafts in the Galician rías (Méndez Martínez *et al.* 2011). Most of these are family-owned and -managed, a factor that contributes significantly to low operational costs and ultimately to the great success of mussel culture in the region. The industry, with an annual turnover of over €340 million, employs more than 10 500 people; 8 000 of these are farmers and the rest are employed in supplier companies, purification and processing plants and canning companies (Caballero Miguez *et al.* 2009).

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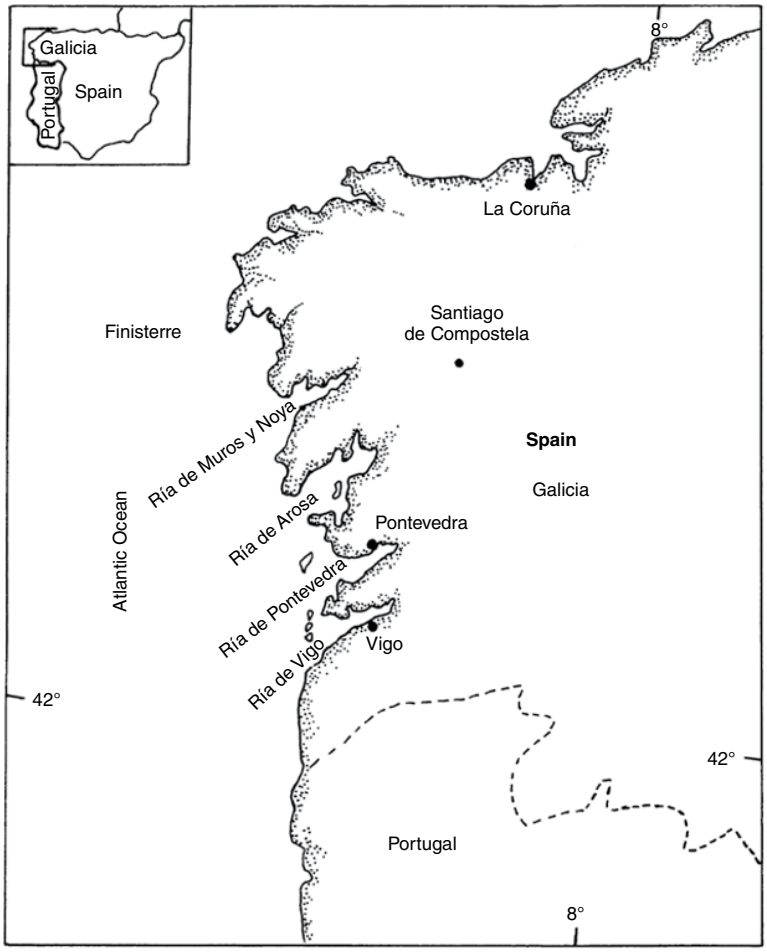


Figure 9.7 Galician rías in northwest Spain, the major raft culture region for *Mytilus galloprovincialis*. From Caceres–Martinez and Figueras (1997).



Figure 9.8 A Galician mussel raft with others in the background.
Photo courtesy of A. Ovejero, Dpto. Geociencias Marinas y Ordenación del Territorio, Edificio Ciencias Experimentales, Universidade de Vigo, Vigo, Spain.

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The culture procedure is divided into five phases: collecting the seed, attaching the seed to the ropes, thinning the ropes, on-growing and harvesting. Mussel farming requires about 9000 t of seed per year to support the current production rate (Filgueira *et al.* 2007). Farmers obtain the seed either from natural settlement (66%) on exposed rocky shores at the mouth of the rías or from collector ropes hung from rafts during March and April. About 4500 t of seed (mean 2 cm shell length) is collected from exposed shores every culture season. The seed is kept moist and wrapped by hand or by machine onto the ropes using water-soluble rayon netting that dissolves within a few days (Figure 9.9). By that time the small mussels will have attached themselves by byssus to the ropes suspended from the rafts. The average weight of seed per metre of rope is 1.5–1.7 kg, about 14 kg per rope. Wooden or plastic pegs are inserted in the rope every 30–40 cm to prevent subsequent slippage of mussel clumps. Ropes are moved to the raft as quickly as possible and attached at a density of 2–3 ropes m^{-2} of raft. The second method, collection on ropes, is likely to be used more frequently since studies show that the seed grows faster than seed originating from rocky shores (Babarro *et al.* 2003 and references). Galician law restricts the number of ropes to a maximum of 600 per raft so there is increasing interest in the development of new designs for artificial collectors in order to improve seed gathering (see Filgueira *et al.* 2007). To ensure that the grower maintains continuous production, there are normally ropes for collecting seed, ropes for producing half-grown mussels and ropes on which marketable mussels are growing, all on the same raft. A major problem for the mussel farmer is how to distinguish between these categories when there are hundreds of ropes hung at different times on a raft. The company Concepción Suárez Fernández, working with the University of Vigo in Galicia, has devised a way of doing this by fitting each rope with a RFID (radio frequency identification) tag. A software programme in the tag tracks information about the mussels on that rope from seeding to harvesting (PREMO Group 2009).



Figure 9.9 Galician seed mussels being loaded into socks, which are subsequently wrapped by machine onto culture ropes. © A. Figueras.

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After 5–6 months when mussels are half-grown (4–5 cm shell length) and the average weight per rope is 46 kg, the mussels are removed from each rope, cleaned, and redistributed onto two or three new ropes. Thinning accomplishes two things: it decreases the risk of clumps of mussels falling off the ropes and it increases growth rate. In general, thinning is automated, which means that it requires less than 17 h to thin 500 ropes of 12 m length. The process is repeated once more to ensure that all mussels reach a similar size before harvesting. Mussels can reach market size (8–10 cm shell length) in 13–16 months, which is half the time it takes for mussels to reach this size in the rest of Europe (Caballero Miguez *et al.* 2009). Apparently, in the past this size was reached in 8–9 months when mussel rafts were at a lower density than they are today. Other factors that affect growth rate are season, location of raft and depth of cultivation. Growth is slowest during summer and highest during winter because water stratification in summer causes a scarcity of phytoplankton feed for the mussels. Mussels situated on rafts at the ocean end of the ría have faster growth than those located further in. Fuentes *et al.* (2000) have shown that depth of cultivation is a more important factor in influencing growth than position of the culture rope on the raft, or stocking density; mussels in the upper part of the water column, above the thermocline (2.5 m), were significantly larger and heavier than those cultivated in deeper water (7.5 m).

In general, mortality of mussels is low on the rafts. Occasionally, extremely heavy rainfall may lower salinity to such an extent that mussels on the upper 0.5–1.0 m of ropes die. Predation from starfish or crabs is minimal as ropes are off the bottom, and disease or parasites do not appear to be a problem. Fouling by algae, tunicates and barnacles is more of a problem, as these organisms must be removed manually during on-growing and at harvesting before the mussels are sent for processing or depuration.

At harvest time, usually October to March when mussel condition is best, the ropes of mature mussels are lifted by crane and a large wire basket is lowered under the rope before both are winched onto the boat. A vigorous shake of the rope removes the mussels, which are then cleaned and graded. Those less than the minimum market size (8 cm shell length) are wrapped onto new ropes for rehanging, while marketable mussels are packed in nylon bags and transported either to processing plants for canning or freezing or, in the case of mussels for the fresh market, to depuration stations (see Chapter 12). Mean production is about 130 kg m^{-2} of raft area, which for a 500 m^2 raft works out at about 65 t (see Figueiras *et al.* 2002 for details). Losses due to natural mortality and handling are estimated to be about 15% per year. About 65% of harvested mussels are frozen or canned while 35% is destined for the fresh-consumption market. The bulk of production is for home consumption with less than 30 000 t for export, mainly to Italy, France and Germany. The industry generates a large volume of mussel shells as a waste product, which can be used as a food additive, liming agent or fertiliser component.

Government regulations stipulate that growers must have a licence to market mussels. The number of licences granted grew between 1948 and 1976, but since 1976 no additional licences have been granted. The Galician Fisheries Act of 1993 established a spatial legislative regulation on raft culture and reinforced legislation governing property rights of mussel farms. Fisheries Acts 11/2008 and 6/2009 stipulate that a licence is granted for 10 years, but can be extended for further 10-year periods up to a maximum of 30 years (see details in Caballero Miguez *et al.* 2009; Caballero-Miguez *et al.* 2012). The Department of Health constantly screens mussels for algal toxins such as PSP and DSP (see Chapter 12). If toxins are detected the entire ría or a section of it is immediately closed and harvesting is not permitted until the area has been given the all clear. The number of such closures has been increasing in Galicia over the past decade. Álvarez-Salgado *et al.* (2008) suggested that in the context of global warming the intensity of coastal winds are predicted to decrease in the

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region, leading to a decrease in the duration and intensity of upwelling, and consequently to the increasing occurrence of harmful algal blooms (HABs).

Although raft culture of mussels is a lucrative industry, it does have a number of negative impacts. Mussel production causes an increase in sedimentation due to the trapping effect of the ropes and the metabolic activity of the mussels themselves. The annual input per raft to the seabed has been estimated as 69.3 t of sediment, 5219 kg of C, 620 kg of N and 979 kg of Fe (references in Otero *et al.* 2009), causing significant changes in macrobenthic community structure (Callier *et al.* 2009; Ysebaert *et al.* 2009). In addition, mussel rafts cause depletion in phytoplankton levels, as high as 30% (Petersen *et al.* 2008), and depletion levels of 26–77% for different zooplankton groups (Maar *et al.* 2008).

Alternative methods of cultivation

While mussel culture in Galicia is based solely on rafts, there are alternative methods of production (see Table 9.4). These fall into two categories: on-bottom cultivation and off-bottom cultivation. Compared to on-bottom culture, off-bottom cultivation makes better use of the water column and mussels are less accessible to bottom predators. Off-bottom cultivation includes raft culture covered earlier and methods that employ sticks or poles driven into the ground, or longline systems.

In France mussel culture (*Mytilus edulis*) on the Atlantic and Channel coasts is based on the bouchot method, a line of poles of pine, oak, or Brazilian hardwood 25–30 cm in diameter and 4–7 m long with half their length embedded in the seabed (Figure 9.10). Spat bouchots are situated offshore and consist of a parallel row of poles with horizontal coir ropes for seed collection strung between the poles (Figure 9.11). When the seed is a few months old, it is removed from the ropes, loaded into long stockings of natural or synthetic fibre and transferred to bouchots in the intertidal zone for on-growing. The stockings are wound around the poles and secured by nailing each end (Figure 9.12). These eventually disintegrate but by that time the mussel seed has spread to cover the entire post. A typical bouchot operation is placed perpendicular to the shoreline and consists of a single line of 125 poles



Figure 9.10 Buochot mussel culture in France.
Photo courtesy of P. Goulletquer, IFREMER, France.

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Figure 9.11 Bouchot mussel culture: spat-collecting ropes (coconut fibres) covered with *Mytilus edulis*. Photo courtesy of P. Goulletquer, IFREMER, France.



Figure 9.12 Bouchot mussel culture: wrapping tubular nets filled with mussel seed onto wooden poles. Photo courtesy of P. Goulletquer, IFREMER, France.

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running for 50–60 m and spaced 15–25 m from the next line. Most are family-run, each with 15 000–20 000 poles on average. Marketable mussels (>4 cm shell length) are harvested when they are 12–18 months old; an average of 25 kg of mussels is harvested from each pole annually. All mussels are sold fresh but production from bouchots and suspended culture is insufficient to satisfy the home market (see Dardignac–Corbeil 1990 and Goulletquer & Heral 1997 for details).

In the Philippines and Thailand, bamboo poles (6–8 m long) are embedded in the seabed either singly or grouped in a circle and tied at the top to form a wigwam structure. The poles are used both for spat collection and for grow-out of *Perna viridis*. Growth is rapid with a harvest size of 5–10 cm shell length attained in 6–10 months. Each pole yields 8–12 kg of mussels. Alternatively, fixed suspended systems can be used such as the rope-web system or hanging parks (as in the Mediterranean). The former consists of rope strung in a zigzag fashion between two bamboo poles in shallow water, while hanging parks consist of metal posts with wooden horizontal beams on top from which ropes are hung. Both systems use the area between the poles or posts to grow the mussels. Details on this and additional methods are in Aypa (1990). Elsewhere, longline systems are used by countries such as Ireland, the United Kingdom, Greece, France, Canada, the United States, Chile and New Zealand. Each longline consists of a series of floats connected by horizontal lines from which rope or mesh-stocking collectors are hung (Figures 9.13 and 9.14). Seed collection, thinning and grow-out practice is similar to that for raft culture. Compared to rafts, longlines are cheaper, easy to construct and maintain and better suited to harsh winters. Also, the method allows highly mechanized culture and yields 18–20 tonnes ha^{-1} $year^{-1}$.

Growing mussels suspended on longlines in deep water (15–25 m) offshore, rather than in their traditional near-shore habitat, is an inevitable consequence of the increasing demand for shellfish. An offshore mussel farm may consist of more than 1000 km of mussel longline and cover an area of up to 1000 ha. Increase in supply, lower production costs and better growth rates are just some of the advantages mooted for offshore production. To date, offshore developments are well established in France and Italy, and programmes at different stages of development are under way in New Zealand, the United States, Ireland, the United

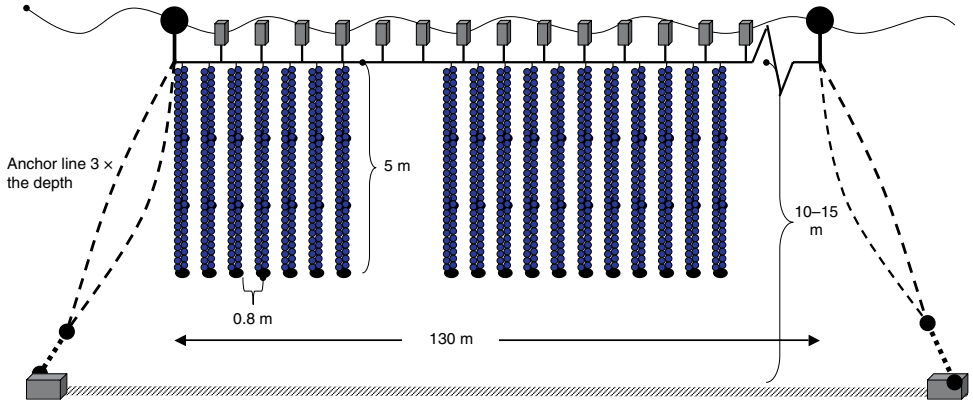


Figure 9.13 A mussel (*Mytilus* sp.) longline system in use in Westernport Bay, Victoria, Australia. The horizontal line is 130 m long with large plastic 150 l floats at each end of the line, and surface floats (25 l drums) regularly spaced between them. The vertical substrates (droppers) are 5 m long and are spaced at intervals of 0.8 m. Between 110 and 150 polypropylene rope droppers (25 mm) are attached to the line and each produces between 15 and 30 kg of mussels over a 12–18 month period.
This drawing, produced by John Mercer, is © State of Victoria, Department of Environment and Primary Industries 2002.

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Figure 9.14 Longline system of mussel culture showing mussel-covered ropes with pegs to prevent slippage.
Photo courtesy of P. Goulletquer, IFREMER, France.

Kingdom, Germany, the Netherlands and Belgium (Holmyard 2008). At the Alfred Wegener Institute for Polar and Marine Research, German researchers are investigating the feasibility of locating longlines within offshore wind farms (Buck *et al.* 2010). Similar trials are ongoing in Wales, UK (http://www.aquafishsolutions.com). Such sites would also be ideal for integrated aquaculture operations, such as mussel, oyster and seaweed cultivation (Buck *et al.* 2004).

Oyster culture

More than 4.4 million metric tonnes of oysters are cultured annually (Table 9.1), 14% of which is contributed by the Pacific oyster, *Crassostrea gigas*. According to official statistics the main producers of this species are Korea, Japan, France, Taiwan and the United States (Table 9.5). China cultivates over 3.5 million tonnes of oysters annually and most of the output comes from three species, the zhe oyster, *Crassostrea* (*Alectryonella*) *plicatula* that accounts for 50–60% of national production; but see Wang *et al.* (2008) who have found using molecular data, that the small oysters in North China are *C. gigas* and not *C. plicatula*; *C. ariakensis* for 20–30% and *C. gigas* for 10–20% (Guo *et al.* 1999), although a more recent paper puts the latter figure closer to 30% (Yu *et al.* 2008). The FAO, however, does not differentiate between oyster species in China, presenting production data under the umbrella of 'cupped oysters' (FishStatJ 1950–2011). It is unclear, therefore, where China stands in terms of global *C. gigas* production. The main species that make up the remaining world production of oysters are the slipper oyster, *Crassostrea iredalei* (Philippines), the eastern oyster, *Crassostrea virginica* (United States and Canada), the European flat oyster, *Ostrea edulis* (France, Spain, UK, Ireland, the Netherlands), and the Sydney rock oyster, *Saccostrea glomerata* (Australia).

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Table 9.5 Yields (live weight, metric tonnes) from the main producers of cultured Pacific oyster, *Crassostrea gigas*, 2009–2011 (FAO FishStatJ 1950–2009).

Country	2009	2010	2011
Korea	240911	267776	281022 (44%)
Japan	210188	200298	165910 (26%)
France	103468	95000E	95000E (15%)
Taiwan	21882	36056	34643 (5%)
United States	38910	29169	29718 (5%)
Others	28965	61036	24352 (4%)
Total	644324	567263	630645

In parentheses is each country's percentage of *C. gigas* global yields for 2011.

Culture of the Pacific oyster, *Crassostrea gigas*, in France

Although France is not the largest global producer of *C. gigas*, it is the largest producer and consumer in Europe. Also, there is a wealth of published information in English on the culture methods used compared to the more major producers Korea and Japan. Therefore, production will be dealt with primarily from the French perspective, with additional information on the methods used in Korea and Japan, where appropriate. The following have been the main sources of information: Héral (1990), Héral and Deslous–Paoli (1991), Goulletquer and Héral (1997), Spencer (2002), Agreste Cahiers (2005), Pillay and Kutty (2005), Choi (2008), Yoon *et al.* (2008) and Buestel *et al.* (2009).

Oyster culture started in France in the seventeenth century with the indigenous species *Ostrea edulis*. Oyster seed was collected from natural beds and grown for 4 or 5 years in rearing ponds or 'claires' at Atlantic coast sites. However, progressive overfishing of the natural beds led to a shortage of oysters, and in 1860 the cupped oyster, *Crassostrea angulata*, was imported from Portugal. This hardy species quickly colonised the west coast of France and by the 1960s the yield was more than three-fold higher than that from flat oysters. However, between 1970 and 1973 massive mortalities from gill disease effectively wiped out the cupped oyster from the French coast. Faced with this crisis France imported commercial quantities of the Pacific oyster, *Crassostrea gigas*, from Canada. These were directly planted into the main cupped oyster bays between 1971 and 1975. In addition, between 1971 and 1977 *C. gigas* spat was imported from Japan to reseed the oyster grounds (Grizel & Héral 1991) and to build oyster reef sanctuaries. The introduction of *C. gigas* was extremely successful, but disaster struck once again in the late 1970s with the appearance of two disease organisms, *Marteilia refringens* and *Bonamia ostreae* (see Chapter 11), that decimated production of *O. edulis* in almost all rearing areas. Production has never recovered and today harvests of *O. edulis* represent only a fraction (1.2%) of those of *C. gigas*. Fortunately, the diseases that have destroyed *C. angulata* and *O. edulis* populations have not so far affected *C. gigas*.

In France seed supply is primarily from natural settlement onto artificial collectors. Spatfall is reliable and regular on the southwest Atlantic coast; the Charente-Maritime region (Marennes-Oléron and Ile de Ré) and the Arcachon Bay furnish the entire French production for natural spat settlement, representing about 90% of the total spat production (Table 9.6). From here seed is transplanted to sites in Brittany, Normandy and the Mediterranean Sea. Although oysters settle on a variety of substrates, the most widely used collectors nowadays are PVC tubes and PVC dishes that have been presoaked and sun-dried to remove hazardous chemicals (Figure 9.15). Recruitment is around five trillion spat in the

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Table 9.6 Characteristics of the six main *Crassostrea gigas* culture areas in France.

Region	Culture method	Yield/area/ businesses	Comments
Normandy, north coast	Off-bottom	20 000 t; 1 025 ha; 168	On-bottom culture not possible due to strong wave movement; 50% of production transferred to claires in Marennes-Oléron Bay
Brittany, northwest coast	Off-bottom (70%) and deep water	31 500 t; 4 500 ha; 490	
Vendée, west coast	Off-bottom	8 600 t; 1 070 ha; 290	Yields are low due to overloading of mesh bags; 50% of production is refined in local oyster ponds; a leading region for hatchery and nursery production
Charente-Maritime (Ile de Ré and Marennes-Oléron Bay), west coast	Off-bottom	33 700 t; 4 400 ha (inc. 2 100 claires); 1 084	Main region for spat collection; supplies spat to many other French regions. 38 800 t marketed from claires
Arcachon, west coast	Off-bottom	7 800 t; 374	~0.5 billion spat produced; harmful algal blooms (HABs) are a major problem
Mediterranean, Thau Lagoon, south coast	Rope culture	9 500 t; 360 ha; 248	Highly productive area with oysters reaching commercial size in 12–18 months; no natural settlement, so spat are brought in from the Atlantic coast and from hatcheries; HABs are a problem

Adapted from text of Buestel *et al.* (2009) with permission from the *Journal of Shellfish Biology*. Production figures are in metric tonnes (t); area of culture is in hectares (ha); claires: oysters refined in ponds; off-bottom: culture in mesh bags on trestles in the intertidal.

Arcachon Bay and double this in the Marennes Oléron Bay, although these figures can vary widely from one year to the next. Since 1988 French hatcheries have begun to produce cultchless spat and larvae for remote setting. This is good news for farmers in areas with no natural recruitment because of low summer water temperatures (<18°C). The share of spat produced by hatcheries is increasing significantly each year. In 2011 about 25% of seed, about 1.5 billion, was produced in hatcheries. This is mainly due to the interest in triploid oysters, which have a faster growth rate and near sterility, giving the oyster a more consistent quality (see Chapter 10). For example, in 2005 about 80% of the 800 million spat sold by hatcheries were triploid.

The three main methods of on-growing are on-bottom culture, off-bottom culture and suspended culture (Table 9.6). On-bottom culture is carried out either in 'parcs' or 'viviers' in the intertidal zone (25% of production), or in deep water (10% of production). The ground is first hardened and the spat (6–10 months old) are then either sown directly on the ground, placed in small mesh oyster bags, or if they are retained on the collectors these are placed on the bottom. In the intertidal area the spat are protected against crab predation by plastic mesh fences (40 cm high) or hedges of twigs or stones. The spat are left for a 'pre-growing' period of 1–2 years after which time if they are still on collectors they are scraped off, graded and put back on the bottom for a further period of 1–2 years. Densities for the pre-growing and maturing phases are on average 5 and 7 kg (total weight) per square metre, respectively, and 1 tonne of spat produces about 20 tonne of market-size oysters (>70 g; note that for oysters it is weight rather than size that is normally used). The oysters

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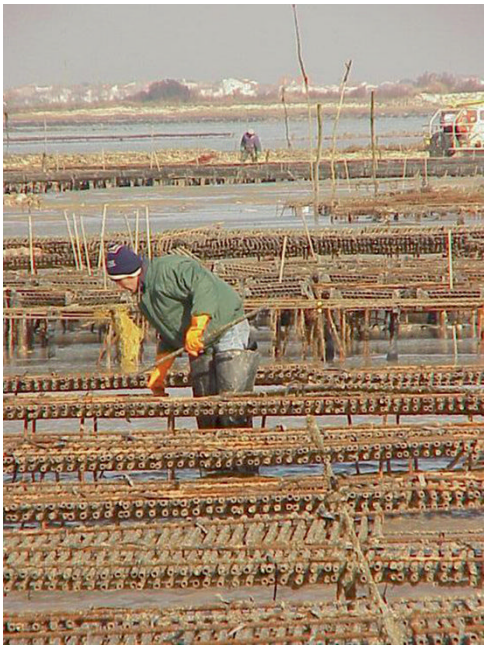


Figure 9.15 PVC collectors used for natural settlement of the oyster *Crassostrea gigas*. The tubes are placed at high density for catching the spat, but the density is subsequently reduced to improve growth of settled spat.
Photo courtesy of P. Goulletquer, IFREMER, France.

are harvested at low water with a rake, put into baskets and at flood tide loaded into boats. Because of high labour costs, this method is being replaced more and more by mechanical harvesting methods.

Off-bottom or rack culture (60% of production) is the most common technique used on the Atlantic and English Channel coasts. Oyster spat are placed in plastic mesh bags (0.5×1.0 m) and tied to metal trestles (3 m long, 0.5 m off-bottom) that are arranged in parallel rows in the intertidal zone (Figure 9.16). As the oysters grow they are thinned and bag mesh size is enlarged to encourage good growth. Biomass per bag varies between 5 and 15 kg depending on the size of the oysters. The bags are turned regularly, often by machine, to produce a well-shaped oyster and to control fouling. Oysters stay in the bags for 1–3 years depending on growth conditions. Advantages of rack culture include good growth and quality, low mortality and ease of access; but overcrowding, fouling and silting under the trestles are some of the disadvantages. These have led to strict controls being imposed, for example, restrictions on the number of trestles per leased area and the removal of trestles during winter to improve silt transport. Oysters are harvested by small boats at high tide, or by tractor at low tide. The boats are often equipped with mechanical washing and grading machines when the oysters are destined for the half-shell trade.

For suspended culture (5% of production) large areas of several hundred hectares are dredged clean and marked out by buoys. Oysters are fixed on ropes or in baskets and hung from permanent structures, called tables, or on lines in the open sea. Densities of planting are either 50 kg/100 m^2 for spat or 70–90 kg/100 m^2 for 2-year-old oysters. Growth is rapid but there is high mortality and a high investment due to dredging costs. In addition, submerged oysters have a weak adductor muscle and do not keep well out of water. They are 'trained' to keep the shell tightly closed by being held for a time in the

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Figure 9.16 Oysters (*Crassostrea gigas*) growing in bags on metal trestles in the intertidal area of the shore.
Photo courtesy of P. Goulletquer, IFREMER, France.

intertidal zone, where they are alternately exposed and submerged as the tide ebbs and flows, a process referred to as 'hardening'. Suspended culture is mainly used in Mediterranean lagoons, where there is good water depth (10 m) and no tide. Spat collectors transported from the Atlantic coast are suspended under the metal tables (11 m × 50 m) driven into the seabed. Some of the oysters are marketed after 12–18 months, while the rest may be individually cemented onto wooden bars or ropes and hung for an additional year to give an oyster of very fine quality. Dredgers harvest the oysters, and average yield per table is 5–7 tonnes. Along the Atlantic coastline several companies are now using longlines for pre-growing oysters.

In salt marsh areas, for example, the Marennes-Oléron Bay on the west coast, oysters are often placed in ponds called 'claires' for fattening (Table 9.6). These small, shallow (0.4 m), earth-bottomed ponds communicate with the sea and are rich in nutrients with consequent high phytoplankton productivity (Figure 9.17). One species of particular interest is the diatom, *Haslea ostrearia*. After death the green pigment from the diatom diffuses into the water and is absorbed by the oyster gills, giving them an attractive dark-green colour, much in demand by the consumer. Oysters fattened for 3 weeks at a stocking density of 3 kg m^{-2} are called 'fines de claires' with a <10.5 fattening index, while those with a >10.5 fattening index are 'speciales de claires'. Moreover, oyster spending more than 4 months at a density below 5 individuals m^{-2} and with a >12 fattening index are called 'pousses en claires'. About 50% of French production is 'fines de claires', while <10% is 'speciales de claires', 'speciales de claires label rouge' and 'pousse en claires label rouge' (higher and certified quality oyster). This fattening technique, however, does not always provide the appropriate quality oyster. The addition of algae (380 000 cells $oyster^{-1}$) to the ponds during autumnal neap tides (when no seawater replenishes the water) has been shown to greatly improve the fattening process (Soletchnik *et al.* 2001). When the oysters are harvested they are transported to a

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Figure 9.17 Aerial view of oyster claires in Marennes–Oréron Bay, W. France. Photo courtesy of P. Goulletquer, IFREMER, France.

processing plant where they are washed and mechanically sorted by weight, packed and brought to market. Almost all oysters are sold fresh to local markets, supermarkets or restaurants. Production is solely for the home market with more than 50% marketed for the Christmas and New Year periods.

Local authorities lease oyster beds for a period of 35 years at a set annual cost per hectare. The state and local agencies manage the dredging of the bays and set regulations governing type of culture, densities, harvesting times and quotas based on yearly stock assessments. Areas are classified into sanitary and unsanitary sectors as stipulated by the Shellfish Waters Directive 2006/113/EC (see Chapter 12), and no production from the latter is permitted. Even when oysters are grown in sanitary waters they must spend 2–3 days in clean, food-free water, mainly to discharge silt particles. The government agency IFREMER (l'Institut Francais de Recherché pour l'Exploitation de la Mer) monitors microbial quality of oysters, toxic algal blooms and chemical contaminants (Buestel *et al.* 2009).

While *C. gigas* culture is free of the disease problems associated with flat oyster production, summer mortality syndrome has become an ever-increasing problem for the industry. The good news is that researchers on the MOREST (Mortalités Estivales) programme at IFREMER have recently developed strains of *C. gigas* that are resistant to the disease (Dégremont *et al.* 2010). Another problem is contamination of oysters by HABs in some regions, which can result in prohibition of oyster sales for several weeks. Pollution can also be a problem for the industry because oysters tend to be cultivated in estuarine areas that are subject to changing bacteriological and chemical input.

The industry in France benefits greatly from having numerous natural sites available for culture, well-educated growers with technical know-how, strong research support and a strong home market for the product. On the other hand, there are several drawbacks: small farms with high production costs, serious overstocking of farms, which leads to a poorer quality of oyster, and numerous natural hazards such as bacterial contamination, toxic algae

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and mortality, which increase production costs and market instability, all major challenges for the future of *C. gigas* culture in France (see Buestel *et al.* 2009 for details).

Korea is the largest producer of oysters. Some 14 species are cultured, but *C. gigas* accounts for 80% of production (Table 9.5). Seed is obtained using collectors of scallop or oyster cultch threaded on plastic lines (1.5–2 m), which are then suspended from wooden racks placed in the intertidal or from boats in subtidal areas. There are two periods of spat collection: 'early spat' in June–July and 'late spat' in August–September. Natural spat collection supplies 90% of demand with hatchery-produced seed providing the remaining 10%. In addition, spat is provided from private hatcheries (3% of national production in 2005), and also imported from Japan and the United States. Shortly after settlement oyster spat is transferred to the intertidal zone for a period of hardening (September–April), a process that eliminates unhealthy and weak individuals (Figure 9.18). The majority (90%) of hardened oysters, now 1–1.5 cm shell height, are transferred onto submerged longlines situated in small semi-enclosed bays (5–20 m deep) on the south coast. In this region about 70 000 longlines, each carrying ~200–250 'strings' of oysters, are spread across >4 500 ha. The grow-out period lasts for 6–10 months after which time the oysters have a shell height of 8–12 cm and a wet weight of 9–15 g. Harvesting, which takes place from September to April, is followed by shucking, size sorting and processing of oysters. There are about 22 000 full-time jobs between farming and processing. Up to 60% of Korean production is exported to Japan and the United States, which in 2004 had a monetary value of $77 million.



Figure 9.18 Hardening newly settled *Crassostrea gigas* spat using racks placed in an intertidal zone in Goseong Bay, Korea.
From Choi (2008). Photo courtesy of K-S Choi, Cheju National University and the Oyster Fisheries Cooperative, Republic of Korea.

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Japan, the second highest producer of *C. gigas* (Table 9.5), uses raft and longline culture methods. Raft culture is centred in Hiroshima Bay, southwest Japan, a sheltered area of high primary productively that accounts for 70% of Japanese oyster production. The Miyagi Prefecture on the east coast is the second largest oyster-producing region (20%), using longlines in coastal inlets and offshore waters; this area produces most (70%) of the country's seed requirements. Spat collection starts in June and lasts about 2 months, after which the settled spat are transferred to longlines and are harvested the following February to May, giving a grow-out period of less than a year. Sometimes, in order to produce larger oysters for the home market (10–20 cm shell height; meat weight 10–30 g), they are grown individually for 6–8 months in net cages suspended from rafts. Alternatively, spat is collected later in the year, and collectors with spat are transferred to racks in shallow water until the following summer for a period of 1 year for hardening. The oysters are then moved to rafts for a final growing period and harvested the following February, a culture period of about 2 years. The industry in Japan and Korea is based on the production of shucked meats; therefore irregularities in shell shape, a feature of oysters grown to market size on cultch, is of little importance compared to the situation in France (see earlier) where the majority of oysters are sold in the shell.

Scallop culture

Currently, about 1.5 million metric tonnes of scallops are cultured annually and most of this figure is produced in China (Tables 9.1 and 9.7). Three main species are cultured in China: the native zhikong scallop, *Chlamys farreri*, and two introduced species, the yesso scallop, *Mizuhopecten yessoensis*, and the bay scallop, *Argopecten irradians* (Table 9.7). Japan is the next largest producer of the yesso scallop (>200 000 t year^{-1}), followed by the Russian Federation and Korea, both of which produce significantly smaller amounts (Table 9.7). The calico scallop, *Argopecten purpuratus*, is cultured in Chile and Peru, with increasing amounts from Peru in recent years (~50 000 t year^{-1}). Small quantities of the great scallop, *Pecten maximus*, are produced in Ireland (Table 9.7). The following sections present more detailed information on two of the main species cultured: *Mizuhopecten yessoensis* in Japan and *Chlamys farreri* in China.

Table 9.7 Yields (live weight, metric tonnes) 2009–2011 for the main cultured scallop species and their principal producers (FishStatJ 1950–2011).

Species	Country	2009	2010	2011
Mizuhopecten yessoensis	Japan	256 695	219 649	118 425
	China*	523 000E	563 000E	523 000E
	Russian Federation	843	854	*725*
	Korea	*548*	453	603
Chlamys farreri	China	500 000E	563 000E	500 000E
Argopecten irradians	China	130 000E	140 000E	130 000E
A. purpuratus	Chile	16 864	8 840	11 018
	Peru	16 047	58 101	52 213
Pecten maximus	Ireland	*55*	*59*	*50*

*Chinese yields in the FishStatJ database are presented under the generic category scallops 'nei' ('not elsewhere indicated'); therefore approximate values for individual species have been estimated (E) using information from Bao (2009) and Guo (2009). Suspended culture for grow-out is used for all species; for *M. yessoensis* on-bottom culture is an additional method in Japan and the Russian Federation.

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Culture of the yesso scallop, *Mizuhopecten yessoensis*, in Japan

The following have been the main sources of information on the culture of *M. yessoensis* in Japan: Ito (1991), S. Ito (1991), Pillay and Kutty (2005), Hardy (2006), Uki (2006) and Kosaka and Ito (2006).

Culture of *M. yessoensis* started because of the collapse in the wild fishery for this species in 1945. Production remained at extremely low levels (<100 t year^{-1}) for the next 25 years. The first attempt at spat collection actually started well before this in 1934 (Kinoshita 1935), and after many years of trials a successful spat collector was invented by a fisherman in Mutsu Bay in 1964. Subsequently, production increased rapidly from a figure of 6 000 t in 1965 to more than 100 000 t by 1985, reaching more than 200 000 t in the mid 1990s, a

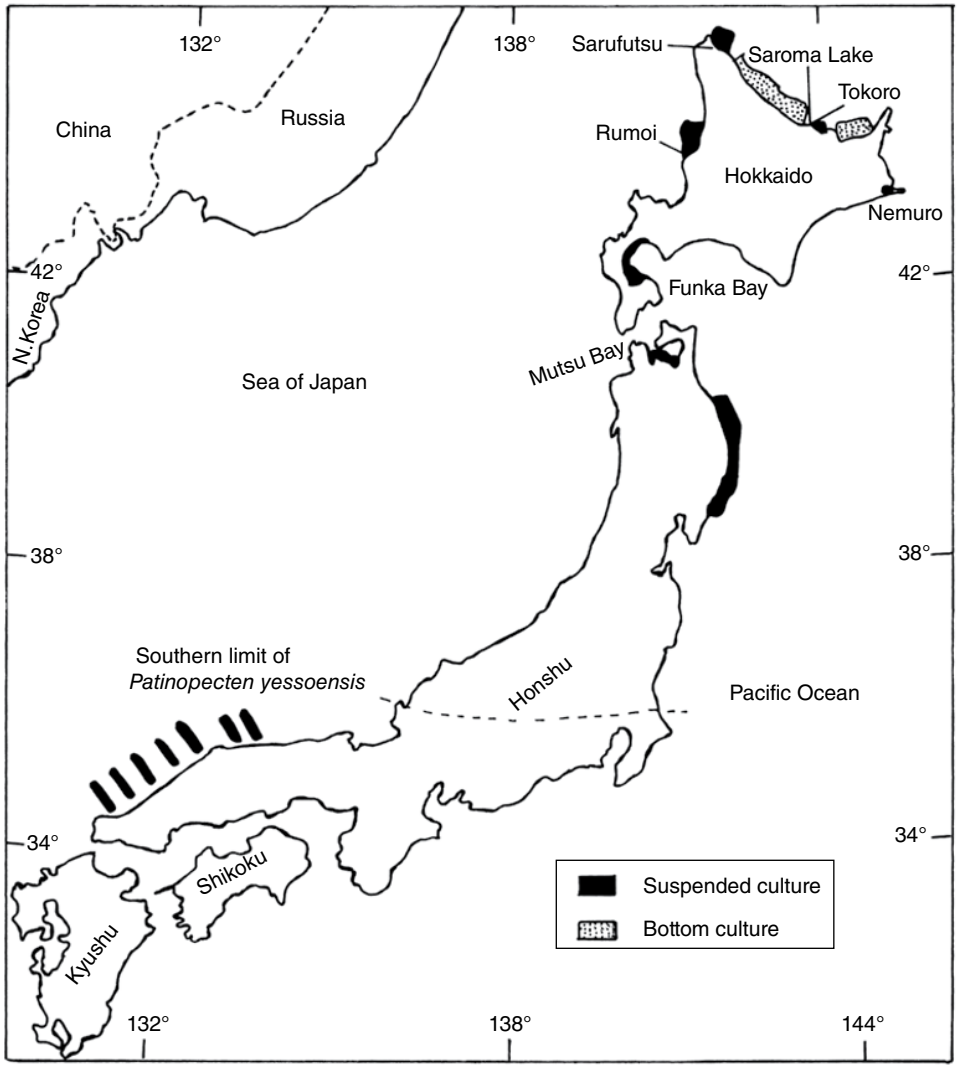


Figure 9.19 Cultivation areas for the scallop *Mizuhopecten yessoensis* in Japan. From text of Fleury *et al.* (1997).

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figure that has remained fairly stable to this day (Table 9.7). The development of innovative methods for collecting, growing and releasing scallop spat paved the way for the restoration of the wild fishery, and landings today are about 300 000 t year^{-1}. However, it is debatable whether the *M. yessoensis* fishery can truly be regarded as a wild fishery as it is now based entirely on aquaculture methods for the capture and rearing of spat for stock enhancement.

The culture of *M. yessoensis* is confined mostly to northern areas of Japan where water temperatures are ideal (10–17°C) for this cold-water species (Figure 9.19). Culture is equally divided between suspended and bottom production. The industry is based on natural spat that settle on collectors hung from longlines. Various types of collectors are used but the most common type is a mesh bag loosely filled with plastic netting or used gill netting. The mesh size must be large enough to allow the water with settling larvae through but small enough to prevent clogging of the meshes by silt and escape of spat when they eventually detach from the netting. The bags, ~60 cm long × 35 cm wide, are attached at intervals to a longline 50–100 m in length (Figure 9.20a). The number of spat range from over 10 000 to a low of 300 per collector. In Mutsu Bay (Figure 9.19) spat collectors are in place from mid April to the beginning of August, but where water temperatures are lower, for example, Hokkaido, spat collection starts and ends later.

Between mid July to mid August, when the scallops are of 5–10 mm shell height, they are removed from the collectors and placed in pearl nets (2–7 mm mesh; 30 cm^2 floor) suspended from longlines that are 7–15 m below the water surface (Figure 9.20b). This stage is referred to as intermediate or nursery culture. The initial stocking density is 100 spat per net but by September–October this is reduced to 20 spat per net if adults are for suspended culture, and 50 spat per net for bottom culture. Scallops do not tolerate as high a stocking density as other bivalves. Growth is fast and juveniles have a shell height of about 4 cm by December and about 5.5 cm by mid March. The survival rate is generally higher than 90%. Juveniles from intermediate culture are used for both hanging (suspended) and bottom culture. In hanging culture, scallops are either grown by 'ear hanging', a traditional Japanese method, or in different types of nets. Scallops need to be contained like this because they can break their byssus and swim off. In the ear hanging method, a small hole, about 1–2 mm in diameter, is drilled in the anterior ear of the left shell valve, or through both the right and left anterior ears. The process is fully automated and each machine can drill and secure one scallop every second. The scallops are tied by nylon thread to a rope (6–8 mm diameter) that is suspended from a longline (Figure 9.20c). Scallops are usually attached in pairs at intervals of 10–20 cm depending on their size. One rope contains about 130 individuals. This method gives a high meat yield, high survival and a well-shaped shell. Fouling can be a problem and if severe can cause the scallops to fall off the ropes. This method is not suitable in areas subject to excessive wave action.

Various types of nets are used is suspended culture, the most common one being the lantern net. This consists of 10 wire hoops, 50 cm in diameter and 15–20 cm apart, enclosed by monofilament netting of 12–25 mm mesh size (Figure 9.20d). Each compartment has an opening through which scallops can be inserted or removed. Several lantern nets can be strung one below the other if there is sufficient water depth. A density of 20 scallops per compartment is optimum for good growth. It usually takes 10–15 months for scallops to reach the market size of 10–11 cm shell height. Although all suspended culture methods are subject to heavy fouling, cleaning must be kept to a minimum and executed rapidly as scallops are very sensitive to being exposed to air and direct sunlight. Fouling can be minimised by deep suspension or by planting the scallops at a lower density in the nets to lessen time before harvest.

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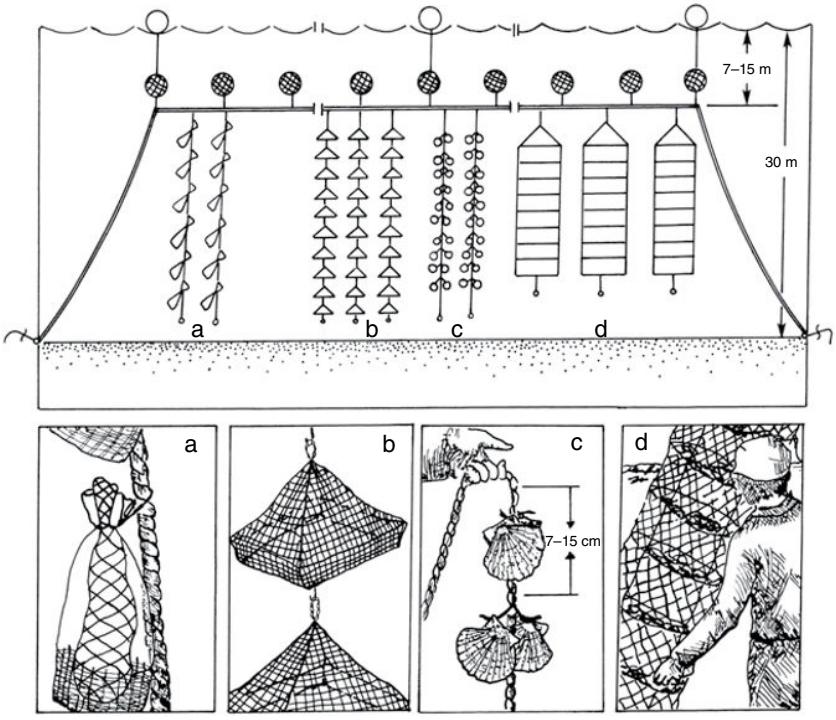


Figure 9.20 Longline system used in the culture of scallops. (a) Mesh bags for spat collection; (b) pearl nets for nursery phase; (c) ear hanging and (d) lantern nets for on-growing phase. Redrawn and modified from Freeman (1988).

The other grow-out method is bottom cultivation, which is mainly concentrated on the north coast of Hokkaido (Figure 9.19). Prior to seeding, predators such as starfish and sea urchins are dredged from the bottom. Seed measuring about 3 cm shell length is sown between December and April at a density of 5–10 individuals m^{-2}, a value based on natural stock density. The seed is obtained either from natural settlement or from hanging culture. It takes between 3 and 4 years for the seed to reach market size. The culture beds are rotated on a regular basis and the Tokoro cooperative will serve as a good illustration of how this operates. The cooperatives situated on the north coast of Hokkaido have divided their coastal area into four units, each averaging 25–30 km^2 with depths ranging from 30 to 60 m. A smaller unit is set aside as a reserve in case of underproduction. Each unit is harvested every 4 years and is followed by a new seeding (density 10 m^{-2}). The spat comes from natural settlement in the nearby Saroma Lake, or from Funka Bay or Mutsu Bay (Figure 9.19). For 300 million spat, the cooperative harvests about 30 000 t live weight of scallop 3 years later. When the cooperative started in 1976 the ratio of adults fished to the number of juveniles seeded was about 1:4, but this ratio has improved to 1:2 due to better management of the beds in terms of stocking, predator control and so on. The scallops are harvested by dredge from June/July to November and each boat has a daily catch quota of 6 t, usually caught over a period of 3–4 h. Between 50 and 60% of Japan's production comes from bottom culture. The Japanese consume most of the scallops they produce and prices are kept low to encourage home consumption. Both the adductor muscle and soft body parts are processed and processing data for Hokkaido over a 27 year period (1975–2002) show the following: fresh scallop 22%; boiled whole body 21%; frozen muscle 36%; dried muscle 15% and canned/other 6%.

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The continuing success of scallop culture in Japan will be guaranteed if there is a reliable seed supply each year and if the carrying capacity of bays used for scallop culture is not overextended. In the context of aquaculture, carrying capacity is generally understood as the standing stock of a particular species at which production is maximised without negatively affecting growth rates (Duarte *et al.* 2003). To date there is no hatchery production of seed scallops, but this situation must change since seed production from Saroma Lake and Funka Bay, two of the main supply areas, will not be sufficient to meet future demands. It is surprising that the Japanese are not producing hatchery seed in view of the innovative culture techniques developed by them in the past, and the fact that other countries have been using the technology now for more than a decade. For example, hatchery-reared *Pecten maximus* spat are now produced on a commercial scale in France (Dao *et al.* 1996), Norway (Torkildsen & Magnesen 2004), the United Kingdom and Ireland (Laing 2002); *M. yessoensis* (Bourne 2000) and *Placopecten magellanicus* (Milke *et al.* 2004) in Canada; *Argopecten irradians* in China (Guo *et al.* 1999); *Pecten fumatus* in Australia (Heasman *et al.* 2002) and *Argopecten purpuratus* in Chile (Uriarte *et al.* 2003).

Many bays used for scallop culture are now overcrowded and offshore culture has, to date, been unsuccessful. Choosing additional appropriate locations for culture would maximise economic return and minimise the risk of environmental impact and conflict between aquaculture and other resource uses. The availability of geographic information system (GIS) and remote sensing (RS) data makes it possible to select environmentally suitable areas rapidly and systematically (see Radiarta *et al.* 2011 for references). In Funka Bay, Hokkaido (Figure 9.19), for example, studies were conducted to identify the most suitable sites for hanging culture (Radiarta *et al.* 2008; Radiarta & Saitoh 2009). Using biophysical parameters (sea temperature, chlorophyll, suspended sediment and bathymetry) extracted using RS, and modelled using GIS, the order of importance of the variables affecting the models was suspended solid > chlorophyll > sea temperature; most areas in the bay had high suitability scores. See Kapetsky and Aguilar-Manjarrez (2007) for a review of how GIS, RS and mapping are playing a central role in the development and management of marine aquaculture.

Culture of the zhikong scallop, *Chlamys farreri*, in China

China has a long history of mollusc culture dating back several thousand years, but it is only in the last 50 years that the industry has expanded exponentially. China is now the top producer of cultured oysters, scallops and clams in the world (Table 9.1). In 2011 China produced more than 10 million metric tonnes of cultured bivalves of which 1.3 million metric tonnes was scallops. The native zhikong scallop, *Chlamys farreri*, accounts for about 40% of this figure, and the introduced species, *Argopecten irradians* from North America and *Mizuhopecten yessoensis* from Japan, account for 10 and 40%, respectively (Table 9.7). Most publications on culture methods in China are in Chinese so the following description of *C. farreri* culture has been heavily dependent on several reports in English: Luo (1991), Guo *et al.* (1999), Guo and Luo (2006), Zhang *et al.* (2006) and Guo (2009).

The zhikong scallop is found in north China (40°N) to Fujian (25°N) where water temperatures do not exceed 25°C (Figure 9.21). It also inhabits the waters of Japan and Korea. The distribution of the species is patchy and the total natural resource is only about 2000 t. Experimental culture of scallops started in the late 1960s and expanded rapidly through the 1980s and 1990s to become the very impressive industry it is today. Culture of the zhikong scallop started in 1974 and initially only hatchery-produced seed

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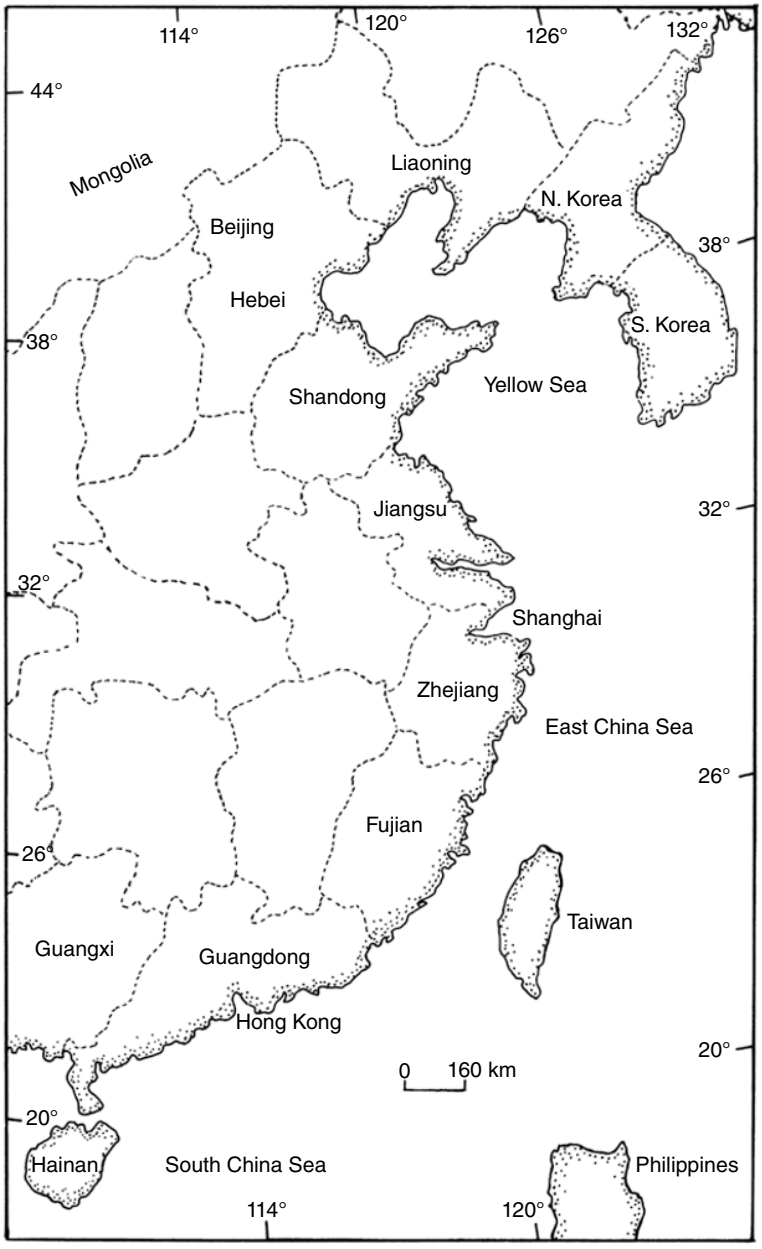


Figure 9.21 The coastal provinces in China where scallops are cultured. *Chlamys farreri* and *Argopecten irradians*: Shandong, Hebei and Liaoning; *Mizuhopecten yessoensis*. Liaoning and Shandong; *Chlamys nobilis*: Fujian, Guangdong and Hainan.
From the text of Guo *et al.* (1999).

was used. But as culture continued there were sufficient adults in culture to provide an abundant source of natural seed, even up to the present day. The most important culture areas for *C. farreri* are Shandong, Hebei and Liaoning Provinces (Figure 9.21), with the former producing about 80% of the national total. Seed is collected in early summer and in the autumn on bags (30 cm × 40 cm, 1.2–1.5 mm mesh) stuffed with about 100 g of

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nylon screens. The bags are strung together, about 10–12 per string, and these are hung (6–8 m depth) from a raft that carries about 500–600 strings. Each bag collects about 100–1000 spat depending on site, season and year. One of the bays in north Shandong produced about 130 billion seed in 1996, all from cultured populations. Most of the seed is collected from the first spawning season that set between late June and mid-July. Spat are left on the collectors until early October when they have reached about 5–10 mm shell height. The seed is put into lantern nets (mesh size 4–8 mm) at a stocking density of 200–300 per layer, giving a total of 2000–3000 per lantern net. The nets are transferred into a nursery area and hung on longlines (80–100 m long) until the following March when shell height is ~30 mm. Lantern nets on suspended longlines is the dominant method used for grow-out for all cultured scallops. The scallops are then thinned to 50–80 per layer for the grow-out phase. By December the scallops have reached the market-size of 60–70 mm shell height, when they are 1.5 years old. In some areas seaweed and scallop culture take place on the same longline system, and sometimes sea cucumbers are cultured in the same lantern nets to keep down fouling, a severe problem particularly near the water surface.

Since 1996 there has been massive summer mortality of zhikong scallops. In 1998 this was so severe that production was reduced by 37% with an estimated loss of revenue of around $360 million. The cause is likely a combination of high temperature, poor circulation in the nets due to overcrowding, fouling and deteriorating water quality (Xiao *et al.* 2005; Zhang *et al.* 2006). The recommended stocking density for grow-out is 30–35 scallops per layer, but farmers often stock at 2–3 times this density (see earlier). Also, the huge expansion in the number of longlines means that the carrying capacity of many areas has been exceeded. Both of these factors result in a low-quality environment for the culture of zhigong scallops, and indeed for other bivalves. Mortality may in fact be due to a viral disease that does not affect bay scallops (He *et al.* 2003).

Dissecting the adductor muscles, placing them in seawater and then drying them in the sun was the traditional method to process scallops. Alternatively, the muscles were first cooked, steeped in seawater for a short while and then dried in the sun. These were marketed as 'dry shellfish' in China and other parts of Asia. However, since the late 1980s most of the harvest is processed into individually quick frozen (IQF) adductor muscles. In the early 1990s more than 60% of these were exported to North America and other regions, but because of the current summer mortality problem, and because the home market is growing rapidly, only a small percentage of scallops are exported. In fact, right now China imports sea scallops *Placopecten magellanicus* from North America.

The other scallop species that accounts for 10% of scallop production is the bay scallop, *Argopecten irradians*, introduced from the United States in 1982. Only 26 of the original imported 128 scallops survived and these were the founders of a very successful industry in the Shandong, Hebei and Liaoning Provinces (Table 9.7; Figure 9.21). Fast growth and the fact that seed is hatchery-produced in spring means that the species reaches market size within 1 year, in contrast to *C. farreri*, which takes 6 months longer to reach market size. In the late 1990s and early 2000s new broodstock were introduced from the United States and Canada to counteract possible inbreeding effects. Another species, *Mizuhopecten yessoensis*, was introduced from Japan in the late 1970s and is grown in the northern provinces of Liaoning and Shandong. The species does poorly in lantern nets but grows well by the ear-hanging method. Although the yield is about the same as the zhikong scallop, it is larger in size and has a higher market value than that species. To date, there are no wild populations of bay scallops in China despite the fact that the species has been cultured on a large scale

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for more than 30 years. While the species spawns in the summer and produces spat, the spat do not survive the first winter. Another species, the huagui scallop, *Chlamys nobilis*, is cultured on the coasts of Fujian, Guangdong and Hainan Provinces in the South China Sea (Figure 9.21), but annual production is extremely low.

To date, there are few commercial selective breeding programmes for scallops in China. However, genetic linkage maps, a prerequisite in mapping quantitative trait loci (QTLs), have been constructed for *C. farreri* (Zhan *et al.* 2009), *A. irradians* (Li *et al.* 2012), and *M. yessoensis* (Xu *et al.* 2008). QTLs are an essential prerequisite for marker-assisted selection (see Chapter 10). Size-related QTLs have been identified in *A. irradians* (Qin *et al.* 2007a), and a shell colour marker closely linked to a shell colour gene has been identified and mapped in this species (Qin *et al.* 2007b). Scallops with brilliant-coloured shells are especially attractive to the consumer and command a higher price. Consequently, red strains of *A. irradians* have recently been developed through selective breeding (Bao 2009). Crossbreeding between geographic subspecies of *A. irradians* and the hybrids show heterosis for shell height, total weight and adductor muscle weight (Zheng *et al.* 2011).

Clam culture

Almost five million metric tonnes of clams (live weight) are cultured each year on a global scale (Table 9.1), with China responsible for 98% of this figure in 2011. Worldwide, there has been more than a nine-fold increase in production since the late 1980s, mostly due to the expansion of culture operations in China, Malaysia, Italy, Taiwan and the United States. A wider variety of clam species are cultivated compared to the other bivalve groups. The main species cultured, in order of importance, are the Manila clam, *R. philippinarum*, the Chinese razor clam, *Sinonovacula constricta*, the blood cockle, *Anadara granosa*, the Japanese hard clam, *Meretrix lusoria*, and the hard (Quahog) clam, *Mercenaria mercenaria* (Table 9.8). These made up >99% of global yields of clams in 2011. The Manila clam, *R. philippinarum*, contributed 81% to annual global yields, and 98% of this figure comes from China (Tables 9.8 and 9.9). The constraints of little or no published information, difficulty in accessing information in the 'grey literature' and language barriers mean that culture of this species will be described from more minor regions in Europe and the United States rather than from the major player in the field.

Table 9.8 Yields (live weight, metric tonnes) 2009–2011 for the main cultured clam species and their principal producers (FishStatJ 1950–2011).

Species	Country	2009	2010	2011
Anadara granosa	China	276742	310380	293200
	Thailand	81959	75611	40526
	Malaysia	64938	78025	57544
Sinonovacula constricta	China	683806	714434	744794
Meretrix lusoria	Taiwan	51820	60325	59764
Mercenaria mercenaria	United States	27000E	29257	28841
Ruditapes philippinarum	China	3192461	3538906	3613349
	Italy	32374	35673	35700E
	Korea	17905	23430	25699

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Table 9.9 Yields (live weight, metric tonnes) from all producers of cultured Manila clam, *R. philippinarum* 2009–2011; Japan is also a major producer but yields are presented under category 'clams nei' (FishStatJ 1950–2011).

Country	2009	2010	2011
China	3192461	3538906	3613349
Italy	32374	35673	35700E
Korea	17905	23430	25699
United States	3507	2722	3429
Canada	1200	1500	1300
Spain	844	1101	1089
France	622	580E	580E
Ireland	162	175	150
Taiwan	138	145	140
United Kingdom	1	15	1E
Total	3249214	3604246	3681437

China is the world's largest producer with 98% of production in 2011.

Culture of the Manila clam *Ruditapes philippinarum*

The main sources of information were Britton (1991), Spencer *et al.* (1991), Jones *et al.* (1993), Pranovi *et al.* (2004), Toba *et al.* (2005), Sladonja *et al.* (2011), FAO (©2005–2012b) and http://www.dfo-mpo.gc.ca/aquaculture/.

Overfishing and irregular yields of the native clam, *Ruditapes* (*Tapes*) *decussatus*, led to the importation of the closely related species, *R. philippinarum*, into northwest Europe in the 1970s and 1980s. The species, a native of Japan, Korea and the Philippines, was introduced accidentally along with oysters into North America during the 1930s, and from there was deliberately introduced as hatchery broodstock into France in 1972 and into the United Kingdom and Ireland in 1980 and 1982, respectively. In contrast, between 1983 and 1984 Italy imported large quantities of seed from a UK hatchery for direct planting into the lagoon of Venice. Within a few years the species had established natural populations on the south coast of Britain, along the Atlantic coasts of France, in coastal lagoons in the northeastern Adriatic, and in North America from British Columbia, Canada, to California. In all regions *R. philippinarum* proved to be hardier and faster-growing than *R. decussatus* and today contributes 90% to European yields of the two species. The techniques used to culture *R. philippinarum* work equally well with *R. decussatus.*

The main culture areas in Europe are the coasts of the northern parts of the Adriatic Sea, Italy; the Atlantic coasts of France from Normandy to Arcachon; the Atlantic coasts of Ireland, Galicia and the Basque region of Spain; and Poole Harbour on the south coast of England. In North America the main areas are on the coasts of British Columbia, Washington State and California. While recruitment of the Manila clam occurs in some regions, seed supply from natural recruitment is generally not sufficiently high to sustain the industry. Therefore, for the most part, the industry is dependent on hatchery-produced seed. California is the leading global supplier of Manila clam seed. The seed is held until individuals are of 2–10 mm shell height and are either sold on to growers or transferred into the sea for grow-out. The size at which to purchase seed depends on the rearing system to be used. The lower cost of smaller seed can easily be offset by higher mortalities. Many growers prefer to start with larger seed, which has higher survival rates, making production more predictable. In 2008 seed producers in California sold over $1.1 million worth of clam seed.

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Each hatchery has its own tried and tested methods for broodstock conditioning, induction of spawning and larval rearing (see earlier). In the spring, when clams have reached 2–3 mm shell length, they are transferred outdoors to the nursery. In France this can be a land-based upwelling system (Figure 9.6) where the clams remain until they have reached 10–15 mm shell length, a period of 3–4 months. Alternatively, slightly larger clams (6–7 mm) may be enclosed in 4 mm mesh bags (1.5 m × 2 m) at a density of 3000 m^{-2}. The bags are placed on the seabed in spring and by summer the seed measures 13–15 mm. In some areas of France small clams are reared all the way to market size (35–50 mm shell length) in 400 m^2 coastal ponds at a density of 300 m^{-2}, and a sprinkling system delivers seawater and live algal food. In Italy the clam-growing areas are situated in the upper Adriatic Sea, the most important being the lagoon of Venice. The industry is almost completely dependent on the gathering of wild clam seed in nursery areas where there are high densities due to environmental conditions that favour setting, survival and growth to a size large enough for successful transplantation (Pellizzato *et al.* 2011). The gathered seed (4–5 mm shell length) is placed at a density of 10 000 m^{-2} in wooden frames (1 m × 5 m) that are covered in plastic netting (2–4 mm mesh), and stacked underwater until the seed is 10–12 mm, a period of 3–4 months.

In Ireland seed (2 mm) is placed in mesh-covered wooden frames (3 m × 1 m) for 12 months, or in mesh bags on trestles, around the low spring tide area of the shore (Figure 9.22). Stocking density is initially high at 300 000 per frame but once the seed has reached 6 mm it is thinned to 30 000 per frame for overwintering. Due to Ireland's northerly latitude it takes until the spring of the following year for the seed to reach 9–10 mm shell length. During the nursery stage clams must be regularly graded and cleaned. In the field a high-pressure pump is used to clean the meshes/frames of silt and fouling organisms but careful hand cleaning is necessary to remove filamentous weed from clumped seed. Care must be taken to ensure



Figure 9.22 Mobile seed trays for nursing clams (*Ruditapes philippinarum*). Photo courtesy of Kevin O Kelly, Lissadell Shellfish Co. Ltd., Sligo, Ireland.

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that small crabs and mussel spat are removed from the bags or frames and that there are no tears or openings through which crabs can enter. Even very small crabs can do a lot of damage by nipping off the siphons of feeding clams. Crabs are usually only a problem from March to October as they migrate offshore during the winter. Another problem is mussel spat that compete with clams for food, bind them in byssus clumps, very quickly outgrow them and thus retard clam growth rate.

When the clams have reached about 10 mm shell length they are ready for seeding in the substrate. Ideally, the seeding site should be sheltered, with a salinity of 20–34 psu, the substrate should be free from rocks, boulders and gravel and firm enough for machinery, there should be good access to the site and trials should show evidence of good clam growth and survival. The chosen site should be monitored over a period of 1 year for changes in substrate, shifting of estuarine channels, water coverage on different tides and under different weather conditions and settlement patterns of potential competitors and fouling organisms. Prior to the 1990s two main methods were in use, the French parc system and the plot system. Parcs consisted of a fenced-off area of the shore. The fence was made from wooden posts about 80 cm high and was designed to prevent crabs from entering the parc. Baited crab traps were used inside to remove the crabs that did manage to breach the fence. The floor of the parc was covered by mesh to protect against bird predators such as oystercatchers. This system was expensive and difficult to maintain and has since been superseded by the plot system of cultivation. In Ireland, before constructing a plot all large stones, weeds, mussels and crabs are removed from the surface of the seeding area. Crabs in the substrate are killed by a crab killer, a device with vertical tines that penetrates to a 75 mm depth, killing all crabs, and in the process loosening the sand to help the young clams bury themselves. Clams of 10–14 mm shell length are seeded at a density of ~300 m^{-2} and covered with a 4 mm aperture netting (1–1.5 m wide and up to 300 m long). A 0.5 m gap is left between each net to allow a tractor to pass over the plot without harming the clams. A planting machine ploughs in the netting and sows the seed simultaneously. Deflector plates backfill the trenches, burying the edges of the net up to 100 mm (Figures 9.23 and 9.24). Ideally, the net strips should be laid parallel to the prevailing wind direction and also parallel to the main run-off. Seeding is generally carried out in spring to maximise growth and to avoid the main period of crab predation (July–September). The nets should be cleaned routinely of fouling organisms and sediment and checked for holes and for predators under the mesh. In Ireland a ganged static brush assembly mounted on a tractor is drawn over the nets to clean them. This is done once or twice every spring tide depending on the season. The United Kingdom and France use much the same method as in Ireland with slight variations in terms of plot size, type of netting and so on. In Italy the plots are sown by manually scattering seed (~200 individuals m^{-2}) over the prepared area at low tide.

Estimated growth of Manila clams at yearly intervals in UK plots is shown in Figure 9.25. A clam placed in a ground plot at 10 mm shell length should grow to 30, 42 and 51 mm after 1, 2 and 3 years, respectively. Similar growth rates are observed in Ireland. Bearing in mind that it takes 1 year of hatchery and nursery rearing before clams are seeded in the ground, the length of time it takes to produce minimum market size (35–40 mm shell length) clams in the United Kingdom and Ireland is about 3 years. This size is reached in Italian plots in 16–18 months (Mattei & Pellizzato 1997) and in France in less than 2 years. However, in all countries the optimum market size is 45–50 mm as demand and price is better for these larger clams.

Various factors influence survival rate of clams in ground culture systems. Data from the United Kingdom show that small clams survive less well than larger clams in ground plots. Clams put in the ground at 3 mm had a survival rate of only 34% compared to

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Figure 9.23 Seeding clams (*R. philippinarum*) with a mechanical net layer. The roll of netting has just run out.
Photo courtesy of Kevin O Kelly, Lissadell Shellfish Co. Ltd., Sligo, Ireland.



Figure 9.24 Clams (*R. philippinarum*) planted under nets.
Photo courtesy of Kevin O Kelly, Lissadell Shellfish Co. Ltd., Sligo, Ireland.

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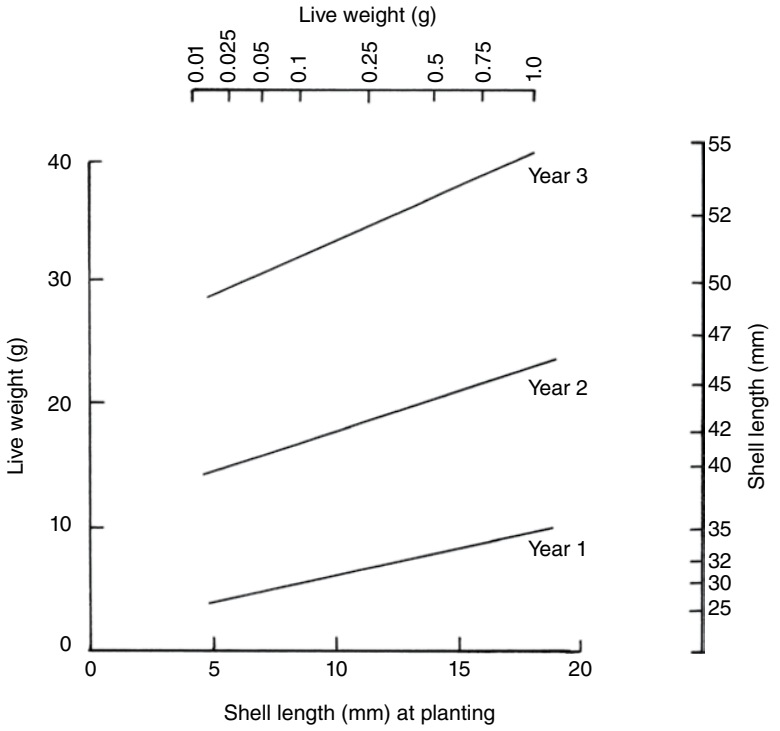


Figure 9.25 Estimated growth of *R. philippinarum* spat after 1, 2 and 3 years in the ground. From Spencer *et al.* (1991) with permission from the Office of Public Sector Information (OPSI), UK.

10 mm clams with a 60% survival rate. Larger clams of 24 mm had a 77% survival rate, but the gain in survival is outweighed by the higher cost of rearing clams to this size. Various characteristics of the plot, for example, silt content of the water and water coverage, are important to survival. In UK trials Manila clams had a 70% survival in plots with up to 30% exposure (low water neap tide) to air, but at 50% exposure higher on the shore no clams survived due to smothering by moving sediment. Winter storms can also be a problem in that clams fill up with sand particles during turbulent weather when there is a lot of sand in suspension. The clams cannot expel the sand so they come to the surface for oxygen, probably use up their energy reserves, gape and eventually die. Fouling of netting, predation and, in particular, diseases (see later) are other factors that can cause high mortality in cultured clams.

Before harvesting, the plots must be sampled to make sure that the clams are of marketable size. In small operations clams are harvested manually by raking them out of the substrate. However, most farms use either a suction dredge or an elevator dredge operated off the back of a tractor (Figure 9.26). In Italy, clams are harvested mainly by 'rusca', which consists of an iron cage, an outboard engine propeller that produces a water flow directed onto the bottom, suspending sediments and fauna, and a net bag where the clams are collected (Pranovi *et al.* 2004). Once harvested clams are stored in boxes or bags and transported for mechanical grading. They are usually stored in water to purge grit and sand before processing and marketing. In some countries depuration may also be a requirement before sale. In France and the United Kingdom all cultured clams are sold fresh to local markets and restaurants. In Ireland there is little demand for clams on the home market and

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Figure 9.26 Harvesting clams (*R. philippinarum*) sown under nets.
Photo courtesy of Kevin O Kelly, Lissadell Shellfish Co. Ltd., Sligo, Ireland.

so almost all of the harvested clams are exported fresh to France and Spain. In Italy about 70% of production is sold fresh for the home market while the remainder is exported to European countries, primarily Spain.

There are a number of concerns facing producers. Undoubtedly, the major one is the potential impact of disease on production. For example, since 1993 the protistan parasite, *Perkinsus olseni*, has caused recurring mortalities in Korean clams beds, resulting in a decline in annual production from 55 000 t in 1992 to ~18 000 t in 2009. Brown ring disease (BRD) also causes mass mortalities in Manila clams in Korea, France, Spain, Italy, Ireland and the United Kingdom (see Chapter 11), and, recently, BRD, which so far was known to affect only *R. philippinarum*, was reported for the first time in 2005 in Arcachon Bay, France, the most important production area for the species. BMD causes progressive necrosis of the posterior adductor muscle, resulting in valve gaping, migration of clams to the sediment surface and death (Dang *et al.* 2009), but so far the causative agent has not been identified (Binias *et al.* 2014). It is crucial that individual countries enforce zoosanitary measures to limit disease expansion. Another concern is the potential risk of the introduced European green crab (*Carcinus maenas*) for commercial production of clams on the Pacific coast of North America (Grosholz *et al.* 2011). In France, low growth and poor juvenile recruitment has been reported in Arcachon Bay, a situation that makes the sustainability of the stock very precarious, especially with current levels of extensive fishing activity in that region (Dang *et al.* 2010). In Italy, the lagoons are heavily polluted with heavy metals, hydrocarbons and polycyclic hydrocarbons (PAHs) from industrial sources and pesticides, fungicides and nutrients from agricultural activities. Intense fishing pressure and frequent illegal harvesting inside the culture lagoons are additional problems.

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Bivalve culture and the environment

Bivalve production from aquaculture has increased from 8.5 to 12.5 million tonnes in the 10 years between 2001 and 2011 (Table 9.1). This increased production has been achieved through the expansion of areas of land and water under culture. Fortunately, bivalve culture does not impact on the environment in the same way that intensive fish culture operations do. Indeed, fish farms with their high inputs of wastewater, feeds, fertiliser and chemicals are now counted as potential polluters of the aquatic environment, and the cause of degradation of wetland areas (reviewed by Pearson & Black 2001; Pillay & Kutty 2005; Holmer 2010). As a result, more and more restrictions are being imposed on fish farming ventures. For example, in most countries environmental impact assessments (EIAs) are essential before permission is granted from designated authorities to start a fish farm. Currently, there is no legal requirement to undertake EIA for shellfish aquaculture, although, with increasing expansion of the industry, it is highly likely that this situation will change in the near future.

If anything the environment has a more adverse effect on bivalve culture than vice versa. For example, HABs occur when environmental conditions favour algal growth often due to an increase in levels of nutrients into coastal waters. Filter-feeding bivalves accumulate the algal toxins and if the bivalves are consumed can cause paralytic (PSP), amnesic (ASP), diarrhetic (DSP) and neurotoxic (NSP) poisoning in humans (see Chapter 12 for details). Monitoring programmes for biotoxins are well established in more than 50 countries, but to date there is little, if any, monitoring carried out in developing countries (Anderson *et al.* 2001; Anderson 2009). When bivalves are declared toxic harvesting is prohibited until such time as the bivalves are pronounced safe to eat. Long-term closures obviously have serious repercussions on the livelihood of the growers. For example, in New England, USA, a 3 month shellfish closure in 2005 resulted in an estimated loss of $15 million in Massachusetts alone (Anderson *et al.* 2005). Toxic blooms can have even more drastic, long-term effects. Repeated occurrences of DSP outbreaks forced Norway and Sweden to abandon mussel rearing as a commercial venture for a number of years (Stewart 1997). In recent years there is increasing evidence that exposure to toxic algae can depress bivalve physiological functions such as clearance rate and absorption efficiency, leading to reduced scope for growth (Li *et al.* 2002). In addition, consumption of toxic algae causes a depression of immune functions in bivalves, primarily through a decrease in phagocytosis, reactive oxygen species production and cell adhesion (Hégaret *et al.* 2011; Chapter 11). It is interesting that toxic species ingested by bivalves can be introduced into new environments through shellfish translocations (Hégaret *et al.* 2008).

Another adverse impact of the environment on aquaculture is when bivalves are grown in water that is contaminated with pollutants, for example, trace metals and pesticides, or with high bacterial or viral concentrations. In the case of bacterial and viral contamination bivalves must be purified before they are marketed, either by relaying them in clean water or by depuration (see Chapter 12 for details). Complete elimination of most bacterial types normally occurs within 48–72 h. In contrast, viruses are released much more slowly. For example, in the oyster, *Crassostrea gigas*, it takes 2–7 days for most viruses present to be removed, and some residual viral contamination may remain even when moderately contaminated bivalves are depurated (Lee *et al.* 2008). Relaying, but not depuration, is used for the elimination of pollutants (Chapter 12).

The negative effect of fish farming on the environment (see earlier) is often further compounded by the frequent use of chemicals, which in turn can have serious repercussions when bivalves are cultured in the vicinity of fish cages or pens. A case in point has been the use of tributyl tin (TBT), used as an anti-fouling paint on sea cages, boats and ships, and

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which is probably the most toxic substance ever introduced into the marine environment. At concentrations as low as 1 ppm TBT is lethal to the larvae of many commercially important bivalve species, and at lower concentrations causes shell malformation and reduced shell and tissue growth in larvae and juveniles (Widdows & Donkin 1992). In 2001 the International Maritime Organization adopted the International Convention on the Control of Harmful Anti-Fouling Systems on Ships (AFS Convention), calling for a global prohibition on the application of organotin compounds. The AFS Convention came into force in September 2008, banning globally both the application and presence on ships hulls of TBT-based compounds. Also, the European Union banned the application of TBT-based paints on EU-flagged vessels and as of 1 January 2008 made it an offence for any ship visiting an EU port to have TBT present on its hull. While there is evidence of a progressive decline in TBT concentrations in seawater and sediments, there are continued hot spots of TBT contamination in Europe, for example, large shipping ports, harbours and marinas. TBT has now been substituted with copper in anti-fouling paints, and to date there are no restrictions on the use of this substitute in most countries, with the exception of Sweden, Canada and the United States. Another chemical, dichlorvos, the active ingredient in the delousing agent Aquagard SLT®, kills bivalve larvae (and adult shrimp and crab) at concentrations that are several orders of magnitude lower than the dosing concentration used on salmon farms (Duggan 1990). The use of the chemical is controlled in EU countries and the United States, but is banned in Denmark, Sweden and Indonesia.

Another major concern for aquaculture is the introduction of alien (non-native) species. These may compete with native species for the same resources and may also carry pests, predators and diseases to which native species are more vulnerable (Kaiser 2001). Deliberate introductions, for example, *Crassostrea gigas* and *R. philippinarum* into Europe, and *Argopecten irradians* and *Mizuhopecten yessoensis* into China, have proved remarkably successful from the culture viewpoint. However, deliberate introductions or stock transfers within a region often bring with them exotic 'hitchhikers' (see McKindsey *et al.* 2007 for review). The introduction of *C. gigas* into Europe in the mid-1960s was accompanied by the inadvertent introduction of the seaweed *Sargassum muticum*, which has spread along the coasts of France and England forming dense obstructive beds. In a simulated transfer of *C. gigas* in the Thau Lagoon, France, a total of 57 macroalgal taxa, including 16 not indigenous to the Thau Lagoon, were recorded, demonstrating that oyster transfers are effective as vectors in macroalgal introductions (Mineur *et al.* 2007). In the nineteenth century two predatory gastropods, the oyster drill, *Urosalpinx cinerea*, and the slipper limpet, *Crepidula fornicata*, accompanied the introduction of the oyster, *Crassostrea virginica*, into Europe. Phytoplankton (toxic and otherwise) may be transferred on the bivalve shell, or as resting cysts in the digestive tract or faeces. To illustrate, in 1993 more than 60 species of phytoplankton were identified on oysters imported from France into Ireland (O'Mahony 1993). Disease organisms can also be introduced through transfers from one country or region to another. For example, *Bonamia*, which is responsible for the decline in flat oyster populations in Europe and elsewhere (see Chapter 11), is believed to have initially been spread by movements of oysters in the United States from California to Maine and Washington, and overseas to France and Spain in Europe. To avoid indiscriminate introductions strict criteria for selecting species is governed, for example, in EU countries by the Aquaculture Animals and Fish; Placing on the Market and Control of Certain Diseases Regulations (1996); in Canada by the National Code on Introductions and Transfers of Aquatic Organisms (2002) and the Federal Fish Health Protection Regulations (FHRP) under the Fisheries Act (1985); in Australia by the Livestock (Restrictions on Entry of Aquaculture Organisms) Notice (2005), under the Livestock Act (1997, as amended in 2000); and in China the Entry and Exit of Animal and Plant Quarantine Law (1991), Regulations for the Protection of Aquatic

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Wildlife (1993) and the Law on Animal Diseases (1997). The United States has state-specific laws governing aquatic species introductions (http://www.invasivespeciesinfo.gov/laws/ut.shtml).

There is an increasing awareness of the impact of bivalve culture itself on the environment. Most adverse effects are largely concerned with the on-growing phase of cultivation, although there are some minor negative effects associated with earlier phases of culture. In on-bottom clam or oyster culture, the addition of gravel or shell to intertidal plots, and the use of protective netting, causes localised changes in sediment composition and benthic community structure some of which are rapidly reversed as in the case of netting or mesh bags, but not with addition of gravel and shell material, which cause more persistent long-term effects (see Kaiser 2001 for details). Density can also have environmental impacts as shown by the results of a study (Washington State, USA) where native eelgrass (*Zostera marina*) density declined with oyster density, likely as a result of direct competition for space (Tallis *et al.* 2009). Some of the negative impacts of suspended culture, for example, raft culture of mussels in northwest Spain, have already been covered earlier. The main detrimental effect in the grow-out phase of culture is the damage done by dredging apparatus. Because clams are grown in direct contact with the substratum, mechanical harvesting is necessary, which inevitably leads to physical disturbance of the sediment habitat. The immediate effect of suction dredging is to reduce the number and abundance of non-target species by ~80%, but recovery occurs 12 months after harvesting (Spencer *et al.* 1998). In contrast, by comparing historical and contemporary data in the Venice Lagoon, Italy, Pranovi *et al.* (2006) showed that mechanical harvesting of *R. philippinarum* caused a significant decrease (~40%) in species diversity. The harvesting fleet of about 600 boats equipped with dredges operate a sort of 'open access regime' without any kind of management strategy, thus causing heavy stress on bottom communities and the whole lagoon (Pranovi *et al.* 2006).

Ecosystem approach to bivalve culture

The overexploitation and collapse of an increasing number of global catch fisheries has resulted in the decline of total annual capture amounts over the past few decades (Worm *et al.* 2006). Concomitant with this has been the increasing demand for seafood from an ever-increasing population, and aquaculture is perceived as having the greatest potential to fulfil this demand. Global production of fish (finfish, crustaceans and molluscs) grew from 49.9 in 2007 to 70.5 million tonnes in 2013, an increase of more than 40% (FAO 2014). But this rapid increase in global production has the potential to affect ecosystem functions and services, with adverse environmental, social and economic consequences. Also, the industry faces risks from other human activities such as contamination of coastal waters by agriculture and industrial activities (see earlier). Therefore, there is pressure on regulators from consumers, the public, NGOs, pressure groups and scientific expert groups to understand the consequences of aquaculture in order to enlighten and refine regulations and codes of practice (Black 2001). Over the past two decades there has indeed been significant progress in formulating legislation that, if applied, should facilitate sustainable development of the aquaculture sector.[1] But regulations on their own do not provide a comprehensive framework for ensuring sustainable use of aquatic environments. This will only happen when aquafarming is treated as an integral process within the ecosystem (FAO 2007).

In 1991 the Committee on Fisheries (COFI), a subsidiary body of FAO, recommended that new approaches to fisheries and aquaculture management, embracing conservation and

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environmental as well as social and economic considerations were urgently needed. Subsequently, the Code of Conduct for Responsible Fisheries (CCRF) was unanimously adopted in 1995 by over 170 member governments of the FAO Conference. The Code provides principles and standards applicable to the conservation, management and development of all fisheries. It also covers the capture, processing and trade of fish and fishery products, fishing operations, aquaculture, fisheries research and integration of fisheries into coastal area management (FAO 1995). The Code also deals with aquaculture more specifically through Article 9, which requires states to 'establish, maintain and develop an appropriate legal and administrative framework to facilitate the development of responsible aquaculture'. While the Code is voluntary certain parts of it are based on relevant rules of international law.

In 2006 the Aquaculture Management and Conservation Service (FIMA) of the FAO Fisheries and Aquaculture Department decided to look into the development and application of an ecosystem approach to aquaculture (EAA). The EAA was to build on the framework developed a few years earlier for the ecosystem approach to fisheries (EAF). As defined by FAO (2010):

An ecosystem approach to aquaculture (EAA) is a strategy for the integration of the activity within the wider ecosystem such that it promotes sustainable development, equity and resilience of interlinked social–ecological systems.

The EAA is based on the principles of sustained development, which encompass ecological, economic and social considerations; these are tightly linked, so that disturbance to one is likely to cause a disruption or change in the other (FAO 2010). To implement the EAA strategy relevant policy goals should be translated into operational objectives and actions, using the best available information and broad stakeholder participation (Figure 9.27). The following spatial scales have been proposed for EAA implementation: (i) at the farm level; (ii) at the water body and its watershed/aquaculture zone; and (iii) at the global, market trade scale. Also, productive (small to intensive, large-scale) and temporal scales should be considered (Soto *et al*. 2008). At the farm level numerous research and pilot-scale commercial initiatives have adopted an EAA, with many of these utilising an integrated multi-trophic aquaculture (IMTA) system. This is a practice in which by-products (wastes) from one species are recycled as inputs (fertilisers, food and energy) for another. An example could be a fish/shellfish/seaweed combination (Costa-Pierce 2008; reviewed in Barrington *et al.* 2009). All stages of an EAA are carried out in accordance with current international (binding and nonbinding) and national agreements, regulations, codes of practice and guidelines that pertain to aquaculture. In the EU, North America, Australia and other regions, legislation and guidelines for an EAA already exist; but many other regions, ironically those with the fastest growth in aquaculture, have the least number of regulatory mechanisms (details in Bermúdez 2008; Soto *et al.* 2008; FAO 2010).

A range of tools, which varies from simple to complex, supports the analysis and management of an EAA. To illustrate, the EU-funded project, ECASA (Ecosystem Approach to Sustainable Aquaculture), is evaluating the ability of quantitative ecosystem indicators to discriminate between aquaculture and other anthropogenic sources of perturbation in the marine environment (http://www.ecasa.org.uk/). To date, the project has evaluated 53 indicators of ecosystem change, and is conducting fieldwork to select the best environmental indicators in many ecological/ecosystem categories (Borja *et al.* 2009). ECASA is also developing a range of tools, particularly predictive models, that are being tested in conjunction with indicators, at a number of sites spread over a wide geographic range, each with different aquaculture impacts. The key deliverable of ECASA is a virtual toolbox, which contains 'tools' to aid owners and operators of fish and shellfish farms in selecting farm sites, and operating farms, so as to minimise environmental impact and ensure the

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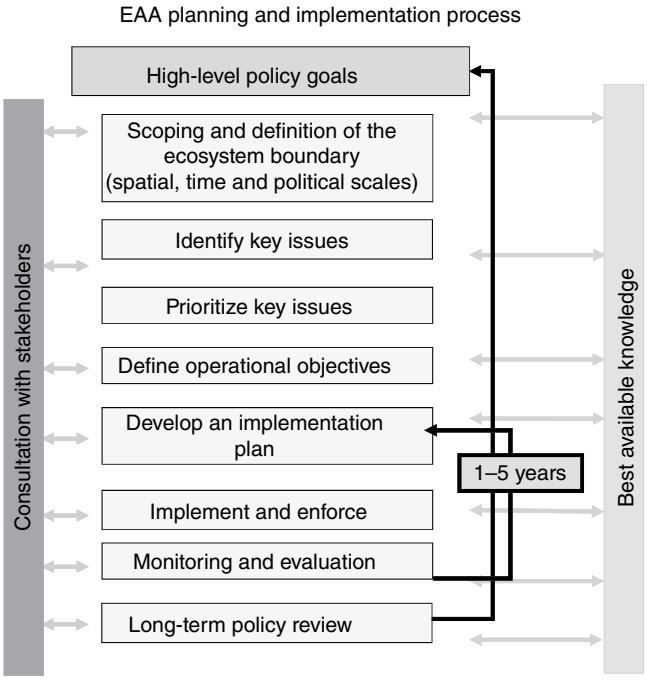


Figure 9.27 A schematic view of the EAA planning and implementation process. All stages of the process are covered in detail in FAO (2010). EAA can start at any stage of the process, for example, policy formulation, monitoring and evaluation, implementation.
Figure adapted from APFIC (2009) and FAO (2010). Reprinted with permission from the Food and Agriculture Organization of the United Nations (FAO).

sustainability of sites and water bodies for aquaculture (http://www.ecasatoolbox.org.uk/). Nunes *et al.* (2011) have recently shown how a range of complementary tools, with different levels of complexity and scope, can be combined for integrated assessment of aquaculture in a coastal system. The study was carried out in Killary Harbour, a fjord-like inlet on the west coast of Ireland, where longline mussel culture production is ~1600 t year^{-1}. The tools included a well-tested system-scale ecological model for coastal waters, EcoWin2000 (Ferreira 1995); a local-scale carrying capacity and environmental effects model, FARM (Ferreira *et al.* 2009); and a management-level eutrophication-screening model, ASSETS (Bricker *et al.* 2003), which is capable of qualifying system-scale trophic status. The EcoWin2000 and FARM models used ShellSIM to simulate individual shellfish growth. Required information for EcoWin2000 includes hydrodynamic and biogeochemical boundary data (land and/or ocean) and information on species and culture practices. FARM requires input data on farm layout and dimensions, species and their stocking densities, suspended food entering the farm and environmental parameters. Quantitative and qualitative data to evaluate trophic status is used by ASSETS to provide information on how aquaculture impacts eutrophication, both at the system and at the farm level. The tools were used to analyse the relationship between shellfish productivity and food sources, the impacts of changes to stocking densities of shellfish and an overall assessment of the ecological status of Killary Harbour. The results from the combined application of these tools showed that the eutrophication status of Killary Harbour is Moderate Low, with a future trend of No Change. There is a strong longitudinal and vertical circulation of phytoplankton, with imports into the harbour from the ocean, and exports of primary production from the harbour to the

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ocean. The maximum production of the system is 4200 t year^{-1}, which is about 2.6 times above the present yearly production value. Achieving this level would result in lower harvest weights and longer growth cycles. But the lower stocking densities (–47%) proposed for the system should lead to lower mussel production, compensated by potential benefits of higher mussel weight at harvest and/or shorter growth cycles.

While bivalve culture is potentially one of the most sustainable forms of aquaculture, requiring no artificial food input, the rapid growth of the sector has inevitably raised questions of carrying capacity and sustainability (Nunes *et al.* 2003). The concept of 'carrying capacity' is of fundamental importance within an EAA. It has been described as the relationship between the size of a population and change in the resources on which it depends, assuming that there is an optimal population size that can be supported by the resource (Inglis *et al.* 2000). Four functional categories have been defined for bivalve culture: physical carrying capacity, the total area of farms that can be accommodated in the available physical space without undue conflict with other users of the space; production carrying capacity, the stocking density of bivalves at which harvests are maximised without undue impact on the environment; ecological carrying capacity, the stocking or farm density above which there are unacceptable ecological impacts; and social carrying capacity, the level of farm density above which there are unacceptable impacts (Inglis *et al.* 2000; FAO 2010). The calculation of carrying capacity relies on models and tools such as GIS and RS. Estimation of physical carrying capacity uses the type of data stored in a GIS, for example, nutrient concentrations and bathymetry, while production carrying capacity uses a wide range of modelling approaches, focusing mainly on hydrodynamics, food availability and production, bivalve feeding and physiology, and the influence of husbandry practices on crop production, as well as the interactions among these factors (see McKindsey *et al.* 2006 for review on carrying capacity models). It is noteworthy that in a study on carrying capacity for bivalve culture in South Island, New Zealand, using a mass-balance approach and the ECOPATH model, Jiang and Gibbs (2005) found that while production carrying capacity of the area was 310 t km^{-2} year^{-1}, the ecological carrying capacity was about five times lower at 65 t km^{-2} year^{-1}, the level of culture above which there would be major changes in energy fluxes with the system's food web.

As mentioned earlier, global aquaculture production is set to increase substantially over the next few decades. By 2030 the earth's population is forecast to reach 8.3 billion, and assuming that capture fisheries production remains constant, aquaculture will have to increase annual production by 30 million tonnes to maintain the current annual per capita consumption of 17 kg (FAO 2011). Adopting a global ecosystem approach is potentially the most sustainable way to achieve this goal. Costa-Pierce (2010) predicts that:

> *the most sustainable growth trajectories for aquaculture are to change dramatically the prevailing aquaculture development model and move rapidly toward more sustainable, social–ecological approaches to development; to shift patterns of production and consumption patterns from global to bioregional food production and job creation; and to develop the indigenous human and institutional capacities that clearly demonstrate to society that aquaculture is culture.*

Note

1 Information pertaining to aquaculture legislation in individual countries can be found on the FAO website: http://www.fao.org/fishery/nalo/search/en

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10 Genetics in aquaculture

Introduction

For about 10 000 years humans have modified useful species over many generations and as a result, through a process of artificial selection, we now have thousands of varieties of crop plants and domesticated livestock. The early attempts at domestication were achieved without any real knowledge of genetic principles and concepts. Nevertheless, the procedures employed then still form the basis of genetic management programmes today: breeding from selected parents, culling inferior offspring, crossbreeding with other species and producing and crossing different strains within a species.

The application of these procedures to aquatic organisms is still in its infancy. Up to three to four decades ago, aquaculture management procedures were largely concerned with the physical constraints inherent in rearing organisms in restricted bodies of water. With husbandry problems largely sorted for a wide variety of fish, bivalve and crustacean species, attention is now increasingly focused on the application of genetic techniques, such as selective breeding, chromosome manipulation and, more recently, gene transfer, to the aquaculture industry. Several features of the life cycle make aquatic organisms more amenable than domesticated livestock to these procedures, for example, shorter generation time, higher fecundity, external fertilisation and larval development, and more plastic sex determination. In addition, the amount of variation for genetic manipulation is greater in fish and bivalves than in domesticated livestock, which are already considerably improved by a long history of artificial selection. It should be pointed out at this stage that because of their higher market value genetic advances in aquaculture are much further ahead for fish than for bivalve species.

The first part of this chapter deals with quantitative genetics and the various types of breeding schemes used in the selective breeding of bivalves. Protein and DNA molecular markers and their use in quantifying genetic variability will then be discussed, as will the technology of chromosome manipulation in the production of triploids, tetraploids and gynogens. More detailed information on methods and applications can

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be found in Neira and Diaz (2005), Vandeputte *et al*. (2007), Na-Nakorn *et al*. (2010) and Dunham and Hulata (2014).

Quantitative genetics

Populations exhibit an enormous amount of variation and much of this is inherited. In a selective breeding programme the success of selection depends upon the extent to which the variation for a particular trait in the population from which the parents were chosen is inherited. The traits of most interest, from a production viewpoint, are growth rate, survival, food conversion efficiency, meat yield and disease resistance. Quantitative traits, as they are called, exhibit continuous variation in populations and their variance usually reflects both genetic and environmental influences. Typically these traits are controlled by a large number of genes on different chromosomes, and collectively referred to as quantitative trait loci (QTLs).

Genetic analysis of quantitative traits involves working with means and variances, trying to partition the phenotypic variance of the trait (V_P) into the variance due to genes (V_G), to the environment (V_E) and to interaction between the two ($V_G \times V_E$):

$$V_P = V_G + V_E + V_{G+E}$$

The genetic component can itself be partitioned into additive and non-additive components:

$$V_G = V_A + V_D + V_I$$

Additive effects result from the cumulative contribution of alleles at all the loci governing a quantitative trait, and as such are important because they contribute to the breeding value of individuals and are passed on to progeny in a predictable manner. Non-additive genetic effects are due to dominance and epistasis. Dominance effects (V_D) result from interactions among alleles at the same gene locus, while epistatic effects (V_I) are due to interactions among loci. Neither of these is passed on to progeny, due to segregation of parental alleles at meiosis.

The ratio of the additive genetic variance to the total phenotypic variance for a trait is called the narrow-sense heritability,[1] denoted by h^2.

$$h^2 = \frac{V_A}{V_P}$$

One of the simplest methods for estimating the heritability of a trait is to compare the mean phenotypic value of full-sibs (individuals that share the same two parents) to the mean phenotypic value of their parents (mid-parent mean) in a regression analysis. In a randomly mating population the slope of the line obtained from a regression of full-sib means on mid-parent means is the heritability (Figure 10.1). Another method for estimating heritability is comparing full-sibs and half-sibs using analysis of variance (Falconer & Mackay 1996). Values of h^2 may theoretically range between zero, where V_P is entirely due to environmental effects, and one, where all the variance is due to additive genetic effects. The response to selection of a particular trait can be predicted from the heritability estimate and the phenotypic variance, and selection methods are chosen on the basis of these values. Table 10.1 presents h^2 estimates for some quantitative traits in bivalves.

One must be aware when estimating heritability that estimates are only valid for the population and specific environmental conditions in which measurements are made

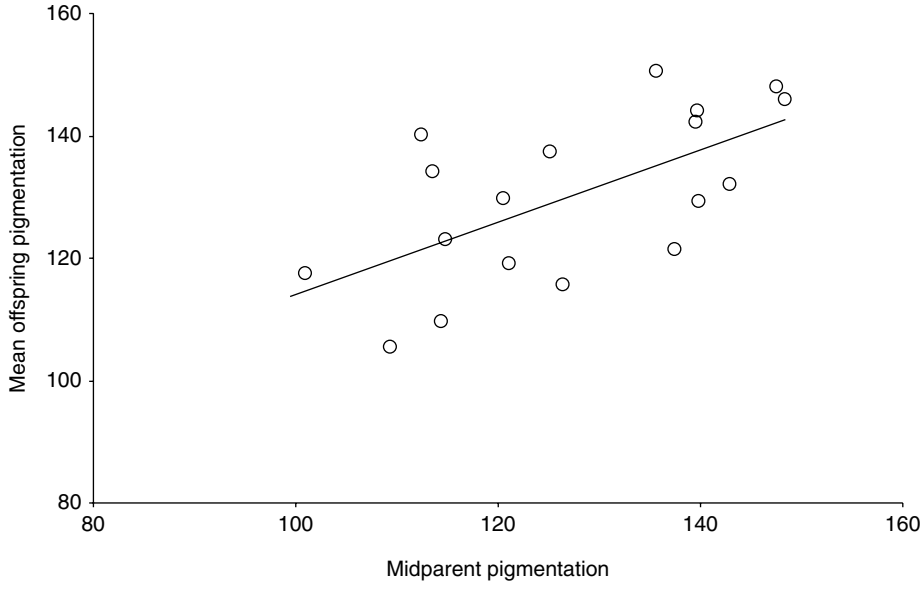


Figure 10.1 Mid-parent–offspring regression of total left-shell pigmentation in the Pacific oyster, *Crassostrea gigas*, based on 18 full-sib families. The narrow-sense heritability of total left-shell pigmentation is derived from the slope of the midparent–offspring regression ($b = h^2 = 0.59$, SE = 0.19, $P < 0.01$).
From Evans *et al.* (2009). Reproduced with permission of Elsevier.

Table 10.1 Narrow-sense heritability estimates with standard error in brackets for various quantitative traits in bivalves.

Species	**Trait**	h^2	**Reference**
Crassostrea gigas	Larval growth	0.24 (0.57)	Ernande *et al.* (2003)
	Larval survival	0.55 (0.40)	
	Juvenile resistance to summer mortality	0.83 (0.40)	Dégremont *et al.* (2007)
	Adult shell pigmentation	0.59 (0.19)	Evans *et al.* (2009)
Pinctada maxima	Adult wet weight	0.16 (0.004)–0.21 (0.02)	Kvingedal *et al.* (2010)
Mytilus chilensis	Larval valve length (25 days)	0.23 (0.30)	Alcapán *et al.* (2007)
	Larval valve length (40 days)	0.33 (0.003)	
	Larval valve length (300 days)	0.001 (0.076)	
	Larval valve weight (300 days)	0.030 (0.102)	
Macoma balthica	Juvenile shell shape	0.23 (0.08)	Luttikhuizen *et al.* (2003)
Argopecten purpuratus	Escape responses	0.36 (0.17)–0.57 (0.18)	Brokordt *et al.* (2012)

(Ernande *et al*. 2003); there are large standard errors associated with h^2 estimates (Table 10.1) unless large numbers of individuals are used; and, frequently, different traits are correlated so that an increase in value for one trait will be accompanied by a decrease in value for another (Beaumont 2000).

Selective breeding

The four objectives in a breeding plan are to ascertain the breeding objectives, identify the broodstock, choose a mating scheme for the reproduction of selected broodstock and identify how the selected stock can be expanded for production purposes.

Breeding objectives

Breeding objectives are generally set by the industry and the consumer, and should be precisely defined at the start of a breeding programme. Frequently, the objectives differ from one species to the next, from one country to another and even between different regions of a country. In a questionnaire on 22 prospective breeding goals in oysters Mahon (1983) found that all respondents gave a high rating for growth rate and survival from settlement to market size. Despite this consensus some countries did give a high rating to additional traits. For example, Irish and Spanish respondents working with the oyster *Ostrea edulis* gave a high score to proportion of larvae that settle, while those from northwest Europe who worked with the oyster *Crassostrea gigas* assigned a high score to resistance to low temperature. Not surprisingly, respondents from North America working with *Crassostrea virginica* gave particularly high scores to resistance to disease (see later). Respondents suggested additional prospective breeding goals, for example, fast larval growth, good food conversion efficiency, ability to use non-algal feeds, shell hardiness to withstand handling, good appearance and flavour of meat. Twenty years later the main breeding objectives had not changed, as is evidenced from a report from a workshop on genetic improvement in the Australian aquaculture industry (Lymbery 2000; Table 10.2).

Table 10.2 Biological traits included in the breeding objective for different aquaculture species groups (freshwater and marine crustaceans, finfish and edible molluscs (oysters *Crassostrea gigas, Saccostrea commercialis* and abalone *Haliotis* spp.).

Trait	Ranking
Size at harvest	4
Survival to harvest	4
Meat yield at market	3
Feed efficiency	3
Size uniformity	2
Disease resistance	2
Taste	2
Flesh colour	2
Reproductive output	1
Temperature tolerance	1
Survival to (live) market	1
Shell shape	1
Claw size	1
Peelability	1

From Lymbery (2000). Reproduced with permission of John Wiley & Sons. Participants ($N = 61$) in the survey were split into four groups depending on their major commercial or research interest. Ranking refers to the number of groups (out of four) that placed that trait in the breeding objective.

Broodstock establishment

It is inevitable that some of the natural variability of the species is lost when individuals are chosen as broodstock, since they represent but a small fraction of the population. In addition, over the following generations the offspring of these parents will be bred together, with possible inadvertent loss of variation through inbreeding (see Table 10.3). Bentsen and Olesen (2002) used a series of replicated stochastic simulations to determine the effect of the number of breeders selected (4–100 pairs), the number of progeny tested (5–150 progeny per pair), and the magnitude of the heritability (0.1–0.4) on the rate of inbreeding, and the response to selection through 15 generations of mass selection (see later). They found that to keep inbreeding rates low (about 1% per generation), a minimum of 50 pairs of breeders should be selected and the number of progeny tested should be restricted and standardised to not less than 30–50 progeny per pair; the gain by testing larger numbers was limited.

Even if 50 pairs of breeders are used there may be an unequal contribution between males and females. The effective population size in a spawning broodstock is estimated using the following equation:

$$N_e = \frac{4N_f N_m}{N_f + N_m}$$

where N_e the effective number of spawners and N_f and N_m are the number of females and males, respectively. If there is a difference between the numbers of spawning males and females then N_e will vary depending on the extent of this difference. Also, bivalves have a large variance in reproductive success, reducing effective population numbers to a small fraction of breeding numbers (Li & Hedgecock 1998; Lallias *et al*. 2010). Generally speaking, N_e in hatchery populations may be many orders of magnitude smaller than actual numbers of breeders (Launey *et al*. 2001 and references therein). In order to increase N_e Appleyard and Ward (2006) suggested that broodstock numbers should be increased and several minispawnings between equal numbers of males and females should be implemented.

Table 10.3 Effects of inbreeding on genotype frequencies at a single locus.

	Genotype frequency			Allele frequency	
Generation	**AA**	**Aa**	**aa**	**A**	**a**
P_1	0.25	0.5	0.25	0.5	0.5
F_1	0.375	0.25	0.375	0.5	0.5
F_2	0.4375	0.125	0.4375	0.5	0.5
F_3	0.46875	0.0625	0.46875	0.5	0.5
F_4	0.48437	0.03125	0.48437	0.5	0.5
F_5	0.49218	0.015625	0.49218	0.5	0.5
F_6	0.49609	0.007812	0.49609	0.5	0.5
F_7	0.49804	0.003906	0.49804	0.5	0.5
F_8	0.49902	0.001953	0.49902	0.5	0.5
F_9	0.49951	0.000976	0.49951	0.5	0.5
F_∞	0.5	0.0	0.5	0.5	0.5

From Tave (1986).

Note that inbreeding increases homozygosity, but does not change allele frequencies. The following matings occur each generation: AA × AA, Aa × Aa and aa × aa. Due to rounding error genotype frequencies do not always sum to 1.0 in a given generation.

Breeding schemes

Several different breeding techniques will be covered in this section. Some of these, for example, individual selection and family selection, make use of additive genetic variation for improvement of stocks, while others such as hybridisation make use of non-additive genetic variation. Techniques such as line crossing and outcrossing can be used to reduce inbreeding in broodstock.

Individual selection

Individual selection (mass selection) is the simplest, easiest and oldest form of selection and in many cases yields the most rapid response. The best individuals, based on their phenotypic performance, are selected from the population for breeding and the rest are discarded (Figure 10.2A). This can be repeated in each generation until the desired phenotypic change is achieved. The difference between the mean phenotypic value of the population and the mean phenotypic value of those individuals selected for breeding is called the selection differential (S) (Figure 10.3). The response to selection (R) is the difference between the mean of the progeny from the selected parents and the mean of the original population, and is given by:

$$R = h^2 S$$

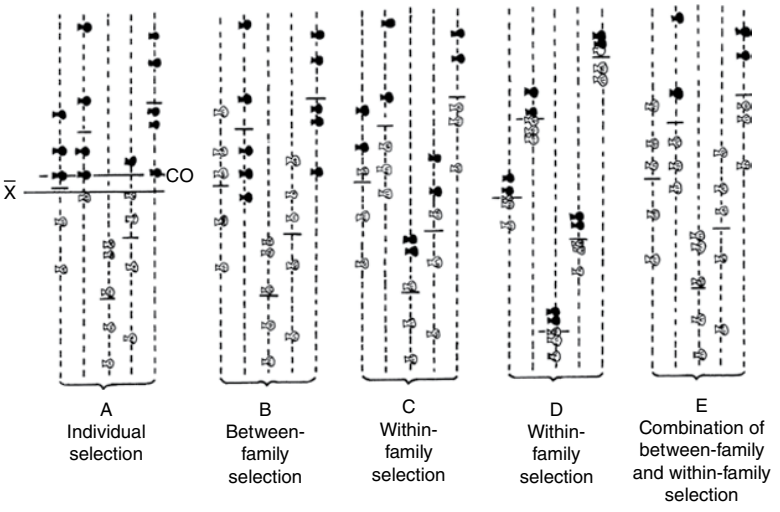


Figure 10.2 Schematic representation of different selection methods. There are five families (represented by vertical dashed lines) each with five individuals; A, B, C, D & E represent identical arrangements of the same 25 individuals. A small cross-bar shows the mean of each family. Individuals are plotted on a vertical scale of merit; the black individuals, those with the best phenotypic values, are selected, while the rest are culled. In individual selection (A) family means are ignored and individuals are compared to the population mean ($\bar{X}$). A cut-off (CO) value is arrived at based on the population mean and all individuals equal to, or larger, than the CO value, are selected, and the rest are culled. In between-family selection (B) the population mean is ignored and only families (two) with the highest mean phenotypic values are selected. The remaining families (three) are culled. In within-family selection (C and D) the population mean is ignored, and individuals within each family are compared only to their family mean. A proportion of the best individuals are selected (2/5 in C and D) and the rest are culled. This method of selection is most useful in situations where the variation between families is large compared to the variation within families, as in D. Between-family and within-family selection (E) may be combined; the best families are initially selected and then the best individuals within those families are selected.
From Tave (1993). Reproduced with permission of Springer Science + Business Media B.V.

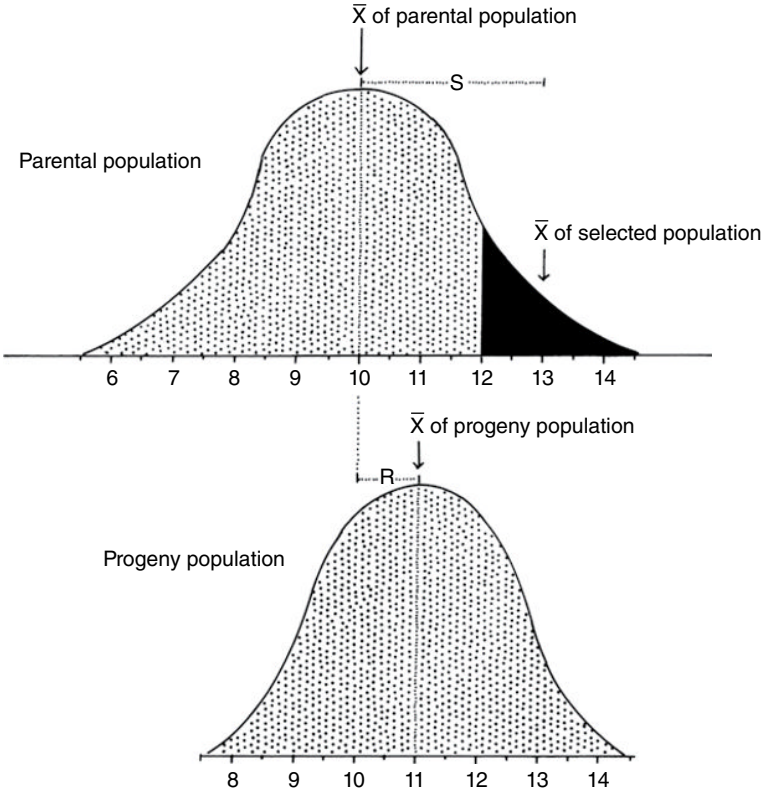


Figure 10.3 Response to individual (mass) selection. A population of fish have a mean weight of 10 g at 6 months of age. The adults selected for breeding (dark section of parental distribution) have a mean weight of 13 g at 6 months of age. The selection differential $S = 13 - 10 = 3$ g. The heritability for weight at 6 months of age is 0.33; therefore the predicted response to one generation of selection is $R = h^2S = 3 \times 0.33 = 1$ g.
From Kapuscinski and Jacobson (1987). Reproduced with permission of Maryland Sea Grant, Maryland, USA.

It is important that the heritability value for the trait under selection has been determined in the same environment and for the same population on which selection is being carried out. Otherwise, the predicted response to selection may be unreliable. Individual selection is particularly appropriate when single traits with high heritability (>0.30) are chosen, and where the population is large so that large selection differentials can be used. A selective breeding programme to improve growth in the bay scallop, *Argopecten irradians*, using individual selection is described in Zheng *et al*. (2004).

Family selection

When the heritability for a trait is low (<0.3) the phenotype of an individual is not an accurate estimate of the breeding value. Then it is more efficient in terms of gain per generation to base selection on family performance rather than on individual performance. Entire families, usually groups of full-sibs or half-sibs (individuals that share one parent), are selected for breeding, based on their mean phenotypic value (Figure 10.2B). Environmental variance among family means is kept low by raising families in the same environment, and by averaging over a large number of individuals in calculating the family mean. If a reasonable intensity

of selection is to be achieved, and at the same time inbreeding is to be avoided, then the number of families raised and measured has to be two to four times greater than the number of families selected for breeding (Falconer & Mackay 1996). Until recently, family selection was costly in terms of space, because each family had to be maintained separately. The use of highly variable microsatellite (MS) DNA markers allows the genetic tagging of individuals within a specific family, which means that many families may now be reared communally. See Dégremont *et al*. (2010a) for details on the use of full-sib family selection for resistance to summer mortality in Pacific oysters on the coast of France.

Within-family selection

This type of selection is useful when phenotypic differences among families are mainly due to environmental factors. The best individuals, those that exceed the mean phenotypic value of the family to which they belong, are selected for breeding (Figure 10.2C and D). If two members of every family are chosen to be parents of the next generation then the variance of family size is zero, and provided that the sexes are equal in numbers, $N_e = 2N$ (Falconer & Mackay 1996). This means that the population size N_e (the number of breeding individuals in the population) is twice the actual, and thus the rate of inbreeding is half that under individual selection.

It is possible to combine family selection with within-family selection by choosing the best individuals from the best families for breeding (Figure 10.2E). The advantage of combined selection is an increased response because the additive genetic variance among individuals, as well as within families, is exploited.

Inbreeding, crossbreeding and hybridisation

Inbreeding is defined as the mating of individuals who are more closely related to each other than individuals mating at random within a population. Pairs mating at random are more closely related to each other in a small population than in a large one. In fish and bivalve hatcheries the populations used are finite populations, and it is therefore not surprising that inbreeding can occur especially if too few parents are used as broodstock, or if the sex ratio departs from 1:1.

The extent of inbreeding in a population is measured by the coefficient of inbreeding, F, which is the probability that uniting gametes contain identical alleles derived from a common ancestor. The value of F increases, as alleles are lost from the population through inbreeding, or genetic drift (random changes in allele frequencies through natural sampling errors that occur in each generation). Values of F range from 0 to 1; an F value of 0 indicates no inbreeding and therefore maximum heterozygosity in the population, while a value of 1 indicates complete inbreeding and total homozygosity. In the hatchery when broodstock is first established it is, by convention, considered to have an inbreeding coefficient of zero. After that, the inbreeding coefficient should be calculated for subsequent generations to track inbreeding and drift. The rate of inbreeding (ΔF) depends on population size:

$$\Delta F = \frac{1}{(2N_e)}$$

N_e is the actual numbers of individuals contributing to the next generation. The calculated ΔF value for each generation is added to the inbreeding coefficient of the preceding generation to yield the new inbreeding coefficient.

The primary effect of inbreeding is an increase in the level of homozygosity in a population (Table 10.3). This is usually detrimental since many recessive deleterious alleles, which normally remain concealed by the presence of their dominant allele, are expressed. Inbreeding results in what is known as inbreeding depression, normally measured as the average performance difference between an inbred population and the base population for a particular trait(s). Inbreeding depression of performance characters such as yield, growth rate, survival have been documented mainly for oyster (Wada & Komaru 1994; Bierne *et al*. 1998; Naciri-Graven *et al*. 2000; Evans *et al*. 2004 and references therein) and scallop (Ibarra *et al*. 1995; Zheng *et al*. 2008; Liu *et al*. 2011) species. The universal practice of culling slow-growing larvae in the hatchery has been shown to reduce effective population size leading to inbreeding depression in the Pacific oyster, *C. gigas* (Taris *et al*. 2007). Self-fertilisation, an extreme form of inbreeding, is a common occurrence during spawning in the bay scallop, for which most instances of inbreeding depression have been reported. Over time, populations with a high level of inbreeding are expected to exhibit a low level of inbreeding depression because selfing should purge deleterious recessives (Zheng *et al*. 2008), resulting in the production of highly inbred lines. When two such lines, homozygous for different alleles at every gene locus, are crossed, the progeny are completely heterozygous at all loci, and are genetically identical. Such progeny should be superior to the parents and display what is known as 'hybrid vigour' or heterosis. Indeed, heterosis for growth, yield and survival has been demonstrated in experimental crosses between inbred lines of *C. gigas*, which would suggest a putative role for crossbreeding in commercial improvement (Hedgecock *et al*. 1995; Bayne *et al*. 1999; Hedgecock & Davis 2007). However, so far there has been little interest by the aquaculture industry in the production of inbred lines for crossbreeding.

An alternative technique for producing offspring with hybrid vigour is to cross individuals from strains, or closely related species or subspecies. This method has been extensively employed in fish aquaculture but has not, to date, been fully explored by bivalve breeders although results are encouraging from a few published studies. For example, broodstock from four geographical subpopulations (stocks) of sea scallops (*Placopecten magellanicus*) on the Atlantic coast of Canada were used to produce 4 parental and 12 crossbred cohorts (Wang & Côté 2012). While there was no significant difference in growth among stocks some outperformed others in terms of survival. Results from inter- and intrapopulation crossing showed that two 'hybrid' strains had superior growth and survival rates, in both hatchery and adult grow-out stages, over purebred and other hybrid groups, which could have immediate application in sea scallop culture. Similar results have been reported for other species, for example, the bay scallop, *A. irradians* (Zhang *et al*. 2007; Wang & Li 2010) and the pearl oyster, *Pinctada martensii* (Zhifeng *et al*. 2011). However, crosses between different populations of *Mercenaria mercenaria* do not produce superior offspring (Manzi *et al.* 1991; see also Camara *et al*. 2006), although, surprisingly, the hybrid from the cross between *M. mercenaria* and *M. campechiensis* was reported to show improved growth rates and tolerance to a wider range of environmental variables than either species (Menzel 1962, 1989). A later report, however, showed that hybrids had growth characteristics that were intermediate to the two parent species (Arnold *et al.* 1998). Allen *et al.* (1993) have shown that although hybrids of *C. virginica* and *C. gigas* can be readily produced they are inviable after 8–10 days. In contrast, when *C. gigas* and *C. rivularis* were crossed they did produce hybrid spat, although there was no evidence for hybrid superiority (Allen & Gaffney 1993). Similar results have been obtained in crosses between the oyster species *C. gigas* and *C. hongkongensis* (Zhang *et al*. 2012b), and *C. ariakensis* and *C. sikamea* (Xu *et al*. 2009). Overall, there is no clear indication that crosses between different bivalve species produce superior hybrids.

Bivalve breeding programmes

Bivalves are prime candidates for selective breeding programmes for a number of reasons: their high economic value in temperate regions of the world; increasing control over the complete life cycle of many species, particularly oysters; high levels of genetic variability as evidenced from genetic marker studies (see later sections); and high fecundity–of the order of 10^6 eggs per female per season. At present the bulk of the research is being conducted in universities and research institutes and it will probably take several decades more before commercial concerns take charge of their own research programmes. It is crucial, however, that personnel at these institutions should maintain constant two-way relations with private industry to ensure that the goals of any programme are pertinent to the producers.

There are several breeding programmes in the United States, almost all involving oysters (*Crassostrea* sp.). The single most extensive one is based in the Aquaculture Genetics and Breeding Technology Center (ABC), established at the Virginia Institute of Marine Science (http://www.vims.edu) in 1997. Initial efforts focused on the development of disease-tolerant strains of *C. virginica*, whose population in Chesapeake Bay had been devastated by the diseases MSX and Dermo (Chapter 11). The Center has produced, through individual (mass) selection, two strains or lines that show high resistance to Dermo disease and low to moderate resistance to MSX. Both strains, DBY and XB, which also exhibit fast growth, were derived from Louisiana oysters with innate Dermo resistance. These lines not only survive but also thrive in diseased waters under commercial culture conditions. Recently, a tetraploid disease-resistant line, produced through crossing DBY and XB, has been used to produce triploids (see later), which are also disease-resistant but because they are sterile have the added advantage of significantly increased growth. Currently, the focus of ABC's efforts is to provide genetically superior brood stock to industry, with the emphasis on improving other production traits such as growth, meat yield and time to market, in addition to further increasing disease resistance. See ABC (2009) for details. Two strains that are highly suitable for aquaculture production in northeastern United States are the NEH strain, which is strongly resistant to MSX and Dermo, and the FMF strain, which is resistant to juvenile oyster disease (JOD; Chapter 11) and also has superior growth. Rutgers University, New Jersey, produced the former strain, while the Frank M. Flowers Company, New York, produced the latter (Guo *et al*. 2008a). The University of Maine has developed the UMFS line, which is resistant to Roseovarius oyster disease (ROD; Chapter 11), and has a high growth performance under the colder, high salinity waters of northern New England (Rawson *et al*. 2010).

Another programme (http://hmsc.oregonstate.edu/projects/mbp/) is the US Department of Agriculture (USDA)-funded Molluscan Broodstock Programme (MBP), which focuses on genetic selection of Pacific oysters (*C. gigas*). The MBP is based at the Hatfield Marine Science Center, Oregon State University, and works in partnership with the oyster industry at sites in California, Oregon, Washington and Alaska. MBP's main focus is selecting for yield, and it has achieved an average increase in yield per generation of approximately 20% (whole live weight) over unselected oysters. The programme is also investigating breeding for oyster shell and mantle colour, as well as shell shape.

In 1990 the Department of Industry and Investment established a breeding programme for faster growth in the Sydney rock oyster, *Saccostrea glomerata* (= *commercialis*), at the Port Stephens Research Centre, New South Wales (NSW; http://www.dpi.nsw.gov.au/research/). The programme was expanded in 1997 to include selection for resistance to two major diseases, winter mortality and QX (Queensland unknown). As a result, oysters breeding lines were produced that are capable of reaching harvest size 11 months earlier, with others which as well as fast growing are resistant to QX or winter mortality.

In consultation with the Fisheries Research and Development Corporation (FRDC) and Industry and Investment NSW, the two industry associations (Oyster Farmers' Association of NSW and NSW Farmers' Association, Oyster Section) combined in 2004 to form the Select Oyster Company P/L to organise production and distribution of stock from the improved breeding lines and to ultimately take control of the management and future development of the current breeding lines (Dove & O'Connor 2009; Dove *et al*. 2013 and references therein).

Scientists from CSIRO (Commonwealth Scientific and Industrial Research Organization; www.csiro.au) and the Tasmanian Aquaculture and Fisheries Research Institute (TAFI) at the University of Tasmania began selectively breeding Pacific oysters for faster growth in 1997, with funding from the FRDC and the former Cooperative Research Centre for Aquaculture. The initial target for improvement was growth rate, but later shifted to optimising shell shape while maintaining growth rate gains (Swan *et al*. 2007). After four generations of mass and family selection the mean weight was approximately twice that of unselected controls, while all selected oysters showed a superior shape. The project had received considerable support from industry, and in 2003 the Tasmanian and South Australian oyster industries established a new company, Australian Seafood Industries, to ensure the continuation of the breeding programme and to facilitate technological transfer to industry. Currently, about 20% of industry production uses the selectively bred oyster lines. See details in Ward *et al*. (2005).

In New Zealand, selective breeding programmes are under way for the Greenshell™ mussel (*Perna canaliculus*) and the Pacific oyster (*C. gigas*) at the Cawthron Institute, Nelson, South Island (http://www.cawthron.org.nz). Since 2002 family-based selection in which tagged animals are grown on mixed-family droppers after separate-family rearing in the hatchery and nursery has been used to improve growth (shell length at harvest) and meat weight in *P. canaliculus*, and after two generations of selection both show strong responses to selection in excess of 20%. Other traits of interest include shell shape, gonad condition and food conversion efficiency, and the breeding programme has recently developed a bioeconomic model to prioritise target traits. The programme also adopted the BLUP (best linear unbiased prediction) animal model for genetic evaluation in order to utilise all available phenotypic information and predict multitrait breeding values adjusted for genetic correlations and systematic non-genetic effects. Currently, hatchery production of the mussel species is limited but this is expected to change as a result of a new public/private venture. Cawthron initiated its oyster-breeding programme in 1999 with the initial objectives of breeding high-surviving, fast-growing oysters using a combination of between- and within-family selection on families reared separately from fertilisation to harvest. After two generations of selection, the mean live weight showed an increase of 22% and survival was high (>80%). Since 2003 other selection criteria (e.g. good shell shape, high meat-to-shell ratios and meat coverage on the half-shell) have been incorporated into the breeding programme. In 2010, mass mortalities of both cultured and wild oyster populations caused by a new variant of the oyster herpes virus OsHV-1 made survival of oysters during viral outbreaks an additional breeding objective. Initial trials in 2012 found large differences in survival between families, and family ranking across sites was consistent. Because the top-surviving families were derived from single-parent crosses between wild parents, the current challenge for oyster breeding at Cawthron is to combine the OsHV-1 resilience of wild families with prior genetic gains for product quality traits. The programme is currently investigating a range of approaches, including hybridisation/backcrossing strategies between families selected for quality traits and wild-derived families with high survival, using mixed-family batches to minimise common environmental effects and impose high selection intensity on both survival and quality traits (see Sonesson *et al*.

2011). Information on breeding programmes was kindly provided by Mark Camera, Senior Scientist-Shellfish Genetics at the Cawthron Institute.

In Europe, where both natural and hatchery-propagated *C. gigas* spat are farmed there are no selective breeding programmes on the scale of those mentioned earlier. In France smaller projects, coordinated by IFREMER (l'Institut Francais de Recherché pour l'Exploitation de la Mer) are investigating resistance to stress and disease in the Pacific oyster, *C. gigas*. MOREST (MORtalité ESTivale), a national multidisciplinary project (http://www.ifremer.fr/lern/Pages/Programme/morest.htm) was initiated to investigate the causes of summer mortality in juvenile oysters and to reduce its impact on oyster production. Several lines resistant (high survival) and susceptible (low survival) to summer mortality have now been produced and tested in the field. Realised heritability estimates are high (0.55 ± 0.18 to 0.81 ± 0.13) so that selective breeding to improve survival in juveniles should lead to rapid gains (details in Dégremont *et al*. 2010a, b and c).

Protein and DNA markers

That large amounts of genetic variation are present in natural populations has been known since Darwin's time. But the ability to measure this variation only became possible in the 1950s with two developments: the elucidation of the structure of the DNA molecule, which ultimately clarified the direct relationship between genes and proteins; and the development of gel electrophoresis, an analytical method of protein separation, which permitted rapid and reliable identification of protein variations.

In gel electrophoresis of proteins a piece of tissue from an individual organism is ground up to disrupt cells, centrifuged to remove insoluble material and the resulting protein solution is inserted into the gel (usually starch or polyacrylamide) on a piece of filter paper. An electric current is applied for a fixed time and different proteins migrate at different rates, depending on their charge and configuration. The gel is sliced and each slice is treated with appropriate chemical solutions to visualise the position of protein or specific enzyme (allozyme) variants, which appear as discrete zones or bands on the gel. The usefulness of the method lies in the fact that the genotype of an individual with respect to the gene locus (loci) coding for a particular protein, can be inferred from the banding pattern on the gel: homozygotes are single-banded, while heterozygote are either double-, triple- or quintuple-banded phenotypes, depending on whether the enzyme is composed of one, two or four polypeptide chains, respectively. By counting all the different homozygotes and heterozygotes in a sample of individuals one can estimate the number of alternative genes (alleles) coding for a particular protein, and also the frequency of the different alleles. One can calculate the genetic variability, or heterozygosity, of a population by first obtaining the frequency of heterozygotes at each locus, and then averaging these frequencies over all loci to get a mean heterozygosity value.

The electrophoretic technique made it possible to compare allele frequencies and levels of heterozygosity within and between different populations of a species, between different species, and so on. The technique has a number of advantages: a large number of loci and individuals can be examined in a relatively short time, and at moderate expense; and environmental effects on banding patterns are usually unimportant. However, the fact that only the products of structural and not regulatory (non-protein coding) genes are detected means that the technique underestimates overall genomic variability. In addition, because protein expression is two steps away from coding DNA, a change in a DNA base will not necessarily result in an altered amino acid sequence of the protein (because of the degeneracy of the genetic code), or altered electrophoretic mobility. Also, fresh or freshly frozen tissue in

relatively large amounts is needed, which invariably means killing the specimens. Up to the end of the twentieth century allozymes were the marker of choice but due to the availability of a variety of more sophisticated DNA markers (see later) these have largely, but not entirely, been replaced.

DNA is found in two locations within animal cells: mitochondrial DNA (mtDNA), which is about 15–20 kilo bases in length, and nuclear DNA (nDNA), which makes up about 99.9% of all cellular DNA, most of which is non-coding–either single copy or repetitive sequences. There are several additional features which distinguish mtDNA from nDNA: the genome is haploid, that is, each mitochondrion generally contains only one type of mtDNA; the mode of inheritance is primarily through the maternal line and therefore all individuals with a particular mtDNA genotype probably belong to the same maternal clone (for exceptions see Chapter 5); unlike the nuclear genome there are no repetitive sequences; most mutations are selectively neutral and mtDNA evolves about 5–10 times faster than nDNA. Because of its higher mutation rate it is more likely to show differences between populations and species.

After extraction from fresh, frozen or alcohol-preserved tissue, both types of DNA can be cut at specific sites by special enzymes called restriction endonucleases. Cleavage results in a series of DNA fragments called 'restriction fragments' whose number and size are quantified by gel electrophoresis. DNA fragments can be detected by direct staining, usually with ethidium bromide, radio end-labelling or Southern blotting. Polymorphisms in the nucleotide sequence at a restriction enzyme recognition site result in varied numbers of DNA fragments, while polymorphisms in the length of DNA fragments result in an altered electrophoretic mobility of a DNA fragment through a gel matrix (Figure 10.4). These are called

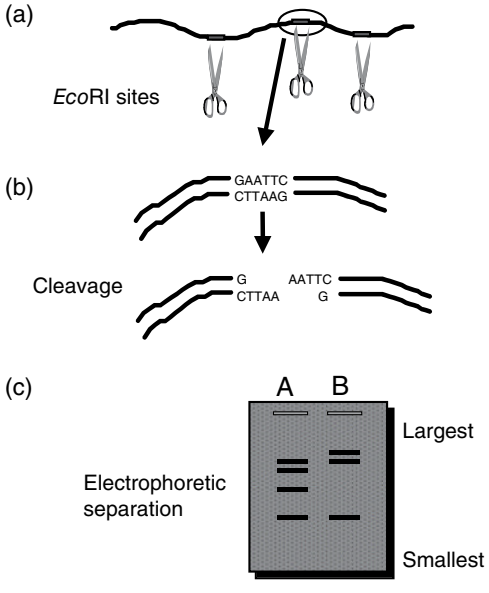


Figure 10.4 Restriction enzyme digestion and separation of resulting DNA fragments by electrophoresis. There are three restriction sites in the DNA molecule, each marked with open rectangles in (a). The restriction enzyme *Eco*RI cuts the DNA molecule at every position where the sequence GAATTC occurs (b), thus producing four DNA fragments. These are separated on an agarose gel [lane A in (c)], with the smallest fragments migrating fastest. The loss of a restriction site, circled in (a), means that the two middle fragments in A profile will not be cleaved and will be seen as the single larger more slowly migrating fragment in the B profile.
From Park and Moran (1995). Reproduced with permission of Springer Science + Business Media B.V.

restriction fragment length polymorphisms or RFLPs. In practice, when individuals within or between populations are being compared a number of different restriction enzymes are used to increase the probability of detecting RFLPs. Digestion of nDNA with most enzymes results in too many fragments, but the technique works well on the smaller mtDNA genome, which can be extracted from an individual in high copy number without cloning[2] or polymerase chain reaction (PCR).[3] The most important advantage of RFLP analysis is that it provides additional genetic markers for population characterisation (see later).

Most nDNA does not code for any gene product, and even within the DNA sequences that are recognised as genes there are non-coding regions, called introns. One class of non-coding DNA consists of a variable number of tandem repeat (VNTR) short nucleotide sequences. The repeat unit can be anywhere from one to several hundred nucleotides long; repeat units from ten to a hundred nucleotides long are referred to as minisatellites, while shorter repeat units of one to four nucleotides long are called microsatellites (MS). Each VNTR locus (tandem repeat array) is flanked by a unique sequence and if these sequences are known primers can be synthesised that are complementary to them. Using the primers the tandem array of the locus can be amplified by PCR. The high rate of mutation in these loci, detected as length variants on gels (Figure 10.5), leads to extensive allelic variation and makes them particularly useful as genetic markers for numerous applications in aquaculture and fisheries (Dunham 2011). For example, they are very useful in detecting variability in situations where conventional markers such as allozymes or mtDNA markers have revealed low interpopulation or species variability. MS markers have also been used to identify individuals and family groups so that they can be reared communally (Wang *et al*. 2010c), or to quantify reproductive contributions of individual males and females in laboratory crosses (Boudry *et al.* 2002).

Other approaches to detect nDNA polymorphisms include DNA fingerprinting where many loci are visualised simultaneously; random amplified polymorphic DNA (RAPD), where DNA polymorphisms are detected by randomly amplifying multiple regions of the genome using single arbitrary primers; amplified fragment length polymorphisms (AFLPs),

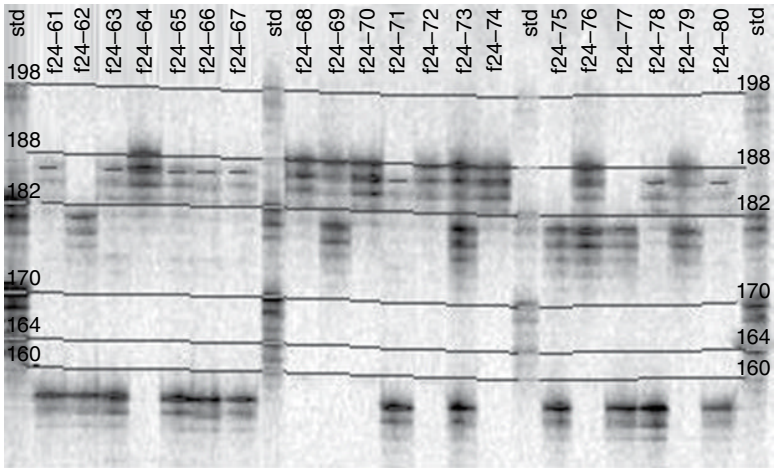


Figure 10.5 Genetic variability at the *Oedu*U2 microsatellite locus in 20 individuals (f24–61 to f24–80) of the European flat oyster, *Ostrea edulis*. Individuals were genotyped with reference to a size standard (std) of pooled PCR products from three individuals of known genotype, chosen to cover the allele size range for this locus. There are four genotypes for these oysters: 156/180 (*N* = 3), 186/188 (*N* = 5), 156/186 (*N* = 8) and 180/188 (*N* = 3); f24–73 has three bands, probably due to two individuals in the same tube.
Photograph by Delphine Lallias, Bangor University, UK. Reproduced with permission.

based on selective PCR amplification of restriction fragments from DNA digestion; and nucleic acid sequencing of a target DNA sequence, for example, a gene, or sequencing of an RNA transcript of a gene. Single-nuclear polymorphisms (SNPs) are the newest addition to the molecular toolbox and are the most common form of genetic variation. They represent DNA sequence variation occurring when a single nucleotide A, T, C or G in the genome, through mutation, differs between individuals. For example, two sequenced DNA fragments from different individuals, ATGGCAT and ATGGTAT, contain a difference in a single nucleotide. In this case we say there are two alleles, C and T. Almost all common SNPs have only two alleles. SNP genotyping through DNA sequencing is rapidly becoming a powerful tool that can be applied to a wide range of population studies, from individual identification to population structure and taxonomy. However, because they are considerably less variable than MS markers, most SNP studies have focused on gene mapping, QTL analysis and functional genomics (see Liu & Cordes 2004; Lowe *et al*. 2004; ICES 2008; Beaumont *et al*. 2010; Chauhan & Rajiv 2010 for details). Expressed sequence tags (ESTs) also play an important role in gene mapping. Complementary DNA (cDNA) libraries representing expressed genes are used to generate ESTs. An individual EST is a small piece (200–500 bp in length) of a cDNA sequence that is generated by sequencing either one or both ends of the expressed gene. ESTs can be used as 'tags' to fish an unknown gene out of a portion of chromosomal DNA by matching base pairs. ESTs are submitted to a general database, for example, GenBank (http://www.ncbi.nih.gov./Genbank/), or one specific to a species, for example, http://www.ifremer.fr/GigasBase/, for the oyster, *C. gigas*. Such collections can form the basis for subsequent microarray design, SNP and MS detection, and placement of novel markers on genetic maps (see later). Table 10.4 provides an evaluation of the various genetic markers with regard to their practical applications in fisheries and aquaculture.

Those wishing to explore how population genetic principles are applied to molecular marker data should consult textbooks Gillespie (2004), Hedrick (2009) and Hamilton (2009).

Genetic variation and population structure

Several evolutionary forces affect the amount of genetic variation, and hence the degree of differentiation, between populations. Factors such as geographic distance and physical barriers lead to reduced contact between populations. Subsequently, limited gene flow in combination with random genetic drift and adaptation act to increase genetic differentiation between the fragmented populations. Molecular markers provide a direct assessment of the pattern and distribution of genetic variation between populations, species and higher order taxonomic groups. Also, increased computational power and mathematical models have enhanced the scope of conclusions that can be drawn from genotype data, for example, information on effective population size, genetic bottlenecks, assignment of migrants (Chauhan & Rajiv 2010).

Intraspecific genetic variation

Most studies using genetic markers are concerned with estimating genetic variation and population subdivision within a species, and bivalves are no exception. Addison *et al*. (2008) tested for genetic differentiation among six populations of California sea mussels (*Mytilus californianus*) sampled across 4000 km of its geographical range by comparing patterns of variation at four independent types of genetic markers: allozymes, single-copy nuclear DNA markers and DNA sequences from the male and female mitochondrial genomes. They found no significant differentiation between localities, and concluded that genetic homogeneity was due to a combination of extensive gene flow, through a long planktonic larval phase lasting up to 45 days, and the lack of exposure, at the post-settlement stage, to strong

Table 10.4 Evaluation of molecular markers with regard to their practical applications in fisheries and aquaculture (Okumuş & Çiftci 2003 and references therein; Morin *et al.* 2004).

			Major applications				
Marker	**Tissue requirements**	**Sacrifice of specimens**	**Population structure**	**Stock/strain identification**	**Individual identification**	**Parentage and pedigree analysis**	**Gene mapping**
Allozymes	Stringent	Often	Moderate	Moderate	Low	Low	Low
mtDNA RFLPs	Moderate	Often	Moderate/high	Moderate/high	Inappropriate	Inappropriate	Low
mtDNA sequence	Relaxed	No	Moderate/high	Moderate/high	Cumbersome	Low/moderate	Low
RAPDs/AFLPs	Relaxed	No	Low	Low	Moderate	Moderate	Moderate-RAPD High-AFLP
Microsatellites (VNTRs)	Relaxed	No	High	High	High	High	High
Expressed sequence tags (ESTs)	Relaxed	No	Low	Low	Low	Low	High
Single-nucleotide polymorphisms (SNPs)*	Relaxed	No	High	Moderate	Low	Low	High

*'Low' and 'Moderate' evaluations may change when larger numbers of polymorphic SNPs are used. Morin *et al.* (2004) suggest that at least two to six times more SNPs may be necessary to achieve the same resolution as achieved by microsatellites.

selective gradients across its range. A similar lack of differentiation, using RAPDs, has been reported for the mussel, *M. chilensis*, at seven of eight locations sampled along 1900 km of the Chilean coast (Toro *et al*. 2004). Genetic homogeneity was ascribed to extensive gene flow due a long larval dispersal phase enhanced by oceanographic features such as the north-flowing West Wind Drift (WWD), and several coastal currents along this coast. The significant differentiation observed between the most southerly sample and all the rest was probably due to the southerly flowing Cape Horn current, a branch of the WWD, which precludes larval transport towards the northern populations. Significant genetic homogeneity, using MS markers, has also been reported for natural populations of the Pacific oyster (*C. gigas*) throughout its European range (Meistertzheim *et al*. 2013). To some extent this is due to a long dispersal larval phase, but repeated transfers from same seed stocks across Europe are probably a major factor, thus highlighting the significance of aquaculture practices on the genetics of this species. In contrast, Xiao *et al*. (2010) reported significant genetic heterogeneity among 10 wild populations of *C. ariakensis* sampled from its native distribution range (the coasts of southern Japan, South Korea and northern and southern China) using MS markers. The genetic differentiation among populations followed an isolation-by-distance (IBD) pattern, with no significant differentiation among populations within small geographic scales (<200 km), but strong structure among populations hundreds to thousands of kilometres apart. Similar IBD patterns have been reported for other marine bivalves (references in Xiao *et al*. 2010). In another study, also using MS markers, genetic variation was analysed in six populations of the Zhikong scallop, *Chlamys farreri*, from different marine gyres in northern China (Zhan *et al*. 2009a). Two populations sampled from the same gyre had no detectable genetic differentiation. However, in contrast to the results of these three studies, the remaining four populations collected from different marine gyres or separated by strong marine currents showed significant genetic differentiation. It is likely that such currents are the factor most likely responsible for the fine-scale genetic structure observed in this species. In the eastern oyster, *C. virginica*, mtDNA haplotypes[4] form two major assemblages, Atlantic (Gulf of St. Lawrence to Cape Canaveral) and Gulf Coast (Hare & Avise 1996). A similar break has been reported from allozyme analyses (Cunningham & Collins 1994), and in other marine species. The post-Miocene ecological history of the region suggests that reduced precipitation levels in an enlarged Floridian peninsula may have created discontinuities in suitable estuarine habitats for oysters during glacial periods and that today such population separations are maintained by the combined influence of ecological gradients and oceanic currents on larval dispersal (Reeb & Avise 1990). The degree of divergence between Atlantic and Gulf oysters using nuclear markers was less well known. Hence the study by Hoover and Gaffney (2005) examined patterns of variation in four nuclear loci, using restriction fragment analysis of amplified DNA, in samples collected from 16 locations spanning the geographic range of *C. virginica*. While they observed marked differentiation between Atlantic and Gulf samples, the pattern was less clear-cut than for mtDNA. Smaller differences were also observed between North Atlantic and South Atlantic populations, and in a subsequent study there was also evidence for population subdivision in Gulf Coast oysters (Varney *et al*. 2009).

Genetic markers are also used to discriminate between wild and hatchery strains or subpopulations of a species. Yu and Li (2007) used MS markers to characterise five hatchery and two wild populations of *C. gigas* from China and Japan, respectively. Pairwise F_{ST} (a measure of population differentiation) values and heterogeneity tests of allele frequencies showed significant differentiation between all populations. Assignment tests correctly assigned high percentages (97–100%) of individuals to their original populations. The differentiation between hatchery and wild populations was likely a result of geographical separation of the ancestral strains, and the results of different founder populations and

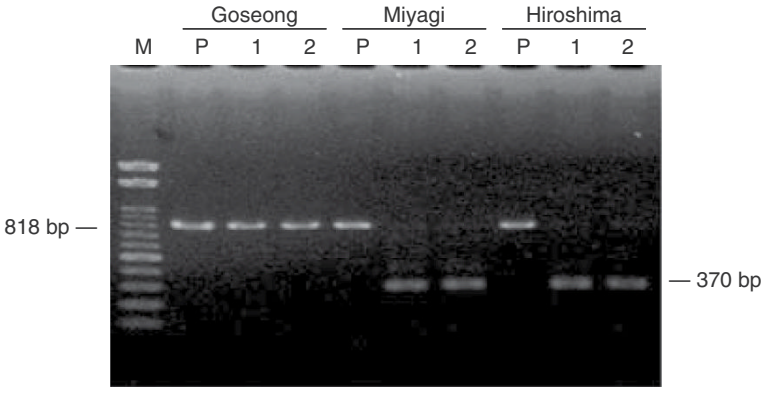


Figure 10.6 PCR-RFLP analysis of Pacific oyster, *Crassostrea gigas*, mtDNA non-coding region (NCR) from Miyagi, Hiroshima and Goseong populations with AluI restriction enzyme digestion. M, P and numbers indicate 100 bp plus ladders, PCR product without digestion and individual specimens, respectively.
From Okimoto *et al.* (2008). Reproduced with permission from Springer Business and Media.

different selection procedures in the hatchery populations. The significant differentiation between the two wild populations was likely due to the large geographic distance (~900 km) between the two samples. Similar studies have been carried out on different cultured strains of *C. gigas* (Okimoto *et al*. 2008; Figure 10.6), *C. ariakensis* (Zhang *et al*. 2005) and *O. edulis* (Vercaemer *et al*. 2006). It is feasible that unknown oyster samples could be assigned with confidence to their geographic origin using MS markers, a useful forensic tool in the case of an illegal transfer from a diseased area (Vercaemer *et al*. 2006).

Species identification

The taxonomic status of several oyster species has, until relatively recently, remained unresolved. The problem is most pronounced in China, where more than 20 species of oysters are naturally distributed along its coasts. A number of these, mostly *Crassostrea* species, for example, *C gigas*, *C. sikamea*, *C. ariakensis* and *C. hongkongensis*, represent significant aquaculture industries (see Chapter 9). But one of these, *C. ariakensis*, could be sympatric through all or part of its range with as many as 10 different congeners including *C. belcheri*, *C. iredalei*, *C. gryphoides*, *C. madrasensis*, *C. nippona*, *C. angulata*, *C. gigas*, *C. plicatula*, *C. sikamea* and *C. hongkongensis*, making species identifications difficult because of a lack of distinguishing morphological characters among many of the species. It is important that reliable methods for distinguishing these species are developed, particularly in view of the planned introduction of *C. ariakensis* into Chesapeake Bay, US. In an effort to develop an effective marker for oyster identification Wang and Guo (2008b) studied length variation in internal transcribed spacers (ITS1 and ITS2) between major ribosomal RNA genes in 12 common oyster species. Among species the length of ITS1 and ITS2 was polymorphic and provided a unique identification of eight species or species pairs: *C. ariakensis*, *C. hongkongensis*, *C. sikamea*, *Ostrea conchaphila*, *C. virginica*/*C. rhizophorae*, *C. gigas*/*C. angulata*, *Saccostrea echinata*/*S. glomerata* and *O. angasi*/*O. edulis* (Figure 10.7). At the same time Cordes *et al*. (2008) developed an identification key for nine *Crassostrea* species (*C. virginica*, *C. belcheri*, *C. iredalei*, *C. madrasensis*, *C. gryphoides*, *C. gigas*, *C. sikamea*, *C. ariakensis and C. hongkongensis)* based on RFLP analyses of nuclear (ITS1) and mitochondrial (cytochrome oxidase) DNA markers. Seven of the nine species were

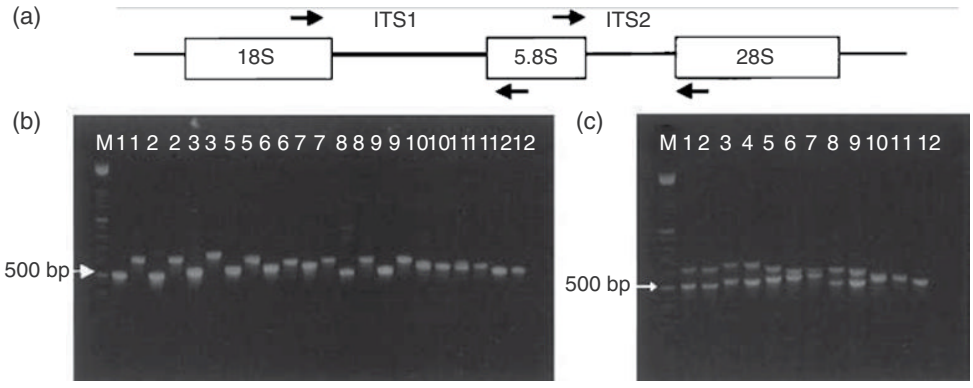


Figure 10.7 Amplification of ITS (internal transcribed spacer)1 and ITS2 in 12 species of oysters. (a) A schematic presentation of primer positions for ITS1 and ITS2; (b) separate amplification of ITS1 (first) and ITS2 (second) in 12 species; (c) simultaneous amplification of ITS1 and ITS2 in one PCR. Species codes are as given: M, 100 bp marker; 1, *Crassostrea virginica*; 2, *C. rhizophorae*; 3, *C. gigas*; 4, *C. angulata*, 5, *C. sikamea*; 6, *C. ariakensis*; 7, *C. hongkongensis*; 8, *Saccostrea echinata*; 9, *S. glomerata*; 10, *Ostrea angasi*; 11, *O. edulis*; and 12, *O. conchaphila*. Note: *C. angulata* is absent in (b).
From Wang and Guo (2008a). Reproduced with permission of the Journal of Shellfish Research.

unambiguously differentiated using the ITS1 marker and all nine species using the cytochrome oxidase1 (CO1) marker (see also Wang & Guo 2008a). The objective is to establish a database that will contain protocols, additional gel images and updates as new species, intraspecific variants and hybrids are added to the key (http://www.vims.edu/env/research/dna/).

Molecular markers have also been used to verify/refute the systematic status of individual *Crassostrea* species. For example, based on information from nuclear and mtDNA sequences Wang *et al*. (2008) found that the small oysters on intertidal rocks in northern China are *C. gigas* and not the zhe oyster, *C. plicatula*, as widely assumed. They also confirmed that the large subtidal oysters are *C. ariakensis* and not *C. rivularis* (see also Wang *et al*. 2004). Sequence analysis of mitochondrial genes has also shown that the small, cupped oyster in southern China is the same species as *C. angulata* in Portugal (Wang *et al*. 2010a). Its wide distribution and high genetic diversity in China suggests a historical transfer of *C. angulata* from China to Europe.

Comparison of hatchery and wild stocks

The ability of a population to respond to selection depends on there being sufficient genetic variation present in the population for the traits being selected. Artificial production of bivalves in hatcheries can lead to a reduction in genetic variability through small effective population size (see earlier). By comparing the number and distribution of alleles and level of heterozygosity of derived and source populations one can get a measure of the loss, or otherwise, of genetic diversity as well as effective population size (N_e) of the hatchery stock. Results from allozyme studies have shown a significant loss of genetic diversity following hatchery culture in *C. gigas* (Gosling 1982; Hedgecock & Sly 1990; but see English *et al.* 2000), *C. virginica* (Vrijenhoek *et al.* 1990) and *M. mercenaria* (Dillon & Manzi 1987). Loss of diversity has generally been manifested by loss of rare alleles rather than by any overall reduction in heterozygosity. However, continuation of

poor hatchery practice over many generations will eventually lead to substantially reduced heterozygosity. In recent years MS markers have replaced allozyme markers in tracking the genetic consequences of current hatchery practices. Yu and Guo (2005) have used MS markers and AFLPs to examine genetic variability in four selected strains and one wild population of *C. virginica*. There was no significant reduction in heterozygosity in the four strains although the number of alleles per locus for both marker types was considerably lower in the strains than in the wild population. Similar results were reported for the same species (Carlsson *et al*. 2006) and for *C. gigas* (Miller *et al*. 2012), using MS markers. However when hatchery populations of *C. gigas* from China were compared with wild populations from Japan there was no significant difference in allelic richness or heterozygosity between them (Yu & Li 2007). This may be because in China, hatcheries typically use hundreds of males and females from different oyster farms in a spawning (Yu & Li 2007). A significant reduction in allelic diversity and heterozygosity has also been observed between hatchery and wild populations of *C. ariakensis* (Zhang *et al*. 2005; Xiao *et al*. 2011), *Ostrea edulis* (Vercaemer *et al*. 2006) and the scallop, *A. irradians* (Wang *et al*. 2007; Qin *et al*. 2007a).

Genetic tagging and other applications

Another way in which molecular markers have proved useful are as tags. For example, large holding facilities are required for the separate rearing of individual families in selective breeding programmes. The use of genetic markers to tag the parents means that identity of offspring can be unambiguously assigned. This has the advantage that many families can be reared *en masse* under identical environmental conditions, thus reducing undesirable environmental variance. Parental assignment in the progeny is mostly dependent on polymorphism and the number of loci scored. At a particular locus, if an allele is carried by a single potential parent, then it can be used directly to assign any offspring carrying that allele to that parent, and the rarer an allele is, the more informative it is. MS markers provide the best result for parental assignment since they are highly polymorphic, making it feasible to obtain unique genotypes for every individual in a study. Using a multiplexed system of three highly polymorphic (~40 alleles/locus) MS markers, the parentage of 93% (1224 out of 1318) of *C. gigas* progeny was successfully determined in a factorial cross between 3 females and 10 males. The inability to genotype the remaining 7% was due to larval DNA degradation rather than assignment uncertainty (Taris *et al*. 2005). From a suite of 49 MS loci isolated from the mussel, *P. canaliculus* four to five loci were sufficient to confidently assign greater than 95% of offspring to the correct parent pair (MacAvoy *et al*. 2008).

As already mentioned, MS markers can also be used to quantify reproductive contributions of individual males and females in laboratory crosses (Boudry *et al.* 2002). In addition, by comparing genotypic ratios of marker loci with Mendelian expectations, estimates of selective mortality, within and among families, may be made (Gaffney 1990).

Identification of seafood products when features such as skin, scales, shell have been removed makes them impossible to identify with traditional taxonomic approaches. Therefore, characterisation of DNA that remains in the product after the early stages of processing is widely used in forensic species identification. Identification for forensic purposes is well advanced for fish species, but to date there are only a few publications on bivalves, and these all deal with scallop species (see Kenchington *et al*. 1993; López-Piñón *et al*. 2002; Marshall *et al*. 2007). The most recent one focuses on *P. magellanicus* and *Chlamys islandica*, which for many years have been fished commercially on the Atlantic coasts of Canada (Chapter 8). Processing is carried out at sea, with only the adductor muscles landed to conserve weight and volume. There is a size difference between the adductor

muscles of the species that is evident in the aggregate, but not reliable individually. Currently, the *P. magellanicus* fishery is open periodically but closed during the breeding season like other shellfish fisheries. The unscrupulous tactic is to obtain a license for an alternative scallop species whose season is open (e.g. *C. islandica*), conduct a directed operation for the closed species and hope you do not get caught Therefore, fishery authorities are concerned with possession and retention of *P. magellanicus* as a bycatch in the directed *C. islandica* fishery. In their study species identification was based on simultaneous *in vitro* amplification of a portion of the mitochondrial Cytochrome Oxidase I locus with a PCR anchor primer for a sequence identical in both species, and two alternative species-specific primers that selectively amplify either a 619-bp in *P. magellanicus* or a 459-bp DNA fragment in *C. islandica*. Analysis of more than 900 scallops from samples seized from two fishing vessels (Marshall *et al*. 2007) showed that the samples contained 65 and 99%, respectively, of the species from the closed fishery. The method is rapid and cost-effective and can easily be adapted to other loci and species. Poaching of *P. magellanicus* has now largely stopped with prosecutions based on their evidence (Steven M. Carr, personal communication, May 2013).

Genetic markers also play an important role in the construction of high-resolution genetic linkage maps for aquaculture species; in identifying genes involved in QTL for marker-assisted selection (MAS) and in the assessment of successful implementation of genetic manipulations such as polyploidy and gynogenesis (see sections later).

Bivalve genomics

The genome comprises the total DNA complement of genetic material in the cell nucleus (nDNA) and mitochondria (mtDNA), and genomics is the discipline that deals with genome structure, function and evolution. Genome size in bivalves is between 700 and 2000 million base pairs, which is comparable to values reported for other cultured marine species (Saavedra & Bachère 2006).

Genome mapping

Genome mapping is the creation of a genetic map whereby DNA fragments are assigned to specific chromosomes. This can be achieved in two different ways, through genetic (linkage) mapping or physical mapping. The main difference between these two approaches is that the former provides the position of known genes or genetic markers relative to each other in terms of recombination frequency, rather than specific physical distance along a chromosome. The greater the frequency of recombination (segregation) between two genetic markers, the farther apart they are assumed to be. Conversely, the lower the frequency of recombination between the markers, the smaller the physical distance between them. The frequency of recombination is estimated from the analysis of the progeny of experimental crosses between parents of known genotype. The physical mapping approach provides the true distance, measured in the number of DNA base pairs between two markers (Canario *et al*. 2008; Beaumont *et al*. 2010). The most commonly used markers in genetic mapping are AFLPs, MS markers and SNPs. Of these, the last two are superior in that MS markers are highly polymorphic and easily mapped, while SNPs are very abundant in the genome, easily identified using high-throughput technology, and can be developed from any gene, which offers the chance of identifying and mapping functional polymorphisms. They are also codominant markers (both alleles can be identified at a locus) unlike AFLPs, which are dominant markers (no distinction between homozygotes and heterozygotes). However, the first genetic maps for many aquaculture species were constructed using AFLPs because they

Table 10.5 Linkage maps in bivalves.

Species		No. and type of marker used for mapping	Map length (cM)	Number of linkage groups	Average distance between markers (cM)	Reference
Pacific oyster	*Crassostrea gigas*	96/119*	758/1031	10/11	8.8/9.5	Li and Guo (2004)
		88/86†	616/771	11/12	8.0/10.4	Hubert and Hedgecock (2004)
Eastern oyster	*Crassostrea virginica*	114/84*,†,‡	647/904	12/12	6.3/12.6	Yu and Guo (2003)
European oyster	*Ostrea edulis*	104/117*,†	471/450	9/10	4.9/4.2	Lallias *et al.* (2007a)
Pearl oyster	*Pinctada martensii*	98/110*,†	1323/1416	16/15	16.1/14.9	Shi *et al.* (2009)
Blue mussel	*Mytilus edulis*	116/121*	825/863	14/14	8.1/8.0	Lallias *et al.* (2007b)
Japanese scallop	*Mizuhopecten yessoensis*	155/163*,†	1882/2185	20/20	13.9/15.3	Chen *et al.* (2012)
Bay scallop	*Argopecten irradians*	95/96†	696/739	18/16	9.0/9.2	Li *et al.* (2012)
Noble scallop	*Chlamys nobilis*	108/117*,†	2054/2235	16/16	16.5/16.8	Yuan *et al.* (2010)
Zhikong scallop	*Chlamys farreri*	100/110†	1064/1211	20/19	14.6/13.2	Zhan *et al.* (2009b)
	C. farreri	2025/1861§	1175/1155	19/19	0.59/0.62	Jiao *et al.* (2014)

The first value for a parameter refers to the male map, and the second to the female map. One centimorgan (cM) corresponds to about 1 million base pairs in humans.
Types of genetic markers used are as follows:
*Amplified fragment length polymorphism (AFLP).
†Microsatellites.
‡Single strand conformation polymorphism (SSCP).
§Single-nucleotide polymorphisms (SNPs).

are cheaper to develop, and a large number of markers can be obtained quickly without prior knowledge of DNA sequences (Guo *et al*. 2008b).

The most common method used to construct physical maps is by using bacterial artificial chromosome (BAC) fingerprinting. The donor's DNA is cut using restriction enzymes into large fragments, 100–300 kb in length, and these are inserted into BACs. The BACs, along with their inserted DNA, are then taken up by bacterial cells, and as the bacterial cells grow and divide, the BAC DNA is amplified, thus producing what is known as a BAC library. Restriction enzymes are used to cut each inserted fragment, and the pattern of restriction-cut sites along the fragment are matched with the patterns on other fragments from the BAC library. Areas of overlap between fragments are identified and thus the restriction profiles of BAC clones are assembled in the correct order to construct a map (Beaumont *et al*. 2010). To date, only two BAC libraries have been reported in bivalves, one for the oysters, *C. gigas* and *C. virginica* (Hedgecock *et al*. 2005; Cunningham *et al*. 2006), and the other for the scallop, *C. farreri* (Zhang *et al*. 2008). The first BAC-based physical map was recently constructed for this species (Zhang *et al*. 2011). Other methods used to construct physical maps are radiation hybrid (RH) mapping, cytogenetic mapping by fluorescent *in situ* hybridisation (FISH) and whole genome sequencing (see Canario *et al*. 2008, for review).

Genetic maps are the most common type of maps available for fish and shellfish, largely because they are affordable and not too complex to prepare. The very first linkage map was constructed for the eastern oyster, *C. virginica*, and shortly after that for the Pacific oyster, *C. gigas*. The *C. virginica* genetic map was constructed using mainly ALFPs along with a few MS markers. Subsequently, more than 250 markers (153 MS and 104 SNPs) were developed for this species and some of these have been added to the ALFP-based map (Guo *et al*. 2008b). Most of the MS markers were developed from ESTs, while SNPs were developed by mining the EST database and resequencing. A large set of MS markers were also developed for *C. gigas*, and these have been used for the construction of the first MS-based oyster map (Hubert & Hedgecock 2004). Genetic maps have also been constructed for several mussel and scallop species, but so far not for any clam species (Table 10.5). Recently, an international team of 75 researchers sequenced and assembled the genome of *C. gigas* (Zhang *et al*. 2012a), and in the same year a draft version of the pearl oyster (*Pinctada fucata*) genome was published (Takeshi *et al*. 2012). Also, complete mitochondrial genomes are now available in GenBank for more than 30 bivalves. There is enormous variation in mtDNA genome size, gene content and arrangement among bivalve taxa, and this is serving as a useful tool in phylogenetic studies (Wu *et al*. 2012; Yuan *et al*. 2012). For a clear and concise description of the construction of a genetic map see Beaumont *et al*. (2010).

QTL mapping and MAS

QTLs are unidentified genes that affect performance traits, for example, growth rate, disease resistance, that are important to breeders. The relative positions of QTLs on a chromosome are identified by first constructing a genetic (linkage) map for a species (see earlier), which is then used in conjunction with studies of breeding and assessment of quantitative traits to identify markers that are closely associated (linked) to QTL of interest, thus allowing the QTL to be positioned on the linkage map. The information is then used to inform breeders what cultured strains/families should be crossed to maximise the specific desirable trait through MAS (Liu & Cordes 2004). Significant factors affecting the power of QTL detection include number of individuals per family, number of families, number and type of markers, genome coverage of markers, accuracy of phenotyping and genotyping, and heritability of the trait (Canario *et al*. 2008; ICES 2008). The majority of studies on QTL detection have been on cultured fish species, and most of these are on growth-related traits,

Table 10.6 Published quantitative trait loci (QTL) studies in bivalve species.

Species		Traits studied	Number of QTLs detected	Reference
Pacific oyster	*Crassostrea gigas*	Growth	4	Hedgecock *et al.* (2004)
			5	Hedgecock *et al.* (2007)
Pacific oyster	*Crassostrea gigas*	Growth	3	Guo *et al.* (2012)
		Sex	1	
Pacific oyster	*Crassostrea gigas*	Shell colour	1	Curole and Hedgecock (2007)
		Shell shape	2	
Bay scallop	*Argopecten irradians*	Size	1	Qin *et al.* (2007b)
Zhikong scallop	*Chlamys farreri*	Growth, sex determination	3	Jiao *et al.* (2014)
Eastern oyster	*Crassostrea virginica*	*Perkinsus marinus* resistance	10	Yu and Guo (2006)
Pacific oyster	*Crassostrea gigas*	Summer mortality resistance	5	Sauvage *et al.* (2010)
European oyster	*Ostrea edulis*	*Bonamia ostreae* resistance	9	Lallias *et al.* (2009)
		Bonamia ostreae susceptibility	6	

followed by disease resistance traits (Liu & Cordes 2004; Wenne *et al*. 2007). There have been far fewer studies on cultured bivalves, although numbers are increasing. Similar to fish, bivalve QTL studies focus mainly on growth and disease resistance traits, and almost all deal with oyster species (Table 10.6).

When QTLs have been identified, the QTL-linked marker information can be incorporated into breeding programmes. This is referred to as marker-assisted selection (MAS), which is an indirect selection method because the quantitative trait of interest is selected not on the basis of the trait itself but on a genetic marker that is linked to it. MAS significantly increases the efficiency of breeding programmes compared to non-MAS programmes (>50% in stochastic simulations; Sonesson 2007), but this is dependent on the heritability of the trait, the proportion of genetic variance associated with marker(s) and the selection scheme used. MAS is of particular value for traits of low heritability. If the gene affecting a trait is known, gene-assisted selection (GAS) can be used and is expected to be even more effective than MAS. Despite the fact that a number of QTL have been identified, particularly in fish, MAS (or GAS) have not been applied to any aquatic species to date (ICES 2008). Before this can happen QTLs need to be validated and mapped at high resolutions. Currently, studies are under way for the validation and fine mapping of disease-resistance genes in the oyster, *C. virginica* (Guo *et al*. 2008b). See Martinez (2007) for a review of MAS in fish and shellfish breeding schemes.

Functional genomics

By using the vast wealth of data produced by genome sequencing projects, functional genomics attempts to answer questions about the function of DNA at the levels of genes, RNA transcripts and protein products. In other words, functional genomics aims to bridge

the gap between the blueprint (genome sequence or genotype) and the living organism (trait or phenotype) under various environmental conditions (Cogburn *et al*. 2007).

Gene expression is the term used to describe the transcription of the information contained within the DNA into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. The entire RNA content of a cell is referred to as the transcriptome, and because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time. The recent availability of large EST collections is one of several tools that are used for the study of differential gene expression and the identification of genes involved in specific biological processes (Romero *et al*. 2012). EST collections contain genes that are modulated in response to environmental, chemical or biological stimuli and can be used to design probes in microarrays. Microarrays consist of a small membrane or glass slide containing samples of many genes arranged in a regular pattern. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to the DNA template from which it originated. By using an array containing many DNA samples, the expression levels of hundreds or thousands of genes within a cell can be determined by measuring the amount of mRNA bound to each site on the array, thus generating a profile of gene expression in the cell (NCBI 2007).

A cDNA microarray based on EST libraries from several different tissues of *C. gigas* and *C. virginica* that were exposed to metal, hypoxia, hyperthermia and immune challenges has been constructed and is publicly available to the research community (Jenny *et al*. 2007). Using this microarray Lang *et al*. (2009) found that the response of *C. gigas* gill to heat shock caused an immediate increase in the transcription of genes that encode heat shock proteins, and an increase in transcription, after 6 h, of genes whose products synthesise lipids, participate in cellular immunity and influence reproductive activity. Wang *et al*. (2010b) developed a microarray, which they used for the analysis of gene expression profiles during interactions between the pathogen, *Perkinsus marinus* (Chapter 11), and its oyster host, *C. virginica*. Significant gene expression regulation was found at day 30 post-challenge, and putative identities of the differentially expressed genes revealed a large set of genes likely involved in an active mitigating response to oxidative stress and apoptosis (programmed cell death) induced by *P. marinus*. The first microarray for mussels (*Mytilus galloprovincialis*) was enriched for genes involved in response to pollutants (Dondero *et al*. 2006). Subsequently, this group developed the MytArray 1.0, which includes 1714 probes (Venier *et al*. 2006) and also an array using RNAs from mussels injected with the pathogen *Vibrio splendidus* (Venier *et al*. 2011). Another microarray was developed using 4488 different genes from *M. galloprovincialis* and *M. trossulus* to analyse the effects of heat stress on gene expression (Lockwood *et al*. 2010) and to study the transcriptional responses of the two species to an acute decrease in salinity (Lockwood & Somero 2011).

The proteome is the entire complement of proteins expressed in a given organism, tissue/organ or cell at a certain point in time; and proteomics is the large-scale study of the expression, identification and interactions of those proteins. Studying the proteome is a challenging task since, unlike the genome, which is relatively static, the proteome changes constantly in response to tens of thousands of intra- and extracellular environmental signals. As a consequence, a particular cell type may make different sets of proteins at different times, or under different conditions. Furthermore, any one protein can undergo a wide range of post-translational modifications.

High-resolution two-dimensional electrophoresis (2-DE), liquid chromatography (LC), mass spectrometry (MS), matrix-assisted laser desorption/ionisation (MALDI), electrospray ionisation (ESI) and various combinations of these, as well as image analysis and bioinformatics approaches, are the major techniques used in proteomic analysis

(see Sheehan & McDonagh 2008 for details). While the majority of proteomic studies are concerned with the identification of human biomarkers for disease diagnosis and drug design, those on bivalves focus on the identification of biomarkers for environmental monitoring. Environmental proteomics is concerned with recognising specific biomarker patterns (or protein expression signatures, PES) in response to environmental stressors. The mussel has been the bivalve of choice in such studies, mainly because of its role as an ideal sentinel organism in marine monitoring programmes. Shepard *et al*. (2000) were the first to analyse the proteome of *Mytilus edulis* exposed to copper, polychlorinated biphenyls (PCBs) and salinity stress, albeit under laboratory conditions. Using 2-DE and quantification analysis of protein abundance by gel image analysis, they got clear separation of 500–600 protein spots, and ultimately obtained a unique PES for each stressor. Since then proteomics has been used to examine PES in response to a wide range of environmental contaminants (xenobiotics), for example, metals (Chora *et al*. 2009; Leung *et al*. 2011; Thompson *et al*. 2011), oil (Monsinjon *et al*. 2006; Apraiz *et al*. 2009), organochlorides (Dowling *et al*. 2006), phthalates (Apraiz *et al*. 2006), as well as other stressors, for example, thermal stress (Tomanek & Zuzow 2010), algae-derived shellfish toxins (Nzoughet *et al*. 2009) and cyanobacteria (Puerto *et al*. 2011). Campos *et al*. (2012), in their review of proteomic research in bivalves, present a comprehensive list of protein biomarkers of xenobiotic toxicity, which comprise enzymes involved in amino acid and fat metabolism, ATP synthesis, electron transport, xenobiotic metabolism and cell maintenance. Proteomics has also been used to compare PES in mussels grown in different habitats (López *et al*. 2001), to identify protein factors linked to oocyte quality in oysters (Corporeau *et al*. 2012) and to search for species-specific larval proteins (López *et al*. 2005).

More detailed information on general genomics can be found in Campbell and Heyer (2006), Gibson and Muse (2009), Liu (2011) and Lesk (2012), and on functional genomics in Hunt and Livesey (2000) and Saroglia and Liu (2012).

Chromosomal genetics and ploidy manipulation

The genes of an organism are located on chromosomes that reside in the cell nucleus. In somatic cells (cells that are not eggs or sperm) chromosomes occur in homologous pairs, consisting of one chromosome from each parent bearing essentially the same genes in the same order. The total number of chromosomes in a somatic cell is called the diploid number ($2N$). This number is normally constant within a species but may vary between species; for instance, mussels have $2N = 28$, oysters have $2N = 20$ and most scallops and clams have $2N = 38$. The number of chromosomes in the egg or sperm is half that in somatic cells and is called the haploid (N) number. When the egg is fertilised by a sperm the diploid number is restored.

Within a species there can be variation in the ploidy of individuals, but this is usually associated with a decrease in fitness, for example, reduced viability and fertility, sterility and deformity. Individuals with more than two sets of homologous chromosomes are called polyploids; an organism is triploid ($3N$) if it contains three sets, tetraploid ($4N$) if it contains four sets and so on. Polyploidy is very common in some groups, such as flowering plants and cereals, but is rare in animals. This is because it upsets complex gene interactions that are crucial in animal development.

For an organism to grow, cells must divide to produce more cells. This type of cell division, called mitosis, involves duplication of whole chromosome sets in order to produce two daughter cells that are diploid and genetically identical to each other and to

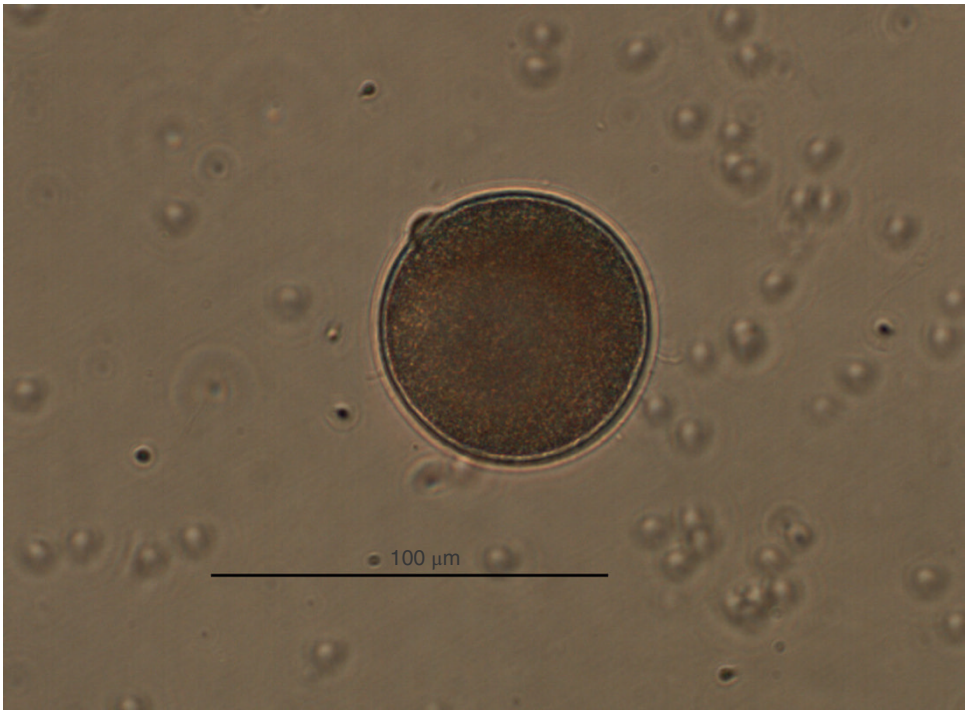


Figure 10.8 First polar body extrusion at meiosis I in an egg of *Mytilus edulis*.
Photograph by Helen Gurney-Smith, University College Cork, Ireland. Reproduced with permission.

the cell that gave rise to them. The type of cell division that produces gametes is called meiosis. This process involves two meiotic cell divisions in a diploid germ cell: meiosis I which produces two new cells each with half the number of chromosomes as their precursor; and meiosis II, which is essentially a mitotic division, and results in four haploid cells. Male germ cells produce four functional gametes, whereas female germ cells divide unequally at meiosis I to produce one large haploid cell and a small, attached first polar body, PB1 (Figures 10.8 and 10.9). Meiosis II produces the egg cell (oocyte) and a second attached polar body, PB2. Occasionally, in some species the first polar body undergoes meiosis II, giving three attached polar bodies, none of which contribute genetic material to the oocyte.

With the advent of a worldwide interest in fish and bivalve culture came the realisation that polyploids, produced through manipulation of chromosome sets, could have economic implications for the industry. The technique of ploidy manipulation was first applied to fish in the early 1970s (Purdom 1972) and a decade later to bivalve species (Stanley *et al.* 1981). For shellfish genetics and breeding the most relevant constructs are triploids, tetraploids, gynogenetic diploids and aneuploids. The following section describes how each of these types is produced.

Triploidy, tetraploidy, gynogenesis and aneuploidy

In bivalves eggs and sperm are released directly into the sea, where fertilisation takes place. Although the sperm are haploid, the eggs are arrested in the early stage of meiosis I, and meiosis I is not completed until the egg is activated by the sperm at fertilisation. Triploidy

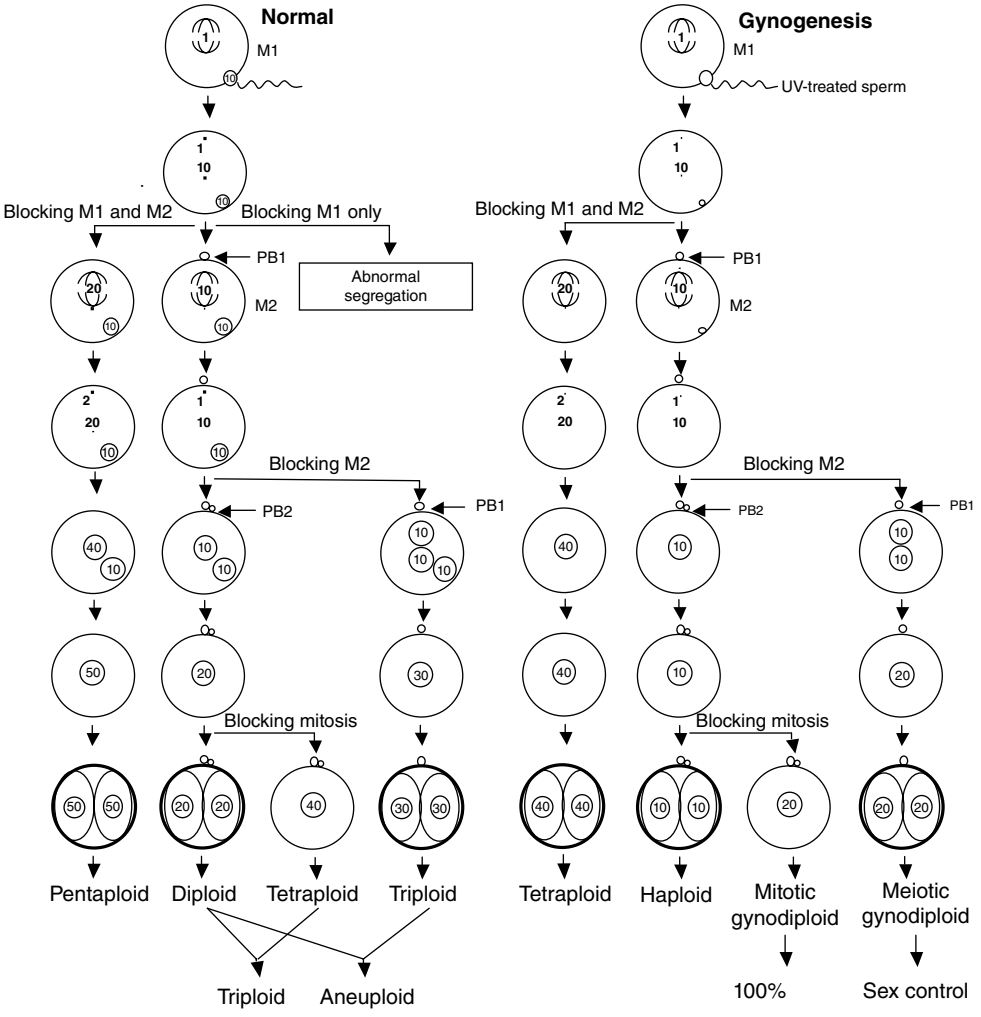


Figure 10.9 Schematic presentation of chromosome set manipulation in shellfish. Left: in normal zygotes, blocking meiosis I (M1) and meiosis II (M2) produces pentaploids (5N), blocking M2 produces triploids, blocking mitosis I produces tetraploids and blocking M1 produces abnormal segregation and aneuploids. Right: in gynogenetically activated eggs, blocking M1 and M2 produces tetraploids, blocking M2 produces meiotic gynodiploids and blocking mitosis I produces mitotic gynodiploids. Bottom: crossing diploids and tetraploids produces triploids, and crossing diploids and triploids produces aneuploids. From Guo *et al.* (2009). Reproduced with permission of Woodhead Publishing.

can be induced in eggs by inhibiting either meiosis I or II by chemical or physical shock treatment. If meiosis I is targeted PB1 is not extruded and homologous pairs of chromosomes are retained in the egg; meiosis II proceeds normally and the result is a diploid egg which, when fertilised by a haploid sperm, results in a triploid zygote (Figure 10.9). When meiosis II is targeted PB2 is not extruded and once again the result is a diploid egg (Figure 10.9). If treatment is administered during the first cleavage (mitotic) division of the fertilised egg the result is a cell with twice the diploid chromosome number. Subsequent divisions should produce a tetraploid embryo (Figure 10.9). Tetraploids can also be produced by blocking meiosis I and II in gynogenetically activated eggs (Figure 10.9). Once

produced, tetraploids can be crossed with normal diploids to produce 100% triploids (Guo *et al*. 2009). Fertilising normal eggs with diploid sperm from a tetraploid male can also produce triploids. Currently, most of the commercial production of triploid oysters in the United States and Europe are produced in this way.

Triploids can be crossed with diploids to create aneuploids (Figure 10.9). These have an abnormal chromosome number due to the loss (hypoploidy), or gain (hyperploidy), of one or more individual chromosomes. High percentages of aneuploids may also be produced when meiosis I is blocked for the production of triploids or tetraploids (Guo *et al*. 1992). Non-disjunction of chromosome pairs during mitosis or meiosis is a major cause of aneuploidy, and is a common phenomenon in bivalves in the wild (Teixeira de Sousa *et al*. 2012 and references therein).

Sperm can be irradiated to destroy the DNA, and this genetically inactive sperm can then be used to stimulate the parthenogenetic development of the egg. The result is a haploid egg that contains genetic information only from the mother. If first cleavage is inhibited this results in retention of an extra functioning set of maternal chromosomes in the egg (Figure 10.9). This compensates for the missing set from the sperm, and thus development can proceed normally. The result is a diploid gynogen that is homozygous at all gene loci, and particularly useful for the creation of inbred lines.

Cell division suppression methods

Suppression of cell division is achieved by using physical or chemical treatments. The former involves pressure shock, hypotonic shock (20 psu), heat shock or cold shock, while the latter uses chemicals such as cytochalasin B (CB), 6-dimethylaminopurine (6-DMAP), caffeine or colchicine. Heat shock is the most effective physical treatment, with often a high percentage (80–100%) of triploids produced. For example, in juvenile dwarf clams, *Mulinia lateralis*, a heat shock of 35°C applied at 8–11 min post-fertilisation to inhibit extrusion of PBI, produced 86–97% triploids (Yang & Guo 2006a). CB and D-MAP are the most effective chemical agents, with CB being the most frequently used. Both inhibit polar body formation but chromosome movement is unaffected by the treatment. A 10–20 min treatment with 0.5 mg/l CB, applied when 60% of the eggs release PB1, or as soon as PB2 is observed, can give 20–100% triploid production (see Guo *et al*. 2009 for details). However, CB is highly toxic so 6-DMAP is a good alternative. For example, using this agent, Vadopalas and Davis (2004) obtained a triploid yield of 92% in the geoduck clam *Panopea abrupta*, comparable to the rate observed for CB treatment in the same study.

The first viable tetraploids were produced using eggs from triploid *C. gigas* that were fertilised with haploid sperm, with subsequent suppression of PB1 extrusion with CB (Guo & Allen 1994a). However, this method is limited by the small numbers of eggs that can be obtained from triploids, and by the finding that some species, such as *Mya arenaria*, *Chlamys nobilis*, *Saccostrea commercialis* and *M. edulis*, do not produce or spawn mature eggs. Also, only a small proportion of offspring are actually tetraploid (reviewed in Piferrer *et al*. 2009). Therefore, heat shock was tested in the dwarf clam, *M. lateralis*, to search for optimal methods for tetraploid production (Yang & Guo 2006b). To inhibit mitosis I and mitosis II they applied a heat shock of 35°C to fertilised eggs at 35–37 min and 50–57 min post-fertilisation for about 20 min, producing up to 83 and 44% tetraploid larvae, respectively. However, most of these were abnormal and did not develop to the early larval D-stage. McCombie *et al*. (2005) used another approach whereby eggs from diploids were fertilised with diploid sperm from tetraploids, and then the extrusion of PB2 was suppressed. Although triploids were also produced some tetraploids were

identified at the 6-month stage. This method offers a way of introducing new genetic material into tetraploid broodstock, thus reducing the potential risks of inbreeding effects (Beaumont *et al*. 2010).

For gynogen production UV irradiation is the most common treatment used to inactivate sperm DNA. Dosage time ranges from 1.5 to 6 min at intensities of 620–1400 μW cm^{-2} s^{-1}, depending on the species (references in Guo *et al*. 2009). This breaks up the chromosomes but does not affect the ability of sperm to activate the egg. The egg then continues through meiosis I, meiosis II, and first cleavage to produce a haploid embryo that is non-viable. Chromosome doubling of the activated egg is achieved either by blocking meiosis II to produce meiotic gynodiploids or by blocking first cleavage (mitosis I) to produce mitotic gynodiploids (Figure 10.9).

All methods, but in particular chemical methods, are accompanied by high early larval mortality. This is particularly true for CB treatment, where Gerard *et al.* (1994) reported a mortality of 64% for CB treatment, but a lower mortality of 36% when 6-DMAP was used. Attention to dosage, timing, duration and temperature of treatment, as well as to factors such as egg quality and larval rearing techniques can substantially increase survival rates. Generally, once the D-larval stage of development has been reached, triploids and diploids exhibit similar survival rates (Downing & Allen 1987).

Ploidy verification

Since no method of polyploidy is 100% efficient it is important to have a quick, reliable and cost-effective means to check the ploidy level at every stage in the life cycle. The main methods are flow cytometry (FCM) analysis of DNA cell content, polar body counts, chromosome counting, nucleus sizing and scoring genetic markers. FCM is probably the technique of choice because it is fast, accurate and can be used on a variety of tissues that can be sampled without killing the animal. A fluorescent dye which bonds specifically to nucleic acids is added to a small sample of cells from fresh or frozen haemolymph, mantle, gill, siphon or foot tissue (Allen 1983). The cells take up the dye in proportion to their DNA content and a flow cytometer measures the fluorescent intensity and registers nuclei of different ploidies in different peaks (Figure 10.10). However, FCM is not sensitive enough to

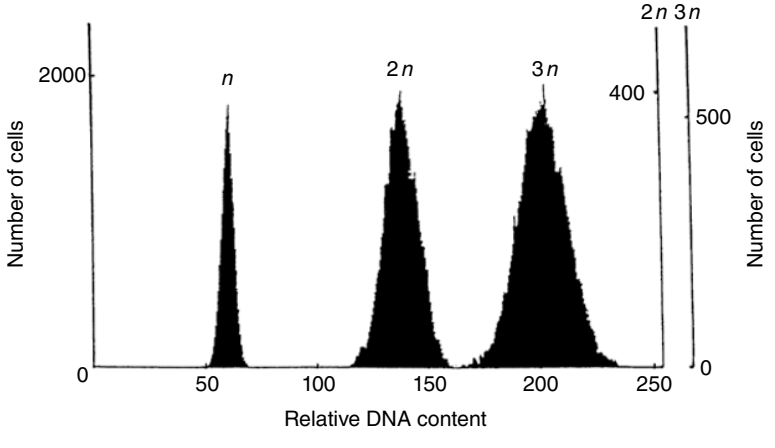


Figure 10.10 Histograms of fluorescence intensity in spermatozoa and diploid and triploid scallop (*Chlamys nobilis*) haemocytes analysed by flow cytometry.
From Komaru *et al.* (1988). Reproduced with permission of Elsevier.

detect the small variations in DNA content associated with aneuploidy (Guo *et al*. 2009). Because fluorescence signals can vary considerably, it is important that diploids are always used as a reference and should be prepared in the same way as test samples (Guo *et al*. 2009).

The success of meiosis I or II can be determined by fluorescent staining of polar bodies and nuclei (Scarpa *et al*. 1994). Chromosome segregation patterns and the presence of polar bodies are observed by using DAPI (4′-6-diamidino-2-phenylindole), a fluorescent dye that binds preferentially to the adenine–thymine base pairs of DNA. One disadvantage of polar body counts is that the method can only be used in embryos, and therefore an alternative method is required to confirm the ploidy of juveniles and adults. Chromosome counts can be carried out at the embryo as well as at later stages of development (Thiriot-Quiévreux & Ayraud 1982). Although the technique is time-consuming and involves a certain degree of expertise, it is a reliable and direct method of ploidy verification.

Due to the extra DNA content the nuclei of triploid cells are 1.5 times greater in volume and therefore have a larger diameter than diploid nuclei. The difference in diameter between diploid and triploid cells can be measured microscopically (Child & Watkins 1994). Generally, stained cells from gill tissue or haemolymph are used, and in the case of the latter the cells can be extracted without killing the animal. This method is fast, accurate and can be used by anyone trained in the use of a microscope. However, the technique does depend on the animals being large enough so that samples of tissue, such as gill, can be easily obtained.

Polymorphic genetic markers such as MS and allozymes can be used to confirm ploidy, and uniparental inheritance in gynogens (details in Beaumont *et al*. 2010).

Results from ploidy manipulations

Triploids

Most of the performance data on triploids have been obtained from triploids produced by meiosis inhibition, with few data, so far, on natural triploids created from crossing diploids and tetraploids.

Growth

Because triploids have a considerably reduced reproductive potential compared to diploids (see later), they should exhibit faster growth rates once maturity has been reached. Results on many bivalve species support this. Guo *et al*. (2009) have collated results from nearly 20 species and in almost all cases triploids grow significantly faster than diploids, with superior growth most pronounced in species of oysters. For example, in the oyster *C. virginica*, CB-induced triploids grew 12–67% faster than diploids, and natural triploids grew considerably faster (82–192%). Interestingly, in the commercial clam species analysed to date, triploids were either smaller or the same size as diploids, and so far the reason for this is unknown (El-Wazzan & Scarpa 2009; Guo *et al*. 2009). Environmental conditions also impact on the growth differential between diploids and triploids. For example, when triploid and diploid *C. gigas* were grown at slow-growing sites, triploids were 30–66% heavier than diploids, but when grown at a fast-growing site this figure was considerably higher at 165–180% (Wang *et al*. 2006). Similar findings have also been reported for the mussel, *M. edulis*, (Brake *et al*. 2004). However, in the lion-paw scallop, *Nodipecten subnodosus*, which is in great demand because of its large adductor muscle, no difference in the growth of the muscle was observed between triploids and diploids grown in a rich food environment (Racotta *et al*. 2008). The lack of superiority of triploids, in terms of adductor muscle growth, may be that diploids in this environment do not need to mobilise reserves to sustain gametogenesis.

Three hypotheses have been put forward, attributing the faster growth of triploids to increased heterozygosity, sterility or enlarged cell size. Since triploids have three instead of two copies of each chromosome in the cell nucleus they are expected to be genetically more variable than diploids. Triploids that are produced by blocking meiosis I are expected to be more heterozygous then meiosis II triploids (Figure 10.9), and triploids produced from diploid × tetraploid matings are expected to be more heterozygous than meiosis I and meiosis II triploids. However, while several studies have indeed shown that triploids are more heterozygous than diploids, meiosis I triploids were not significantly more heterozygous than meiosis II triploids (Margoulas *et al*. 2000; Guo *et al*. 2009 and references therein). Wang *et al*. (2002) found, however, that natural triploids were more heterozygous and grew faster than induced triploids. But overall, there is not strong support for the heterozygosity hypothesis.

The sterility hypothesis predicts that because triploids are sterile they allocate more energy into somatic growth rather than into gonad development. However, in several studies triploids have been found to be significantly larger than diploids well before sexual maturation. For example, *C. virginica* natural triploids are 165–180% larger than diploids at 3 months, well before their first reproductive season (Wang *et al*. 2006). Also, in the dwarf clam, *M. lateralis*, Guo and Allen (1994b) found that triploids were 72% heavier than diploids even though triploids in this species do not show reduced gonadal development, suggesting that sterility or energy relocation is not the major cause of increased growth of triploids. The cell size hypothesis proposes that triploid cells are larger than diploids and should therefore lead to an increase in organ and body size. However, the fact that triploids are often greater than 50% larger than diploids would seem to argue against this hypothesis because cell size increase in triploids should be proportional to the increase in cell DNA content, that is, about 50% (Guo *et al*. 2009). So, no one hypothesis provides an adequate explanation for faster growth in triploids.

Sterility

Since triploids have three sets of homologous chromosomes, there is difficulty in pairing during meiosis and consequently gonad development is impaired resulting in fully or partially sterile triploids. Sterility is the biggest advantage of triploidy because metabolic energy normally utilised in gonad development is instead available for increased somatic growth. Another advantage is biological containment in the culture of non-native species. Because of full or partial sterility, triploids may have improved meat quality during the spawning season. Indeed, this was the factor, not superior growth, that was the initial impetus for commercial production of triploid oysters (*C. gigas*). Gonad growth impairment, however, varies considerably between species. For example, in *C. gigas*, the fecundity of triploid females is about 2% of normal diploids, but there is wide variation in reproductive effort with some females showing no mature oocytes and others with up to 21 million oocytes, a value equivalent to the mean fecundity of normal diploid females (Guo & Allen 1994c). Interestingly, Normand *et al*. (2008) have found that gonad development is enhanced when triploid *C. gigas* are reared in the field under unusually warm conditions. Functional gametes and sometimes spawning, albeit at a low rate, have been reported in the pearl oyster *P. fucata martensii*, the clam species *M. lateralis* and *Ruditapes philippinarum* and in the oyster species *C. gigas*, *C. virginica* and *C. ariankensis* (references in Piferrer *et al*. 2009). Indeed, the production of first-generation tetraploids depends on obtaining eggs from triploids! In contrast, no formation of eggs or spermatozoa has been reported in the scallops *C. nobilis* and *Argopecten ventricosus*, in the mussels *M. edulis* and *M. galloprovincialis*, in the oyster *Saccostrea commercialis*, and in the clam species *M. arenaria* and *M. mercenaria* (references in Piferrer *et al*. 2009).

Disease and survival

Only a small number of studies have compared the responses of triploids and diploids to disease challenge. Cheney *et al*. (2000) found that during outbreaks of summer mortality disease in Puget Sound, US, survival was consistently lower in triploid oysters (*C. gigas*) than in diploids planted in comparable plots. However, in France, triploids were reported to have a higher resistance to summer mortality disease than diploids (Boudry *et al*. 2008). In the eastern oyster, *C. virginica*, triploids and diploids are equally susceptible to Dermo (Barber & Mann 1991), but are less susceptible than diploids to MSX (Matthiessen & Davis 1992; Chapter 11). Also, for the same species, triploids show better survival against ROD. Guo *et al*. (2009) suggest that since this disease affects juveniles, triploids probably grow fast enough to reach the refuge size earlier than diploids. In contrast to the findings so far, triploids (*C. gigas*) showed lower survival than diploids to two pathogenic *Vibrio* species with an affinity for gonadic tissue, a surprising result in view of the reduced gonadal development of triploids (De Decker *et al*. 2011). While there have been few studies on physiological performances of triploid bivalves there is the suggestion that triploids may have greater stress resistance than diploids due to their sterility and higher heterozygosity, both of which are associated with lower metabolic energy costs, and consequently there is more energy available for growth and survival under stressful conditions (see Hawkins *et al.* 2000).

Triploids and aquaculture

In North America commercial production of triploid *C. gigas* started in 1985 in the Pacific Northwest of the United States and in British Columbia, Canada. The industry rapidly embraced triploid and tetraploidy techniques, facilitated by its almost total dependence on hatcheries for the supply of seed. Today, triploids represent around 50% of production, and most of this relies on crossing tetraploid males and diploid females to produce triploids, according to the patented Guo and Allen (1994a) method (see later), which has an efficiency of 100%. In Europe, nearly 20% of the production is based on triploid oysters, almost all of which are produced by the Guo & Allen method. In France, tetraploid lines are being produced and held in quarantine by the government agency IFREMER under strict environmental constraints and controls (Piferrer *et al*. 2009). Semen with diploid sperm is being sold to the hatcheries to fertilise the eggs from their diploid maternal broodstocks. Triploid oysters grow faster, can be sold year round and provide a firmer, more palatable product compared with diploids. As triploids are virtually sterile the risk of their potential reproduction in the sea with triploids or diploids is estimated to be very low, given their poor reproductive performances. Although the commercial benefits of triploidy have been evaluated in the Pacific oyster, eastern oyster, Sydney rock oyster and European flat oyster, so far the production technique has only been commercialised for Pacific oysters (Nell 2002).

Tetraploids

Most of the methods used for triploid induction are rarely 100% effective due to poor larval survival rates. Therefore, the method that ensures 100% triploidy, by crossing diploids with tetraploids, was greatly welcomed by commercial hatcheries in the 1990s. As already mentioned, tetraploids can be produced by inhibition of mitosis I, inhibition of meiosis I, inhibition of meiosis I and II in gynogenetically activated eggs (Figure 10.9), and also by fusion of two diploid cells, or inhibition of meiosis I in eggs from triploids fertilised by normal sperm. Guo *et al*. (2009) have examined the success rate of the various induction methods, and while the majority of these produce viable 4N embryos (~36% over 30

treatments), survival rates are so low that no post-embryonic 4N individuals survive, the exception being the last of these methods, which produces a large number of viable *C. gigas* tetraploids (Guo & Allen 1994a). Using this method, which is patented in the United States with licences for EU use (US Patent #5824841; 20 October 1998), viable tetraploids have been produced in the oysters *C. virginica*, *C. ariankensis* and *P. martensii* and in the scallop *A. ventricosus* (references in Guo *et al*. 2009). Another method, which has been patented (European Patent ♯CN101677524; 23 April 2010), involves inhibition of meiosis I in eggs from diploids fertilised with sperm from tetraploids. Because larvae with different ploidy levels are produced by this method, the patent includes details on how tetraploids can be isolated and raised from the obtained larvae. The method offers a means of direct introgression of genetic characters from selected diploid to tetraploid lines, thus avoiding a triploid step (McCombie *et al*. 2005).

To date, large numbers of tetraploids are only available in three *Crassostrea* species (see earlier). Initially, first-generation tetraploids in *C. virginica* tend to be smaller than diploids and triploids, but by the fourth generation they are significantly larger than diploids, which may be due to rapid adaptation during the first few generations (Guo *et al*. 2009). Tetraploids are fertile with a fecundity similar to that of normal diploids (Guo & Allen 1997). They have an approximately 1:1 sex ratio, and males produce mostly diploid sperm with some aneuploids. The main advantage of tetraploids is that mating them with diploids produces almost 100% triploid offspring.

Gynogens and aneuploids

Diploid gynogens contain two copies of the maternally inherited chromosome set and are almost always female. Mitotic gynodiploids are completely homozygous at all loci, while meiotic gynodiploids are less inbred (Figure 10.9). Because they are highly inbred, gynogens suffer inbreeding depression resulting in higher mortality and slower growth than diploids. However, if they survive to maturity they can provide inbred lines for later outcrossing with other inbred lines to produce highly heterozygous F_1 offspring (Beaumont *et al*. 2010). Meiotic gynodiploids have been successfully produced in a number of bivalve species and have been used for centromere mapping, but so far mitotic gynodiploids have not been reported in shellfish (Guo *et al*. 2009).

Aneuploids originate from the non-disjunction of chromosomes during mitosis or meiosis, or from premature centromere division. In the hatchery they can be produced by crossing triploids with diploids with or without inhibition of meiosis (Figure 10.9). But aneuploidy is also a natural occurrence in many animal and plant groups, including bivalves. The effects are generally lethal in higher animals but are less deleterious in plants and lower animals, and even less so in bivalves. Hypoploid cells, which lack of one or more individual chromosomes, have often been reported in several populations of bivalve species, with values as high as 39% observed in the clam, *Ruditapes decussatus* (Teixeira de Sousa *et al*. 2011). Environmental factors such as herbicides and heavy metals may increase the incidence of aneuploidy in bivalve populations (Bouilly *et al*. 2004, 2006, 2007).

In hypoploids chromosome loss is non-random with 3 (1, 9 and 10) of the 10 chromosome pairs in *C. gigas* and *C. angulata* preferentially affected by the loss of one homologous chromosome (Leitão *et al*. 2001a; Teixeira de Sousa *et al*. 2012). A negative correlation between growth rate and aneuploidy has been reported in several bivalves with fast-growing individuals showing a lower incidence of aneuploidy compared to slow growers (Figure 10.11) (Zouros *et al*. 1996; Leitão *et al*. 2001b; Batista *et al*. 2007; Teixeira de Sousa *et al*. 2011). The causes of this are unclear, although a genetic basis has been suggested by Zouros *et al*. (1996), who proposed that the relationship is due to the unmasking of

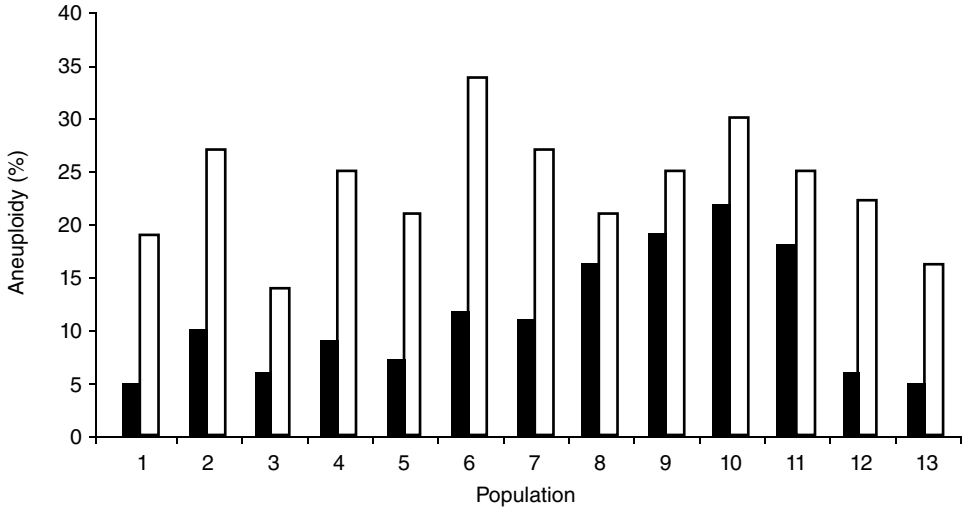


Figure 10.11 Comparison of percentage aneuploidy between fast- (black) and slow-growing (white) oysters (*Crassostrea gigas*) in 13 hatchery-produced populations in France. See tables 1 and 2 in Leitaõ *et al.* (2001b) for identity of populations.
From Leitaõ *et al.* (2001b). Reproduced with permission of Elsevier.

deleterious recessive genes caused by the progressive haploidisation of somatic cells. This, in turn, will result in the unmasking of mutations affecting growth rate and may cause a retardation of growth (Leitão *et al*. 2001b). It is important that the aneuploidy–growth relationship is taken into account in bivalve selective breeding programmes.

Transgenics

The injection of foreign DNA into animal ova has been used since the early 1980s to produce what are known as 'transgenics'. The first attempt at transgenic production was in the early 1980s when the rat growth hormone gene was injected into mice, producing a dramatic increase in growth rate (Palmiter *et al.* 1982). These experiments paved the way for the production of transgenic farm animals and towards the end of the 1980s the technology was applied to several commercially important fish species (reviews by Iyengar *et al*. 1996 and Devlin 1997). The principal genes that have been used in fish transgenic studies to date are fish growth hormone gene, antifreeze protein genes and disease resistance genes (Rasmussen & Morrissey 2007), chosen primarily because of the potential commercial contribution of the produced transgenics to the aquaculture industry. Briefly, the gene with flanking sequences, such as promotor and tissue enhancer sequences, is obtained from a gene library. To provide a high copy number many million copies of the acquired gene is inserted into a DNA vector, grown up in a suitable bacterial strain and then harvested. About 1 million copies of the gene are introduced close to the nucleus of the fertilised egg. The major transfection methods are microinjection, lipofection (liposome mediated), electroporation, whereby eggs are subjected to electrical pulses that increase membrane porosity and facilitate the uptake of the DNA, particle bombardment (biolistics), sperm-mediated transfer and viral vectors (Cadoret *et al*. 2000; Beaumont *et al*. 2010). The next step is to ascertain whether the transgene has been integrated into the host's genome and, if so, whether it is expressed. Expression can be established either by assaying for the appropriate mRNA or,

more commonly, by assaying directly for the translated protein product. Finally, it must be established whether the gene has been incorporated into the germline (details in Gama Sosa *et al*. 2010).

Transgenic, farmed fish include Atlantic, coho and Chinook salmon, rainbow trout, common and silver carp, channel and African catfish, and Nile tilapia (Hulata 2001; Dunham 2004a). Initially, most of the literature on fish transgenics was concerned with the technical aspects of the method, but over the past decade there have been increasing numbers of reports on the actual phenotypic effects of gene transfer. Almost all deal exclusively with the effect of growth hormone on fish growth, with all reporting an improvement in transgenic versus untreated fish from 10% to an incredible 30-fold (Dunham 2004a and references therein).

Although there is a large body of research on gene transfer in finfish, there are few reports of successful transfer in bivalves. The small size of gametes and embryos, together with selection of transformed larvae, makes transfection a difficult task in bivalves. The first successful introduction and expression of foreign DNA was in the dwarf surf clam, *Mulinia lateralis*, a species that is often used as a model organism because of its fast generation time, short life cycle and high fecundity. A retroviral vector, containing a foreign virus gene sequence, was introduced into fertilised eggs (Lu *et al*. 1996). Subsequently, expression of β-galactosidase, encoded by the virus, was detected in transgenic but not control embryos. This gene transfer method has since been patented in the United States (US Patent #5969211; 19 October 1999). A subsequent study successfully introduced a foreign gene into *C. virginica* larvae, the goal being the development of gene transfer techniques to counter disease-related problems in the oyster farming industry (Buchanan *et al*. 2001). The gene used was the bacterial gene aminoglycoside phosphotransferase II, which confers resistance to neomycin and related antibiotics. Transfected larvae exposed to the antibiotic G418 demonstrated significantly increased survival rates compared to non-transfected larvae. A more recent study introduced the p-GeneGrip construct, which encodes green fluorescent protein, into spermatozoa from the mussels *Mytilus galloprovincialis* and *M. chilensis*, and the clam *Chamelea gallina* (Guerra *et al*. 2005). The efficiency of transfection was 59–70% in the species used, indicating that sperm-mediated transfer could be useful for other gene constructs in bivalves.

While the technology has reached the stage of commercial application for fish species there is still quite a long way to go before farming transgenic bivalves becomes a reality. A major problem for shellfish farmers is the high losses often sustained due to protozoan and bacterial pathogens, particularly in oysters (Chapter 11). While selective breeding programmes to produce resistant strains are under way in several countries (see bivalve breeding programmes, earlier), transgenesis could be a fast and efficient method for producing disease-resistant lines in bivalves. Several anti-microbial peptides have already been isolated, and identification of the genes coding for some of these peptides, as well as their regulatory mechanism, has been accomplished (see Chapter 11).

The major problems facing this new technology are not just technical, but have more to do with ethical considerations, non-acceptance by the consumer, environmental concerns and the lack of specific guidelines for experiments with transgenic organisms. Over the past 15 years concerns on food safety, environmental risks, government regulations and intellectual property rights regarding transgenic fish have been discussed at length in several scientific articles (Hallerman *et al*. 1999; Hulata 2001; Aerni 2004; Dunham 2004b). But 'well executed public education may be necessary to gain broad consumer acceptance of transgenic fish from an environmental standpoint, a food safety standpoint and perhaps in relationship to how "organic" a transgenic fish may be' (Dunham 2004a). Despite concerns, the US Food and Drug Administration (FDA) is about to approve transgenic Atlantic salmon for human consumption (Ledford 2013). The fish, which are sterile, have been engineered



Figure 10.12 Genetically engineered Atlantic salmon (top) grows twice as fast as its wild counterpart of the same age (bottom).
Photo courtesy of AquaBounty Technologies Inc., Maynard, Massachusetts, USA.

to grow twice as fast as unaltered individuals (Figure 10.12), and have been at the centre of an 18-year US$60-million battle by AquaBounty Technologies of Maynard, Massachusetts, to bring them to the dinner table. However, environmental and consumer groups are preparing to do battle to prevent the sale of these fish in the United States. The latest news is that the Canadian Government has granted AquaBounty permission to export up to 100 000 genetically modified (GM) salmon eggs from a hatchery in Canada to a site high in the Panamanian rainforest, where the GM fish will be reared (Goldenberg 2013). However, in Canada GM fish or eggs are not currently approved for human consumption.

Notes

1 Broad-sense heritability ($H = V_G/V_P$) captures the proportion of phenotypic variation due to genetic values that may include effects due to dominance and epistasis. It is the narrow-sense heritability that is used by breeders to adopt appropriate selection strategies and to predict rate of phenotypic change.
2 A DNA sequence, such as a gene, that is transferred from one organism to another and replicated by genetic engineering techniques.
3 The polymerase chain reaction is a revolutionary technique used to produce thousands to millions of copies of a short (0.1–10 kb) piece of DNA.
4 A haplotype is a combination of alleles (for different genes) that are located closely together on the same chromosome, and that tend to be inherited together.

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11 Diseases and parasites

Introduction

Because of the considerable economic importance of bivalves, there is a wealth of information available on diseases that affect them. To quote Otto Kinne, who said in 1983, 'We now know more about the diseases of bivalves than of the diseases of all other marine invertebrate groups together'. Thirty years later this still applies.

The major disease-causing agents of marine bivalves are viruses, bacteria, fungi, protozoans, helminths and parasitic crustaceans. Because of the considerable amount of information available, only key bivalve pathogens within each group will be described, and readers are referred to Ford and Tripp (1996), Ford (2001), McGladdery *et al*. (2006), Renault (2008a) and Bower (2010a) for further information. The different defence mechanisms utilised by bivalves to combat disease are also considered (see Song *et al*. 2010 for review).

Viruses

Viruses are ultramicroscopic infectious agents (~20–350 nm long) which can only multiply inside living cells. Once infected, the host cell begins to degenerate and is soon destroyed, liberating new virus particles in the process. These in turn infect new cells.

As filter feeders, bivalves may accumulate viruses from humans and other vertebrates, thus acting as carriers of enteroviruses, such as hepatitis A or polio for vertebrate hosts that feed on infected individuals (see Chapter 12). Many viruses, however, cause serious disease in bivalves themselves and are responsible for mass mortalities in the aquaculture industry. Table 11.1 presents some examples of viruses implicated in disease outbreaks. In most cases, however, only a tentative link can be made between a particular virus and the pathology observed. In order to demonstrate that a virus is the causal agent it must first be isolated from a diseased host and passed via a cell-free extract to a susceptible host in which the same disease is later manifested. This is not an easy task because there is seldom conclusive evidence that the pathology is virus-related; there are also problems in identification,

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Table 11.1 Examples of some disease-causing viruses in bivalves (Renault & Novoa 2004; Renault 2008a; Dang *et al.* 2009; Lynch *et al.* 2012).

Virus type	Host species	Disease	Effects on host	Distribution
Irido-like	*Crassosrea angulata*, *Crassostrea gigas* (cultured in France)	Gill necrosis virus (GNV)	Destruction of gill filaments	France, Portugal, Spain, UK
	C. angulata, *C. gigas* (cultured in France)	Haemocyte infection virus (HIV)	Virus infected haemocytes	France, Spain
	C. gigas larvae	Oyster velar virus disease (OVVD)	Larval movement affected through loss of infected epithelial cells from velum	Washington State, USA
Herpes-like	Mainly hatchery-reared larvae of *Crassostrea* and *Ostrea* spp.	Herpes virus infection	Velar and mantle lesions; swim in circles.	USA, Mexico, Europe, Australia, New Zealand
Picorna-like	Mussel, oyster, scallop and clam species		Various depending on host species (haemocytosis; digestive gland cell necrosis; granulocytomas; muscle degeneration)	Denmark, France, Spain, Japan, French Polynesia, New Zealand
Papova-like	*Crassostrea* and *Pinctada* spp.	Viral gametic hypertrophy	No or limited impact but may affect gametes and consequently fecundity	Canada, USA, Korea, Australia, France, Ireland, Germany

isolation and characterisation of the virus; experimental transmission of the virus is virtually impossible due to the absence of mollusc cell lines and there is often uncertainty as to whether the virus is a primary pathogen or secondary invader.

However, over the past decade specific and sensitive diagnostic methods, for example, polymerase chain reaction (PCR), in situ hybridisation (ISH), immunochemistry, have been developed to detect herpes-like viruses, a group associated with high mortalities in bivalve larval and spat culture (Arzul *et al.* 2002). One of these viruses, collected during a mortality outbreak in France in 1995 in *Crassostrea gigas* larvae, was characterised and named as *Ostreid herpes virus1* (OsHV-1) (Minson *et al.* 2000), and its gross genome sequence was completed later (Davison *et al.* 2005). The pathogenicity of the virus has been demonstrated by experimental transmission of the virus to sterile larvae. Recently, Schikorski *et al.* (2011) developed an experimental protocol of horizontal transmission based on cohabitation between healthy and experimentally infected oysters.

Eradication of viral diseases will prove difficult because of the high-density production systems used in commercial hatcheries and nurseries, further exacerbated by stock movement, one of the major risks in the spread of disease (Renault 2008a). When a viral disease is diagnosed there are at present no specific chemotherapies or vaccines available to treat it. But one encouraging finding is that some species may have innate immunity against viral infection. In a study of OsHV-1 in *C. gigas* an antiviral substance was reported in adult haemolymph, which indicates that adults could be more resistant than larvae and juveniles to infection. This substance may contribute to oyster survival despite continuing exposure to OsHV-1 (Olicard *et al.* 2005). Antiviral activity against a wider range of viruses has since been demonstrated in the haemolymph of additional species (Carriel-Gomes *et al.* 2006;

Defer *et al*. 2009). Purification of antiviral substances could contribute to the development of new approaches for the control of viral infections and to a greater understanding of mollusc defence mechanisms. Recently, Normand *et al*. (2014) have examined the expression profiles of 17 previously identified candidate genes in the context of mass mortalities of juvenile *C. gigas*.

Bacteria

The aquatic environment harbours a rich bacterial flora and bivalves, because of their efficient filter-feeding mechanism, can ingest many different kinds of microorganisms. While the majority of marine bacteria are not harmful, unless present in excessive numbers, some can be pathogenic to their host. In some cases bivalves can act as passive carriers of human pathogens, without themselves contracting a bacterial disease (see Chapter 12). Much of the information on bacterial diseases comes from studies on cultured larvae, which are more susceptible than adults to infection (Table 11.2). This is because larvae are reared in static systems at elevated temperatures and high densities. Also, ocean warming and biological invasions of exotic species may bring with it the appearance and spread of new and/or extant bacterial diseases in bivalves (Paillard *et al*. 2004). Information on bacterial infections and their treatment in hatchery-reared larvae and juveniles is in Chapter 9.

Table 11.2 Examples of some pathogenic bacteria in bivalves.

Bacteria	Disease	Species	Symptoms	References
Vibrio anguillarum, V. tubiashii, V. alginolyticus, V. splendidus, V. aestuarianus, V. neptunis	Larval and juvenile vibriosis	Wide range of hatchery-reared species	Up to 100% larval mortality*	Gómez-León *et al*. (2005); Prado *et al*. (2005); Labreuche *et al*. (2006a, b); Elson *et al*. (2008) and Bower (2009a)
Vibrio tapetis	Brown Ring Disease (BRD)	*Ruditapes philippinarum*	Brown deposit on shell; degeneration of digestive gland followed by metabolic disorder and death	See text for references
Roseovarius crassostreae	Roseovarius Oyster Disease (ROD)	*Crassostrea virginica* juveniles <25 mm shell length; USA	Mantle lesions; internal nodules; up to 90% mortalities	Maloy *et al*. (2007) and ICES (2011a)
Nocardia crassostreae	Pacific Oyster Nocardiosis (PON) or summer mortality	*Crassostrea gigas* and *Ostrea edulis* cultivated near infected *C. gigas*	Up to 35% mortalities	Bower (2006a)
Cyanobacteria spp.	Blue-green alga shell infestation	*Perna perna* and *Mytilus galloprovincialis*	Shell damage; lowered reproductive output; mortalities up to 50%	Bower *et al*. (2002)

*In the Thau Lagoon, France, the ostreid herpesvirus OsHV-1 and, secondarily, bacteria of the group *Vibrio splendidus* are responsible for mass summer mortality of *C. gigas* during their first 2 years (Pernet *et al*. 2012).

Most bacterial diseases of bivalves are caused by a range of *Vibrio* species (Table 11.2). One of these diseases, brown ring disease (BRD), in clams will now be described in some detail. This is one of the few well-documented bacterial diseases that affect adults as well as juveniles.

Brown ring disease

During the spring and summer of 1987 mass mortality of adult and juvenile *Ruditapes philippinarum* was reported from culture beds in the northwest of France. All of the moribund clams exhibited a brown deposit on the inside of the shell, between the pallial line and the shell margin (Figure 11.1). The deposit forms a characteristic ring, hence the name brown ring disease or BRD (Paillard & Maes 1989). The etiological agent responsible for the disease is *Vibrio tapetis*. While BRD affect clams in the genera *Ruditape*s, *Tapes* and *Venerupi*s, *R. philippinarum* is the most sensitive species and the only one to exhibit BRD-related mortalities (Maes & Paillard 1992). The disease causes mass mortalities in cultured clam beds, but has a lower impact in wild populations (Paillard 2004). The most likely mode of transmission is through direct contact with infected clams (Martinez-Manzanares *et al*. 1998). Since 1987 the disease has been reported from additional areas of France, and in Spain, Portugal, Italy, the United Kingdom, Ireland, Norway and, more recently, in Korea (Bower 2010b).

The disease disrupts the normal deposition of periostracum at the shell edge and instead it is deposited on the inner shell, resulting in an accumulation of brown organic material (details in Paillard (2004)). As the disease progresses the bacteria penetrate through the mantle epithelium and into the soft tissues, causing severe damage. There is degeneration of the digestive diverticula manifested in an inability to store reserves, mainly glycogen, and distribute them to other tissues. Growth, weight and condition indices are subsequently affected (Plana *et al.* 1996). Flye-Sainte-Marie *et al*. (2008) have shown, experimentally, that severely diseased clams show a reduction in clearance and respiration rates leading to a

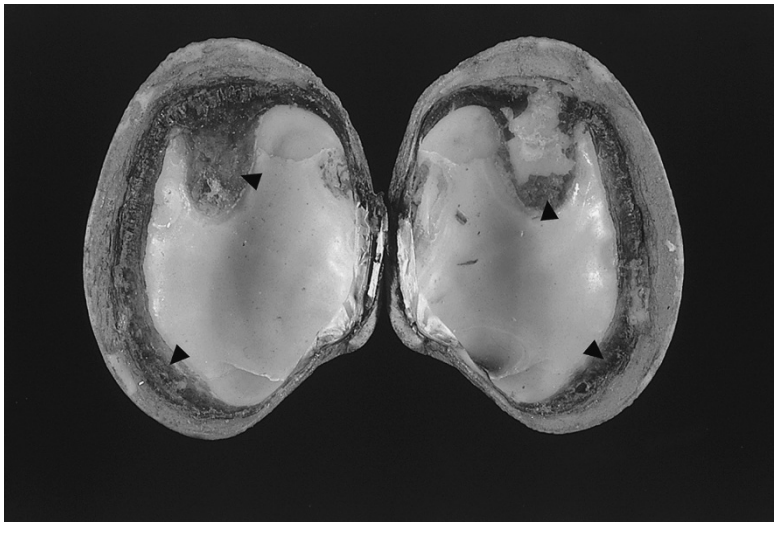


Figure 11.1 Characteristic brown ring disease organic material (arrowed) on the inner shell surface of the Manila clam, *Ruditapes philippinarum*.
Photograph by C. Paillard, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, France. Reproduced with permission.

decrease in energy acquisition. This, along with the high maintenance costs associated with mounting a defence against the pathogen, may be the actual cause of clam death. Clams can, however, recover from the disease by covering the organic brown deposit by shell material, a process called nacrezation (see later). Indeed, this is the principal mechanism implicated in the development of BRD resistance in hatchery-reared stocks of *R. philippinarum* in France (Trinkler *et al*. 2010).

Several methods are used to diagnose BRD. The extent of the disease can be assessed by macroscopic examination of the brown deposits on the inner shell using the classification scheme of Paillard & Maes (1994). These authors also proposed a shell repair classification scheme, which was later expanded (Paillard 2004). Light and electron microscopy as well as indirect immunoflorescence (IIF) have been used to identify and localise *V. tapetis* in tissue sections (references in Paillard 2004). Viral concentrations in extrapallial fluid and haemolymph are estimated using an enzyme-linked immunoassay (ELISA) (Allam *et al*. 2002). Bacterial techniques are used to isolate potential *V. tapetis* strains; purified strains undergo phenotypic, serological and molecular characterisation, and are then injected into the pallial cavity of clams to evaluate their capacity to produce clinical signs of BRD. A PCR assay, based on dot-blot hybridisation and a species-specific primer that targets the 16S rDNA gene, is a fast and direct method that is routinely used to detect the pathogen in larvae and diseased clams and also in asymptomatic clams that later developed BRD (Paillard *et al*. 2006). However, no one technique is sufficient on its own to detect the pathogen. Inspection of shell valves in combination with the PCR assay is regarded as the most sensitive and rapid method (Drummond *et al*. 2006).

Laboratory-based studies show that temperature and salinity are important factors in modulating BRD development and immune responses (see Section 'host–parasite interactions'). The pathogen does not grow and survive at temperatures higher than 27°C, and is rapidly killed at 30°C in seawater (Paillard 2004). Not surprisingly, the disease tends to be more frequent in the spring and winter, and is absent in areas with high summer temperatures. In challenge experiments Reid *et al*. (2003) showed that disease prevalence (number of individuals infected) was highest at a salinity of 20 psu, and significantly fewer clams presented with symptoms at a salinity of 40 psu (see also Binias *et al*. 2014). Rough handling of clams can also lead to a significant increase in disease prevalence (Jean *et al*. 2011); this practice can lead to mechanical disruptions of the periostracal lamina and/or chipping of the shell margin, thus facilitating entry of the pathogen. Large sediment grains becoming lodged in the shell openings may also induce such disruptions (Flye-Sainte-Marie *et al*. 2008). All these factors must be considered when devising strategies for the control of BRD. In addition, assessing the health of juvenile clams before planting out and reducing the density of planting might be beneficial (Flassch *et al*. 1992).

Before concluding this section on bacterial diseases a few points are worth noting:

- Bacteria that are pathogenic in larval rearing systems may or may not be pathogenic in the wild. Adults in culture are frequently unaffected by concentrations of bacteria that are pathogenic to larvae (reviewed by Paillard *et al*. 2004).
- Very often bacteria are not the primary causative agent in disease; stress factors can lower resistance, and thus increase the probability of infection.
- The genus *Vibrio* consists of many strains, and pathogenicity varies depending on the host species. It is essential to be able to distinguish between pathogenic and non-pathogenic strains. In the past decade methods for strain isolation, characterisation and screening for virulence have been developed (Estes *et al*. 2004). Strains are isolated using microbiological methods; they are then characterised using PCR amplification of specific genes, and virulence is tested in challenge experiments by injection into healthy individuals

(Gay *et al*. 2004; Guisande *et al*. 2008; Saulnier *et al*. 2009). Marine *Vibrio* spp. have been shown to produce an array of extracellular products (ECPs), some of which are known pathogenicity factors (Hasegawa *et al*. 2008).

- Remedial measures such as the use of antibiotics in culture systems are not always successful; elimination of certain species of bacteria may favour the appearance of other bacteria, and reduce bivalve resistance to infection by pathogenic forms. Also, there is the danger that with persistent use of antibiotics resistant strains of bacteria will appear. Alternatives to systematic use of antibiotics are UV treatment of incoming waters, maintenance of bacterial-free algae cultures and good hygiene practice (Prieur *et al*. 1990). However, the most promising approach is the use of probiotics, live bacteria that have a beneficial effect on the host (see Chapter 9). To date, a multitude of bacterial strains have been tested for their antibacterial activity (Prado *et al*. 2009). Strains belonging to the genus *Phaeobacter* show the strongest inhibitory activity against aquaculture pathogens, especially against members in the genus *Vibrio* (Prado *et al*. 2009; Romalde & Barja 2010).

Fungi

Few fungi are pathogenic to bivalves. Those that are confine themselves to external parts such as shell or byssus. It is the larval stages that are most prone to disease, although it is not always clear whether the fungus is a primary pathogen or secondary invader.

There is one disease, however, maladie du pied, or shell disease, which has severe effects on *Ostrea edulis*, *C. gigas*, *Saccostrea cucullata* and *Crassostrea angulata*, although the latter only suffers light infections (Alderman & Jones 1971a). The disease has been reported in Western Europe, India and both the Pacific and Atlantic coasts of Canada (Bower 2001a). The causative agent, *Ostracoblabe implexa*, lives in the shell (Alderman & Jones 1971b). Initially, small white spots appear on the inner shell surfaces but as the fungus proliferates these develop into greenish rubbery warts of conchiolin, particularly in the region of the adductor muscle attachment. In the advanced stages of the disease the valves become grossly deformed, and shell closure may become impossible, leading to the eventual death of the oyster.

The disease was first reported in Holland in 1902, but was not serious in that only a small percentage of oysters were infected. However, with increased oyster production in the 1930s, and subsequent widespread importation and dispersal of oysters, the disease has spread through Western Europe. Progress of the disease is usually slow but high water temperatures (above 19°C) favour the spread and virulence of the fungus (Lauckner 1983). Incidences can be as high as 70%, and young oysters are more susceptible than older individuals; apparently, the latter may recover from an infection under good feeding conditions.

Protistans

These single-celled organisms are the most common cause of disease in bivalves. Most belong to the phyla Haplosporidia, Dinoflagellata or Cercozoa (*Marteilia* spp.), and infect mainly oyster and clam species. The enormous literature on these pathogens is a reflection of the extent of damage that they cause to commercial production. Undoubtedly, they are also significant disease-causing organisms in non-economic species. The most important protozoan pathogens are listed in Table 11.3 and some of these are considered here.

Table 11.3 Important protistan pathogens of oysters.

Pathogen	Disease name	Host	Distribution	Pathology
*Bonamia ostreae**, *B. perspora*, *B. exitiosa*, *B. roughleyi*	Bonamiasis	Wide range of oyster species	Atlantic coasts W. Europe, east and west coasts USA, west coast Canada, New Zealand and SE Australia	Yellow discolouration of tissue, extensive gill lesions, breakdown of connective tissue and loss of condition; mortalities up to 90%.
Marteilia refringens	Digestive gland (or Aber) disease	*Ostrea edulis* and *Mytilus galloprovincialis*	In *O. edulis* on western Atlantic coasts from UK to Morocco; in *M. galloprovincialis* on Adriatic Sea coasts	Pale digestive gland, severe emaciation, tissue necrosis, cessation of growth; 50–90% mortality rate summer and autumn.
Marteilia sydneyi	QX (Queensland unknown) disease	*Saccostrea glomerata*; possibly other *Saccostrea* spp.	E. Australia	Necrosis of digestive gland, loss of condition, gonad absorption; mortalities up to 90% in summer.
QPX (Quahog Parasite unknown)	QPX	Hatchery and wild-caught *Mercenaria mercenaria*	East coast of N. America from New Brunswick to Virginia	Disruption of whole-body connective tissue, mantle lesions, impaired growth and lower condition index; year round mortalities up to 100% in some cultured stocks.
*Haplosporidium nelsoni**	MSX (Multinucleate Sphere X)	*Crassostrea virginica* and *C. gigas* to a lesser extent	East coast of North America from Nova Scotia, Canada to Florida, USA. *C. gigas*: Korea, Japan, west coast USA, France	Reduced condition, and fecundity; maximum mortalities (up to 90%) in summer.
*Haplosporidium costale**	Seaside organism (SSO) disease	*C. virginica*	New York to Virginia, USA in high salinity waters (>25 psu)	Maximum mortalities (≤40%) in May–June.
*Perkinsus marinus**	'Dermo' disease	*C. virginica*	Gulf of Mexico northwards to Delaware Bay, Cape Cod, and Maine, USA	Severe emaciation, loss of condition; mortalities as high as 80% depending on temperature and salinity.
Mikrocytos mackini	Denman Island disease	*C. gigas*, *C. sikamea*, *Ostrea lurida*	West coast of Canada; Californian coast, USA	Focal lesions up to 5 mm diameter within body wall, adductor muscle or surface of labial palps or mantle. Mortalities up to 30%.

*Indicates pathogen dealt with in text.
Details on Aber disease in Bower (2011 c, d), on QX in Rubio *et al.* (2013), on QPX in Bower (2010c) and on Denman Island disease in Bower (2013).

Bonamiosis

Bonamiosis is a disease caused by a group of haplosporidian protists (phylum Haplosporidia, class Haplosporea) in the genus *Bonamia*. The pathogen infects the haemocytes of oyster species and causes significant and widespread mortalities (>90%) in both the northern and southern hemispheres (Figure 11.2). The first reports of the disease were from the coasts of France where *Bonamia ostreae* was identified in *O. edulis* (Comps *et al.* 1980). The pathogen has also been found in other species of flat oyster: *Ostrea angasi*, *Ostrea chilensis* (=*Tiostrea chilensis*, =*Tiostrea lutaria*, =*Ostrea lutaria*), *Ostrea conchaphila* (=*Ostrea lurida*), *Ostrea puelchana*, and also in the Asian oyster, *Crassostrea ariakensis*. Since the late 1970s the disease has spread from France southwards to Spain and Morocco, and northwards to Denmark, the United Kingdom and Ireland, and is also on the west coast of Canada and both coasts of the United States. A second species, *Bonamia perspora*, has been found in *Ostrea equestris* at a single location on the east coast of the United States (Carnegie *et al*. 2006). In the southern hemisphere *Bonamia exitiosa* infects *O. chilensis* in New Zealand, while *Bonamia roughleyi* parasitises *Saccostrea glomerata* in southeastern Australia (references in Culloty & Mulcahy 2007).

To date, no agreement has been reached regarding the complete life cycle of *B. ostreae*. The parasite probably enters the oyster by filtration, proliferates in both epithelial cells and haemocytes by binary fission, and because free parasite cells have been observed in the haemolymph it is believed that infected haemocytes eventually die and release their contents. The freed parasites possibly then infect other haemocytes and gill epithelial cells, or, alternatively, enter the water column where they subsequently infect other oysters (Montes *et al.* 1994). Uninfected oysters introduced in contaminated areas contract the disease after about 3 to 5 months and this has been experimentally demonstrated in field and laboratory studies

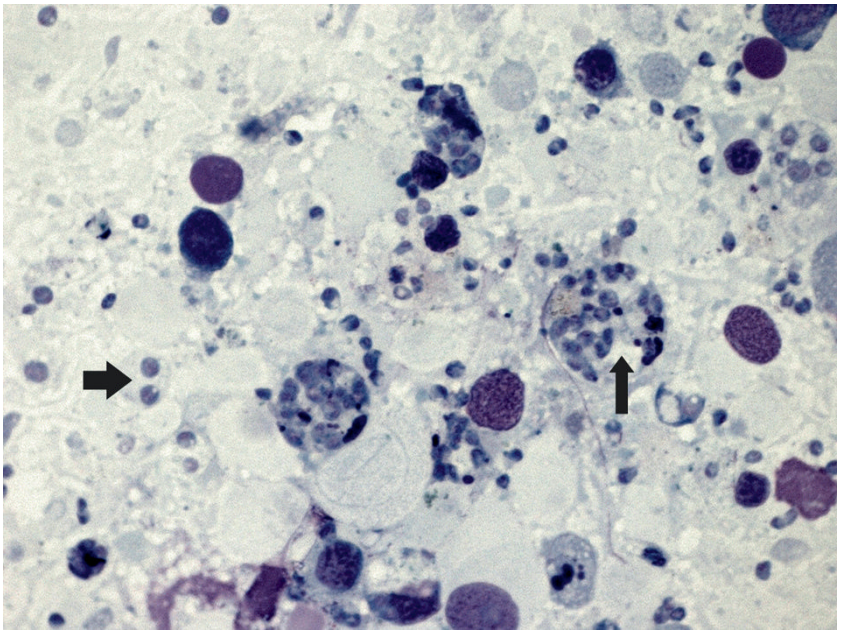


Figure 11.2 Heart smear showing extracellular (large arrow) and intracellular (small arrow) *Bonamia ostreae* cells within oyster, *Ostrea edulis*, haemocytes.
Photograph by S. Culloty, University College Cork, Ireland. Reproduced with permission of the Marine Institute, Dublin, Ireland.

(Lallias *et al*. 2008). It is worth mentioning that other species, for example, *Mytilus edulis*, *Mytilus galloprovincialis*, *R. philippinarum*, *Ruditapes decussatus* and *C. gigas*, cannot be infected either naturally or experimentally, and therefore do not appear to act as vectors or intermediate hosts for the parasite (Culloty *et al*. 1999). However, the possibility of a carrier/reservoir host cannot be dismissed (Bishop *et al*. 2006; Lynch *et al*. 2010).

In heavily infected oysters the disease is manifested as yellow discolouration, extensive lesions of the gill, mantle and digestive gland and mantle, breakdown of connective tissue and loss of condition. Oysters more than 2 years old are the most heavily infected (Culloty & Mulcahy 1996; Engelsma *et al*. 2010), but young prespawning spat can show a high prevalence and moderate intensity of infection (Lynch *et al*. 2005). Also, results of a recent study showed that even larvae can be infected, and could contribute to the spread of the parasite during their planktonic life (Arzul *et al*. 2011). The parasite is present and transmissible throughout the year, although the prevalence of infection is generally higher in spring than in autumn (Figure 11.3; Engelsma *et al*. 2010). Such seasonality may be influenced by temperature as evidenced by a study of parasitism in *C. ariakensis* in North Carolina, USA, where *Bonamia* appeared in oysters in late spring when water temperatures approached 25°C, with high prevalences (~70%) and rising to 100% by summer; by early autumn mortality had fallen to 17–82%, and by late autumn, when temperatures were less than 20°C, there were no infections at all (Carnegie *et al*. 2008).

To date it has not been possible to culture *B. ostreae*, but Mialhe *et al.* (1988) have developed a method for the isolation and purification of parasitic cells from infected oysters. In addition, primary cultures of haemocytes can be prepared with relative ease. Therefore, it has been possible to carry out in vitro studies on interactions between *B. ostreae* and haemocytes (see Section 'host–parasite interactions'). Purified parasites are also used to test the effect of various environmental factors on survival of the parasite (Arzul *et al*. 2009).

Microscopic and molecular techniques have been developed to detect the presence of the parasite (Culloty & Mulcahy 2007; Bower 2011a). Light microscopy is used to examine haemolymph smears from heart tissue, or stained sections or smears from heart, digestive gland and gill. Transmission electron microscopy (TEM), while time-consuming, is a useful technique when *Bonamia*-like parasites are described in a new host species, and can help to differentiate *B. ostreae* from other members of the genus, for example, *B. exitios*a (Lohrmann

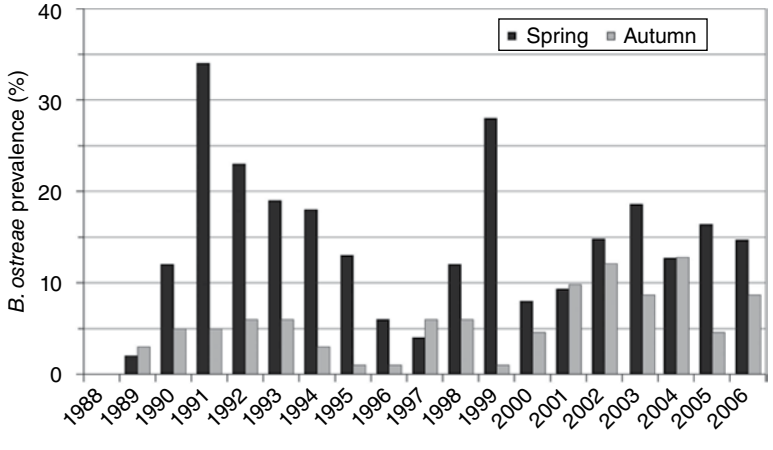


Figure 11.3 Prevalence of *Bonamia ostreae* in the oyster, *Ostrea edulis*, from Lake Grevelingen, the Netherlands, in spring and autumn during the period 1988–2006 as detected by histopathology. Engelsma *et al.* (2010). Reproduced with permission of Inter-Research, Germany.

et al. 2009). Various DNA probes have been developed that amplify segments of the small subunit ribosomal RNA (SSU rRNA) gene, the 18S rRNA gene, the internal transcribed spacer (ITS) region between the SSU rRNA and large subunit (LSU) rRNA genes and two actin genes. PCR assays are more sensitive than light microscopy methods, especially in the detection of light infections (Carnegie *et al*. 2000; Lynch *et al*. 2005; Balseiro *et al*. 2006). PCR assays must be thoroughly tested for sensitivity and specificity, and ultimately validated against a technique, typically histology, which allows visualisation of the parasite in host tissues (Burreson 2008). One limitation of conventional PCR is that it is not quantitative. In contrast, real-time TaqMan® PCR assays have been developed that are rapid, sensitive and can quantify the parasite load in samples (Corbeil *et al*. 2006; Marty *et al*. 2006). Restriction fragment length polymorphism (RFLP) analysis of PCR products of the SSU rRNA gene has been used to differentiate species of *Bonamia* (Hine *et al*. 2001; Cochennec-Laureau *et al*. 2003a). Another technique, ISH, is used to locate light infections of the parasite within histological sections of gill and digestive gland tissue (Carnegie *et al*. 2003). ISH is particularly useful in tracking infection during the latent period of the disease (Culloty & Mulcahy 2007). A number of factors are important when considering which technique to select for screening. Ease of use, speed and cost must be weighed against reliability and sensitivity of the test. Also, reproducibility of methods between different laboratories is important. The OIE (Office International des Epizooties) *Manual of Diagnostic Tests for Aquatic Animals* (2009) recommends the following:

- Tissue imprints, histopathology and PCR for screening
- Tissue imprints and PCR for presumptive diagnosis
- PCR-RFLP, sequencing and TEM for confirmatory diagnosis.

Field trials to investigate the ability of oysters exposed to *B. ostreae* to develop resistance have started. Note that the term 'resistance' refers only to improved survival, and implies nothing about host–parasite interactions (Naciri–Graven *et al.* 1999). In a study of *O. edulis* populations in Ireland one population exposed to the parasite for more than 10 years performed better than other Irish populations in terms of survival and prevalence of infection, indicating that some level of resistance had built up in this particular population (Culloty *et al*. 2004). A few selective breeding programmes to increase resistance to bonamiosis have also been initiated. In France the government agency IFREMER (l'Institut Francais de Recherché pour l'Exploitation de la Mer) has produced several hatchery-propagated populations of *O. edulis* that are resistant to the parasite (Naciri–Graven *et al.* 1998, 1999). However, there is evidence for low genetic variability in these populations due to high relatedness and the small effective number of breeders used, which will have important implications for the future management of these populations (Launey *et al*. 2001; Lallias *et al*. 2010). Another breeding programme is selecting for resistance to *Bonamia roughleyi*, the causative agent of winter mortality disease in Sydney rock oysters, *S. glomerata* (Nell & Perkins 2006). The programme, based in southeastern Australia, is simultaneously selecting for resistance to QX (Queensland unknown) disease caused by the haplosporidian protist, *Marteilia sydneyi*. Results from the programme are promising and winter mortality disease-resistant breeding lines are available for commercial production; since 2007 mortality from the disease has been reduced by 50% through selective breeding (Nell 2007). Quantitative trait loci (QTLs; gene loci controlling quantitative traits such as growth, disease resistance) and ultimately identifying candidate genes involved in this trait play an increasingly important role in improving the efficiency of selective breeding programmes in aquaculture (see Chapter 10). So far, QTLs linked to bonamiosis resistance have been mapped (Lallias *et al*. 2009) and five potential candidate genes have also been identified (Morga *et al*. 2011a).

Bonamiasis continues to spread, both within and between countries, through movement of infected oysters. Transmission of the disease within infected areas seems to depend on proximity of oyster stocks and local hydrography. Since there are no known eradication measures, the emphasis of disease management is on prevention and control. There are a number of control measures in use to minimise the consequences of bonamiosis in an area (Culloty & Mulcahy 2007; Bower 2011a):

- Mortalities can be reduced using suspension culture (>2 m water depth) or subtidal growing areas, avoiding stress factors, and lowering stocking densities.
- Oysters can be cultured successfully from contaminated areas if they are marketed before they are 2 years old, the age when mortalities from bonamiosis rise sharply.
- Oyster seed from natural settlement should be avoided because these oysters tend to be significantly more parasitised than seed produced by hatcheries.
- Oyster beds free of the disease should be maintained as single-species areas, and movement of other mollusc species into these areas should be avoided to minimise the danger of introducing *Bonamia* spp. or other pathogens.
- Boats and equipment, which can carry and passively transfer pathogens, including *Bonamia*, should be used in one area only, and not be moved between areas.

In Europe the Directive 2006/88/EC deals with animal health requirements for aquaculture animals, and the prevention and control of certain diseases (European Commission 2006). There is no equivalent directive on disease control in the United States. A number of agencies, for example, Fish and Wildlife Service (FWS), Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), are permanent members of the National Aquatic Animal Health Plan (NAAHP) and the Subcommittee on Aquatic Animal Health (SAAH), which set health policy for all US aquaculture and allied maricultures, and work with international partners on import and export regulations (F. W. S. Joel Bader personal communication).

MSX disease

Haplosporidium nelsoni (phylum Haplosporidia, class Haplosporea) is a parasite that causes the disease MSX (multinucleate sphere X), an often fatal disease of *Crassostrea virginica*. Unknown prior to 1957, MSX killed 90–95% of this species in Delaware and lower Chesapeake Bays, United States, between 1957 and 1960 (Haskin *et al.* 1966; Andrews & Wood 1967). The parasite has since spread northwards as far as Maine and Nova Scotia, and southwards to Florida. It has also been reported from cultured *C. gigas* in California and Washington, USA, France, Korea and Japan, but mortalities are low in this species (references in Burreson & Ford 2004). A closely related species, *Haplosporidium costale*, also infects *C. virginica* causing seaside organism disease (SSO). The parasite is found in high salinity waters (>25 psu) on the east coast of the United States, and can kill 20–50% of oysters in an affected population annually (ICES 2010).

Initial infections occur in the gill epithelium, where the parasite proliferates, eventually breaking through the basement membrane into the circulatory system and is spread to all organs. Infections confined to the gill epithelium are not lethal and have little measurable effect on the oyster (Ford *et al.* 1999). However, the effects of systemic infection on the host are severe in that food intake is reduced and parameters such as condition index and fecundity are affected. It is still not known how MSX actually kills its host. Highly susceptible oysters die rapidly and in 'good condition', possibly killed by a toxin that disrupts some key metabolic process. More resistant oysters take longer to die, and when they do, they are emaciated, indicating that the metabolic burden of prolonged parasitism finally killed them (Ford & Tripp 1996).

The parasite is thought to enter its host as a uninucleate spore that divides to produce a multinucleate plasmodium, from 5 to <30 μm in diameter depending on the number and size of nuclei they contain (Figures 11.4 and 11.5). This stage is found throughout all tissues.

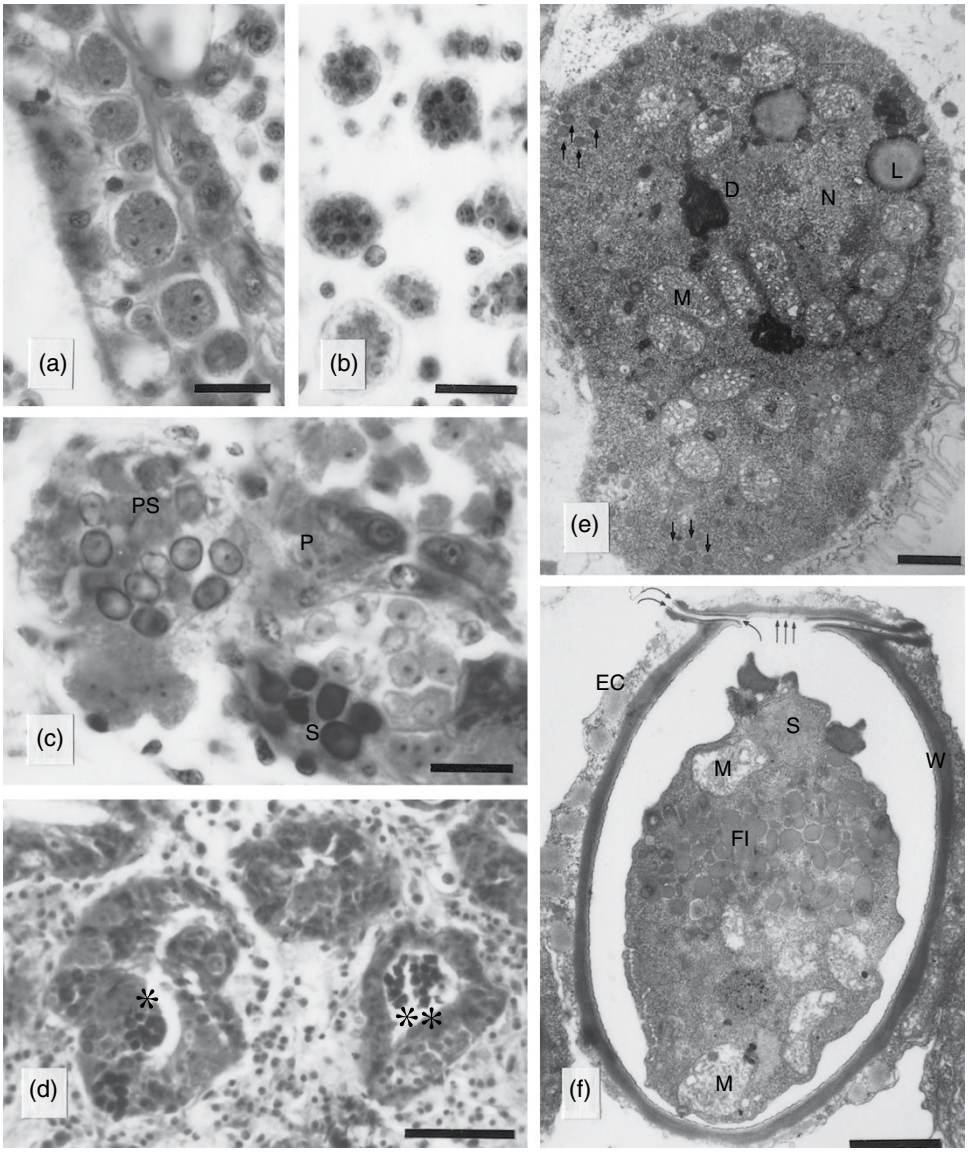


Figure 11.4 Stages of *Haplosporidium nelsoni* found in *Crassostrea virginica*. (a) Plasmodia in gill epithelium (earliest recognised statge). (b) Plasmodia in haemolymph vessels. (c) Plasmodial [P], prespore [PS] and spore [S] stages. (d) Sporulating stages in epithelium (*) and lumen (**) of digestive tubules. (e) Plasmodium showng mitochondria [M], nucleus [N], lipid body [L], digestive lamellae [D] and haploid spores (arrows). (f) Mature spore showing spherule [S], mitochondria [M], spore wall [W], operculum [straight arrows], juncture between operculum and lip of spore wall [curved arrows], formative inclusions [FI] thought to produce haplosporosomes and epispore cytoplasm [EC]. Scale bars =10 μm in (a to c); 50 μm in (d); 1 μm in (e and f). Reprinted with permission from Maryland Sea Grant and Elsevier. Photos courtesy of S. Ford, Institute of Marine and Coastal Sciences, Rutgers University, New Jersey, USA; (e) from Scro & Ford (1990); rest from Ford & Tripp (1996).

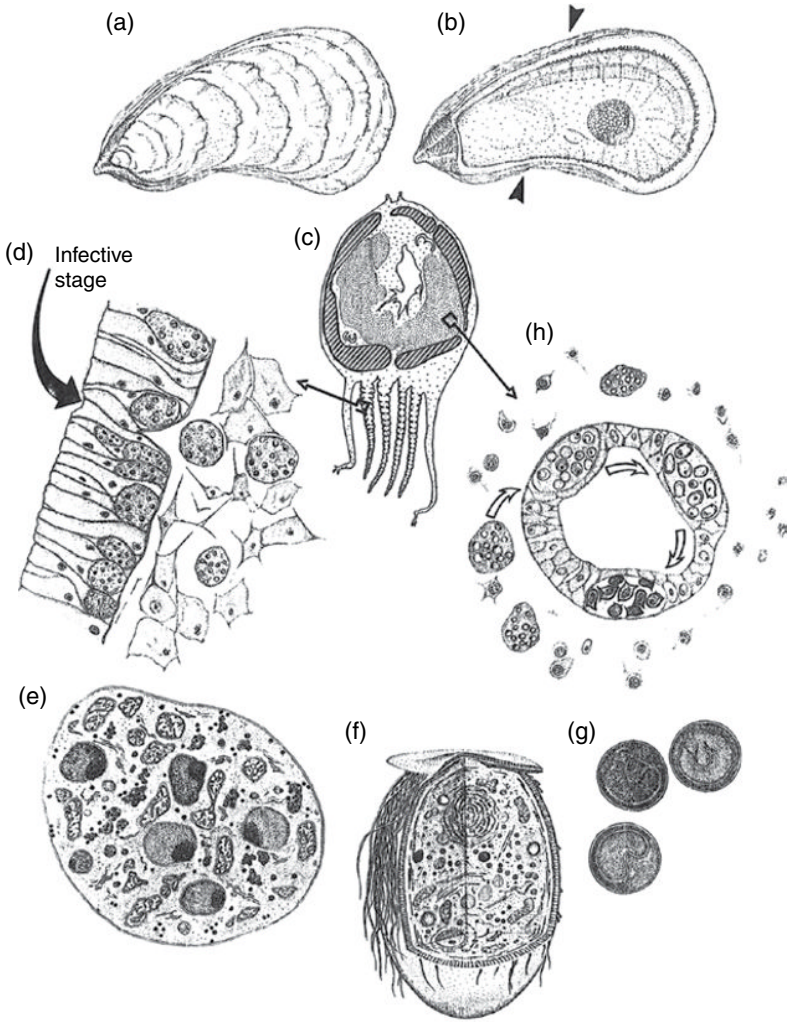


Figure 11.5 Diagram of stages of *Haplosporidium nelsoni* seen in *Crassostrea virginica:* (a) intact oyster; (b) oyster with right valve removed showing plane [arrows] of section in (c); (d) gill epithelium (entry site) showing plasmodia between cells and in spaces beneath epithelium; (e) plasmodium; (f) spore; (g) haploid spores; (h) development of plasmodia into sporocysts in epithelium of digestive tubules [open arrows].
Ford and Tripp (1996). Reproduced with permission of Maryland Sea Grant. Original drawing by Tim Littlewood.

Spore stages are rare in adults but are regularly formed in juveniles (20 mm) with advanced infections. The reason may be that juvenile oysters with their higher energy reserves provide a superior internal environment for sporulation than do adult oysters (Ford *et al.* 1999). Sporulation is confined to the epithelial lining of the digestive tubules and mature spores (5.5 × 8 μm) are shed from live or dying oysters. Transmission in the field does not depend on the presence of infected oysters and to date transmission has not been achieved experimentally. It is not known whether spores are directly infective to oysters, or whether they infect an alternate host, a suggestion supported by model simulations (Burreson & Ford 2004).

In the field new infections are acquired from June through to October each year (Figure 11.6a and b). Maximum mortality occurs in the summer, but declines as water temperatures decrease in the autumn. Infection levels do, however, remain high during the winter months, and parasite activity and oyster mortality resume when water temperature starts to increase the following spring (Barber *et al.* 1991). Superimposed on this pattern there is a periodicity to infection with peaks spaced 6–8 years apart; low disease activity appears to be associated with unusually low winter temperatures in the preceding year (Ford &

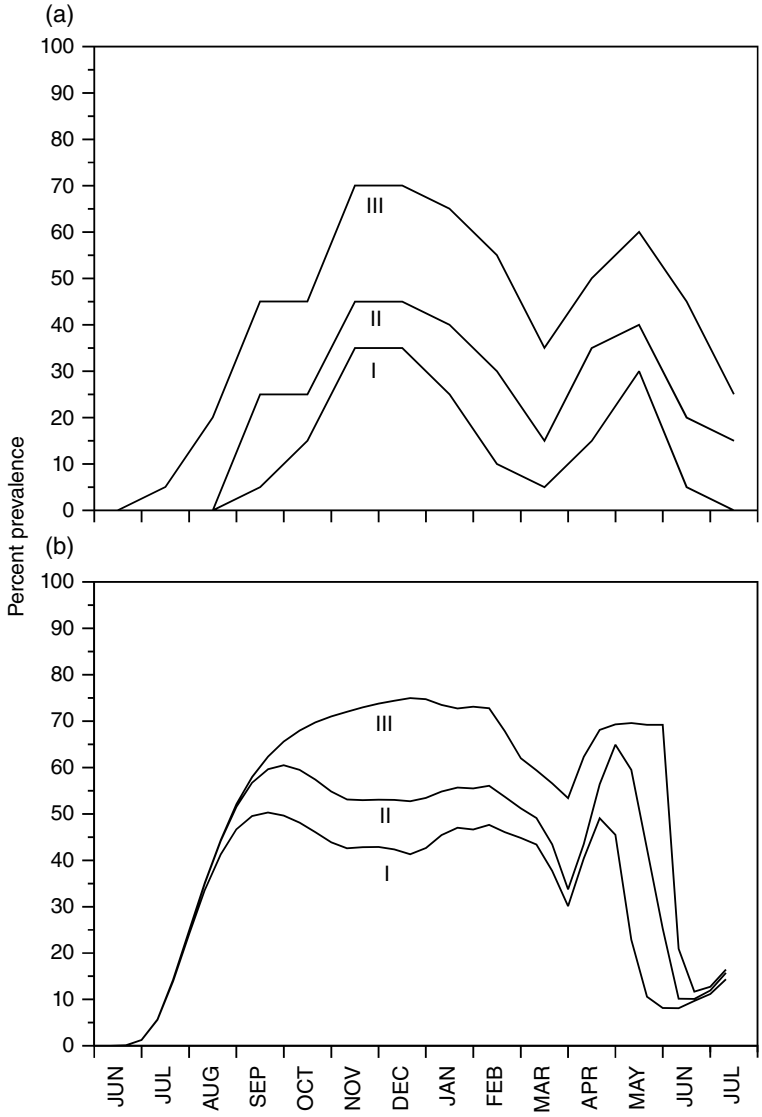


Figure 11.6 (a) Typical observed annual cycle of *Haplosporidium nelsoni* infection prevalence in Delaware Bay (DB), New Jersey; (b) model simulations using a temperature and salinity time series from lower DB, where salinity is always high enough to favour *H. nelsoni* activity, reproduced the observed annual infection cycle at DB, indicating that under a favorable salinity regime, the annual temperature cycle is the primary influence on seasonal prevalence patterns. Roman numerals designate increasingly advanced infection categories: I: Epithelial infections; II: Subepithelial, local infections; III: Systemic infections. Burreson and Ford (2004). Reproduced with permission of Cambridge University Press.

Haskin 1982). Salinity is another important controlling factor in the distribution of MSX. *C. virginica* is found in salinities from 5 to 30 psu but infections are extremely rare at salinities below 10 psu, and the disease does not become epizootic (spreading rapidly) at salinities below 15 psu. Results from laboratory and field studies indicate that this is most probably due to the physiological inability of the parasite to tolerate salinities below 10 psu (Sprague *et al.* 1969; Haskin & Ford 1982), rather than to enhanced effectiveness of host defence mechanisms (Ford & Haskin 1988). By holding infected oysters in cold, low-salinity waters (<15 psu) for as long as possible, and reducing the length of grow-out time in high salinity, warm water infection levels can be reduced; elimination of infection can be achieved by exposing oysters to mean salinities of less than or equal to 10 psu and temperatures greater than 20°C (Bower 2007). Complex mathematical models based on experimental and field data have been developed to describe the transmission of the disease and host–parasite–environmental interactions (Ford *et al.* 1999; Paraso *et al.* 1999; Powell *et al*. 1999; Hofmann *et al*. 2001, 2009). These provide a quantitative framework to guide future laboratory and field studies, as well as management efforts in disease control (Bower 2007).

Gross clinical signs cannot be used to diagnose either MSX or SSO, so histological sections are routinely used to detect the plasmodial and sporocyst stages of these two diseases. However, in areas here *H. nelsoni* and *H. costale* co-occur, the plasmodial stages of the two parasites, in the absence of the spore stage, cannot be reliably distinguished in stained tissue sections. Therefore, PCR assays using primers that target different regions of the SSU rRNA gene have been developed to differentially diagnose the two species (Burreson & Ford 2004). ISH allows visualisation of species-specific probes in mixed plasmodial infections of *H. nelsoni* and *H. costale* (Stokes & Burreson 2001). A multiplex PCR assay that simultaneously tests for *H. nelsoni*, *H. costale* and *Perkinsus marinus* (see later) in a single test reaction has also been developed (Penna *et al*. 2001). This assay was subsequently modified to include an internal standard control for DNA quality control, and results from field trials have indicated that the assay is a reliable alternative to histopathological examination (Russell *et al*. 2004). The OIE *Manual of Diagnostic Tests for Aquatic Animals* (2003) recommends histology for screening, histology and PCR for presumptive diagnosis, and ISH for confirmatory diagnosis.

The effects of the parasite on the oyster industry in the United States have been devastating. At least half of all oyster deaths in lower Delaware Bay from the early 1960s to the early 1980s were attributed to MSX (Ford & Haskin 1982). However, since about 1990 there has not been much mortality in the Bay. The parasite is still present because it continues to infect and kill susceptible oysters brought into the area. Using PCR technology and oysters and mussels as particle collectors, Ford *et al*. (2009) showed that the parasite indeed persists throughout Delaware Bay, although it is rarely detected in histological sections. It seems that the native Delaware and Chesapeake Bay oysters have in a relatively short space of time become highly resistant to the parasite (Carnegie & Burreson 2011; Ford & Bushek 2012). Resistant oysters are infected but are able to contain and localise infections, preventing them from becoming systemic (Ford & Haskin 1982). In addition, infected resistant oysters are able to tolerate parasite numbers that would be fatal to susceptible oysters (Ford & Haskin 1987). Also, dual resistance to *H. nelsoni* and *P. marinus*, the causative agent in Dermo disease, (see later) has been achieved through four generations of selection on the east coast of the United States, where both diseases are prevalent (Ragone Calvo *et al*. 2003). These findings stimulated the setting up of several selective breeding programmes to create disease-resistant oyster strains. All programmes use oysters that have first undergone extensive selection by *H. nelsoni*-caused mortality, either as wild stocks or in a selective breeding programme in a hatchery (Burreson & Ford 2004). Two lines, CROSSBreed (XB) and DBY, produced at the Virginia Institute of Marine Science (VIMS), USA, show high to moderate

tolerance to MSX and Dermo disease, respectively (ABC 2009). Resistance to *H. nelsoni* may, according to Ford and Haskin (1987), involve a combination of failure to provide a completely suitable environment for the parasite (i.e. being insusceptible), which inhibits the ability to complete its life cycle, and a capacity to tolerate parasitism while continuing to carry out life processes while parasitised. A range of parasite burdens are found in unselected oysters indicating that individuals vary in their ability to provide a suitable environment for plasmodial growth (Ford 1986).

Dermo (Perkinsosis) disease

A third serious protistan parasite, *P. marinus*, causes Dermo disease in *C. virginica*, and to a much lesser extent in *C. gigas*, *Crassostrea rhizophorae* and *Crassostrea corteziensis* (ICES 2011b). Originally placed within the phylum Apicomplexa, phylogenetic analyses of SSU rRNA gene sequences support a closer affinity between the parasite and members of the phylum Dinoflagellata (Villalba *et al*. 2004). The pathogen, first described from the Gulf of Mexico (Mackin *et al*. 1950), was found all along the southeast coast of the United States as far north as the Virginia portion of Chesapeake Bay during the late 1940s and early 1950s (see Ray 1996, for a lively account of the history of the disease). In the mid 1980s it spread northwards within Chesapeake Bay, and between 1990 and 1992 it was found from Delaware Bay to Cape Cod, a distance of about 500 km (Ford 1996). The parasite is now firmly established in oyster populations throughout the Gulf of Mexico and all along the east coast of the United States, and causes mortalities over most of that range. Typical prevalence rates are in the range 50–100% (Karolus *et al.* 2000). Other species, such as *Perkinsus olseni* (=*Perkinsus atlanticus*), parasitise several clam species throughout Australia, Korea, China, Japan and Europe (details in Villalba *et al*. 2004; McCoy *et al*. 2007). The disease affects a wide variety of bivalve species worldwide resulting in severe economic losses. Not surprisingly, considerable research effort has been, and continues to be, focused on the study of *Perkinsus* parasites.

The earliest recognised stage in oysters is unicellular trophozoites that proliferate by multiple fission intra- or extracellularly throughout the host tissue (Figure 11.7). This process may occur in any tissue but is most often found in connective tissue, between epithelial cells of the gut, gill, digestive gland and in lesions of the gut and gill epithelia (Perkins 1996). Under anaerobic conditions, or in necrotic tissue, trophozoites develop into hypnospores, a dormant stage characterised by enlarged size and thick walls (Villalba *et al*. 2004; Choi & Park 2010). When the hypnospores are placed in seawater, zoosporulation begins and hundreds of biflagellated zoospores are produced that are eventually discharged through a tube (Figure 11.7). The liberated zoospores swim to, or are pulled into, an oyster's mantle cavity, thus setting up new infections. Primary infection is most likely through the gill, mantle and gut epithelia. The parasite destroys the epithelium, lyses the basement membrane and is distributed via haemocytes to all parts of the body. Early infections occur in the digestive tract, gills, palps and mantle. Advanced infections are associated with diffuse systemic parasitism, pronounced haemocytosis and tissue lysis, with a consequent reduction in feeding, growth and reproduction, leaving oysters weak and emaciated. Mortalities may range from 5 to 30% in the first year of a disease outbreak, rising to 60–80% by the end of the second year (ICES 2011b).

Transmission is direct from one oyster to another and all life stages have been shown experimentally to cause infection (see Section 'host–parasite interactions'). Viable cells are released in host faeces or on death of the host (Park *et al*. 2010). Temperature appears to be the major large-scale controlling factor in the epizootiology of the disease, explaining about 40% of the variability in prevalence and intensity (Burreson & Calvo 1996). In Chesapeake and Delaware Bays infections and accompanying mortalities rise during the summer months,

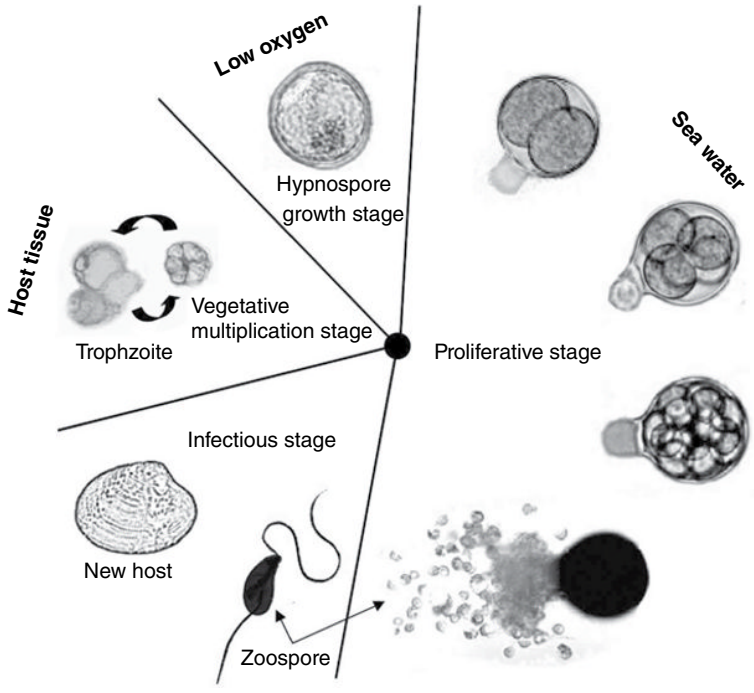


Figure 11.7 Life cycle of *Perkinsus olseni*, a parasite of the Manilla clam, *Ruditapes philippinarum*. Choi and Parks (2010). Figure by S-K Choi, School of Marine Biomedical Science, Jeju National University, Jeju, Republic of Korea. Reproduced with permission of Terra Scientific Publishing Company (TERRAPUB), Japan.

peak in early autumn and decline dramatically over the late winter and early spring. However, some parasites remain and proliferate as temperatures rise in the spring (references in Ford & Tripp 1996). The sudden appearance of the disease between 1990 and 1992 at sites from Delaware Bay to Cape Cod has been ascribed to above-average winter rather than summer temperatures (Ford 1996; Cook *et al.* 1998). Undoubtedly, long-term changes in climate could have a significant impact on *P. marinus* prevalence (Powell *et al.* 1992; see also Soniat *et al*. 2006). Salinity is another important controlling factor in the incidence of the disease, and within estuaries (i.e. on a local scale) is probably as important as temperature. Prevalence and intensity of infections are highest at salinities above 12 psu, but once established in a low-salinity area the parasite persists and can tolerate salinities below 5 psu for periods longer than 3 months (Villalba *et al*. 2004). It would appear that the interaction of temperature and salinity are more important in regulating *P. marinus* epizootics than either factor acting alone (Calvo & Burreson 1994; Chu 1996). This synergistic interplay could possibly explain the periodic nature of outbreaks that are so characteristic of this disease. Prevalence also varies with oyster age. Death rates are low during the first year of life but increase with age and size of the oyster. Other factors such as food availability (Hofmann *et al.* 1995) and oyster density (Andrews 1965) have been cited as being important in the incidence of the disease. Several studies have reported a link between environmental contaminants and disease development (Wilson *et al.* 1990; Chu & Hale 1994; Anderson *et al.* 1996; Fisher *et al.* 1999), although a study of the impact of several common anthropogenic contaminants on in vitro proliferation of *P. marinus* showed that only a herbicide (Weed-B-Gone®) had a significant negative effect, and then only above the manufacturer's recommended application

rate (Bushek *et al*. 2007). Ford and Tripp (1996) contend that while some contaminants may accelerate disease development there is no relationship between the distribution of the disease in nature and pollution.

There are no gross clinical signs or behavioural changes that are specific to infection with *P. marinus*. Initially diagnosis of *P. marinus* infections relied on examination of haemolymph smears and tissue sections, but since the early 1950s Ray's fluid thioglycollate medium (RFTM) assay (Ray 1966) became the detection method of choice because it is simple, inexpensive and very sensitive, but not species specific (OIE 2009). When infected oyster tissue is incubated in the medium *P. marinus* trophozoites are induced to differentiate into large thick-walled hypnospores that are easily stained with Lugol's iodine. The assay is also used on haemolymph to detect circulating parasites (Gauthier & Fisher 1990) and to isolate and quantify hypnospores from oyster tissue (Choi *et al*. (1989). *P. marinus* cells are easily cultured in a variety of media (OIE 2009), although freshly isolated parasites are much more virulent than those propagated in culture (Ford *et al*. 2002). An ELISA assay using polyclonal antibodies raised against purified extracellular proteins, recovered from *P. marinus* cell culture, has been shown to be more sensitive than the traditional RFTM assay (Ottinger *et al*. 2001). PCR assays based on primers pairs that specifically target *P. marinus* sequences such as the nontranscribed spacer (NTS) or ITS regions of the rRNA gene have been developed (see Bower 2011b for references). General primers have also been designed to detect virtually all *Perkinsus* species (Casas *et al*. 2002a). Also, a sensitive and efficient real-time polymerase chain reaction assay that quantifies *P. marinus* and *Perkinsus* spp. cell density in oyster tissue is now available (Gauthier *et al*. 2006). However, before molecular assays become the diagnostic method of choice it is important to validate them against more traditional methods. In one such study De Faveri *et al*. (2009) showed that a quantitative RT-PCR assay developed by them was a sensitive and specific alternative to the RFTM assay. Species-specific DNA probes that target LSU rRNA gene sequences have been developed for ISH diagnostic tests (Moss *et al*. 2006). The OIE *Manual of Diagnostic Tests for Aquatic Animals* (2009) recommends RFTM, tissue assay for screening, PCR for presumptive diagnosis, and ISH for confirmatory diagnosis.

There are indications that oysters exposed to *P. marinus* for several decades have developed some resistance to the parasite. Bushek and Allen (1996) found that in four genetically distinct populations each with different natural histories of exposure to the pathogen, oysters showed levels of resistance roughly corresponding to the duration of exposure. Resistance/tolerance to infection has since been shown to have a significant genetic component and to depend on the host genotype and genotype–environment interactions (Oliver *et al*. 2000). Several genetic markers associated with resistance have now been identified (Sokolova *et al*. 2006; Yu & Guo 2006). A number of selective breeding programmes are ongoing in Chesapeake and Delaware Bays, but while strains of *C. virginica* with improved resistance to *H. nelsoni* have been produced, progress in the development of *P. marinus*-resistant lines has been slow. This is particularly problematic for areas where the two parasites co-occur. However, breeding programmes at VIMS, USA, have produced two strains or lines, Lola and Hana, that show high resistance to Dermo disease and low to moderate resistance to MSX (Table 11.4). Both strains, which also exhibit fast growth, were derived from Louisiana oysters with innate Dermo resistance (ABC 2009). As mentioned earlier, VIMS have also produced DBY and CROSSBreed (XB) strains that show dual resistance to both parasites (Ragone Calvo *et al*. 2003). Recently, a tetraploid disease-resistant line, produced through crossing DBY and XB, has been used to produce triploids, which are also disease resistant, but because they are sterile have the added advantage of significantly increased growth (ABC 2009). Additional disease-resistant lines of *C. virginica* have been produced (details in Rawson *et al*. 2010) by Rutgers University (NEH line; MSX/Dermo), Frank M Flower & Sons Inc.,

Table 11.4 Composite lines of *Crassostrea virginica*, the site of their selection with either high (unshaded) or low (shaded) disease pressure and attributes.

Line	Selection site	Attributes	Comments
DBY-H	YR & LR	Disease tolerance • High for MSX • Moderate for Dermo	General all-purpose line across a range of salinities
CROSBreed-H (XB)	YR & LR	Disease tolerance • High for MSX • Moderate for Dermo	Seems to favour higher salinities
Hana	YR & LR	Disease tolerance • Moderate for MSX • High for Dermo Fast growth	Derived from several imports of Louisiana wild oysters
DBY-L	YR first, then two generations in K	Disease tolerance • High for MSX • Moderate for Dermo	Recently selected for only low salinity
CROSBreed-L (XB)	NJ first, then YR, then two generations in K	Disease tolerance • High for MSX • Moderate for Dermo	Recently selected for only low salinity
Lola	K	Disease tolerance • Low-moderate for MSX • High for Dermo Fast growth	After two generations of MSX pressure, selected in low salinity for growth

ABC (2009), with permission from S. Allen, VIMS.
YR, York River, Virginia (VA); LR, Lynnhaven River (VA); K, Kinsale (VA); NJ, New Jersey, USA.
These lines are available from the Virginia Institute of Marine Science (VIMS), USA.

New York (Flower line; MSX/ROD, Table 11.2), the University of Maine (UMFS; ROD) and the State of Connecticut Bureau of Aquaculture (Clinton line; MSX/Dermo/ROD).

The continued decline in oyster populations, especially on the Atlantic coasts of the United States, has led to an increased need for effective management strategies. In oyster-growing areas culture schedules have been adapted to reduce the impact of the disease. Some measures currently employed are particle filtration (1-μm filters) and UV irradiation of water coming into and exiting hatcheries, chemotherapeutants in hatcheries, low-density planting, early harvest at minimal commercial size, harvesting or moving oysters to low-salinity areas (<9 psu) before water temperatures increase to greater than 15°C, planting of large seed oysters in high-salinity water as late as possible in the growing season to avoid the acquisition and development of new infections and utilisation of low-salinity growing areas (Villalba 2008; Bower 2011b; ICES 2011b). Other strategies include the use of disease-resistant strains (discussed earlier), interline crossing of disease-resistant strains and disease-resistant triploids, which are sterile and thus grow faster and have a better taste than diploids. Various simulation models have been developed based on known factors influencing *P. marinus* disease dynamics. Such models may be useful predictive tools and thus useful in designing management strategies for affected oyster beds (Villalba 2008).

Porifera

Boring sponges (*Cliona* spp.) have been reported globally in the shells of mussels, oysters and scallops, especially those growing on the bottom substrate. The sponge excavates into the shell and in heavy infestations may even penetrate through to the inner shell. If

perforation is extensive the shell is weakened and affords little protection from crabs and other predators. In addition, infestation may interfere with adductor muscle attachment, impede feeding and cause mortality. Sponges bore to obtain shelter and do not obtain nutrients directly from their hosts. In the pearl oyster, *Pinctada maxima*, pearl production and quality may be seriously affected in heavy infestations. This is because oysters expend energy in depositing thickened nacre as a protection against the invader at the expense of depositing nacre around the inserted pearl nuclei (see Chapter 2). Growing bivalves in suspended culture is the simplest method to reduce shell damage (Bower 2001b). However, Carver *et al*. (2010) reported a 25–30% infestation rate by *Cliona celata* in off-bottom cultured *C. virginica* in New Brunswick, Canada. A comparison of various treatment strategies indicated that a 6-min brine dip (>90% NaCl saturation) every second year was completely effective at eliminating the sponge without harming the oysters.

Helminths

The main helminth parasites of bivalves are trematodes, cestodes and nematodes. The first two belong to the phylum Platyhelmintha, while the latter are in the phylum Nematoda. In terms of their disease-causing potential helminths are nowhere near as important as protozoan parasites. However, an enormous literature is available on this group, chiefly because they are visible to the naked eye, often have complex life cycles with several hosts, and in some cases may be passed on to humans through consumption of infected shellfish.

Since trematodes, in particular the larval stages, are more important as bivalves pathogens than cestodes and nematodes, most of the following section will be devoted to them. For more details see Bower (2010a) and the extensive review by Lauckner (1983) on helminth parasites.

Trematodes

Trematodes may use bivalves as the first host (sporocyst, redial and cercarial stages) or as the secondary host (metacercarial stage), or the bivalve may be the host for all stages of the life cycle (Figure 11.8). An example of the latter is *Proctoeces maculatus*, which is widely distributed in temperate and tropical marine waters. The larval stages (sporocysts and cercariae) have been found in mussels such as *Mytilus* and *Brachidontes* species, while the metacercariae have been reported in a range of molluscs, polychaetes, echinoderms, and cephalopods and in more than 50 marine fish species (references in Aldana *et al.* 2009). Cercaria infect mussels and numerous sporocysts, as many as 28 000/mussel develop in the vascular tissue of the mantle, giving it an orange hue (Machkevski 1985). Such numbers can seriously reduce energy reserves resulting in disturbance to gametogenesis, and possible castration and death. In some regions, for example, western Portugal, castrated mussels do not die despite heavy infections, which indicates possible host adaptation to the parasite (Teia dos Santos & Coimbra 1995). In other areas heavy infections (>60%) have been shown to limit the culture of mussels to a seasonal product (Sunila *et al*. 2004). In wild populations Calvo-Ugarteburu and McQuaid (1998) found that *Perna perna* in S. Africa had infection rates as high as 62%, but that introduced *M. galloprovincialis* was free of the parasite. This may have conferred a competitive advantage to *M. galloprovincialis*, thus explaining the rapid, invasive spread from its introduction on the west coast in the early 1970s onto the south and east coasts of South Africa in the 1990s. There is a strong likelihood that *M. galloprovincialis* will replace *P. perna* on the south coast as it has done with *Aulacomya* on the west coast (Bownes & McQuaid 2006).

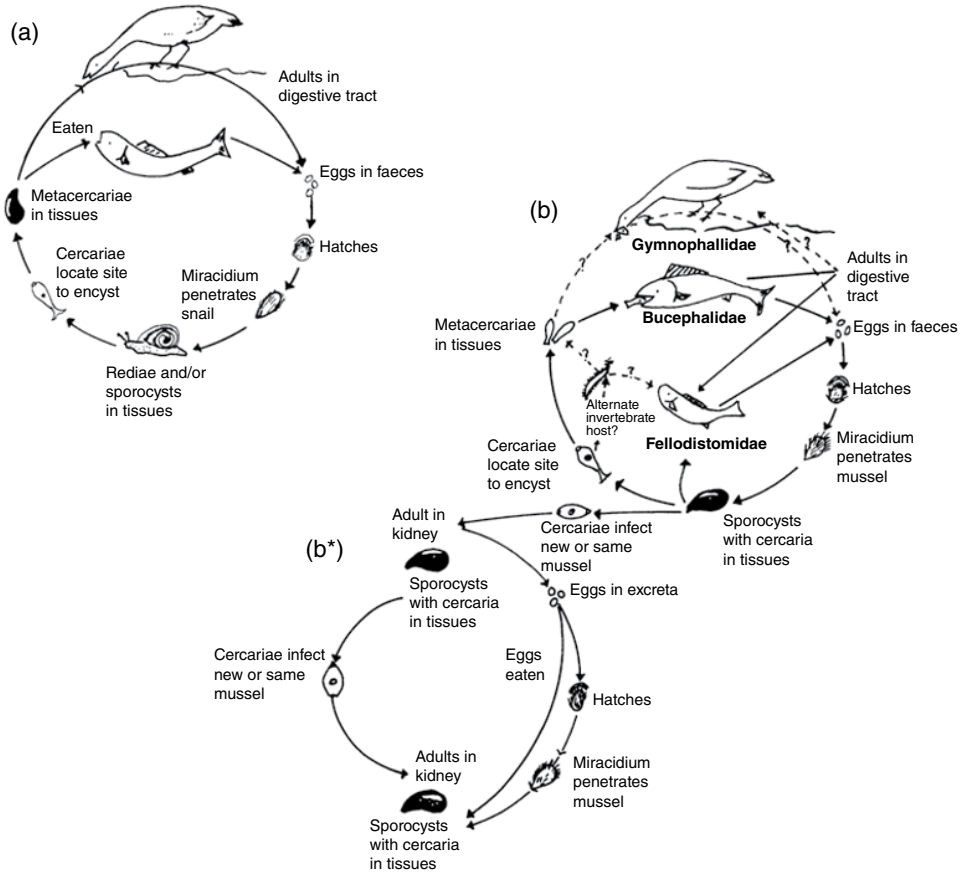


Figure 11.8 Generalised life cycles of trematodes that infect mussels (*Mytilus* sp.). (a) Represents the life cycle of the majority of trematode species in the marine environment, where the metacercariae encyst in, or on, mussels as well as other animals and/or plants. (b) Represents the life cycle for members of the family Fellodistomidae, Bucephalidae and possibly Gymnophallidae as indicated. (b*) Represents the abbreviated development of *Proctoeces maculatus*, which is capable of completing its entire life cycle in mussels. However, like other Fellodistomidae, adult forms also occur in the intestinal tract of fishes (usually tropical mollusc-eating fishes of the family Sparidae and Labridae) as illustrated in (b). Bower (1992). Reprinted with permission from Elsevier Science.

Other species of trematode use mussels as secondary or final hosts but unlike *P. maculatus*, these are nonpathogenic although infection can cause organ deformation, reduced byssal production and induction of pearl formation. Pearls are formed because of an infection, probably by *Gymnophallus bursicola*. The fluke enters the mussel as a small larva and lodges in the mantle epithelium. As a protectant the mussel encapsulates the fluke with layers of nacreous shell material, and so a pearl is formed. Heavy infestation does not seem to harm the mussel, but it seriously reduces the commercial value of a mussel population. This problem can be eliminated if mussels are grown to harvest over a shorter period of time, for example, on ropes; this means that the mussels are marketed before the pearls reach a detectable size (Lutz 1980).

Trematode parasites can have more wide-ranging effects, affecting not just their hosts but also the benthic community structure in the host habitat. For example, burrowing and movement ability of the New Zealand cockle, *Austrovenus stutchburyi*, is reduced when infected by echinostome trematodes. Heavily infected cockles accumulate on the sediment surface where they are subject to increased bird predation. Mouritsen and Poulin (2010) found that

cockle parasitism positively affected species abundance and diversity, probably governed, either directly or indirectly, by reduced sediment disturbance, increased surface structural complexity and availability of larval trematodes as an additional food source.

Information on other bivalve trematode parasites is presented in Table 11.5. Several points are worth noting from this table: *Bucephalus* species are the most common parasites of commercial bivalves; the adult stage of the trematode life cycle is completed in fish or bird species; the main symptoms of infection are tissue destruction and castration; and, while mortalities are generally low, heavy infestation in some instances may be responsible for population crashes.

Table 11.5 Some trematode parasites of bivalves.

Parasite	Host species	Locality	Pathology	References
Bucephalus cucullus	*Crassostrea virginica* and *Mugil* sp. of fish	Atlantic coasts of N. America	Infection of gonad, digestive gland and eventually all organs; castration; up to 30% prevalence	Hopkins (1957)
B. longicornutus	*Ostrea lutaria* and various fish species	New Zealand	Responsble for a decline in oyster numbers	Millar (1963) and Howell (1967)
B. varicus	*Pinctada martensii* and *Caranx* species of fish	Japan	Loss of condition leading to death; low quality pearls in surviving oysters	Sakaguchi (1967) and (1968)
B. haimeanus (=*cucullus*?)	*Crassostrea madrasensis*	India	Retarded growth and castration	Samuel (1976)
Bucephalus sp.	*Pecten fumatus*	Australia	Castration; prevalence up to 60% in scallops >80 mm shell height	Heasman *et al.* (1996)
Bucephalus sp.	*Pecten alba*	Australia	Castration; loss of energy reserves	Sanders and Lester (1981)
Gymnophalloides tokiensis	*Crassostrea gigas* and *C. virginica* and marine birds	Japan	Retardation in growth and inhibition of reproduction; prevalences up to 100%	Hoshina and Ogino (1951) and Bower (2009b)
Himasthla quissetensis	*Mya arenaria*, *Argopecten irradians*, *Cardium edule* and gastropods *Nassarius reticulatus*, *Cyclope neritea* and herring gull *Larus argentatus*	Maine, USA; Atlantic and Mediterranean coasts of France	Infects gills and palps; up to 100% prevalence	Getchell (1991), de Montaudouin *et al.* (2005), and McGladdery *et al.* (2006)
H. elongata	*C. edule*, *Mytilus edulis*, gastropods *Littorina littorea* and *L. saxatilis* and gulls	N. Europe	Infects the foot; up to 100% prevalence; seriously affects burrowing in *C. edule* and byssal production in *M. edulis*, leading to reduced survival	Lauckner (1983) and de Montaudouin *et al.* (2005)
Postmonorchis donacis	*Donax gouldi* and various fish species	California, USA	Sterilisation; trematode may be responsible for population crashes	References in Sindermann (1990)

Cestodes

Marine bivalves are hosts for the pre-adult stages of the tapeworm, while adults are parasites in the intestine of elasmobranch fish (sharks, skates and ray). Infection in bivalves is therefore most common in tropical and subtropical waters, where elasmobranchs constitute an important proportion of the vertebrate fauna (Lauckner 1983). Larval cestodes of the genus *Tylocephalum* occur, very often in great numbers, in a variety of marine bivalves, especially oysters (*Crassostrea*, *Pinctada*). Bivalves are initially infected when they ingest free-swimming larval coracidia or cestode eggs excreted by an elasmobranch. Once inside the host they develop and encyst as metacestodes in the tissue of the digestive system. The host's response is to secrete a thick fibrous capsule around each metacestode. Infestation can be heavy with up to 200 metacestodes reported from a single *Pinctada margaritifera* (Lauckner 1983). Despite heavy infestation, larval cestodes elicit few ill effects on their host. For example, among 60 heavily infected *C. virginica* none was weak or moribund, or exhibited any significant loss of body volume and weight (Cake & Menzel 1980). Other cestode parasites include species of *Echeneibothrium*, *Parachristianella*, *Acanthobothrium* and *Rhinebothrium* (Lauckner 1983; Sindermann 1990). There is no evidence that any of these can infest humans.

Nematodes

Nematodes are uncommon parasites of marine bivalves, but two are worth mentioning: *Echinocephalus sinensis* in oysters and *Sulcascaris sulcata* in scallops and clams. The larval stages of both species are found in bivalves, while the adult stages are found in elasmobranch and turtle species. Coiled larvae of *E. sinensis* inhabit the gonoduct lumen of *C. gigas* and cause tissue damage, possibly due to the movement of the spiny anterior end of the worm. It is not clear whether *E. sinensis* disrupts reproduction, or whether there is higher mortality among heavily infected oysters. What is particularly interesting about this parasite is that it is a potential health hazard to humans as experiments have shown that the larvae are capable of infecting cats and monkeys (Ko 1976). *S. sulcata* is a parasite of the clam, *Spisula solidissima*, on the east coast of the United States from Virginia southwards (Bower 2001c). It inhabits all tissues and sometimes gives a brown colour to the meats, due to the presence of another parasite, a haplosporidian *Urosporidium spisuli*, infecting the worms. In the 1970s an epizootic of *U. spisuli* on *S. sulcata* occurred along the mid Atlantic coast of the United States causing loss of revenue due to closure of the clam fishery. However, this has not reoccurred, although *S. sulcata* continues to be a significant parasite of *S. solidissima* but with generally low prevalence (<3%). *S. sulcata* also parasitises several scallop species (McGladdery *et al.* 2006) but is restricted to the adductor muscle. High infection levels – values of 64% have been reported in *Amusium balotti* – can reduce the commercial value of scallops.

Annelids

Several species of the polychaete annelid *Polydora* burrow into the shells of bivalves, and excavate U-shaped tunnels that subsequently become filled with compacted mud, causing 'mudblisters' on the shell. Oyster shells containing them are unsuitable for the lucrative half-shell market, as mudblisters are unsightly and, if punctured, can release sediments, faecal deposits and anaerobic metabolites such as hydrogen sulphide (Handley & Bergquist 1997). The burrows also weaken the shell, thereby increasing susceptibility to predation, especially in thin-shelled species such as mussels, although crabs have been shown to show

preference for non-infested mussels (Ambariyanto & Seed 1991). In oysters this weakening can cause problems during shucking, packing and transport. Other effects of the parasite include atrophy and detachment of muscle when burrowing occurs in the region of the adductor muscle, loss of condition, retarded growth and mortality (Lauckner 1983; Riascos *et al*. 2008). Heavy infestations, up to 300 worms per host, exacerbate these effects. Mortalities as high as 84% in the scallop *Patinopecten yessoensis* were attributed to heavy infestations by *Polydora websteri* (Bower 1992).

Various methods have been tested to try to eradicate the parasite in bivalve-growing areas. Infestations are responsive to factors such as temperature, salinity, host density and intertidal level of cultivation. Handley and Bergquist (1997) have found that increasing the aerial exposure of oyster stocks (*C. gigas*) significantly decreased the incidence of new infections. Nel *et al.* (1996) exposed infested oysters of the same species to freshwater for 12 h, or to heated (70°C) seawater for 40 s. Oysters were then placed in the field for a 2-month recovery period. *Polydora* infestation was significantly reduced by both treatments, with heat treatment yielding the lowest average infestation (~1 worm/oyster) compared to the untreated control (~3/oyster).

Crustaceans

Compared with bacterial or protozoan parasites, crustaceans are only mildly pathogenic to bivalves. The best-documented disease organism is the copepod *Mytilicola intestinalis*, found in European waters. The mussels *M. edulis* and *M. galloprovincialis* are the primary hosts, although oyster and clam species can also be infested (Bower 2009c). Another copepod, *M. orientalis*, originally confined to Japan, has spread to mussels (*Mytilus trossulus* and *Mytilus californianus*) on the Pacific coast of North America, and has been introduced into France with imported oysters (*C. gigas*). It is also found in *M. edulis* and *M. galloprovincialis*, where dual infections with *M. intestinalis* occur (Bower & Figueras 1989; Goater & Weber 1996 see also Lauckner 1983 for details on the spread of *Mytilicola*). The copepod is red coloured and large; females reach a size of 7–8 mm and males 3–4 mm. Mature females carry paired egg sacs (Figure 11.9). The eggs hatch in the host and are expelled into the water column. The infective stage is a free-swimming larva that enters the mussel by the inhalant siphon. Infestation is a passive process depending on the chance encounter by the larvae with the host's field of filtration, and also on the strength of the inhalant current (Gee & Davey 1986). Not surprisingly, infestation success is dependent on host size and density. In addition, intensity of infestation is negatively correlated with exposure, that is, mussels higher up the shore are less likely to be infected than those lower down. Prevalence of 100% is common, and more than 30 copepods may be dissected from a single mussel.

M. intestinalis has been blamed for widespread mortality and loss of condition in European populations of mussels. For example, the crash of the Dutch and German mussel fisheries in 1949 and 1950 was attributed to the copepod (Korringa 1951). For the next 30 years or so conflicting reports on the pathogenicity of the copepod appeared. Some of these reported serious effects on the health, growth and condition of parasitised mussels (Cole & Savage 1951; Bayne *et al.* 1978; Theise 1987; Robledo *et al.* 1995), especially when parasite burdens exceeded 25 individuals per host (Gee *et al.* 1977). However, in no instance was the copepod deemed to be responsible for the death of its host. In contrast, others have reported no loss of condition in parasitised mussels (Campbell 1970; Figueras *et al.* 1991; Gilek *et al.* 1992), even when parasite numbers exceeded 20 individuals per host (Dethlefsen 1975). It now seems likely that the *Mytilicola–Mytilus* relationship is commensal rather than parasitic (Davey 1989), with the copepod feeding on host gut contents rather than on host tissue (Moore *et al*. 1978). Reported negative effects of this, and other *Mytilicola*, species on

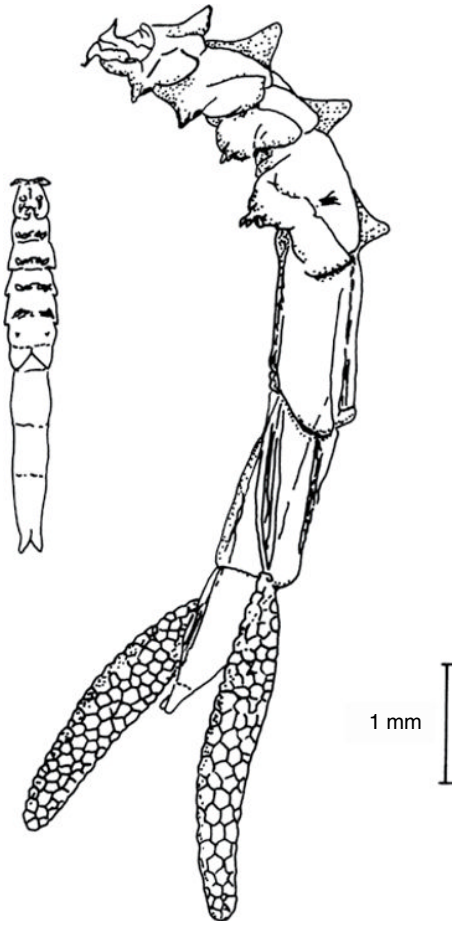


Figure 11.9 The copepod *Mytilicola intestinalis* from the intestine of the mussel *Mytilus edulis*. Adult male (left, ventral aspect) and female with egg sacs (right, dorsolateral view).
Lauckner (1983), after Hockley (1951). Reproduced with permission of Inter-Research, Germany.

bivalves may be explained by environmental variables, host gametogenic stage and even the presence of microscopic pathogens. In culture conditions, growing mussels on stakes, fences or ropes is believed to reduce intensity of infections. Restricting the density of farmed mussels and using seed less than 20 mm shell length from less infected areas are additional control methods (see Bower 2009c, for references).

Another crustacean, the pea crab (*Pinnotheres* spp.), inhabits the mantle cavity of many bivalve species. The crab feeds on material collected by the gills of the host, and causes gill erosion, weight loss through reduced filtration, loss of condition and impaired gametogenesis (Pregenzer 1981 and references therein; Lauckner 1983; Obeirn & Walker 1999). Increased mortalities have not been reported in infected hosts. Indeed, pea crabs may actually protect their hosts from predation! Campbell (1993) has found that in selection experiments starfish prefer to feed on uninfected mussels, although pea crabs usually abandon their host during attacks. Because no free-living mature females have been found in nature, their association with a host is believed to be true parasitism, while for males the association is seen as amensalism as they can survive outside of their host (Haines *et al*. 1994).

Neoplasia

Two predominant types of malignant neoplasia are common in marine bivalves: the first, haemocytic neoplasia (HN), or 'disseminated neoplasia', is characterised by proliferative growth of abnormal haemocytes, while the second, gonadal neoplasia, is typified by proliferation of undifferentiated germ cells. HN is progressive and fatal; as abnormal cells multiply there is progressive displacement, compression and necrosis of gill, gonad and connective tissues, culminating in widespread degeneration and necrosis of tissue, ultimately ending with death of the host (Barber 2004) (Figure 11.10). The disease has been reported in at least 15 bivalve species worldwide (details in Bower 2006b, c; Bower & McGladdery 2006).

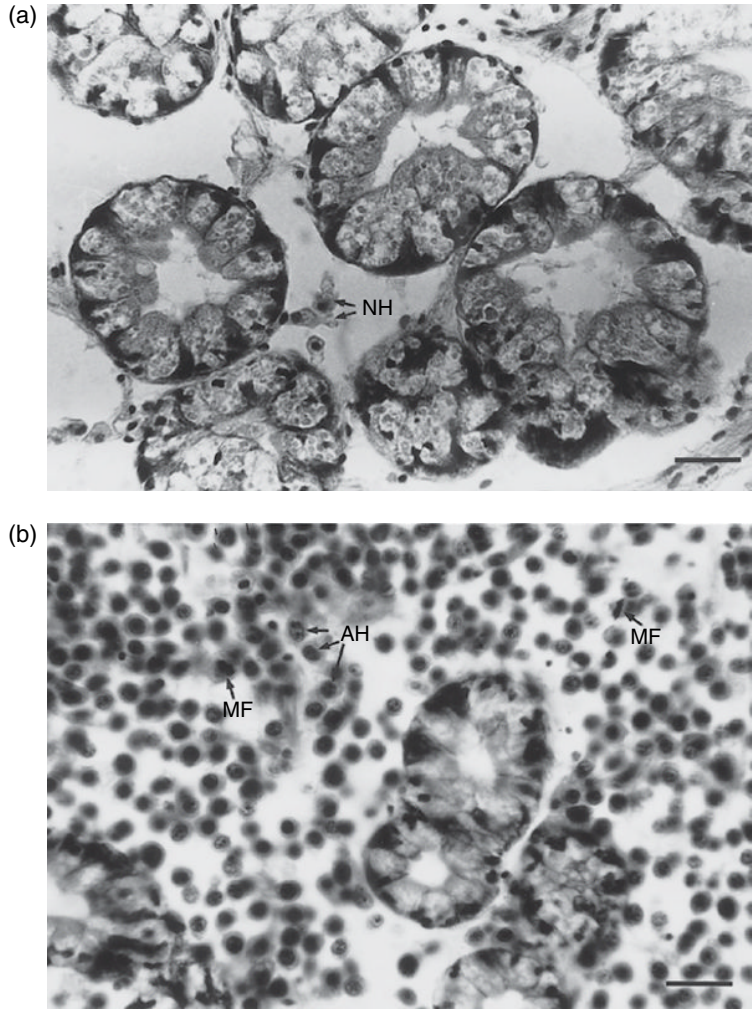


Figure 11.10 Histological sections of the digestive gland of *Mytilus trossulus*, illustrating (a) a few normal haemocytes (NH) in the spaces between the tubules of a normal mussel, and (b) numerous neoplastic haemocytes, some with a large nucleus containing two or more nucleoli and a small amount of peripheral cytoplasm (AH), and some undergoing mitosis (MF) in the distended spaces between the digestive gland tubules. Haematoxylin and eosin stain. Scale bar = 50 μm.
Photographs by S. Bower, Pacific Biological Station, Nanaimo, British Columbia, Canada. Reproduced with permission.

In most cases prevalences are low (<5%) but prevalences as high as 40 and 90% have been reported for *M. trossulus* and *Mya arenaria*, respectively (Ciocan & Sunila 2005). The second type, gonadal neoplasia, has been reported in several clam species on the Atlantic coasts of Canada and the United States, and is characterised by multiplication of germ cells to form gonadal germinomas (tumours), leading to abnormal gametogenesis and reduced fecundity, sometimes ending in the death of the host (Bower 2006d). Prevalence is generally low (2–3%) but can be as high as 50% in some populations, with females generally having higher prevalence of the disease than males (Barber 2004). It is interesting that hybrids had a significantly higher incidence of gonadal neoplasia (22%) than either of the parent species, *Mercenaria mercenaria* (7%) or *Mercenaria campechiensis* (12%) (Bert *et al*. 1993).

The etiology of both types of neoplasia remains unknown. Factors such as environmental contaminants, biotoxins, physiological stressors or a retroviral agent (but see Manso *et al*. 2012) have all been discounted (Krishnakumar *et al*. 1999; Alonso *et al*. 2001 and references therein; Barber 2004; Walker *et al*. 2011; AboElkhair *et al*. 2012). Bivalve species differ in their susceptibility to neoplasia. For example, *M. edulis* and *C. gigas* appear to be resistant to HN, yet the closely related species *M. trossulus* and *C. virginica* are susceptible; therefore a genetic etiology has been suggested (Van Beneden *et al*. 1999). The p53 family proteins play a central role in tumour suppression and embryonic development in vertebrates, and have also been discovered in a number of invertebrate species (see Muttray *et al*. 2008 for references). The tumour suppressor activity can be neutralised by mutation or interaction with other cellular proteins (Díaz *et al*. 2010). Mutant p53 was detected in haemolymph cells of cockles (*Cerastoderma edule*) with moderate to heavy HN, while it was not detected in cockles with no or light neoplasia. Also, the higher the intensity of HN the higher the activity of heat shock proteins (HPSs), usually synthesised in response to a variety of stress factors (Figure 11.11). This association in neoplastic cells could prevent p53 from carrying out its functions, as has been shown in human cancers. Transmissibility of HN has been demonstrated by injecting neoplastic cells, whole haemolymph or ova from diseased bivalves into healthy individuals (Collins & Mulcahy 2003; Barber 2004 and references). Also, the disease is transmitted when healthy individuals of a species are placed in close proximity to infected individuals (Elston *et al*. 1988). So far, cross-transmission between species has not been clearly demonstrated (Bower 2006b). In contrast to HN, it has not been possible to transmit gonadal neoplasia through injection of neoplastic cells or proximity experiments; reasons for this are unclear at present.

Histology and haemocytology have been the traditional methods in HN diagnosis. Neoplastic cells in HN contain more DNA than normal cells and thus their presence in a haemolymph sample can be quantified using a flow cytometer that counts and sorts cells based on DNA content. This method provides a more accurate and rapid diagnosis than more traditional methods (Delaporte *et al*. 2008). Gross examination of the gonad, in conjunction with histology, is the main method used in the diagnosis of gonadal neoplasia. Since neoplastic germs cells are not prevalent in haemolymph, this eliminates haemocytology as a diagnostic method (Barber 2004).

Defence mechanisms

All metazoan animals are protected against invading organisms or foreign substances by an internal defence system. The system is classified into two subsystems: the innate and adaptive immune systems. Innate immunity allows the animal to combat foreign components without any previous contact with them. Adaptive immunity provides rapid and selective protection against a specific foreign body (antigen), but requires previous exposure to the

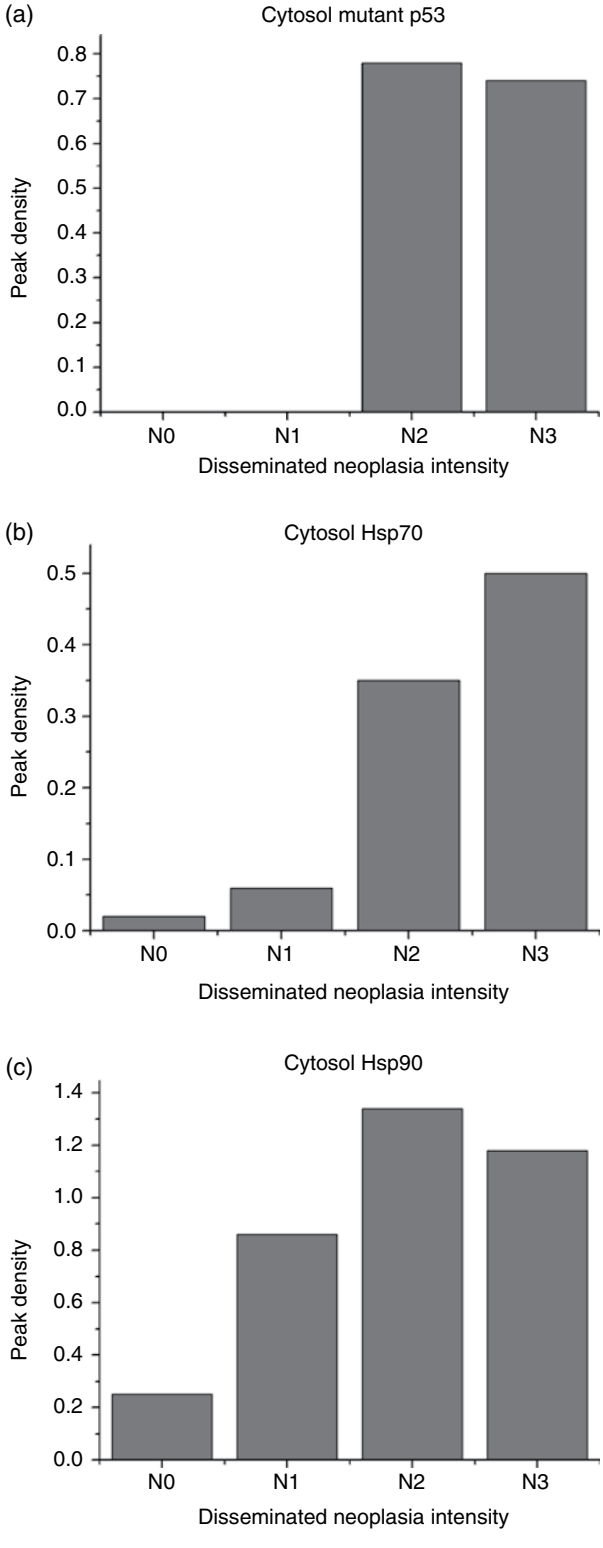


Figure 11.11 Intensity of (a) mutant p53, (b) Hsp70 and (c) Hsp90 protein expression measured by densitometric scanning (AU: arbitrary units) in haemolymph cells of the cockle, *Cerastoderma edule*, distributed in disseminated neoplasia intensity classes: non- disseminated neoplasia (N0); low-severity disseminated neoplasia (N1); moderate-severity disseminated neoplasia (N2) and high-severity disseminated neoplasia (N3). Diaz *et al*. (2010). Reproduced with permission of Inter-Research, Germany.

antigen. A second contact with the same antigen produces an enhanced response in the form of specific antibodies, produced as a result of memory formation (Prieur *et al.* 1990). This type of immunity is only present in vertebrates; but both types, innate and adaptive, rely on cellular (whole cell) and humoral (cell product) mechanisms. In bivalves phagocytosis and haemocytic infiltration are the primary cellular responses, while a multitude of haemolymph factors such as agglutinins (e.g. lectins, various antimicrobial peptides (AMPs) and lysosomal enzymes – either normally present or induced) constitute the humoral defence mechanism. Haemocytes are the first line of defence against parasites and pathogens. Although there is no single system of haemocyte classification, detailed morphological studies, along with investigations based on molecular characterisation of haemocyte cell surfaces, for example, lectin-binding and antigenic properties, or on haemocyte production of specific chemicals or peptides, have gone some way in helping to identify subpopulations of these cells in bivalves (Xue & Renault 2001; Wootton *et al*. 2003a; Aladaileh *et al*. 2007; García-García *et al*. 2008).

Cellular defence mechanisms

When a pathogen invades a host, a number of reactions are set in motion, initiated both by the pathogen in its attempt to survive and by the host which tries to destroy the invader (reviewed in Gestal *et al*. 2008). There are four types of defence mechanisms utilised by bivalve haemocytes: haemocytosis, phagocytosis, encapsulation and nacrezation. Haemocytosis is a first response to infection and involves a measurable increase in the number of circulating haemocytes, which then infiltrate infected or injured tissues. Phagocytosis is the next step in the defence process and can be divided into a number of stages: chemotaxis, recognition, adhesion, endocytosis and destruction. Chemotaxis, still poorly understood in bivalves, involves the directed movement of haemocytes towards the target, probably through chemo-attractant substances secreted by the target. For example, in the hard clam, *M. mercenaria*, haemocytes migrated towards peptidic as well as *N*-formyl-methionyl-leucyl-phenylalanine (*N*-FMLP) attractants released by bacteria (Fawcett & Tripp 1994), but in the mussel, *M. edulis*, while liposaccharide molecules from bacteria stimulated chemotaxis, *N*-FMLP stimulated random (cytokinetic) cell migration (Schneeweiß & Renwrantz 1993). Canesi *et al*. (2002) suggest that these peptides may function as a universal haemocyte-activating signal, at least in bacterial infections. After non-self-recognition, adhesion between haemocytes and foreign materials is principally through cell surface receptors such as integrin- and lectin-like proteins (Humphries & Yoshino 2003). At the site of adhesion the foreign material is taken into the cell by endocytosis (Figure 11.12) and is enclosed in a vesicle called a primary phagosome. This fuses with a lysosome to form a secondary phagosome or phagolysosome. The fate of ingested particles varies. Numerous enzymes within the phagolysosome degrade digestible material, and nutrients then diffuse into the cytoplasm, thus providing the host with a supplementary source of nourishment. Indigestible material is stored within haemocytes or else physically removed through migration of haemocytes out of the host (references in Sindermann 1990). Phagocytosis is accompanied by a diversity of killing methods and these will be covered later in the Section 'humoral defence mechanisms'.

When the invading organism or particle is too large to be phagocytosed then encapsulation by haemocytes, mainly granulocytes, is the defence method of choice. A capsule of haemocytes encloses the foreign body and cytotoxic products such as degradative enzymes and free radicals are released by the haemocytes. Disintegration of the foreign material, followed by resorption of cellular debris, may follow. Non-specific electrostatic forces and humoral plasma factors (see later) have a synergistic role in haemocyte attachment and

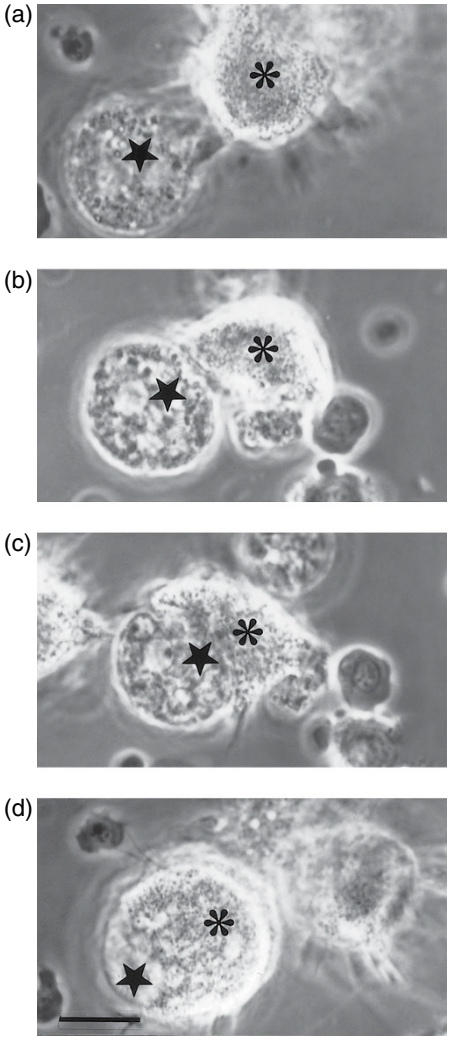


Figure 11.12 In vitro encounters between haemocytes from the mussel *Geukensia demissa* and *Haplosporidium nelsoni* plasmodia. (a) Granular haemocyte (asterisk) contacts plasmodium (star) with extended filipodia. (b) Granular haemocyte (asterisk) adheres to plasmodium (star). (c) Granular haemocyte (asterisk) extends cytoplasm around plasmodium (star). (d) Haemocyte (asterisk) has almost completely engulfed plasmodium (star). Photomicrographs were taken of two encounters; the time between initial contact and complete engulfment ranged from 2 to 4 min. Scale bar = 10 μm. Ford *et al.* (1993). Photomicrographs courtesy of S. Ford, Rutgers University, USA. Reprinted with permission of Allen Press Publishing Services.

encapsulation. In bivalves, positively charged targets are more vigorously encapsulated by haemocytes with their predominance of negative charges than negatively charged or uncharged targets (Wootton *et al*. 2006; Jayaraj *et al*. 2009; Meena *et al*. 2010). Another defence mechanism, and one which is distinct from encapsulation, is nacrezation whereby nacre is laid down around parasites or foreign material that invades the space between mantle and shell. An example of nacrezation is pearl formation by *Mytilus* in response to invasion by the trematode parasite *G. bursicola* (see earlier discussion and Chapter 2).

Humoral defence mechanisms

Circulating haemocytes secrete a variety of biologically active molecules into the haemolymph, and these play an important role in the humoral defence system. Such molecules are generally classified into two categories: serologically active, for example, lysozymes, agglutinins (lectins) and antimicrobial factors; and enzymes of lysosomal origin, such as aminopeptidases, β-glucuronidases, acid phosphatase, alkaline phosphatase, α-mannosidase, esterases and peroxidases (Gestal *et al*. 2008). This latter category plays a key role in degradation of foreign material in secondary phagosomes (see earlier discussion). Lysozyme is a widely distributed antibacterial molecule in animals. To date, different lysozymes have been identified in bivalves and amino acid and nucleotide sequences are now available for many of these. Lysozyme activity is expressed strongly in digestive gland haemocytes, and is increased by exposure to injected bacterial cells (references in Gestal *et al*. 2008). Lectins are also synthesised by haemocytes and occur on the cell surface as protein receptors for foreign antigens, for example, bacteria. They play a role in eliminating bacteria by promoting their immobilisation, binding and eventual destruction by phagocytosis. Their role in bivalve host defence is well documented, and several lectins as well as other adhesion molecules such as collagen, galectin-, integrin- and fibronectin-like proteins have been isolated and characterised (Bulgakov *et al*. 2004; Kim *et al*. 2006; Tasumi & Vasta 2007; Gestal *et al*. 2008; see Chatterjee & Adhya 2013 for review), and several lectin-coding genes have been identified in the oyster *C. gigas* (Yamaura *et al*. 2008). AMPs are found in both prokaryotes and eukaryotes and so far more than 800 have been isolated. They are not very specific, acting against a broad range of microorganisms, including bacteria, yeast, fungi and in some cases viruses and protistans. Numerous AMPs have been characterised in marine bivalves. The peptides are produced in haemocytes where they are stored as inactive AMP precursors; they are released as active peptides on microbial challenge. AMPs can act at an intracellular level through phagocytosis, and extracellularly after exocytosis (Bachère 2003). In *Mytilus* AMPs have been categorised into three families: defensins, which are akin to arthropod defensins, and mytilins and myticins, which are unique (see Mitta *et al*. 2000a, b). In mussels the three categories are present in dramatically different quantities and genes encoding them have been shown to be differentially regulated depending on the challenging bacteria (Cellura *et al*. 2007). See Figure 11.13 for a schematic presentation of humoral and cellular bivalve defence mechanisms (Soudant *et al*. 2008).

Phagocytic activity by bivalve haemocytes triggers O_2 uptake and the generation of a variety of cytotoxic molecules (the respiratory burst), such as reactive oxygen species (ROS), which include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radicals (OH^-). All of these play an important role in phagoctye-mediated killing of microorganisms (Buggé *et al*. 2007). Another radical, nitrous oxide (NO), is not toxic by itself but when it reacts with O_2^- to form the more potent oxidising species peroxynitrite ($ONOO^-$), this has the potential to cause severe cytotoxic damage (Figure 11.14; Sun & Wu 2009). Some pathogens do not trigger ROS production, for example, *B. ostreae* in the oysters *Crassostrea* and *O. edulis*, also *Perkinus* parasites and various *Vibrio* spp. (references in Gestal *et al.* 2008).

There is now a good body of evidence to show that various factors exert adverse effects on immunity, and this can affect resistance to infection, thus influencing survival. A range of assays based on haemocyte counts, phagocytosis, degradative enzyme levels and release of reactive oxygen metabolites have been developed to measure the competence of the bivalve immune system. Such assays are also used as biomarkers of environmental perturbation (Livingstone *et al.* 2000; Wootton *et al*. 2003b; Brown *et al*. 2004; Duchemin *et al*. 2008; Canesi *et al*. 2010). Polycyclic aromatic hydrocarbons (PAHs), which enter the marine

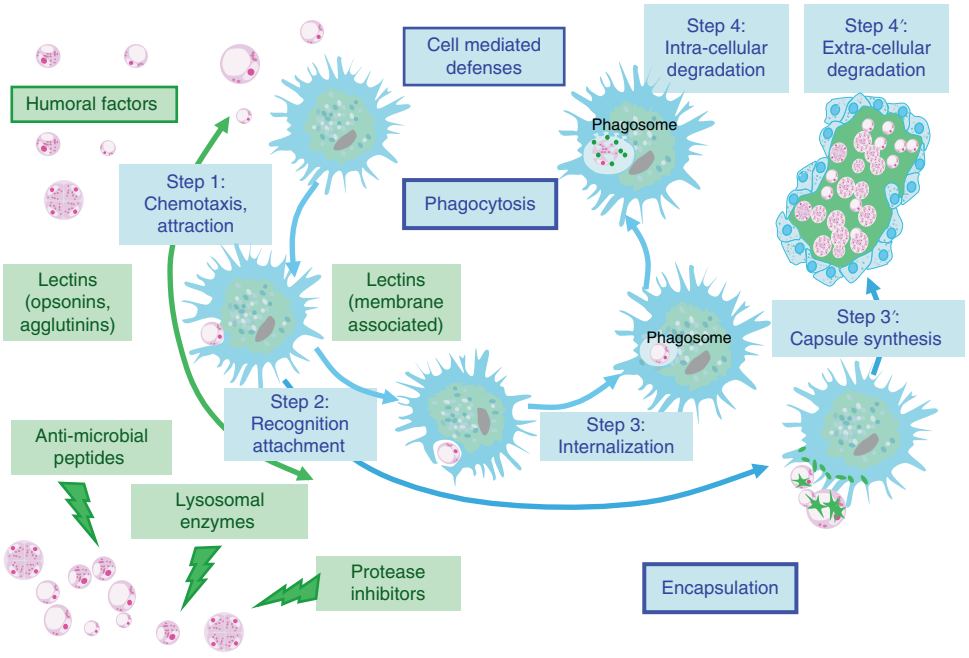


Figure 11.13 Schematic presentation of humoral and cellular responses in bivalve defence mechanisms during bacterial or parasitic infection. Step 1: Chemotaxis, attraction and migration. Step 2: Recognition and attachment of invading microorganisms. These two steps involve the presence and the synthesis of lectins. Step 3: Microorganisms are internalised within a phagosome. Step 4: The microorganism is destroyed by oxygen-dependent and oxygen-independent microbicidal activities. These four steps constitute the phagocytosis process. However, at Step 3′ when hemocytes failed to phagocyte the microorganisms (possibly because they are too big for internalisation), they encapsulate and destroy them externally with lysosomal enzymatic and ROS activities. Step 4′: Eventually, encapsulated microbes are destroyed extracellularly.
Soudant *et al.* (2008). Reproduced with permission of P. Soudant and A.Villalba, Centro de Investigacións Mariñas, Consellería do Medio Rural e do Mar, Xunta de Galicia, Vilanova de Arousa, Spain.

environment from a range of industrial sources, cause an increase in haemocyte numbers, but a decrease in cell viability, membrane stability and adhesion capability, as well as decreased lysozyme and phagocytic activity, depending on the concentration and duration of exposure of the species to the contaminant (reviewed in Girón-Pérez 2010). Similar effects have been reported for other pollutants such as heavy metals, PCBs (polychlorinated biphenyls) pesticides and pharmaceutical drugs. Other factors that modulate the bivalve immune system are temperature and salinity fluctuations (Gagnaire *et al*. 2006; Mosca *et al*. 2013), ocean acidification (Bibby *et al*. 2008), hypoxia (Chen *et al*. 2007; Mosca *et al*. 2013), spawning (Duchemin *et al*. 2007), starvation (Xu *et al*. 2008), injury (Husmann *et al*. 2011), algal diet quality (Delaporte *et al*. 2003), toxic (Ford *et al*. 2008) and even natural algal blooms (Mello *et al*. 2010).

New molecular approaches, such as mRNA differential display and suppression subtractive hybridisation libraries, increase the number of identified ESTs (see Chapter 10), which in turn has facilitated the identification of genes potentially involved in bivalve immune responses (Prado-Alvarez *et al*. 2009). Some examples are genes encoding proteases and protease inhibitors, lectins, AMPs, for example, defensins and isoforms of mytilin and mytocin (details in Gueguen *et al*. 2003; Tanguy *et al*. 2004; Kang *et al*. 2006; Gestal *et al*. 2007, 2010; Costa *et al*. 2009; Roberts *et al*. 2009; Venier *et al*. 2011).

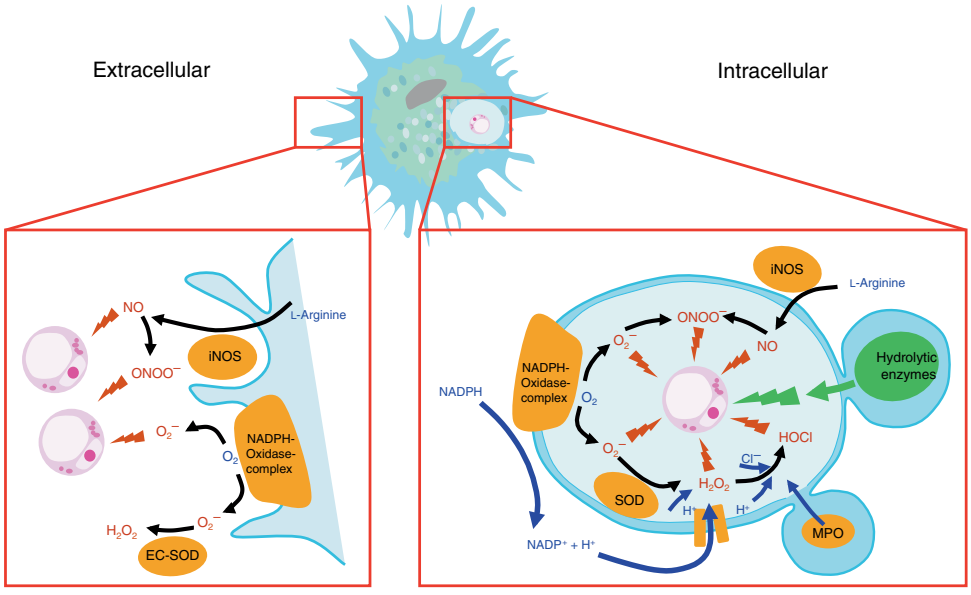


Figure 11.14 Hypothetical presentations of the pathways involved in production of major reactive oxygen species (ROS) and nitrogen species outside the cell membrane upon contact with foreign particles (left) and inside the phagosome upon phagocytosis of foreign particles (right). EC-SOD, Extracellular superoxide dismutase; $HOCl^-$, Hypochloride; iNOS, inductible nitric oxide synthase; MPO, myeloperoxidase; NO, Nitric oxide; $ONOO^-$, peroxynitrite; O_2^-, Anion superoxide; SOD: superoxide dismutase.
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Host–parasite interactions

Several of the major diseases covered earlier will now be discussed with reference to host–pathogen interactions on two levels: host defence reactions initiated by the host against the parasite and mechanisms employed by the parasite to evade the host's defence system. In order to survive in the host pathogens may:

- Scavenge/inhibit the host respiratory burst or destroy toxic compounds generated in the phagocytosis process
- Facilitate invasion and acquire nutrients for growth through secretion of enzymes involved in fat and protein breakdown
- Circumvent the hydrolytic activity of lysosomes
- Evade host immunity by modifying surface integrity using host lipids and fatty acids (Soudant *et al*. 2008).

To illustrate these points, attention will focus on host–parasite interactions in one bacterial (BRD) and two protistan (Dermo disease and bonamiosis) infections. These three diseases are well documented, mostly because of advances in pathogen purification and haemocyte culture methods, although in vitro propagated parasites are often less virulent than those freshly isolated from infected hosts (Ford *et al*. 2002).

The bacterium *V. tapetis* causes BRD and is a serious pathogen of the clam, *R. philippinarum* (see earlier discussion). Unlike other major diseases affecting bivalves, for example, Dermo, MSX, bonamiosis, haemocytes play a determining role in the defence against BRD (Allam & Ford 2006). Experimental challenge with the pathogen induces elevation of

haemocyte numbers in the haemolymph after 24–72 h (Paillard & Maes 1994; Allam *et al*. 2006). Haemocytes migrate towards the bacteria and phagocytose them. This active migration is induced and regulated by soluble molecules called chemotaxins, released either by the foreign agent or by host cells (Donaghy *et al*. 2009). Extrapallial fluids may also play a role in defence, as enzymatic activities of peptidases, hydrolases and lysozyme are at levels comparable to those in haemolymph (Allam *et al*. 2000). On the pathogen's side, in vitro studies have shown that it causes a decrease in haemocyte phagocytic activity and viability, probably as a result of haemolytic and cytotoxic virulence factors, for example, haemolysins, cytotoxins, exotoxins and possibly proteases, secreted by the pathogen (Allam & Ford 2006). Two weeks post-infection there is a decline in circulating haemocyte numbers that is accompanied by an increasing prevalence of BRD in the challenged clams. Simultaneous with this decline is an increase in the numbers of haemocytes in the extrapallial space, probably due to mobilisation of haemocytes from tissue to the inner shell, where they play a role in transportation of calcium and metabolites to damaged sites (Allam *et al*. 2006). An accompanying increase in protein concentration may also be related to the repair process, which requires deposition of an organic matrix primarily composed of protein compounds (Allam *et al*. 2006; Table 11.6). The host also employs an external defence mechanism by embedding bacteria within a brown organic deposit on the inside of the shell, a process similar to but more complex than nacrezation already described. If rapidly activated, this process may prevent pathogen proliferation into tissues (Paillard & Maes 1994). Development of BRD requires not just the presence of the pathogen but also specific environmental conditions. Reid *et al*. (2003) studied the effects of salinity on various immune parameters and disease progression in experimentally infected clams, and found that at a salinity of 20 psu the total haemocyte count was reduced and disease prevalence was highest. But at a salinity of 40 psu significantly fewer clams presented signs of BRD, and this was correlated with increases in the total haemocyte count, phenoloxidase levels and phagocytic activity of haemocytes.

P. marinus is a prevalent pathogen of oysters and causes massive mortalities in oyster (*C. virginica*) populations. The closely related *P. olseni* also causes severe economic losses in many clam populations, particularly *Ruditapes* spp. (see earlier discussion). Upon infection with *P. olseni* haemocytes of its clam hosts, *R. philippinarum* or *R. decussatus*, migrate from the haemolymph to the connective tissue of parasitised organs and secrete a polypeptide, which forms a capsule that encloses, but does not kill, the parasite. Encapsulation effectively removes *P. olseni* from the haemolymph, the main distribution pathway of the parasite.

Table 11.6 Haemolymph parameters (mean ± SEM) measured 3 days following injection of different bacterial strains into the adductor muscle of *Ruditapes philippinarum*.

Measured parameter	**Bacterial strain**				
	SSW	**Vibrio tapetis**	**H-K V. tapetis**	**V. anguillarum**	**R2**
THC	2.5 ± 0.2*	5.3 ± 0.9†	2.2 ± 0.2*	4.1 ± 0.4†	2.3 ± 0.3*
PDC	5.2 ± 0.8*	13.9 ± 4.3†	5.2 ± 0.6*	4.1 ± 1.1*	2.2 ± 1.0*
Protein	3.2 ± 0.2*	5.8 ± 0.7†	3.1 ± 0.3*	4.2 ± 0.4*	3.8 ± 0.3*
Lysozyme	5.4 ± 0.8*	14.8 ± 2.3†	6.3 ± 1.0*	9.0 ± 1.4*	5.8 ± 0.9*

Allam *et al*. (2006). Reprinted with permission from Elsevier.
H-K *V. tapetis*, heat-killed *V. tapetis*; Lysozyme, microgram equivalent chicken egg white lysozyme mg protein^{-1}; PDC, percentage of dead haemocytes; Protein, $\times 10^{-1}$ mg ml^{-1}; R2, non-Vibrionaceae bacterial species; SSW, sterile seawater; THC, total haemocyte counts (10^6 cells ml^{-1}).
* and † represent differences between treatments (Fisher's PLSD post hoc test, $P < 0.05$).
$n = 10$ clams sample^{-1}.

Encapsulation is believed to represent aborted attempts at phagocytosis and is much less frequently observed in *P. marinus* infections of the oyster, *C. virginica*. It might be expected that lysosomal enzymes, acting in conjunction with the respiratory burst, would destroy the encapsulated parasite. This is not the case, as the cell wall of *Perkinsus* spp. is remarkably resistant to proteolysis and may explain why they can survive after encapsulation (Sagristá *et al*. 1995), and after phagocytosis by oyster haemocytes (La Peyre & Faisal 1996). *P. marinus* secretes ECPs, mainly serine proteases, that reduce several haemocyte and humoral immune functions such as lysozyme, agglutination and vibriocidal activity of haemocytes, and ROS production (see Soudant *et al*. 2008 for references). Interestingly, *P. olseni* has little serine protease activity (Casas *et al*. 2002b), which may explain the lower virulence to its clam hosts, compared to *P. marinus* to *C. virginica* (Soudant *et al*. 2008). This oyster species, however, possesses several protease inhibitors although their level is insufficient to prevent infection. *C. gigas*, which is less susceptible to *P. marinus* infection, possesses protease inhibitors with significantly higher specific activities than those in *C. virginica* (Faisal *et al*. 1998). *P. marinu*s can also prevent death of haemocytes, a process called apoptosis, or programmed cell death – a natural part of the animal cell cycle. When *C. virginica* haemocytes were infected with *P. marinus*, haemocyte apoptosis was suppressed (Goedken *et al.* 2005); this may be a strategy on the part of the pathogen to provide more host cells for itself (Sunila & LaBanca 2003).

Bonamia spp. target the haemocytes of several oyster species causing significant mortalities in northern and southern hemisphere populations (see earlier discussion). The parasite enters the cells by phagocytosis, and is then enclosed by a membrane to form a phagosome. Entry of the parasite into haemocytes may also be mediated by the parasite itself as suggested by Morga *et al*. (2009), who reported higher numbers of live compared to dead parasites in oyster haemocytes. Within the phagosome the pathogen is neither injured nor destroyed but proceeds to proliferate, evading the normal degradative process of the phagosome. Morga *et al*. (2009) have shown that the presence of *B. ostreae* within oyster haemocytes exerts a direct impact on host cell hydrolytic enzyme activity and ROS production, which suggests that catalytic enzymes present in the parasite may be able to inhibit hydrolyases and modify ROS production in order to ensure its own intracellular survival (Figure 11.15a and b). It is interesting that although all three haemocyte types (see Chapter 7) contain parasites, agranular cells contain the highest number, which may be related to a lower lysosomal enzyme content in agranulocytes compared to granulocytes and hyalinocytes (Hine & Wesney 1994; Cochennec-Laureau *et al*. 2003b). Molecular analysis has shown an upregulation of several genes potentially involved in entry of the parasite into host cells, and in detoxification and degradation processes (Morga *et al*. 2011a, b; Martín-Gómez *et al*. 2012). In vitro tests on haemocytes of *C. gigas*, a species not susceptible to bonamiosis, showed that they were able to bind more *B. ostreae* than those of *O. edulis* (Fisher 1988). Also, *C. gigas* haemocytes had higher hydrolytic enzymatic activity in haemocytes and haemolymph than *O. edulis* (Xue & Renault 2000). These factors may be pertinent to understanding differences in susceptibility to infection and disease development between the two oyster species (Cao *et al*. 2009; Bower 2011a).

Perspectives

In the coming years there will be an increased use of:

- Reliable, accurate and sensitive molecular diagnostic tools to identify bivalve pathogens of the same and related species over a wide geographic range.
- Simulation models, based on experimental and field data, to describe transmission of disease and host–parasite–environmental interactions (Hofmann *et al*. 2009).

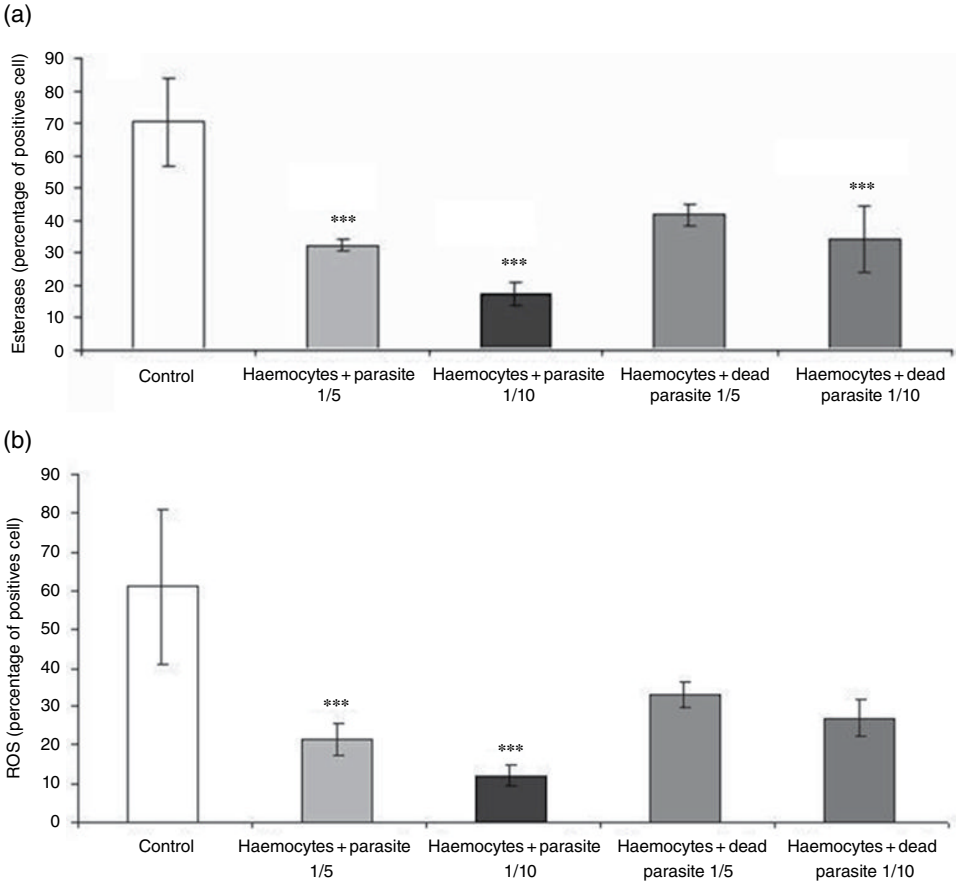


Figure 11.15 Percentage of haemocytes positive for non-specific esterase activity (a) and ROS (b) after 2 h of incubation with live and dead *Bonamia ostreae* at 5:1 and 10:1 ratios. Control = haemocytes alone. Values are means of three replicates and bars represent standard deviation. ***$P < 0.0001$ significant decrease compared to the control.
Morga *et al.* (2009). Reproduced with permission of Elsevier Science.

- Probiotics to inhibit growth of potentially pathogenic bacteria in bivalve hatcheries (Prado *et al*. 2010).
- New approaches combining cellular and molecular techniques, for example, genomics, proteomics, microarrays, to study diseases, which will lead to the development of new concepts on immunity and host resistance, as well as on pathogen virulence (Paillard *et al*. 2004; Renault 2008b; Song *et al*. 2010).
- Multidisciplinary transnational networks of diagnostic practitioners and recognised experts in shellfish pathology, as well as the bivalve industry and its regulators. Activities of the networks should include development of training programmes and cooperative programmes for test validation and laboratory accreditation (Renault *et al*. 2008b).

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12 Public health

Introduction

By virtue of their feeding habit, bivalves concentrate and accumulate material from the environment. This, coupled with the tradition of consuming bivalves raw or partially cooked, means that they act as potential vectors for human infection from water-borne agents such as bacteria, viruses, algal toxins and heavy metals, and thus are high-risk products. Generally speaking, the bivalves are not themselves affected by the microorganisms or toxins, merely serving to concentrate and passively transport the etiological agent. In this section on public health the role of bivalves in transmission of disease to humans will be dealt with under the following headings: bacterial infections, viral infections, biotoxins and industrial pollutants. Methods of bacterial, viral and toxin detection, decontamination procedures and monitoring measures will also be covered.

Bacterial infections

Bivalves living in polluted waters are often subject to contamination from domestic sewage and land run-off, which typically contain pathogenic bacteria such as *Salmonella* spp. and *Vibrio* spp. Before the 1950s the most common illness associated with the consumption of raw bivalves in the United States was typhoid fever caused by *Salmonella typhi* (Rippey 1994). With improved sewage treatment and water quality standards in bivalve-growing areas, the incidence of typhoid has declined, although about 7500 cases are reported annually in the United States, the majority of which are acquired while travelling in the developing world (NCEZID 2013). Since the 1950s non-typhoidal *Salmonella* infections have emerged as a public health problem. Symptoms are acute gastroenteritis, accompanied by diarrhoea, abdominal cramps and fever. Control measures aimed at detecting contamination of shellfish harvest waters, for example, monitoring faecal coliform counts in these waters, have been effective in reducing *Salmonella* contamination of seafood that occurs before harvest (Iwamoto *et al*. 2010). However, shellfish can also become contaminated with

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Salmonella during the storage and processing stages, although adequate cooking and proper storage and handling after harvesting can prevent infections.

There are about 30 species of *Vibrio*, 14 of which are recognised as potentially pathogenic to humans. Their presence is not associated with faecal contamination from human or animal source, and they are not detected by standard monitoring methods, or eliminated from shellfish by standard depuration processes (see later). The most important pathogenic species are *Vibrio parahaemolyticus* and *V. vulnificus*. The former is a normal constituent of the inshore marine flora but its abundance is increased through organic enrichment of coastal and estuarine areas, where bivalve-growing areas are concentrated. Infection is generally confined to gastroenteritis, which lasts 2–5 days. A more important disease organism is *V. vulnificus*, which can cause serious illness and even death in persons with pre-existing liver disease, diabetes or compromised immune systems. Among healthy people, ingestion of the species can cause vomiting, diarrhoea and abdominal pain, which lasts 2–8 days. Cases of *Vibrio* infections have a marked seasonal distribution, mostly occurring in the warmer months of the year, and almost all cases are associated with a recent history of seafood consumption, primarily raw oyster consumption. Since 2007 all *Vibrio* infections are required by US law to be reported to government authorities. Centers for Disease Control and Prevention (CDC) in the United States estimate 45 000 cases (90% CI 23 000–75 000) of *V. parahaemolyticus* infection each year, while the numbers for *V. vulnificus* are much lower at 207 cases (90% CI 138–287). Another serious disease organism is *V. cholera*, the cause of cholera, an acute diarrhoeal disease. Although rare in the developed world, there are three to five million cases a year and more than 100 000 deaths around the world. Infection is acquired through drinking contaminated water or eating contaminated food, including raw or undercooked seafood. Additional information on infective bacteria can be found in Richards *et al*. (2008), Lévesque *et al*. (2010), Campos *et al*. (2013) and the CDC website http://www.cdc.gov.

Faecal indicator organisms (FIO), such as faecal coliforms, *Escherichia coli* and *Enterococcus faecalis*, are routinely used as surrogates for microbial pathogens to assess the microbiological quality of bivalves and the waters they inhabit. The rates of uptake of these bacteria have been shown to be species and temperature dependent. For example, *Mytilus* spp. accumulated coliforms at a higher rate than three other bivalve species, the Pacific oyster, *Crassostrea gigas*, the littleneck clam, *Protothaca staminea*, and the soft shell clam, *Mya arenaria*, and also eliminated these bacteria more effectively (Bernard 1989). In *Mytilus* spp. rates of accumulation were higher at 17°C than at 12°C. A more recent study found that *Mytilus* spp., the cockle, *Cerastoderma edule*, and the Manila clam, *Ruditapes philippinarum*, had equivalent rates of *E. coli* accumulation, which were greater than for the oysters *Ostrea edulis* and *C. gigas*, which in turn were higher than the accumulation rate for the hard clam, *Mercenaria mercenaria* (Younger & Reese 2013). The results from both studies suggest that the use of *Mytilus* spp. alone may provide an adequate index of faecal pollution in shellfish-growing areas. These areas provide favourable conditions (low solar radiation, low temperature, low salinity, low densities of micropredators and high levels of organic matter) for survival of FIO (Campos *et al*. 2013).

Bacterial assays

The culturing and plating method is the oldest bacterial detection technique and remains the standard method used in monitoring for bacterial contamination. However, alternative techniques are necessary because the standard method is time-consuming, taking up to 16 days to yield a result in the case of some species. In addition, the standard method cannot be used to detect *Vibrio* spp. The polymerase chain reaction (PCR) is a sensitive technique that can

rapidly detect and amplify DNA sequences from specific *Vibrio* and other bacterial species genes (see references in Lazcka *et al*. 2007). The method is currently employed to detect different strains of *V. cholerae* (Depaola & Hwang 1995), *V. parahaemolyticus* (McCarthy *et al.* 2000) and *V. vulnificus* (Kim & Jeong 2001). PCR has also been used to detect non-*Vibrio* bacteria, for example, *E. coli* (Gonzalez *et al*. 1999) and *Salmonella* and *Shigella* species (Vantarakis *et al*. 2000). Because PCR amplifies DNA from both living and dead cells a reverse-transcriptase PCR (RT-PCR)[1] method has been developed that only detects viable cells. Several genes that are only active during the growth phase of the bacterium can then be detected (Deisingh & Thompson 2004). PCR may also be coupled to other techniques, for example, real-time (RT)-PCR, PCR-enzyme-linked immunosorbent assay (ELISA), the most probable number counting method (MPN-PCR; see later), or with gene-specific DNA microarrays. For example, the latter coupled with a multiplex PCR protocol was used to detect and discriminate between *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in oyster tissue (Panicker *et al*. 2004). The recent development of biosensors has brought a new and promising approach to pathogen detection. A biosensor that can immediately detect very low levels of *S. typhi* has now been developed (Zelada-Guillén *et al*. 2009). The technique uses carbon nanotubes and synthetic DNA fragments that activate an electric signal when they link up with the pathogen.

Viral infections

Viruses are increasingly recognised as an important cause of food-borne diseases. They can be transmitted directly from person to person, but also indirectly via virus-contaminated water, air, soil, surfaces or food. They need to enter living host tissue in order to replicate. A human enteric virus is one that replicates in the gastrointestinal tract or in the liver and is transmitted mainly by the faecal–oral route. Enteric viruses can persist for months in foodstuffs or in the environment. The two most frequently implicated in food-borne outbreaks are norovirus (NoV) and hepatitis A virus (HAV). NoV is very contagious and infection is through contact with an infected person, contaminated food, such as shellfish or water, or by touching contaminated surfaces. The symptoms of infection, which generally last 1–2 days, are acute gastroenteritis, accompanied by nausea, vomiting, stomach pain, diarrhoea, headaches and fever. At present there is no vaccine available against NoV. There are more than nine million episodes of food-borne illnesses each year in the United States, and NoV is the most common cause with 5.5 million episodes, and about 15 000 hospitalisations and 150 deaths (Scallan *et al*. 2011). See Le Guyader *et al*. (2010) for a comprehensive analysis of one such outbreak in France. Outbreaks show strong seasonality with the peak occurring in December–March in the United States and April–May in Europe. Also, outbreaks are affected by various environmental factors, for example, precipitation, temperature, solar radiation, wind and salinity. Various models can be applied to forecast outbreaks including Bayesian models, process-based models, regression models and artificial neural networks (details in Wang & Deng 2012).

HAV infection is the most serious infection associated with seafood consumption. The virus replicates in the liver and is shed in high concentrations in faeces from 2 weeks before to 1 week after the onset of clinical illness. Symptoms include fever, malaise, jaundice, anorexia and nausea and may last from several weeks to several months. In countries where HAV infection is highly endemic, the majority of people are infected in early childhood and more than 90% of children under 5 years of age show no symptoms, and virtually all adults are immune. But in countries with good standards of public health, HAV infection is less common; very few are infected in early childhood, and the majority of adults remain

susceptible to infection, with a 1% mortality rate in over 60 year olds (Hollinger & Emerson 2007). In developed countries infection is becoming increasingly rare due to an ongoing vaccine campaign. Nevertheless, the virus is exceptionally persistent in the environment and within shellfish tissue. Provost *et al*. (2011) found that in the eastern oyster, *Crassostrea virginica*, phagocytic haemocytes played an important role in the retention of HAV virus particles and are thus a site of virus persistence. Persistence of HAV, and NoV, in the environment is illustrated by the percentages of positive samples for the two viruses in bivalves harvested and distributed across several European countries (Table 12.1). In nine studies NoV was more frequently detected, although in Italy, there was a high prevalence of HAV in the Ionian and Adriatic Seas, where hepatitis A is endemic. There was no correlation between the positive count of human NoVs and the level of bacterial contamination in the growing waters, which agrees with results from several previous studies, for example, Le Guyader *et al*. (2000) and Bosch *et al*. (2009).

Other faecal viruses that are less commonly transmitted by shellfish but have been documented as being associated with bivalve shellfish consumption include Aichi virus (AiV), hepatitis E virus (HEV), sapovirus and astrovirus (references in Kingsley 2013).

Viral assays

Since NoV and HAV strains do not replicate in cell culture (Duizer *et al*. 2004), molecular techniques must be used to detect and quantify them. The most common method for detection of these viruses employs RT-PCR (see earlier), which allows not only qualitative but also quantitative detection of the virus. Protocols for virus extraction, nucleic acid extraction and purification, PCR assays and their limitations are reviewed in Bosch *et al*. (2009, 2011), Lees (2010) and Le Guyader *et al*. (2010). Because of the proliferation of published protocols for detecting NoV and HAV in shellfish, the European Committee of Standardization (CEN) has set up a working group (CEN/TC 275/WG6/TAG4-viruses in foods) to develop a two-part (quantitative and qualitative) standard method for virus detection in foodstuffs, including shellfish, which has the potential to be incorporated into EU legislation (Lees 2010). The first part of the standard (the quantitative method) and the second part (the qualitative method) were presented in 2009 and 2010, respectively, to CEN for technical and formal assessment. The International Organization of Standardization (ISO) published the final version of the methods in 2013 (ISO 2013a; ISO/TS 15216-1:2013; ISO, 2013b; ISO/TS 15216-2:2013).

Biotoxins

Marine biotoxins, produced by dinoflagellates and diatoms, are a naturally occurring phenomenon and not associated with sewage contamination of coastal waters. It is not clear why only about 1% of microalgal species produce these toxins, although under eutrophic conditions, where nutrients are limited, toxin secretion may be a mechanism of inhibiting potential competitors (Granéli *et al*. 2008). Filter-feeding bivalves accumulate the toxic cells in their tissues, and are therefore the main vectors of toxins that cause paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and azaspiracid poisoning (AZP) in humans. There are no suitable post-harvest mitigation strategies against these toxins (Kingsley 2013). Even cooking does not inactivate these toxins, and there is no known antidote. The continuing increase in the number of toxic species, coupled with the increased occurrences of harmful algal blooms (HABs) of these species in coastal waters, poses a constant threat to public

Table 12.1 Percentage of human norovirus (NoV) and/or hepatitis A virus (HAV)-positive seafood samples from European coasts.

Countries of origin	Sampling point	Period	No of samples	NoV (%)	HAV (%)	Reference
France	The Netherlands (R)	2004–2008	58	12	NA	Boxman (2010)
	Switzerland (R)	2001–2002	61	13	0	Beuret *et al.* (2003)
Spain						
Galicia	Area B (C)	2005	24	58	0	Vilariño *et al.* (2009a)
	Area C (W)	2005	17	53	0	
Italy						
River Po delta	B area (D)	2005–2006	96	0	0	Suffredini *et al.* (2008)
Adriatic Sea	B area (D)	2003–2004	235	14	6	Croci *et al.* (2007)
Ionian Sea	B area (D)	2002	29	NA	90	Di Pinto *et al.* (2003)
	C area (RL)	2002	20	NA	30	
United Kingdom	B area	2004–2006	237	57	NA	Lowther *et al.* (2008)
Ireland	Area B	2005–2007	42	54	NA	Flannery *et al.* (2009)
The Netherlands	The Netherlands (R)	2004–2008	126	9	NA	Boxman (2010)
Germany/Denmark	Area A	2004–2008	36	11	NA	Boxman (unpublished) cited in Boxman (2010)
Norway	Area A	2000–2003	681	7	NA	Myrmel *et al.* (2004)

Adapted from Boxman (2010). Reprinted with permission of Springer Media and Business.
Viruses were detected using reverse transcriptase PCR.
C, cultured mussels; D, depurated; NA, not analysed; R1, retail; RL, relayed; W, wild mussels.

health worldwide (Figure 12.1). Therefore, shellfish and shellfish harvest areas are closely monitored for biotoxins using a variety of analytical techniques (see later).

PSP is caused by eating bivalves that contain saxitoxin (STX) and its derivatives (details in Kodama 2010). The toxins, a group of about 20, are bacterial catabolites in dinoflagellate cells of the genera, *Alexandrium*, *Gymnodinium* and *Pyrodinium*. Even when blooms of these species are not apparent, bivalves may ingest resting cysts, which are 10–1000 times more toxic than motile dinoflagellates (Sindermann 1990). The toxins produced by these organisms are the most common and widespread of the shellfish toxins, and are among the most potent neurotoxins known. They act by blocking the passage of sodium ions through cell membranes, thus inhibiting nerve impulse transmission. Typical symptoms in humans

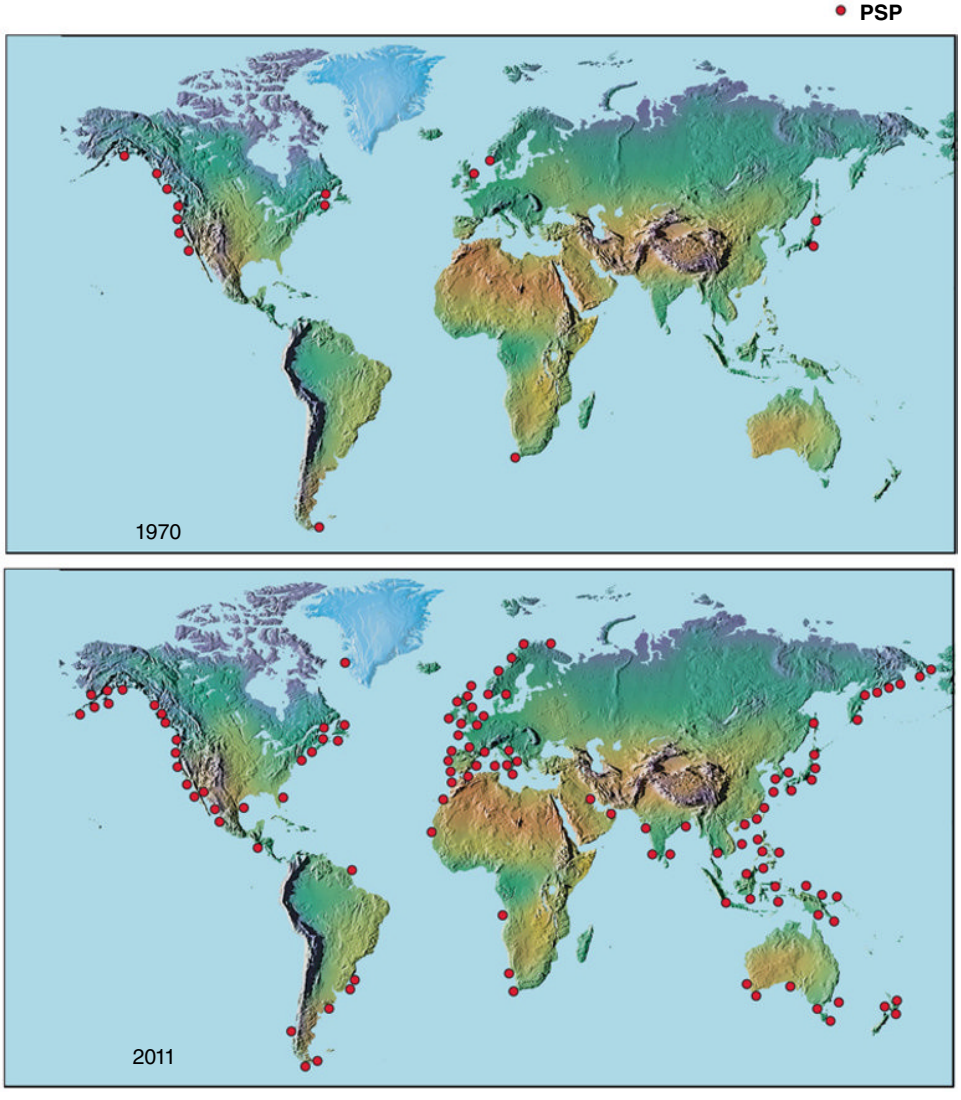


Figure 12.1 Global distribution of paralytic shellfish poisoning (PSP) toxins recorded as of 2011, compared to the distribution recorded in 1970.
Reproduced with permission of the US National Office for Harmful Algal Blooms, Woods Hole Oceanographic Institution, Massachusetts, USA.

range from a slight tingling and numbness of the lips to complete paralysis and death from respiratory failure, depending on the dose. The level at which PSP intoxications occur in humans varies considerably between individuals. Therefore, average doses are not very informative for the purpose of deriving a safe dose estimate. Based on epidemiological data, a dose of 2 μg STX equivalents/kg body weight (bw) may be considered as the lowest observable adverse effect level (LOAEL) and 0.7 μg STX equivalents/kg bw as the acute reference dose (ARfD[2]) (Oshima *et al*. 2011). A value of 80 μg/100 g meat has been established as the safety limit in shellfish (Table 12.2).

ASP is caused when bivalves are eaten that contain domoic acid (DA) and/or its analogues. The toxins are secreted by the diatom *Pseudo-nitzschia multiseries*, and the red alga, *Chondria armata*. DA is an analogue of glutamic acid, a neurotransmitter in the brain. Within 24 h of consuming contaminated bivalves, usually mussels, there is nausea, vomiting and diarrhoea with the following neurological perturbations within 48 h: confusion, memory loss, disorientation and even seizures, coma and death. The severity of symptoms depends on the amount of DA ingested. For example, Botana (2008) found that mild symptoms were observed after consuming 0.9–2.0 mg DA/kg bw, while 1.9–4.2 mg DA/kg bw caused severe neurological symptoms. Assuming a body weight of 60 kg the LOAEL is 1 mg/kg bw, and consumption of 0.33 mg/kg bw produces no ill effects (Tasker *et al*. 2011). The LOAEL value has been divided by a safety factor of 10 to derive a provisional ARfD of 0.1 mg/kg bw. A value of 20 μg DA/g has been established as the safety limit in shellfish (Table 12.2). Occurrences of ASP have been reported from Europe, the west and east coasts of North America, Chile, Australia and New Zealand.

Table 12.2 Maximum allowable levels for poisonous or deleterious substances in shellfish meat in the United States, Canada, EU and Australia.

	Allowable level			
Deleterious substance	**United States**	**Canada**	**Europe Union**	**Australia**
Dieldrin insecticide	0.30 ppm	0.30 ppm	Not specified	Not specified
PCBs	2.0 ppm	2.0 ppm	8×10^{-6} ppm (8 pg/g ww)*	0.50 ppm
Mercury	1.0 ppm	0.50 ppm	0.50 ppm	0.50 ppm
Cadmium	Not specified	Not specified	1.0 ppm	2.0 ppm
Lead	Not specified	Not specified	1.5 ppm	2.0 ppm
PSP	80 μg/100 g	80 μg/100 g	80 μg/100 g	80 μg/100 g
DSP okadaic acid plus derivatives	0.20 $\mu g\,g^{-1}$	0.20 $\mu g\,g^{-1}$	16.0 μg/100 g†	0.20 $\mu g\,g^{-1}$
NSP brevetoxin	0.80 $\mu g\,g^{-1}$	No detectible amount‡	Not specified	20 MU/100 g
ASP domoic acid	20 $\mu g\,g^{-1}$	20 $\mu g\,g^{-1}$	20 $\mu g\,g^{-1}$	20 $\mu g\,g^{-1}$

Values for the United States, Canada, EU and Australia from FDA (2011), CFIA (2013), Commission Regulations (EC) Nos. 853/2004 and 1881/2006 (EC 2004a, 2006a), and Australia New Zealand Food Standards Code (2013), respectively.
*dioxin plus dioxin-like PCBs;
†includes okadaic derivatives, dinophysistoxins and pectenotoxins;
‡presence of any toxin is considered to be hazardous to human health.
ASP, amnesic shellfish poison; DSP, diarrhetic shellfish poison; MU, mouse unit; 1 mouse unit (MU) is the minimum quantity of toxin needed to kill two out of three mice within 24 h of injection; NSP, neurotoxic shellfish poison; PCBs, polychlorinated biphenyls; ppm, parts per million; PSP, paralytic shellfish poison; ww, wet weight.

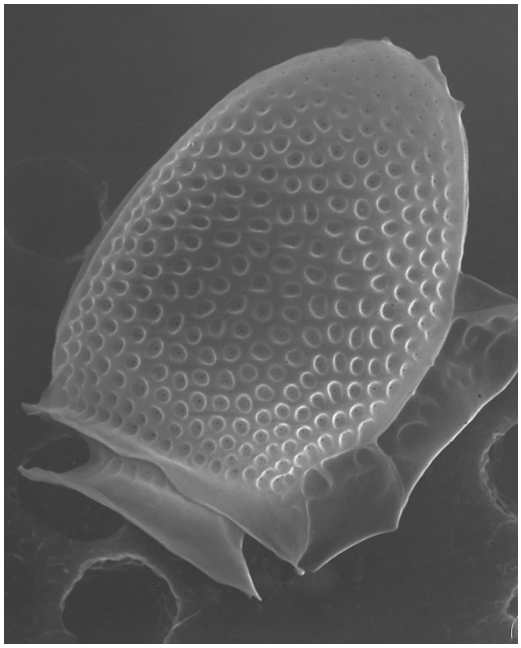


Figure 12.2 Scanning electron micrograph of the dinoflagellate *Dinophysis acuminata*; maximum dimension approximately 50 μm.
Photograph by Caroline Cusack, Marine Institute, Galway, Ireland.

DSP and NSP are much less serious than PSP or ASP. DSP is caused primarily by okadaic acid (OA) and its derivatives, the dinophysistoxins (DTXs), produced by dinoflagellate species of *Dinophysis* (Figure 12.2) and *Prorocentrum*. The main symptoms of DSP are cramps, severe diarrhoea and vomiting and patients usually recover within 3–4 days. Findings that DSP toxins have tumour-promoting and immune-suppressing activity in animal cells is a cause for concern. An ARfD of 0.33 μg OA equivalents/kg bw has been estimated, based on the LOAEL of 1.0 μg OA/kg bw, and a safety factor of 3 because of documentation of human cases including more than 40 persons (Van Egmond 2011). The safety limit for OA in seafood is 0.20 μg/g (Table 12.2). While DSP has a global distribution, confirmed occurrences of NSP have only been reported from the Gulf of Mexico, the southeast coast of the United States and the New Zealand Hauraki Gulf region (Dickey *et al*. 2011). NSP is caused by a group of toxins, the brevetoxins (BTXs), secreted by marine dinoflagellates of the genus *Karenia*. The main symptoms of NSP are headaches, dizziness, muscle and joint pain and difficulty in breathing, and full recovery generally occurs within 48 h. No human fatalities have been reported associated with the consumption of shellfish contaminated with BTXs to date. There is insufficient quantitative data available to derive a human oral ARfD (Dickey *et al*. 2011). The present regulatory limit is set at 0.80 μg g^{-1} of shellfish tissue in the United States and 20 mouse units (MUs) per 100 g in Australia and New Zealand (Table 12.2). The European Union (EU) has specified no safety limit for brevetoxin, and in Canada the presence of any of the toxins is deemed hazardous to human health.

A number of new shellfish toxins, for example, azaspiracids (AZAs), pectenotoxins and yessotoxins (YTXs), have been identified in the past two decades. AZP was detected for the first time in 1995 after consumers in the Netherlands ate blue mussels from Ireland. Symptoms of poisoning are similar to DSP. The toxin is a polyether and there are about 20 different analogues, some of which have been identified from dinoflagellate *Protoperidinium*

spp. To date, AZP has only been recorded in Europe and Northwest Africa (see reviews by Twiner *et al*. 2008 and Ryan *et al*. 2011). The EU has set a provisional ARfD of 0.04 µg/kg bw based on the LOAEL of 23 µg per person and a body weight of 60 kg using a 10-fold safety factor to take into consideration the small sample size to date (Amzil *et al*. 2008). The EU has set a regulatory limit of 160 µg AZA/kg shellfish weight. Pectenotoxin is a polyether also, with about 10 derivatives from PTX-2. All exclusively arise from *Dinophysis* spp. and are always accompanied by toxins from the OA group. The toxins have been reported in microalgae and/or bivalves from Australia, New Zealand, Japan and several European countries, but to date there are no data indicating adverse effects in humans (reviewed by Yasumoto *et al*. 2011). YTXs are sulphated polyethers with a large number of analogues (Amzil *et al*. 2008). The toxins are produced by dinoflagellates *Protoceratium reticulatum* and *Gonyaulax* spp. and have a global distribution. To date there are no reports in humans of ill effects associated with consumption of these toxins. In the absence of human data a provisional ArfD of 50 µg/kg bw has been established (see Speijers *et al*. 2011 for review).

It was generally believed that the toxins do not harm the bivalves themselves, but results from recent studies have shown this to be untrue. Toxic algal species have detrimental effects on feeding responses (Hégaret *et al*. 2007; Robbins *et al*. 2010), digestion (Alexander *et al*. 2008), reproductive effort (Haberkorn *et al*. 2010), larval development (Leverone *et al*. 2006) and larval lipid storage (Przeslawski *et al*. 2008). However, in some cases these effects are transitory due to biotransformation of algal toxins by epimerisation, reduction and hydrolysis within bivalve tissues (Contreras *et al*. 2012 and references).

Harmful algal blooms

Algal blooms are natural events of increased algal cell numbers generally due to elevated nutrients coupled with adequate light conditions. Salinity is also an important factor as it determines the types of bloom species (Terlizzi & Mazzacaro 2010). The occurrence of human poisoning episodes from toxic seafood ingestion, involving ten to hundreds of people in several regions of the world, has focused attention on these HABs. The blooms also kill marine life, and cause losses in millions of dollars per year in closures of harvestable shellfish, lost production and monitoring costs (Garthwaite 2000). In recent years there is growing concern that HABs are occurring more frequently, and lasting longer, and are appearing in areas where they had not been recorded in the past. Increased HAB frequency may, on the one hand, reflect increased awareness and monitoring, but there is compelling evidence that human activities play an important role. Such activities include eutrophication of coastal waters as a consequence of increased aquaculture and fertiliser run-off from agriculture; changes in climatic conditions making some coastal environments more hospitable to toxic algal species and the transportation of toxic algae and their cysts around the world in ships' ballast water or through the movement of shellfish stocks (Hallegraeff 2004; Dale *et al*. 2006; Hégaret *et al*. 2008; Lawrence *et al*. 2011). In addition, a meta-analysis of published data and historical records indicates that loss of species diversity, and the concomitant loss of filtering and detoxification services provided by suspension feeders, has led to an increase in the occurrence of algal blooms in coastal regions (Worm *et al*. 2006). Because of the perceived increase in the number and duration of HABs, advance warning increases the chances of managing these events. One forecasting system is the NOAA (National Oceanic and Atmospheric Administration) HAB Operational Forecast System (HAB-OFS) in the Gulf of Mexico. The system uses satellite imagery, field observations, models, public health reports and buoy data to provide the large spatial scale and high frequency of observations required to assess and predict bloom conditions, location and movements. Current HAB conditions in the Gulf can be viewed on the NOAA link: http://tidesandcurrents.noaa.gov/hab/.

Biotoxin assays

The mouse bioassay (MBA) is the official procedure of the Association of Official Analytical Chemists (AOAC) for measuring PSP toxins. To date, it has not been validated by the AOAC for measuring other toxins, although the American Public Health Association (APHA) officially recognises the assay for detection of NSP toxins. For the MBA, liquid is extracted from putative toxic bivalves and injected into mice; the time-to-death of mice of standard weight (18–22 g) is related to toxin level (details in Oshima *et al*. 2011). It provides an indication of overall toxicity but is not able to differentiate among individual toxins (Campàs *et al*. 2007). A rat bioassay has also been developed, but not validated, for detection of DSP and AZA toxins. The MBA suffers from several drawbacks including a lack of specificity and nontoxic interferences, and is regarded as unethical by defenders of animal welfare. Also, the bioassays for lipophilic toxins (OA, AZA, BTX, YTX and PTX groups) are not quantitative and thus do not lend themselves to effective monitoring practices (Lawrence *et al*. 2011). To develop quantitative and specific methods the availability of pure reference compounds (the toxins themselves) is a major prerequisite. Currently, there is at least one reference group available for every toxin group, but not for all relevant toxic analogues. But as these become available, alternative detection methods to bioassays are being developed.

One such alternative is *in vitro* cytotoxicity assays, cell-based bioassays that measure the effects of toxins on cultured animal or bacterial cells (Flanagan *et al*. 2001). These assays are quick, inexpensive and highly sensitive. For example, a cytotoxicity assay for OA, the major DSP toxin, can detect 0.01 mg of toxin per gram of mussel tissue (Croci *et al*. 2001). Other cytotoxicity tests are neuroreceptor assays based on the binding of PSP and NSP toxins to voltage-sensitive sodium channels in nerve cells. The toxins bind to the receptors with an affinity proportional to their toxic potency (Campàs *et al*. 2007). A glutamate receptor-binding assay has been developed for detection of DA, the toxin responsible for ASP (Van Dolah *et al*. 1997). Methods based on perturbations to cellular parameters, such as enzyme inhibition, have also been developed. One such assay, the protein phosphatase (PP) inhibition assay, detects OA down to 0.1 $\mu g\, g^{-1}$ tissue, which is about twice as sensitive as the MBA test (Mountfort *et al*. 2001). The EU is examining PP inhibition with fluorimetric detection as a possible alternative to the MBA for detection of DSP toxins.

Immunoassay methods, based on the affinity between antibodies and antigens, are also used to detect toxins. The most commonly used method is the ELISA, which has been developed for the PSP, ASP, DSP and NSP toxin groups (references in Campàs *et al*. 2007). The EU is also examining immunoassays as a possible alternative to the MBA because of their higher sensitivities.

As already indicated, many of the toxin groups discussed contain a multiplicity of structural forms, which makes detection a formidable challenge. Chromatographic techniques allow separation, highly selective identification and sensitive quantification of the different toxins present in a sample (reviewed by Quilliam 2003). Liquid chromatography (LC) with fluorescence detection (FLD), LC in combination with mass spectroscopy (LC-MS) and, more recently, LC coupled to electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS; Regueiro *et al.* 2011) are being used to detect STX, DA, OA and its DTXs analogues, pectinotoxins (PTX) and AZAs (Figure 12.3). LC is the official AOAC method for DA and PSP toxins, and has been validated by the New Zealand Food Safety Authority (NZFSA) for detection and quantification of DSP and ASP toxins (Rhodes *et al*. 2013).

A new and promising analytical technique, currently under development, is the use of biosensors for fast, simple, cheap and reliable toxicity screening (Campàs *et al*. 2007; Vilariño *et al*. 2009b; Campbell *et al*. 2011; Van Egmond 2011). Most of the biosensors developed for shellfish toxins are immunosensors. These are devices that use specific

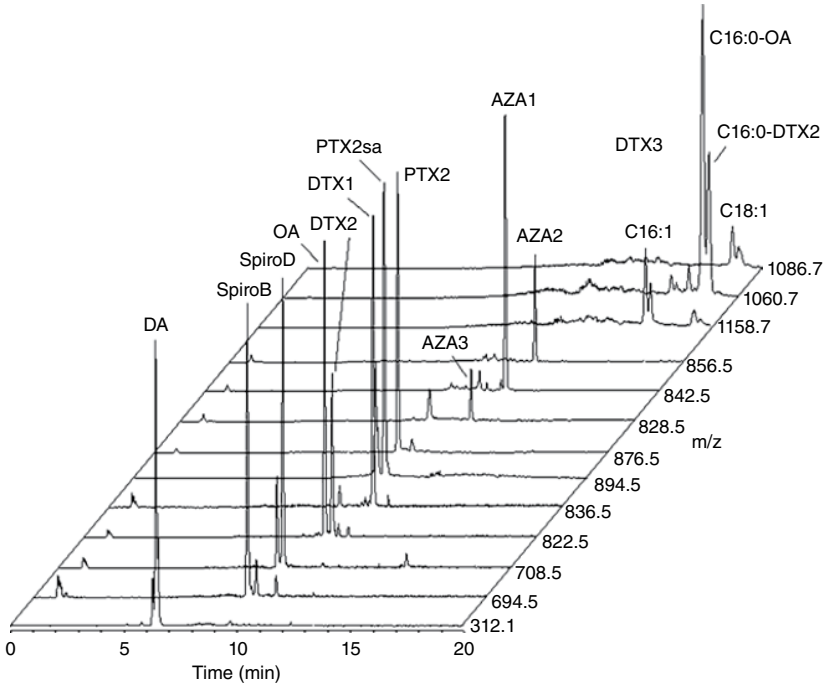


Figure 12.3 Reversed-phase gradient elution LC–MS analysis of a range of toxins, displayed as individual mass chromatograms, from a blend of contaminated mussel tissue extracts. The toxins present include domoic acid (DA), spirolides (SpiroB SpiroD; spirolides are cyclic imines with no apparent adverse effect on humans), okadaic acid (OA), dinophysistoxins (DTX1, DTX2), pectenotoxins (PTX2, PTX2sa), azaspiracids (AZAs) and acyl esters of OA and DTX2 ('DTX3').
Quilliam (2003). Reproduced with permission of Elsevier.

biochemical reactions mediated by antibodies to detect chemical compounds, usually by electrical, thermal or optical signals. A multiplex method for the simultaneous analysis of STX, OA and DA has the potential to analyse up to a total of 16 toxins on a microarray chip. There is also the potential for miniaturisation and portability, for ultimate on-site monitoring (Campàs *et al*. 2007; Anderson 2009).

Industrial pollutants

Bivalves accumulate high levels of pesticides, heavy metals and hydrocarbons from contaminated water and there is ample evidence that these have a severe effect on their physiology and immune system (see Chapters 5, 6, 7 and 11). It is for this reason that bivalves, particularly mussels, are used as sentinel organisms in environmental monitoring programmes (see later). The best-documented case of heavy metal poisoning occurred in Minamata Bay, Japan, when the Chisso Corporation dumped an estimated 27 tonnes of mercury-related compounds into the Bay between 1932 and 1968. The chemicals accumulated in fish and shellfish, and, when eaten by the local people, resulted in mercury poisoning, the effects of which were neurological symptoms such as spasms and blurred vision. To date, over 10 000 people have received financial compensation from Chisso (Tsuchiya 1992). Mercury concentrations continue to increase with a two-fold increase recorded in surface waters over the last century due to increasing industrialisation and energy

production (Mason *et al*. 2012). In the United States alone it has been estimated that more than 300 000 newborns each year are at increased risk of adverse neurodevelopmental effects due to *in utero* exposure to methylmercury (Mahaffey *et al*. 2004). Currently, there are more fish consumption advisories for mercury across the United States than for any other pollutant (US EPA 2011). Also, strategies for decreasing mercury pollution are in place in the United States, and appear to be effective, as atmospheric emissions of mercury have been reduced by 60% since 1990 (see Chen *et al*. 2012). Globally, about 65% of human-induced mercury releases to the atmosphere come from Asia, with North America responsible for about 8%. Recently, more than 140 countries at a meeting of the United Nations Environment Programme (UNEP) Governing Council agreed to negotiate a legally binding international agreement by 2013 to limit mercury emissions. The regulatory limit in Canada, the EU and Australia is 0.5 mg methylmercury per kilogram shellfish weight (0.5 ppm), while in the United States it is twice this level (Table 12.2). The Joint (FAO/WHO) Expert Committee on Food Additives (JECFA 2004) set a provisional tolerable weekly intake (PTWI) of 1.6 µg/kg body weight/week. For a comprehensive review on health effects of mercury contamination, see Guynup (2012).

Other heavy metals of concern to human health through seafood consumption are cadmium (Cd), lead (Pb) and arsenic (As). Cadmium accumulates in the human body negatively affecting organs such as the liver, kidney, lung, bones, placenta, brain and the central nervous system (Castro-González & Méndez-Armenta 2008). The regulatory limit in the EU and Australia is 1.0 and 2 mg Cd/kg shellfish weight, respectively, while no amount is specified for the United States and Canada (Table 12.2). The JECFA has recommended a PTWI of 0.007 mg/kg bw. Short-term exposure to high levels of Pb can cause brain damage, paralysis, anaemia and gastrointestinal symptoms while longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. The most critical effect of low-level Pb exposure is on intellectual development in young children and, like mercury, lead crosses the placental barrier and accumulates in the foetus (FSAI 2009). The regulatory limit in the EU and Australia is 1.5 and 2 mg Pb/kg shellfish weight, respectively but no amount is specified for the United States and Canada (Table 12.2); a PTWI of 0.025 mg/kg bw is recommended. Seafood is a major source of As, ranging between 5 and 250 $\mu g\, g^{-1}$ depending on the species. Fortunately, about 80–95% of total arsenic is present as organic compounds that do not cause any damage to health (Mudgal *et al*. 2010). However, inorganic arsenic is a carcinogen of lung, kidney, bladder, and skin tissue in humans, and a PTWI has been set at 0.015 mg/kg bw. To date, no regulatory limits have been established for seafood.

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), such as dieldrin, are persistent organic pollutants (POPs) that were widely used up to the 1970s. The Stockholm Convention on Persistent Organic Pollutants, an international environmental treaty that aims to eliminate or restrict the production and use of POPs, was signed and took effect in May 2004. As of May 2013, there are 179 states that are party to the Convention; non-ratifying states include the United States, Israel, Malaysia, Italy, Saudi Arabia and Iraq. To date, there is no established link between POP-contaminated bivalves and human disease (Mozaffarian & Rimm 2006), although results of a recent study suggest a link with fertility impairment (Chevrier *et al*. 2013). Despite the ban, POPs continue to be present in the environment, albeit at low concentrations. A recent study by Zhang *et al*. (2012) measured a broad spectrum of PCBs and OCPs in eight bivalve species from a market in Xiamen, a city on the southeast coast of China where seafood is widely consumed by its 3.5 million inhabitants. They found that the estimated intake levels of PCBs and OCPs were several orders lower than allowable daily intake levels (Table 12.2), which is good news for a country that was once one of the largest and most prominent producers and consumers of these chemicals.

Controls on the production and processing of bivalves

Regulatory framework

To safeguard public health, many countries have established sanitation programmes that oversee the production, harvesting and marketing of bivalve species for human consumption. Most of these have signed either the memoranda of understanding with the Food and Drug Administration (FDA) in the United States or agreements with the EU, and have put in place controls equivalent to those of the United States or EU, thus enabling producers in these countries to export bivalves to the lucrative EU and US markets (Murray & Lee 2010). Current EU legislation on live bivalves is included in hygiene legislation concerned with all food of animal origin. Regulation (EC) No 853/2004/EC (European Communities 2004a) covers the hygiene rules to be applied by harvesters/businesses, including those operating depuration centres and relaying operations (see later). Regulation (EC) No 854/2004/EC (European Communities 2004b) applies to live bivalves and specifies controls at all stages of production from monitoring of the quality of harvesting areas through to placement on the market.

The controls only apply to commercial production. Regulation (EC) No 852/2004 (European Communities 2004c) concerns general rules for the hygiene of foodstuffs including hazard analysis and critical control points (HACCP) requirements; and Regulation (EC) No 2073/2005 (European Communities 2005) sets out the microbiological criteria for foodstuffs. The Shellfish Waters Directive 2006/113/EC (European Communities 2006b) aims to protect shellfish-growing waters from pollution. In the United States the National Shellfish Sanitation Program (NSSP), set up in 1925, in response to outbreaks of bivalves-borne typhoid fever and other diseases, is the federal/state cooperative programme recognised by the FDA and the Interstate Shellfish Sanitation Conference (ISSC) for the sanitary control of shellfish produced and sold for human consumption (NSSP 2011). The NSSP operates under a set of guidelines that have been drawn up by state agencies, technical experts and representatives from the bivalve industry itself. Coordination, supervision and evaluation of the programme are the responsibility of the FDA. The guidelines are updated on a regular basis and are published in a Manual of Operations that is readily accessible to interested parties. The Manual gives detailed instructions on how a monitoring programme should be run in any US member state, describes the interrelation of member state programmes, and cites the criteria to be applied by the FDA in evaluation of the programmes. See WHO (2010) on current legislative controls governing shellfish sanitation programmes in Scotland, Canada and New Zealand.

Controlling harvesting areas

The most logical and least onerous approach in applying the principles of bivalves sanitation is prevention of contamination at source, that is, production and harvesting in clean waters. This requires knowledge of local geography, prevailing water currents and the local discharge and treatment of sewage. In addition, monitoring of water quality is essential. The microbiological status of a shellfish-growing area is usually based on the results from monitoring levels of faecal indicator bacteria, such as *E. coli*, using the standard five-tube, three-dilution most probable number (MPN) technique ISO/TS16649. Both EU and US legislation employ a classification approach with standards set for categories ranging from waters with very low contamination levels to those where harvesting is prohibited. In the EU four categories of shellfish waters are recognised based on *E. coli* levels: category A, where products

Table 12.3 EU criteria for the classification of shellfish harvesting areas.

Class	Microbiological standard per 100 g of bivalve flesh and intravalvular fluid*	Treatment required
A	Less than 230 *Escherichia coli*/100 g of flesh and intravalvular fluid†	None
B	Live bivalves from these areas must not exceed, in 90% of samples, the limits of a five-tube, three-dilution most probable number (MPN) test of 4600 *E. coli*/100 g of flesh and intravalvular fluid. In the remaining 10% of samples, bivalves must not exceed 46 000 *E. coli*/100 g of flesh and intravalvular fluid‡	Purification, relaying in a class A area, or cooking by an approved method
C	Live bivalves from these areas must not exceed the limits of a five-tube, three dilution MPN test of 46 000 *E. coli*/100 g of flesh and intravalvular fluid§	Relaying or cooking by an approved method
Prohibited	More than 46 000 *E. coli*/100 g of flesh and intravalvular fluid	Harvesting not permitted

Lee *et al.* (2008). Reproduced with permission of the Food and Agriculture Organization of the United Nations (FAO).
*The reference method is given as ISO 16649-3.
†By cross-reference from Regulation (EC) No 854/2004, via Regulation (EC) No 853/2004, to Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs (European Communities 2005).
‡From Regulation (EC) No 1021/2008, amending Regulation (EC) No 854/2004.
§From Regulation (EC) No 854/2004.

can be placed directly on the market provided that they comply with a standard of <230 *E. coli* in 100 g of shellfish meat in more than 90% of samples; categories B and C where the product must go through depuration, relaying or designated heat treatment prior to reaching the market and category D where shellfish cannot be treated by any of the earlier methods, that is, harvesting for human consumption is prohibited (Table 12.3). In the United States there are effectively five categories of waters recognised based on faecal indicators (Table 12.4). Note that faecal indicators in the EU are measured in shellfish flesh, while in the United States they are measured in shellfish-growing waters. Shellfish from 'approved' waters can be sold directly on the market without prior treatment, while shellfish from 'restricted' areas may only be sold after depuration or relaying. These two classes correspond to class A and B, respectively, in Table 12.3. Areas that are subject to periodic pollution may be 'conditionally approved' or 'conditionally restricted' and during such events, and for a period afterwards, they remain closed to harvesting. Shellfish from 'prohibited' areas may not be sold for consumption. These two classification systems are the principal systems used worldwide and countries that trade with Europe and/or the United States will use either one, or a hybrid of the two systems.

In addition to classification of harvesting areas there is legislation for monitoring of these areas and guidance and protocols for harvesting, and handling following harvesting, documentation of batches, and procedures such as heat treatment, relaying and purification as well as structural and hygiene requirements for dispatch and purification centres (see later). Apart from microbiological criteria there is legislation controlling organoleptic characteristics (sight, smell, touch, taste) of bivalves, biotoxins levels (Table 12.2), wrapping and packaging, identification marking and labelling. For details on EU legislation see Section VII in regulation (EC) No 853/2004, and NSSP (2011) for the United States. Also, Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP) and a well-designed HACCP programme (see later) are needed to prevent contamination and

Table 12.4 US National Shellfish Sanitation Program (NSSP) criteria for the classification of shellfish-harvesting areas.

	Total coliforms/ 100 ml water		Faecal coliforms/ 100 ml water		
Classification	**Geometric mean**	**90% compliance***	**Geometric mean**	**90% compliance***	**Treatment required**
Approved areas†	≤70	≤230	≤14	≤43	None
Restricted areas‡	≤700	≤2300	≤88	≤260	Purification or relaying to an approved area
Prohibited areas	No sanitary survey, or conditions for approved/restricted areas not met§				Harvesting not permitted

Lee *et al.* (2008). Reproduced with permission of the Food and Agriculture Organization of the United Nations (FAO).
*Values for five-tube decimal dilution test – different 90% compliance levels are given for the three-tube MPN and mTEC membrane filtration tests.
†Determination of approved area status must be based on a minimum of 15 samples from each monitoring station.
‡Conditionally restricted (and approved) areas may be declared where these are subject to predictable contamination events; such areas are closed for harvesting during contamination events and for a period afterwards to permit natural cleansing.
§Considerations other than the concentration of contaminants may be used to declare an area prohibited.

ensure a safe product (see *Codex Alimentarius* Code of Practice,[3] 2009; Oliveira *et al*. 2011 and references; and review by Kingsley 2014).

Decontamination procedures

The capacity of bivalves to concentrate and accumulate bacteria, viruses and biotoxins and pollutants means that special decontamination procedures are often necessary before bivalves can be harvested and marketed. There are two methods, relaying and depuration, that are used to purify bivalves contaminated with pathogenic bacteria, viruses or chemicals. See Lee *et al*. (2008) and Lees *et al*. (2010) for compressive reviews.

Relaying

Relaying involves the transfer of harvested individuals to cleaner estuaries or inlets in the natural environment under the supervision of the agency having jurisdiction, and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption (Codex Alimentarius 2009). Individuals may be placed in floats, rafts or directly on the bottom. Relaying is particularly suitable for treating more heavily polluted bivalves where longer periods, usually more than 2 months, are required to remove heavy contaminant loads (Lees *et al*. 2010). Drawbacks of relaying include the lack or availability of suitable clean coastal areas, and of obtaining ownership to those areas. Also, the water quality of relaying areas is difficult to ensure due to the possibility of recontamination by seasonal variations of naturally occurring bacterial populations or transient pollution. In addition, it can be difficult to assess the efficiency of the relaying process because levels of

the indicator microorganisms may fluctuate erratically during the relay period (see Oliveira *et al*. 2011 and references).

Depuration

Depuration (controlled purification) involves placing contaminated bivalves in flowing clean seawater for a set period of time to allow natural cleansing. The main principles of depuration are:

- *The resumption of filtration activity* so that contaminants are expelled through the maintenance of correct conditions of salinity, temperature and dissolved oxygen.
- *The removal of contaminants* by settlement and/or removal by flow away from the bivalves, and by applying correct depuration conditions for an adequate length of time.
- *Avoidance of recontamination* by operating a batch 'all-in/all-out' system, using clean seawater at all stages of depuration, avoiding resuspension of settled expelled material and cleaning the system thoroughly between batches.
- *Maintenance of viability and quality* by correct handling before, during and after depuration (Lee *et al*. 2008).

Depuration systems range from those in which water is static or changed in batches to systems where seawater is flushed through continually or recycled through a steriliser. Sterilisation methods include ozonation, chlorination, UV irradiation and, less commonly, iodophors. Containers for depuration range from small-scale shallow tanks, to multilayer systems, various types of stacking systems, through to the bulk bin system (Figures 12.4, 12.5, 12.6 and 12.7; Lees *et al*. 2010). Table 12.5 gives information in

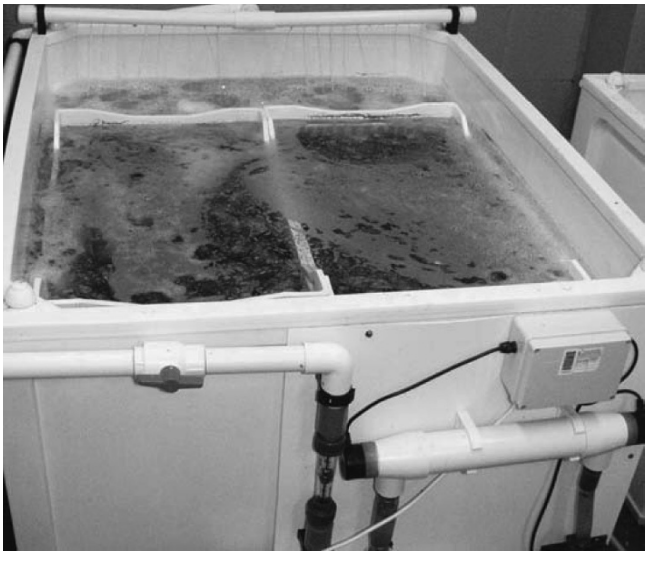


Figure 12.4 Small-scale shallow tank depuration system.
Cefas. © Crown Copyright. Bivalves are held in mesh-type plastic containers placed on battens on the bottom of a shallow tank of approximately 550l capacity. Seawater, after UV treatment, enters the system via water jets above one end of the tank and flows through the containers to a suction pipe or weir across the other end. Water flow rate is relatively low and hence the capacity to supply sufficient dissolved oxygen to the bivalves is also low and the permissible loading (i.e. the stacking) of the system is limited.

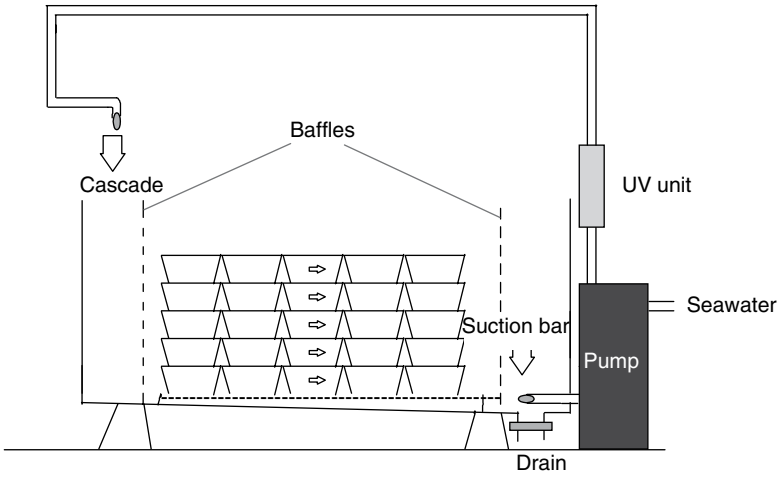


Figure 12.5 Multilayer system.
Cefas. © Crown Copyright. Trays of bivalves are stacked up to six layers high saving a significant amount of floor space compared to the shallow tank system. This system has a high shellfish to water ratio and therefore the flow rate needed to maintain an adequate level of dissolved oxygen in the circulating seawater is relatively high (~200lmin^{-1} for a medium-scale multilayer system of 750kg of bivalves). Two baffles, consisting of a sheet of plastic evenly drilled with holes, through which water passes, are necessary to maintain an even flow of water through the system.



Figure 12.6 Small-scale vertical stack system.
Cefas. © Crown Copyright. Space saving, plus the additional benefit of ready access to individual containers, are the two main advantages of this system. Because it is expensive its use is generally limited to high-value bivalves such as clams and oysters. The system has a water capacity of around 600l, a flow rate of 15lmin^{-1} and accommodates about 2000 oysters.

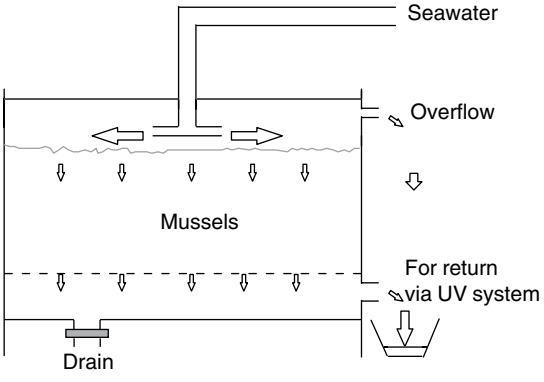


Figure 12.7 Bulk bin system designed specifically for the depuration of mussels Cefas. © Crown Copyright. The mussels are held in a deep layer (~40 cm) on a plastic sheet (false floor), which is 8 cm higher than the base of the bin, and is drilled with holes to permit the flow of water and detritus. Units (bins) can be added or removed to cope with different-sized batches. Each bin has a capacity of about 650 l and the flow rate is high at 108 l min^{-1} to ensure adequate oxygenation of the circulating water.

selected countries on the main species depurated, together with the types of systems and seawater sterilisation methods used.

Legislation governing depuration and relaying processes in the United States and EU are set out in NSSP (2011) and Regulation (EC) No 853/2004 (European Communities 2004a), respectively. The EU Regulation provides details on the construction and general running of a purification plant, such as tank construction and operation, the operation of batch systems and non-mixing of species during purification. The Regulation also covers hygiene requirements of purification and dispatch centres, laboratory testing, packaging, labelling, transportation, wet storage and documentation/traceability requirements. Requirements for relaying operations are also included. Apart from these requirements, purified bivalves, sold live, must comply with an end-product standard of <230 *E. coli*/100 g of flesh and intravalvular fluid (Table 12.3). Approval of plants to operate must be given by the local enforcement authority (LEA) in each member state. For example, the Food Standards Agency is the LEA for the United Kingdom, and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS; http://cefas.defra.gov.uk/) has the delegated responsibility for setting the specific conditions under which the purification system should operate. In addition, CEFAS is the UK-accredited service against the standard ISO/17020 for the purification system inspection service.

Elimination of pathogens and other hazards

The efficiency of depuration is primarily related to bivalve size, filtering activity and physiological status (Jones *et al*. 1991). Efficiency also depends on the nature of the contaminant, the length of the depuration period and the quality of the depuration plant itself. Complete elimination of *E. coli* normally occurs well within 48 h. EU legislation does not stipulate a minimum duration for depuration, and periods employed can be as little as 12 h through to around 48 h, depending on the country. But, even a period of 48 h may not be long enough for elimination of some bacterial types, for example, *Streptococcus faecalis* (Plusquellec *et al.* 1990) and some *Vibrio* species (Marino *et al*. 1999; Croci *et al*. 2002). Indeed, Harrisyoung *et al.* (1995) found that *Vibrio vulnificus* numbers actually increased during UV depuration;

Table 12.5 Depuration in selected European countries.

Countries	Number of approved establishments*	Main species depurated	Types of systems	Main disinfection methods
France	1281	Mussels *Mytilus edulis* and *M. galloprovincialis*; oysters *Ostrea edulis* and *Crassostrea gigas*; and the clam *Ruditapes philippinarum*	Static; recirculating; flow-through	UV light, ozonation, chlorination
Italy	110	*R. philippinarum*, *M. galloprovincialis* and the clam, *Chamelea gallina*	Continuous flow-through	Chlorination, with activated carbon filters used for dechlorination of process water; ozonation
United Kingdom (England, Wales, Scotland and Northern Ireland)	86	*Mytilus* spp., *O. edulis*, *C. gigas*, *R. philippinarum*, *R. decussatus* and the cockle, *Cerastoderma edule*	Recirculating	UV light
Spain†	60	*O. edulis*, *M. galloprovincialis*, the clams *R. decussatus*, *Venerupis pullastra*, *V. rhomboideus*, *Venus gallina* and *C. edule*	Recirculating; flow-through	Chlorination, with some UV light and ozonation
Portugal	24	*M. galloprovincialis*, *Crassostrea angulata*, *R. philippinarum*	Static; recirculating; flow-through	UV light, chlorination
Ireland	16	*M. edulis*, *O. edulis* and *C. gigas*	Recirculating	UV light
The Netherlands‡	21	*M. edulis*, *O. edulis* and *C. gigas*	Recirculating; flow-through	UV, or not disinfected

Lee *et al.* (2008). Reproduced with permission of the Food and Agriculture Organization of the United Nations (FAO).
*As of July 2013 accessed on the EU website: ec.europa.eu/food/food/biosafety/establishments/list_en.htm.
†As of December 2006 in Lee *et al.* (2008).
‡The waters in which shellfish are grown are class A and consequently depuration is generally not required except for imported shellfish.

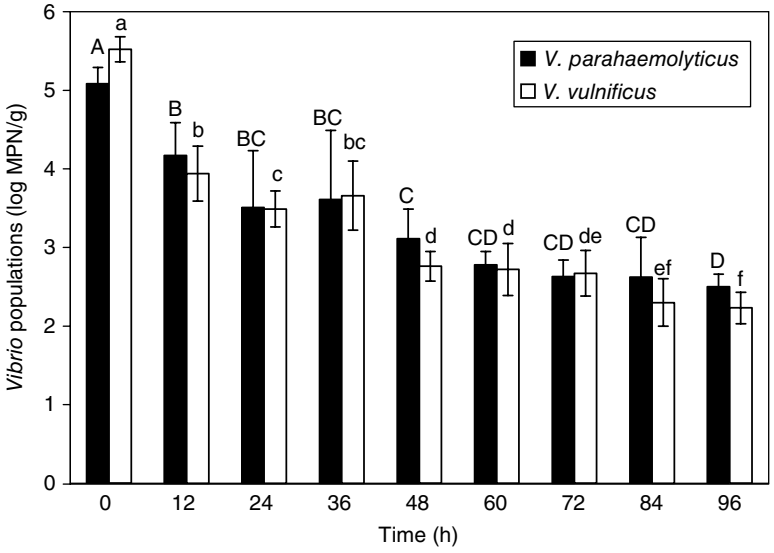


Figure 12.8 Reductions of *Vibrio parahaemolyticus* (solid bars) and *V. vulnificus* (hollow bars) in laboratory-contaminated oysters depurated in artificial seawater at 15°C for 96 h. Data are means of four determinations ± standard deviations. Means with the same letter are not significantly different ($P > 0.05$).
Chae *et al.* (2009). Reproduced with permission of John Wiley & Sons.

it seems that this species employs encapsulation as a way of resisting ingestion and degradation by haemocytes during the purification process. But the temperature at which depuration is carried out may be a crucial factor in effective elimination of *Vibrio* sp. Depuration of oysters (*C. virginica*) at 22°C had limited effects on reducing *V. parahaemolyticus* or *V. vulnificus* (Chae *et al*. 2009). Decreasing water temperature to 15°C increased the efficacy of depuration, and extending the depuration period from 48 to 96 h increased this even more. Depurations at 10 and 5°C were less effective than at 15°C (Figure 12.8).

It is now generally recognised that the main threat to public health from consumption of bivalves is from viral rather than bacterial contamination, especially from species that are eaten raw, for example, oysters. Unlike most bacteria, viruses during the depuration process are released slowly and at different rates depending on the virus, and on the bivalve species being depurated (Richards *et al*. 2010; SARF 2011 and references). For example, at 18°C, levels of male-specific RNA bacteriophage (viral indicator) were reduced to just 2% of initial contamination levels compared with 40% of initial contamination levels for oysters (*C. gigas*) depurated at 9°C (Figure 12.9a). However, even at the higher temperature it was still not possible to eliminate all viruses, even after 7 days of depuration. At light contamination it was possible to reduce RNA bacteriophage to below detection level (<30 plaque forming units (pfu)/100 g bivalve) after only 2 days of depuration at 18°C, whereas depuration at 9°C did not completely eliminate low levels even after 7 days (Figure 12.9b; see also Lee *et al*. 2008; Doré *et al*. 2010; Lees *et al*. 2010). This may be because viruses, such as NoV, bind specifically to digestive tissue receptor sites (Le Guyader *et al*. 2006), or in the case of HAV, are phagocytosed by bivalve haemocytes (Provost *et al*. 2011), thus impeding elimination. Therefore, compliance with faecal coliform end-product standards, the index used to evaluate the efficacy of the depuration process, does not provide a guarantee of the absence of enteric viruses in depurated bivalves (Muniain-Mujika *et al*. 2002; SARF 2011). Consequently, various strategies have been suggested for elimination/inactivation of viruses.

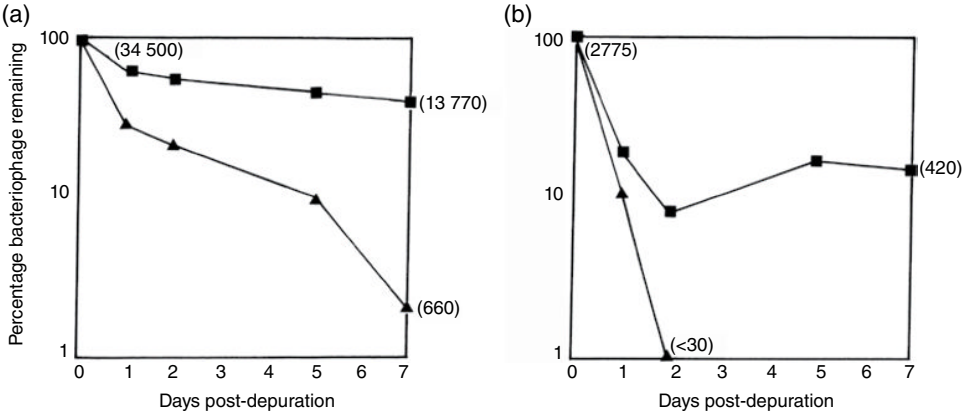


Figure 12.9 Percentage of male-specific RNA bacteriophage remaining in oysters (*Crassostrea gigas*) after depuration at 9 ± 1°C (■) and 18 ± 1°C (▲) in two depuration cycles (a and b). Values in brackets are bacteriophage titres pfu/100 g oyster for initial contamination and post-depuration levels. Doré *et al.* (1998). Reproduced with permission of IWA Publishing.

Very long-term relaying (more than several months) is a possible solution provided that clean waters are available and if the cleanliness of the waters containing the shellfish can be maintained (Richards *et al*. 2010). An alternative strategy is a combination of depuration and relaying. Virus inactivation by heat treatment, for example, cooking, is also used but only in countries where shellfish are sold as a processed product. Evaluation of the method is difficult because of the use of different testing methods, different virus strains or surrogates, and different bivalve species (Richards *et al*. 2010). One heat treatment method approved by the EU is immersion of the bivalve in boiling water for a sufficient length of time to raise the internal temperature of the flesh to not less than 90°C, and maintenance of this temperature for at least 90 s (details in Regulation (EC) 853/2004). A non-thermal method is high-pressure processing (HPP), which inactivates NoV and HAV (Kingsley 2013). The method inactivates the virus by denaturing proteins on its capsid (outer shell), making the virus incapable of binding to its receptor on the surface of the host cell. Other thermal and non-thermal methods include heat pasteurisation, freezing, smoking (details in Richards *et al*. 2010) and electron beam irradiation (Praveen *et al*. 2013). No one method guarantees complete elimination or inactivation of viruses. Richards *et al*. (2010) recommend the use of combined techniques, such as shellfish relaying followed by HPP; pasteurisation followed by freezing or depuration followed by moderate cooking, since multiple methods have an additive advantage in reducing viral contamination.

Bivalves that have accumulated biotoxins are normally left to purge themselves in their natural habitat. However, there is no clear pattern in the detoxification process due to different detoxification rates among toxins (Huss *et al*. 2004), bivalve species (Choi *et al*. 2003; Fast *et al*. 2006; Lindegarth *et al*. 2009; Röder *et al*. 2011) and sites of toxin storage (toxins in the gastrointestinal tract are eliminated much more readily than toxins bound in tissues). Other factors, such as bivalve size and temperature, may influence detoxification rates. For example, DA was eliminated faster in small (45–55 mm) than in large mussels (60–70 mm), and was more rapid at 11°C than at 6°C, although salinity, or whether mussels were fed or starved, had no effect on elimination rates (Novaczek *et al.* 1992). Overall, the elimination period varies from several weeks, as is the case for BTXs (Dickey *et al*. 2011), to many months, for example, 7–8 months for AZAs (Ryan *et al*. 2011). Other methods for accelerating the detoxification process have been tried, particularly for PSP toxins. These include electric shocks, decrease in pH, chlorination and biotransformation of phycotoxins by

enzymes, but none of these have proved effective. A European consortium (SPIES-DETOX project) is currently testing the potential of microencapsulated marine bacteria to eliminate toxins from shellfish. And in France the national CONSAUMOL programme funded by IFREMER includes detoxification trials on bivalves maintained in recirculation systems and provided with 'fodder algae and/or clay' to accelerate toxin elimination (SARF 2011).

In contrast to the naturally occurring biotoxins, the risk from chemical contaminants such as heavy metals, pesticides and drug residues in commercially harvested marine bivalves is low (Huss *et al*. 2000). But, where contamination does occur, uptake and clearance is a passive process, and relaying is the method used in the elimination process. Metallothioneins (MTs), non-enzymatic proteins with a low molecular weight, play a central role in heavy metal detoxification. Induction of MT synthesis is brought about by heavy metals such as Cd, Cu and Hg, and, consequently, MT concentrations in bivalves can be used as biomarkers of heavy metal exposure (review in Amiard *et al*. 2006). Elimination rate of contaminants is markedly affected by the duration of exposure to the chemical. Elimination is rapid and complete following short-term exposure, but long-term exposure results in slower and often incomplete clearance of the chemical (Livingstone & Pipe 1992). Elimination rate also depends on the molecular weight and water solubility of the chemical, as well as on environmental factors such as temperature.

Monitoring and quality control

Every country involved in bivalve production should have an effective sanitation programme that oversees the production, harvesting and marketing of species for human consumption. The basic requirements of such a programme should include (Canzonier 1988):

- An infrastructure, responsible for the monitoring, culture and harvesting activities; also, adequate surveillance of the chain of supply from point of production to point of retail.
- An administrative system for coordinating the activities of the various public agencies responsible for running the programme.
- Appropriate legislation so that the agencies can prosecute those who breach regulations.

To illustrate how such a programme operates an example from the southern hemisphere will be taken.

Regulation of bivalve monitoring programmes in New Zealand

The NZFSA, set up in 2007, reports directly to the Minister for Food Safety. It has two key roles: protection and promotion of public health and safety and facilitation of market access for New Zealand's food and food-related products. About 80% of bivalves grown in New Zealand are exported to over 60 countries. Therefore, it is important that New Zealand has in place regulatory shellfish standards that encompass international best practice. The Greenshell™ mussel (*Perna canaliculus*) and the Pacific oyster (*C. gigas*) are the two most important species, and these are grown either in small 5–7 ha farms or, more recently, in larger offshore (2–10 000 ha) operations. Information in the following section has been primarily gleaned from Busby (2010) and the New Zealand Legislation http://www.legislation.govt.nz) and Food Safety websites (http://foodsafety.govt.nz).

Bivalve production in New Zealand is governed by two pieces of legislation under the Bivalve Molluscan Shellfish Regulated Control Scheme (BMSRCS): the Animal Products (Regulated Control Scheme–Bivalve Molluscan Shellfish) Regulations (2006) and the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice (2006). The main

aim of the Animal Products Regulations (2006) is to identify, monitor, evaluate and manage the risks associated with the commercial growing, harvesting, sorting and transporting of bivalves intended for human consumption. Part 1 of these Regulations also legislates for other related activities or conditions affecting the suitability for processing or fitness for intended purpose of bivalves. In addition, they govern the growing, harvesting, sorting and transporting of bivalves for commercial purposes up until the time that bivalves are received by a wholesaler or retailer or sold direct to the consumer. In Part 2, the Regulations sets out the standards, obligations and requirements governing the suitability of vessels and vehicles used in bivalve transportation; the personnel handling bivalves; identification, labelling and record keeping; obligations of growers and obligations of harvest, relay, transport, sorting shed and depot operators. Also, the requirements for classification, sanitary survey, monitoring and procedures for opening and closing areas are also described. Part 3 addresses the registration, permits and listings of operators, while Part 4 addresses miscellaneous provisions including offences (Busby 2010).

The Animal Products Notice (2006) sets out specifications that are necessary, or desirable, to regulate bivalve production. These deal with growing areas, growing area classification and status, relaying, wet storage (at sea), marine biotoxin control, sampling, control of harvesting, requirements for harvest operators, vessels and vehicles, health of personnel, sorting sheds and depots, transport of BMS, microbiological risk management, marinas, BMS laboratories, calibration and record keeping. Some of these will now be described in more detail.

Growing areas: Sanitary survey and classification

Before a bivalve-growing area is classified, an Animal Product Officer carries out a sanitary survey. This includes a shoreline survey; a survey of bacterial quality of the water in the growing and adjacent areas; a survey of bacterial quality of bivalves in the growing area; and an evaluation of effect of any hydrographic, meteorological and geographic characteristics of the growing area and catchment. The location and number of sample stations must be adequate to allow the effective evaluation and routine monitoring of all actual and potential pollution sources that may have an impact on the bacteriological quality of the growing area. The BMSRCS requires that the sanitary survey and classification status of growing areas be reviewed on an annual basis.

When the survey data are analysed a growing area is classified, on the basis of bacterial standards, as remote approved, approved, conditionally approved, restricted or conditionally restricted, rather similar to the classification system used in the United States (Table 12.4). 'Remote' signifies that there is no human habitation in the catchment or no actual or potential sources of pollution in the growing area. The bacterial standards for remote approved, approved and conditionally approved must meet the bacterial standards at each sample station in the growing area when it is open for harvesting. The faecal coliform median MPN of the water samples must not exceed 14/100 ml and not more than 10% of the samples must exceed an MPN of 43/100 ml; and the *E. coli* median MPN of the bivalve samples must not exceed 230/100 g and not more than 10% of samples must exceed an MPN of 700/100 g.

For 'approved' classification the growing area must be suitable for the trade of bivalves without relay, depuration or post-harvest treatment, and must not be contaminated with pathogenic organisms or toxic substances. For remote approved areas, a minimum of two samples must be collected annually; for approved areas the number is five; and for conditionally approved areas, monthly samples are required. For restricted and conditionally restricted areas the faecal coliform median MPN of water samples must not exceed 88/100 ml and not more than 10% of samples must exceed 260/100 ml;

and the *E. coli* median MPN of the bivalve samples must not exceed 4600/100 g and not more than 10% must exceed 14 100 *E. coli* per 100 g. For 'restricted' classification levels of faecal pollution, human pathogens or toxic substances in the growing area are such that bivalves can be made fit for human consumption by either relaying, depuration or post-harvest treatment. Five samples per year must be collected to maintain the restricted classification, and monthly samples in the case of conditionally restricted classification. Overall, there are clearly evident similarities between bacterial standards used for classifying growing areas in New Zealand and those in use in the EU and the United States (Tables 12.3 and 12.4).

Marine biotoxin monitoring and control

The NZFSA is the state agency overseeing marine biotoxin monitoring and control. Each growing area has a management plan, which is drawn up by an animal products officer (APO) in consultation with the shellfish industry (Table 12.6). As part of this plan the APO designs and implements a monitoring programme for each growing area. Sampling stations are chosen on the basis of the history of marine toxin and phytoplankton activity in the growing and adjacent areas; the hydrography of the catchment and the need to provide spatial and depth coverage of bivalves and toxigenic phytoplankton. Weekly sampling is carried out for some 20 toxigenic phytoplankton species from seawater (e.g. *Alexandrium*, *Pseudo-nitzschia*, *Karenia*, *Dinophysis*) and toxins (e.g. PSP, ASP, NSP, DSP, YTX and AZP) from bivalves. Sampling is more frequent if biotoxins are detected above background level but below maximum permissible level (alert level) or above maximum level in which case the growing area is closed. The maximum allowable levels for PSP, ASP and NSP in New Zealand are identical to those in Australia, and for DSP, they are identical to EU levels (see Table 12.2). The area remains closed until the APO has determined, from comprehensive spatial sampling, that levels of toxigenic phytoplankton in water samples, and biotoxins in bivalves, are decreasing or static for at least two consecutive sampling occasions at least 48 h apart. The APO must ensure that the reopened area undergoes intensive spatial and depth monitoring until such time as the level of biotoxin in the bivalves and the levels of toxigenic phytoplankton are consistently below the background level. For a recent review of HAB monitoring practices in New Zealand, see Rhodes *et al*. (2013).

Table 12.6 Essentials of a management plan for marine biotoxin management in bivalve-growing areas in New Zealand (Animal Products Regulations 2006).

The plan for a growing area must include:
Location and identification number of each farm, the species being cultured, location of phytoplankton and bivalve sampling stations and details on the hydrography of the catchment area.
Agency and personnel contact details at local, regional and national level in relation to regulatory, laboratory, shellfish industry and research matters.
The routine monitoring programme for bivalves and phytoplankton.
Criteria and actions to be taken for increasing sampling during a toxic event.
Marine biotoxin test methods used for individual toxin groups.
Procedures for notification of phytoplankton and bivalve results from the laboratory to APOs and the shellfish industry.
Procedures and draft letters for growing area closure and reopening (see text for details).
The APO must prepare a report on each growing area closure and reopening.
The APO must conduct an annual review of the management plan and append it to the annual growing area review report.

HAACP system

To ensure that a high-quality product reaches the consumer, several quality assurance schemes have been developed such as certification under an International Accepted Standard (ISO 9000 series), Total Quality Management (TQM), GMP, Good Hygienic Practice (GHP) and Hazard Analysis Critical Control Point (HACCP). The aim of the HACCP system is to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. The system was first developed in the late 1960s to develop safe foods for the US space programme. Since then it has become the premier food safety system, not just in the United States, but also in the EU, Canada, Australia and New Zealand, as well as other countries, and it serves as the foundation of the World Health Organization's Codex Alimentarius Commission's General Principles of Food Hygiene (Savage & Lafontaine 2010). Working Groups of the Codex Committee on Fish and Fishery Products have produced revised codes of practice for various seafoods including finfish, crustacea and bivalve molluscs (see Codex Alimentarius 2009).

The system, which can be applied from production to consumption, consists of the following seven principles:

Principle 1: *Conduct a hazard analysis*
Identify any hazards that must be prevented, eliminated or reduced to acceptable levels.

Principle 2: *Determine Critical Control Points*[4] *(CCP)*
Determine the points, procedures or operational steps that can be controlled to eliminate the hazard(s) or minimise its (their) likelihood of occurrence.

Principle 3: *Establish critical limits*
Establish critical limit(s) which must be met to ensure that the CCP is under control.

Principle 4: *Establish a system to monitor control of the CCP*
Establish a system to monitor control of the CCP by scheduled testing or observations.

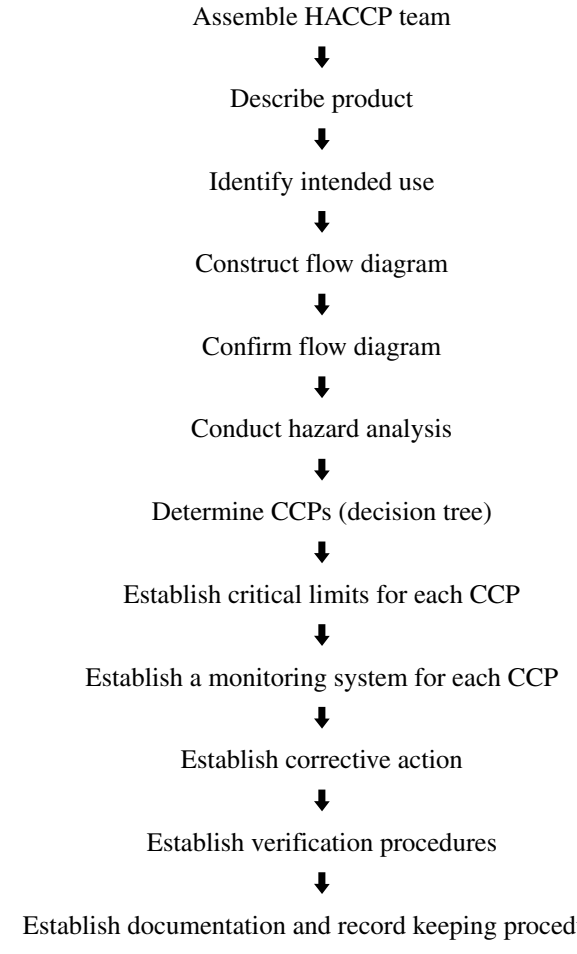
Principle 5: *Establish corrective action(s)*
Establish the corrective action(s) that must be taken when monitoring indicates that a particular CCP is not under control.

Principle 6: *Establish procedures for verification*
Establish procedures for verification, which include supplementary tests and procedures to confirm that the HACCP system is working effectively.

Principle 7: *Establish records and record keeping*
Establish documentation concerning all procedures and records appropriate to these principles and their application.

Application of HACCP

Any food business operator, handling or supplying live bivalves, is required by law to put in place, implement and maintain permanent food safety management procedures based on HACCP principles. Codex Alimentarius recommends the following sequence for the implementation of a specific HACCP plan.



Ideally, the HACCP team should be multidisciplinary, including personnel such as the processing facility manager, a microbiologist, a quality assurance/quality control specialist, buyers and operators. The team must provide a full description of the product (s), which should include name, intended use of product(s), intended consumer, any regulatory limit and other details, for example, packaging specifications, shelf life and storage requirements, labelling requirements. The product profile is a necessary prerequisite for setting appropriate food safety criteria, and for hazard identification and analysis (see MAF 2011 for details). An accurate description of the steps involved in the processing of the product is essential for a proper hazard analysis. This is usually presented in the form of a flow diagram (Figure 12.10). The team must identify and list at each stage of the process all the food safety hazards that may reasonably be likely to occur. In addition, potential quality (non-safety) defects should also be determined. Once hazards and defects have been identified their significance must be determined and an assessment made on whether control measures are available at each step. Most control measures are likely to be covered by Good Operating Practice (GOP). The next step is to determine whether there are any critical Control Points (CCPs) for the process. CCP determination can be facilitated by the use of a decision tree or a table that provides a series of questions that guide the user through the decision-making process (Figure 12.11). When

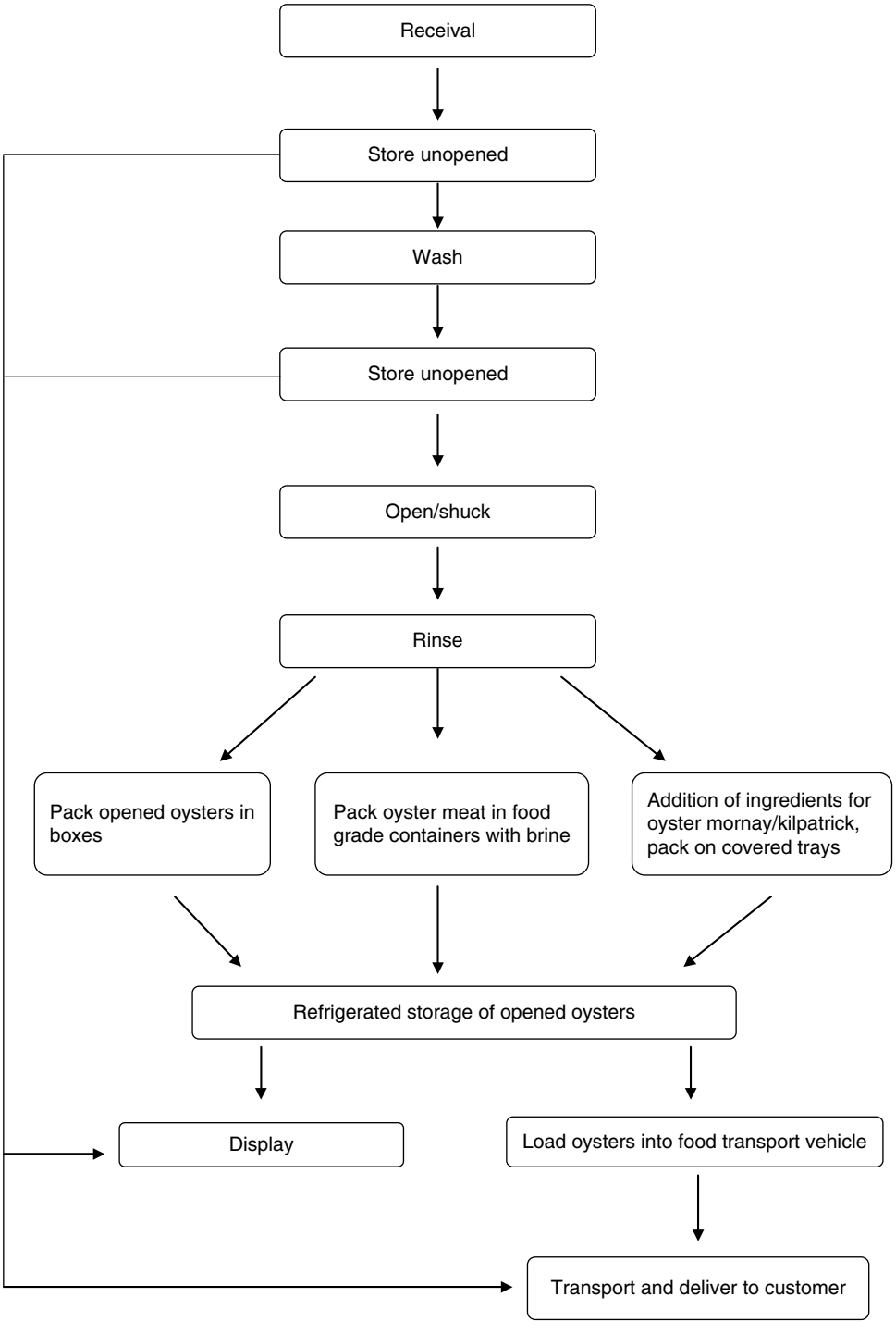


Figure 12.10 Hazard analysis flow chart for Sydney rock oyster (*Saccostrea glomerata*), Pacific oyster (*Crassostrea gigas*) and the flat oyster (*Ostrea angasi*).
NSW Food Authority, April 2012. Reproduced with permission.

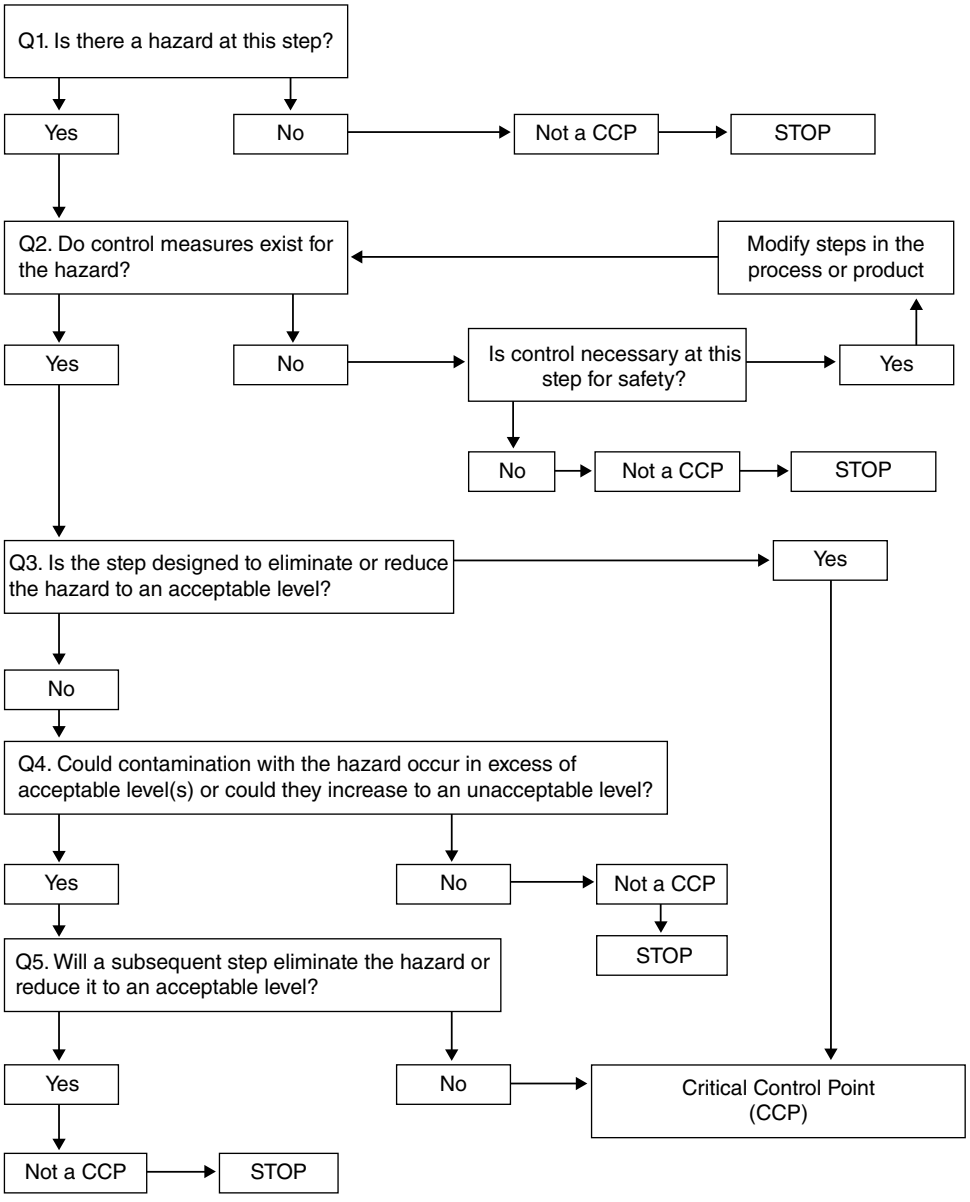


Figure 12.11 Critical Control Point (CCP) decision tree used in hazard analysis for oyster (*Saccostrea glomerata, Crassostrea gigas* and *Ostrea angasi*) processing.
NSW Food Authority, April 2012. Reproduced with permission.

a CCP is identified, the remaining HACCP requirements in the flow diagram must be applied. If no CCPs are identified, verification, documentation and record keeping must still be applied. When a CCP is identified the team must define and justify critical limit(s) for each CCP (Table 12.7). Critical limits must be measurable and should be parameters that can be monitored on an ongoing basis, for example, temperature, time, moisture level, pH. Monitoring procedures should include the person responsible for monitoring, the monitoring method, the monitoring frequency and sampling regime, and records to be kept (MAF 2011). The team must document corrective action procedures to be implemented when a critical

Table 12.7 Hazard analysis for processing Sydney rock oyster (*Saccostrea glomerata*), Pacific oyster (*Crassostrea gigas*) and the flat oyster (*Ostrea angasi*).

Process step	Hazard	Control measures	CCP decision
Receival	Excessive numbers/levels of pathogenic microorganisms or other contaminants in the oysters and other ingredients	Supplier approval programme Oysters fully labelled	Yes CCP
	Growth of microorganisms in the oysters and other ingredients during transport prior to delivery	Temperature control	Yes CCP
Store unopened	Contamination from external source	Oysters protected from contamination during storage	No (support programme)
	Growth of microorganisms	Temperature control	Yes CCP
Wash	Contamination with pathogenic microorganisms	Potable water used	No (support programme)
		Avoid washing methods that cause physical damage to the oyster shell and methods that submerge oysters in water	No (support programme)
Open/shuck	Microbial contamination from dead or sick oysters	Sort and discard dead, damaged, diseased or dying oysters during shucking	No (support programme)
	Contamination from opening equipment/utensils	Clean and sanitise equipment prior to use	No (support programme)
	Contamination from opener	Observe good personal hygiene	No (support programme)
Rinse	Contamination with pathogenic microorganisms	Rinse in running clean, potable water using shower rose	No (support program)
Packing	Contamination from packaging material	Clean and sanitise all equipment prior to use, and use only clean, food grade packaging material	No (support programme)
	Contamination of flesh from other foreign matter	Separate packaged layers using food grade plastic and paper between layers	No (support programme)
	Contamination with pathogenic microorganisms from handlers	Observe good personal hygiene	No (support programme)
	Contamination from addition of brine or other added ingredients	Observe good personal hygiene Potable water used to prepare brine	No (support programme)
	Microbiological growth if mornay sauce stored out of refrigeration	Temperature control	Yes CCP

Refrigerated storage of opened oysters	Growth of pathogenic microorganisms	Temperature control	Yes CCP
	Contamination with pathogenic microorganisms	Ensure oysters are covered	No (support programme)
Display	Contamination with pathogenic microorganisms	Temperature control	Yes CCP
	Contamination from foreign materials Contamination from chemicals	Maintenance and cleaning procedures	No (support programme)
Loading of oysters into food transport vehicle	Growth of pathogenic microorganisms	Temperature control	Yes CCP
	Contamination with pathogenic microorganisms	Ensure oysters are covered	No (support programme)
Transport and delivery to customer	Growth of pathogenic microorganisms	Temperature control	Yes CCP

NSW Food Authority, April 2012. Reproduced with permission.
CCP, Critical Control Point; potable water, water quality consistent with Australian Drinking Water Guidelines 6 (NHMRC, NRMMC 2011); support programme, the HACCP system is based on a solid foundation of support or prerequisite programmes, which are managed separately from the HACCP plan. Examples of support programmes are staff health and hygiene, cleaning and sanitation, equipment calibration and maintenance.

limit is not met, and establish and document operator verification procedures to ensure that the HACCP system is working effectively. Finally, documentation and record keeping should be sufficient to verify that HACCP controls are in place and being maintained. For more information on the implementation of the HACCP system, see Huss *et al*. (2004), Codex Alimentarius (2009), FDA (2011), MAF (2011) and NSSP (2011). For information specifically related to HACCP in shellfish depuration, see Lee *et al*. (2008, 2010) and NSSP (2011).

Notes

1 The RT-PCR qualitative method uses a combination of retro-transcription of RNA to complementary (c) DNA and amplification of cDNA by PCR, not to be confused with quantitative real-time PCR (RT-PCR) method.
2 ARfD is the amount of a chemical, normally expressed on a body weight basis, that can be consumed in a period of 24 h or less without appreciable health risks to the consumer.
3 *Codex Alimentarius* is a collection of internationally adopted food standards, guidelines, codes of practice and other recommendations.
4 Critical Control Point means a point, step or procedure in a food process at which control can be applied, and a food safety hazard can, as a result, be prevented, eliminated or reduced to acceptable levels.

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