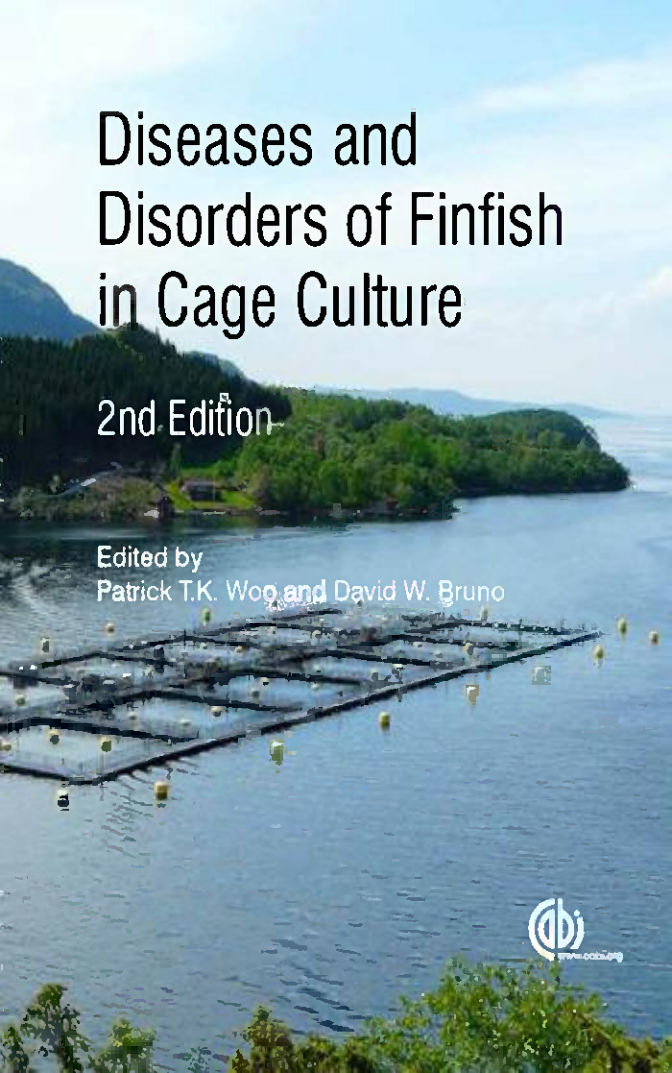
Diseases and Disorders of Finfish in Cage Culture

2nd Edition

Edited by
Patrick T.K. Woo and David W. Bruno



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2nd Edition

Edited by

Patrick T.K. Woo

Department of Integrative Biology
College of Biological Science
University of Guelph
Guelph, Ontario, Canada

and

David W. Bruno

Marine Scotland Science
Aberdeen, Scotland, UK

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CABI
Nosworthy Way
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Fax: +44 (0)1491 833508
E-mail: info@cabi.org
Website: www.cabi.org

CABI
38 Chauncy Street
Suite 1002
Boston, MA 02111
USA

Tel: +1 800 552 3083 (toll free)
Tel: +1 617 395 4051
E-mail: cabi-nao@cabi.org

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Contents

Contributors vii

Preface to the Second Edition ix

Preface to the First Edition xi

1 Overview of Cage Culture and Its Importance in the 21st Century 1
Donald J. Noakes

2 Infectious Diseases of Coldwater Fish in Marine and Brackish Waters 15
Eva Jansson and Pia Vennerström

3 Infectious Diseases of Coldwater Fish in Fresh Water 60
Kenneth D. Cain and Mark P. Polinski

4 Non-infectious Disorders of Coldwater Fish 114
Heike Schmidt-Posthaus and Mar Marcos-López

5 Infectious Diseases of Warmwater Fish in Marine and Brackish Waters 155
Angelo Colorni and Ariel Diamant

6 Infectious Diseases of Warmwater Fish in Fresh Water 193
Gilda D. Lio-Po and L.H. Susan Lim

7 Non-infectious Disorders of Warmwater Fish 254
Florbela Soares, Ignacio Fernández, Benjamín Costas and Paulo Gavaia

8 Sporadic Emerging Diseases and Disorders 287
Simon R.M. Jones and Pedro A. Smith

9 Transmission of Infectious Agents between Wild and Farmed Fish 313
Sonja M. Saksida, Ian Gardner and Michael L. Kent

Index 331

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Contributors

David W. Bruno, Marine Scotland Science, 275 Victoria Road, PO Box 101, Aberdeen, AB11 9DB, Scotland, UK. E-mail: david.bruno@scotland.gsi.gov.uk

Kenneth D. Cain, Department of Fish and Wildlife Science, University of Idaho, 875 Perimeter Drive M51136, Moscow, Idaho 83844-1136, USA. E-mail: kcain@uidaho.edu

Angelo Colorni, National Center for Mariculture, Israel Oceanographic and Limnological Research Ltd., PO Box 1212, Eilat 88112, Israel. E-mail: angelo@ocean.org.il

Benjamín Costas, CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123, Porto, Portugal. E-mail: bcostas@ciimar.up.pt

Ariel Diamant, National Center for Mariculture, Israel Oceanographic and Limnological Research Ltd., PO Box 1212, Eilat 88112, Israel. E-mail: diamant@ocean.org.il

Ignacio Fernández, CCMAR - Centre of Marine Sciences (CCMAR/CIMAR-LA), University of Algarve, Campus of Gambelas, 8000-139 Faro, Portugal. E-mail: ivmonzon@ualg.pt

Ian Gardner, Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada. E-mail: iagardner@upei.ca

Paulo Gavaia, CCMAR - Centre of Marine Sciences (CCMAR/CIMAR-LA), University of Algarve, Campus of Gambelas, 8000-139 Faro, Portugal. E-mail: pgavaia@ualg.pt

Eva Jansson, National Veterinary Institute (SVA), SE-75189 Uppsala, Sweden. E-mail: eva.jansson@sva.se

Simon R.M. Jones, Pacific Biological Station, Nanaimo, British Columbia, Canada. E-mail: simon.jones@dfo-mpo.gc.ca

Michael L. Kent, Oregon State University, Corvallis, Oregon, USA. E-mail: michael.kent@oregonstate.edu

L.H. Susan Lim, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Selangor, Malaysia. E-mail: susan@um.edu.my

Mar Marcos-López, Marine Laboratory, Marine Scotland Science, 375 Victoria Road, Aberdeen AB11 9DB, UK. E-mail: mar.marcos-lopez@scotland.gsi.gov.uk

Donald J. Noakes, Thompson Rivers University, 900 McGill Road, Kamloops, British Columbia, Canada V2C 0C8. E-mail: dnoakes@tru.ca

Gilda D. Lio-Po, Fish Health Section, Aquaculture Department, South East Asia Fisheries Development Center, Tigbauan, Iloilo, Philippines. E-mail: liopo@seafdec.org.ph

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Mark P. Polinski, National Centre of Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Launceston, Tasmania 7250, Australia. E-mail: mark.polinski@utas.edu.au

Sonja M. Saksida, BC Centre for Aquatic Health Sciences, Campbell River British Columbia, Canada. E-mail: sonja.saksida@cahs-bc.ca

Heike Schmidt-Posthaus, Centre for Fish and Wildlife Health, Institute of Animal Pathology, University of Berne, Laenggassstrasse 122, PO Box 8466, 3001 Berne, Switzerland. E-mail: heike.schmidt@vetsuisse.unibe.ch

Pedro A. Smith, Department of Animal Pathology, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile. E-mail: psmith@uchile.cl

Florbela Soares, IPMA - National Institute for the Ocean and Atmosphere, Olhão, Portugal. E-mail: fsoares@ipma.pt

Pia Vennerström, Finnish Food Safety Authority Evira, FI-00790 Helsinki, Finland. E-mail: pia.vennerstrom@evira.fi

Patrick T.K. Woo, Department of Integrative Biology, College of Biological Science, University of Guelph, Ontario, Canada. E-mail: pwoo@uoguelph.ca

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Preface to the Second Edition

The world population was 7 billion in 2011, and at the current rate of increase it will be about 8 billion by 2025. Also, the demand for animal protein as a food source will continue to increase and exert additional pressures on food production which will have to compete with other human activities (e.g. housing, transportation, industry) for the limited usable land. Animal protein contains essential amino acids which are important components of a balanced diet. However, free ranging land animals are no longer a significant source of protein, and the production costs of farm animals continue to escalate. To increase efficiency and to reduce costs animal farms are large and often close to human habitations. Wastes associated with the large scale breeding of mammals and birds can pollute the environment and also increase the risks of disease outbreaks in animals with the subsequent interspecies transmission of zoonotic diseases (e.g. Nipah virus in pigs, avian influenza virus in birds, cryptosporidian parasites in cattle) to humans.

Finfish are an excellent source of protein and many marine species have beneficial PUFA (polyunsaturated fatty acids); however, the capture-fishery is either stagnant or in decline as there are no newly discovered fishing grounds. Also, natural fish stocks in many parts of the world have been significantly reduced due to more efficient fishing technologies, over and/or indiscriminate fishing, and the loss and/or destruction of spawning grounds. Industrial wastes (e.g. heavy metals, organophosphates) discharged into the aquatic environment can affect fish growth, survival and reproduction, and in some areas pollutants have accumulated in fish to the extent they are no longer suitable for human consumption. Cage culture of finfish (especially in-shore) has lower start-up and production costs and it does not have some of the problems associated with the raising of large numbers of warm blooded animals. Intensive culture of fish is one solution to producing more affordable animal protein; however, outbreaks of diseases may occur more frequently because of numerous factors, which include enhanced transmission of infectious pathogens between fish.

A tremendous volume of research has been conducted on the diseases and disorders since the publication of the first edition of 'Diseases and Disorders of Finfish in Cage Culture' in 2002. The aims, philosophy, audience, focus and format have remained unchanged. However, significant changes in the current edition include new contributors for eight of the nine chapters, the addition of a new chapter (on 'transmission of infectious agents between wild and farmed fish'), and the deletion of one chapter (on 'the history of cage culture') have resulted in a more relevant and informative text.

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Our contributors are highly respected international experts from Asia, Australia, Europe, North America and South America. They have practical experience and/or research expertise on diseases/disorders and their diagnosis, and /or solutions to problems associated with cage culture. As with the first edition our primary objective is to produce an authoritative and practical volume for colleagues in the aquaculture industry, especially those associated with the cage culture of finfish. We also hope this volume will alert industry to potential and/or emerging diseases and disorders in specific regions of the world and to point out gaps in our knowledge so as to stimulate further research.

Patrick T.K. Woo and David W. Bruno

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Preface to the First Edition

In many parts of the world the primary source of animal protein for humans is finfish. The intensive culture of finfish has grown significantly since the 1980s partly because of the dramatic decline in the natural fish stocks and the increase in fish consumption by the ever-increasing population. For example, the worldwide consumption of fish between 1990 and 1997 increased by 30% while the capture fisheries increased only by 9%. The demand for fish is expected to continue to increase, especially as the more affluent consumers in the developed countries become more aware of the beneficial effects of fish (e.g. marine fish are an excellent source of polyunsaturated omega-3 fatty acids). Aquaculture is the only solution to the demand as it can provide consistently high quality fish protein year round. The industry is already considered the single fastest-growing food production process in the world.

The cage culture of finfish, especially mariculture, is becoming more popular because there are many economic advantages associated with this approach. However, it also has problems and one of them is disease. Disease outbreaks tend to occur more often when fish are raised under intensive culture conditions, and consequently both infectious and non-infectious diseases are important constraints to the industry.

Our primary objective is to produce an authoritative and practical volume on diseases and disorders of finfish in cage culture. We hope the book will also alert the industry to potential and/or emerging disease problems in specific regions of the world, and to point out gaps in our knowledge so as to stimulate further research. This book is designed for aquaculturalists who are using or intend to use cage culture. It will also be useful to fish health consultants (e.g. veterinarians), microbiologists, parasitologists, fish pathologists, and managers and directors of diagnostic laboratories. Each chapter is written by international experts who have personal experience or expertise on diseases and their diagnosis, and/or solutions to problems associated with the cage culture of finfish.

This book is divided into four parts – the first part is on the cage culture system, the second and third are on diseases/disorders in warmwater fish (water temperature above 15°C) and in coldwater fish, respectively. In each of these parts, there are three chapters – one on infectious diseases in fresh water (zero salinity), one on estuarine and marine diseases and one on non-infectious disorders. The final part on emerging diseases is to alert the industry to potential problems. We hope this division of the book will make it easier for the reader to access information on known diseases/disorders within a group of fish. The arrangement will also help to highlight similarities and differences in disease problems between groups of fish

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(e.g. between marine warmwater and marine coldwater fish). However, such divisions also create some minor problems, e.g. a few pathogens have been isolated from both seawater and freshwater fish, so our authors and editors have worked closely to avoid extensive overlaps in coverage. For example, furunculosis is in Chapter 4, with only brief reference to it in Chapter 3, because it is often seen in freshwater fish. Similarly, important infectious agents (e.g. *Piscirickettsia salmonis*) of marine fish (Chapter 3) are only briefly mentioned in Chapter 4 because of their lesser importance to freshwater fish.

There are books on infectious and on non-infectious diseases/disorders of fish (e.g. Fish Diseases and Disorders, Volumes 1–3, CAB International), but there are none devoted specifically to problems associated with cage culture of finfish. Problems encountered in cage culture are in some ways different from those using other rearing methods. In cage culture, fish may be exposed constantly to ubiquitous pathogens. Also, the stress associated with captive rearing creates opportunities for disease, and to a lesser extent non-infectious disorders, to become significant causes of morbidity and mortality. Transmissions of infectious agents are also enhanced, and fish become more susceptible to disease partly because their immune system may be compromised due to prolonged exposure to pollutants in the water and/or crowding stress. The impact and spread of new and/or emerging diseases are also important, and are influenced by factors that include international trade in eggs or fry, unauthorized transportation of fish, and contact with migratory or naive fish species. Under natural conditions these agents in their natural hosts may not be considered important pathogens, but in an expanded geographical and/or host range, under different environmental conditions or temperatures, they may lead to epizootics with serious consequential economic impact.

As the demand for animal protein increases in the new millennium, we expect a significant increase in cage culture activity in many countries. This will be true especially in countries with limited usable land mass but with relatively long coastlines and/or extensive river–lake systems. We hope this book will fill a niche and be useful to colleagues who are active in the industry.

Patrick T.K.Woo
David W. Bruno
L.H. Susan Lim

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1 Overview of Cage Culture and its Importance in the 21st Century

Donald J. Noakes*
Thompson Rivers University, Kamloops, Canada

Almost half of the fish consumed by humans is the product of some form of aquaculture and the relative and absolute contribution of this important sector will only increase in the future. While there are many different forms of aquaculture, there are currently more than 100 species of fish, shellfish and invertebrates cultured in cages and that number is expected to increase substantially in the future (FAO, 2011). Typically these are high value, fast or relatively fast growing species that not only provide consumers with high quality food but also contribute substantially to local, regional and global trade and commerce. There are also many other socio-economic benefits associated with aquaculture (cage culture and other forms) and they include direct and indirect local employment as well as opportunities for specialized education and training, and for research and development. Indeed, research and development in fish culture and husbandry practices, disease monitoring, detection, and treatment, and optimizing fish feed have driven the development of cage culture worldwide.

Although fish have been cultured for more than 2500 years, the first record of cage culture is from the late 1800s (Eng and Tech, 2002 and references within). Eng and Tech (2002, Table 1.1a, b, c) provide a good summary of the finfish species that have been or are cultured in cages in fresh, brackish and salt water worldwide with some of the species being cultured in more than one of these environments. Although there are some problems with incomplete records and standardized reporting, currently about 10% of the total world aquaculture production or roughly 5 million t comes from cage culture (FAO, 2012a). Salmon and trout (*Salmo salar* and *Oncorhychus* spp.) accounts for approximately half (by weight) of the finfish grown in cages (FAO, 2012b). Given the significant capital investment required to establish and maintain a successful cage culture operation and the number of regulatory and environmental conditions that must be met and addressed, salmon and trout are likely to remain the key species cultured in cages in the next decade (FAO, 2012b).

To fully appreciate the importance of aquaculture now and in the future, it is worthwhile adding both context and perspective by comparing aspects of this sector with traditional fisheries. To that end, four broad areas are considered in this chapter. First, current and past production trends for traditional fisheries and aquaculture are compared as well as expected future trends in both sectors. This includes the importance of cage culture

* E-mail: dnoakes@tru.ca

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in the future where significant overall growth is expected. Second, the production and economic value of the top 15 currently cultured species are discussed with particular emphasis on the importance of and outlook for species being raised through cage culture. Third, an overview of the socio-economic benefits of aquaculture including direct and indirect employment and trade are discussed. Although the focus is on the aquaculture, data for traditional (wild) fisheries are also included for perspective. Finally, there are significant challenges and issues facing aquaculture in general and cage culture in particular that need resolution. A discussion of these issues (sustainability and growth) with specific emphasis on problems facing cage culture is included along with concluding remarks.

Production Trends

Aquaculture has been practised for at least the past 2500 years or more and since it began it has been and continues to be an important source of food production and employment for local communities. It has also contributed substantially to local, regional and global trade and commerce – much more so recently given the significant growth in the aquaculture sector worldwide, an increasing global population, and the continued globalization of the world's economy. Despite recent economic troubles and concerns, there is every reason to believe that the aquaculture sector will continue to grow and contribute substantially to global food security. Demand for high quality fish products (especially for the fresh food market) continues to grow and it is clear that traditional fisheries cannot and will not be able to meet this demand now and in the future. Recent estimates of stock status suggest that about 30% of world fish stocks are over exploited, 50% are fully exploited and the remaining 20% under or moderately exploited. Thus given the current state of world fish stocks, it is unlikely that there will be any real growth in capture fisheries in the near future and there is a real possibility of further declines in stocks (fisheries) in both the short and long term (FAO, 2012a).

Aquaculture is different from traditional harvest fisheries in two very important ways. First, it involves some form of intervention in the production cycle of freshwater or marine fish, invertebrates and shellfish or aquatic plants. The interventions may include the regular stocking of ponds, tanks, cages or other grow-out systems using captured (wild) or hatchery produced juvenile fish or plants and regular feeding of the stocked fish or plants. They may also include monitoring and detection of disease-causing agents and treatment of infections, or a variety of other fish husbandry practices aimed at enhancing the survival and/or growth of the species being cultured. Another very important and essential feature of any aquaculture venture is ownership of the stock. This ensures that benefits accrue to those directly involved with and responsible for the aquaculture enterprise. Stock ownership applies whether the aquaculture operation is being conducted on privately owned land or waterways or on leased or public land or water. This is quite different from capture fisheries where typically participants do not have ownership rights – a characteristic that has frequently resulted in overfishing and depletion of fish stocks (commonly referred to as 'the tragedy of the commons'). Limited entry fisheries where the number of fishers allowed to catch a particular species in a specific area provide more predictable access to fish stocks but only after conservation targets are met and only after those with legitimate fishing 'rights' to access (such as First Nations or Aboriginal peoples) have been allowed their share (often negotiated). Thus, in some years fishers, even those involved in limited access fisheries, may have low or no quota allocated to them. Stock enhancement programmes used to rebuild or supplement traditional fisheries or stocks may employ some of the same types of interventions that are used in the aquaculture sector, such as using hatchery produced juveniles. However, like capture fisheries there is no ownership of the stock. All three of these approaches to fish production (aquaculture, fisheries and stock enhancement) are important for food production and conservation and they are certainly linked economically.

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World aquaculture production, excluding marine plants, was less than 1 million t per year in the 1950s or about 5% of the total world fisheries and aquaculture production (FAO, 2012a). Aquaculture production grew at a very modest rate until about the mid- to late-1980s at which time it was roughly 10 million t per year. The rate of growth in this sector increased substantially through the 1990s and 2000s and between 2001 and 2010, world aquaculture production increased by approximately 6.3% per year or about three times the rate of increase for meat production (beef, poultry and pork) (FAO, 2012a). In 2010, world aquaculture production reached 59.9 million t for fish, shellfish and invertebrates with an additional 19 million t of aquatic plants. By comparison, production from all capture fisheries increased steadily from about 18 million t in the early 1950s until the early 1990s when the annual production from world capture fisheries levelled off at approximately 90 million t. Although the rate of growth in aquaculture production has moderated slightly in recent years, total world aquaculture production is expected to equal or exceed production in the wild capture fisheries within the next decade or two (FAO, 2012a). This may in fact happen sooner than later given the predicted decline in world fish population expected as a result of climate change (IPCC, 2007).

Fish is an important source of animal protein providing almost 4.2 billion people with about 15% of their average annual per capita intake (FAO, 2012a). In 2010, that represented an average per capita consumption of fish of approximately 18.6 kg per person, which is more than double the per capita consumption of fish in the 1960s. Demand for fish for human consumption is expected to substantially increase in the future (given both its significant economic and health benefits) and demand will be further compounded by population growth (FAO, 2012a). While world capture fisheries totalled about 90 million t in 2010, not all of the fish were for human consumption. A substantial fraction of the 90 million t was by-catch and some of the catch was used as fishmeal for feed, and fish oil for animal and fish consumption as well as for use in industry. By contrast, the vast majority (90% or more) of aquaculture production is used for human consumption. The net result was that aquaculture production contributed approximately 47% of the 115 million t of fish, shellfish and invertebrates (excluding marine plants) destined for human consumption in 2010. This disproportionate and very significant contribution from aquaculture is not immediately obvious from production statistics but none the less it is an important and crucial fact (FAO, 2012b). With wild capture fishery production levelling or slightly declining, it is estimated that more than half of the aquatic food destined for human consumption will come from aquaculture sources in the very near future. Thus, the importance of the aquaculture sector to local, regional and global food security now and in the future cannot be overstated.

Major Species and Their Importance by Area and Region

The recent growth in aquaculture production has been the result of significant increases in production in China, which now accounts for about 60% (36.7 million t) of the total biomass (FAO, 2012a). Other Asian countries (including India and a number of other Southeast Asian countries) account for another 30% of the world's production (Fig. 1.1). The growth in production in these areas is clearly driven by the demands of increasing populations in China and other Asian countries as well as their expanding and maturing economies that support healthy export markets. While most aquaculture production is consumed by the producing nation, a portion is also exported to countries such as Japan, the United States and European nations where the demand for fish and fish products is more than can be produced locally either through their capture fisheries or aquaculture ventures. The demand in these markets also tends to be for species such as salmon, shrimp, tilapia and other high value species, particularly for servicing the fresh fish market (FAO, 2012b).

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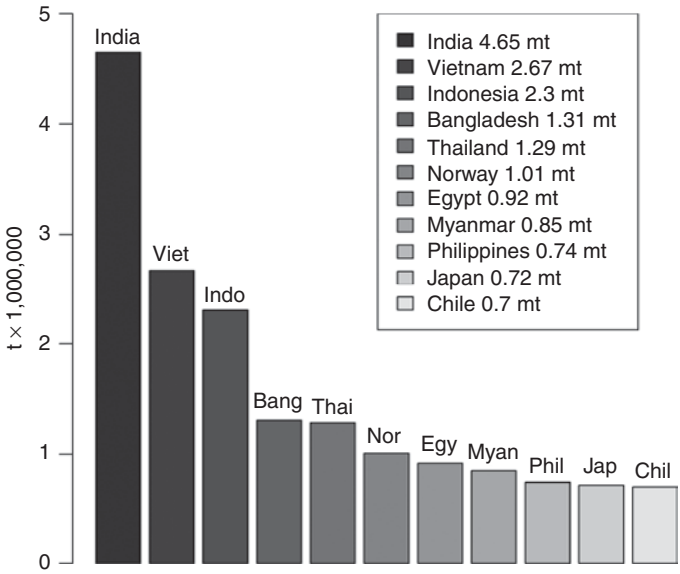


Fig. 1.1. The 2010 production (million t) of cultured fish, crustaceans, molluscs and other non-plant species for nine of the top ten producing nations (excluding China). In 2010, China's aquaculture production was 36,734,200t (excluding marine plants) representing approximately 61% of the total world aquaculture production. Production from these ten countries accounted for nearly 90% of the world aquaculture production. Source: FAO, 2012b, FAO Fisheries and Aquaculture 2010 Statistical Yearbook.

In 2010 and in recent years, approximately 55% of the world's aquaculture production occurred in freshwater (Fig. 1.2) primarily in lakes or ponds or other areas including flooded fields whose primary purpose is growing other crops such as rice (FAO, 2012b). Although some cage culture also occurs in fresh water (approximately 1 million t), this is an area or mode of production that is expected to increase substantially in the future (FAO, 2007). In addition to promoting and expanding co-culture opportunities, there is an increasing trend to create aquaculture operations or facilities (including cage culture) as part of other projects in developing countries both to meet the demand for fish and to ensure the best use of limited space and resources (Soto, 2009). The rate of increase in aquaculture production has been similar for fresh and brackish waters (approximately 5% or 6% growth per year over the last decade) and both are about double the rate of increase in production for species grown in marine waters (Fig. 1.2). In part this is because many freshwater species (such as various species of tilapia and carp, Table 1.2) have been cultured for many years and production is simply being scaled up, whereas the technologies for cultivating many marine species (such as tropical sea bass (*Lates calcarifer*) *Centropomidae* and sablefish (*Anoplopoma fimbra*) are still being developed and refined. Also, a significantly higher capital investment and higher on-going costs are required for marine aquaculture ventures, so expansion in this sector is less rapid than in fresh water. While aquaculture remains an important sector worldwide, Asia currently accounts for about 90% of the aquaculture production by weight and almost 80% of the total value. In both the short- and longer-term, this will likely be the region in which most of the future growth in the industry will occur, although Africa is also an area where significant growth in aquaculture may occur, particularly with freshwater species (FAO, 2011, 2012b).

While freshwater species accounted for just over half the production by weight and value of the aquaculture sector, other species were important both regionally and globally (Table 1.1). For instance, mollusc production in 2010 was 14.1 million t or approximately 40% of the freshwater fish production (by weight). Although as a group molluscs were

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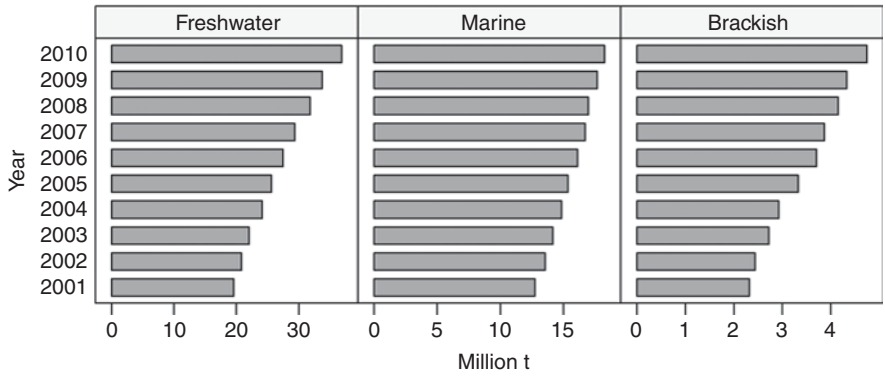


Fig. 1.2. Marine, brackish and freshwater aquaculture production of fish, crustaceans, molluscs and other non-plant species from 2001 through 2010 inclusive. Production increased by approximately 75% over this 10-year period with freshwater aquaculture production accounting for approximately 60% of the total on an annual basis. Source: FAO, 2012b, FAO Fisheries and Aquaculture 2010 Statistical Yearbook.

Table 1.1. Aquaculture production (million t) and value (billion US$) by species group (excluding aquatic plants) in 2010. While aquaculture production was dominated by freshwater fishes, high-valued crustacean and diadromous fish species contributed substantially (US$ 42.7 billion) to the economies of producing nations and international trade. Source: FAO, 2012b, FAO Fisheries and Aquaculture 2010 Statistical Yearbook.

Species group	Quantity (million t)	Value (billion US$)
Freshwater fishes	33.7 (56.4%)	51.5 (43.1%)
Molluscs	14.1 (23.6%)	14.3 (12.0%)
Crustaceans	5.7 (9.6%)	26.9 (22.5%)
Diadromous fishes	3.6 (6.0%)	15.8 (13.2%)
Marine fishes	1.8 (3.1%)	8.0 (6.7%)
Aquatic animals	0.8 (1.4%)	3.0 (2.6%)
Total	59.7	119.5

less valuable per t of production compared to some other species they still contributed over US$14 billion to the aquaculture sector and were an important source of protein for local communities. Conversely, crustacean and diadromous fish production (culture) by weight was much more modest (9.3 million t combined) but these high value species contributed more than US$40 billion (roughly 36% of the total value) to the sector in 2010 (Table 1.1). While some high-valued species (such as shrimp and salmon) are consumed where they are produced, the majority of the production is destined for the fresh fish food markets in developed countries where demand is high and the economies (and per capita income) can support the premium prices for these high quality products (FAO, 2012b). There are also multiplier factors associated with each group (Table 1.1) which would magnify the economic importance of the entire sector and perhaps to a greater degree for those species (such as salmon, shrimp) that are exported rather than consumed locally.

The top 15 species cultivated in 2010 accounted for roughly 60% of the total production or 35.1 million t (Table 1.2). These major species will likely retain their prominence for the foreseeable future, although their individual ranking may change slightly reflecting year-to-year variations in production and/or annual shifts in species preference (FAO, 2012b). Six freshwater carp species dominated the list, each with production in excess of 2 million t annually

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Table 1.2. Top 15 cultured species according to 2010 production. Carp and tilapia species culture accounted for 24,277,264t or roughly 40% of the 59,872,600t of fish, crustaceans, molluscs and other non-plant species grown or cultivated in 2010. Source: FAO, 2012b, FAO Fisheries and Aquaculture 2010 Statistical Yearbook.

Species	Common name(s)	2010 Production (t)
Ctenopharyngodon idellus	Grass carp	4,337,114
Hypophthalmichthys molitrix	Silver carp	4,116,835
Catla catla	Indian carp	3,869,984
Ruditapes philippinarum	Manila clam	3,604,247
Cyprinus carpio	Common carp	3,444,203
Penaeus vannamei	White leg shrimp	2,720,929
Hypophthalmichthys nobilis	Bighead carp	2,585,962
Oreochromis niloticus	Nile tilapia	2,538,052
Carassius carassius	Crucian carp	2,217,799
Salmo salar	Atlantic salmon	1,425,968
Labeo rohita	Rohi or Rohu (carp)	1,167,315
Chanos chanos	Milkfish	808,559
Penaeus monodon	Giant Tiger prawn	781,582
Oncorhynchus mykiss	Rainbow trout	728,448
Sinonovacula constricta	Chinese razor or Agemaki clam	714,434

(Table 1.2). The top three species, the grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*) and Indian carp (*Catla catla*), had a combined production of about 12.3 million t in 2010 (Table 1.2). While these species are cultured worldwide, much of the production is in China, India and other Asian countries. Carp and tilapia are cultured primarily in lakes, ponds or fields (as a component of a co-culture venture) and although most are consumed locally some are also exported (FAO, 2012b). Manila clams (*Ruditapes philippinarum*), white legged shrimp (*Penaeus vannamei*) and Nile tilapia (*Oreochromis niloticus*) round out the list of species with production in excess of 2 million t annually. Shrimp and tilapia are also important species for export (FAO, 2012b).

Atlantic salmon (*Salmo salmar*) and rainbow trout (*Oncorhynchus mykiss*) are cold-water or temperate water species that are raised primarily in cages and tanks in both the northern and southern hemispheres with a combined production of 2.15 million t in 2010 (Table 1.2). Salmon and trout (as well as shrimp and prawns) are high-value species with much of the production being exported to Japan, the United States and a number of European nations (FAO, 2012b). Because the unit production cost for these species is relatively high, these species are usually raised at high density and in cages or tanks. In 2005, salmon and trout accounted for more than 50% (by weight) of the cage culture globally although the data on cage culture were at best incomplete (FAO, 2007). Currently, more than 100 species are cultured in cages worldwide with 10 species accounting for 90% of the production and the remaining species contributing about 10% of the production (Tacon and Halwart, 2007; FAO, 2011). Complete records are not available for all nations but reporting and the statistics from some countries (particularly for China) have improved since 2005 (FAO, 2012a). It's likely that cage culture still only accounts for a small (5% to 10%) fraction of the total production of cultured fish, shellfish and invertebrates (FAO, 2012a, b). Nevertheless, a significant amount of research has been done to diagnose, manage and treat diseases of species raised in cages or tanks in order to maximize production, minimize costs and ensure the highest quality product (Woo *et al*., 2002; Woo, 2006; Eiras *et al*., 2008; Leatherland and Woo, 2010; Noga, 2010; Woo and Bruno, 2011). While much of this work has been directed to resolving issues associated with the culture of salmonids and shrimp, the advances made for these species may be useful or provide guidance for finding solutions for new species being cultured or being considered for culture.

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Not surprisingly, China having the largest freshwater aquaculture industry is also the country with the largest freshwater cage culture sector with a production of approximately 704,000 t in 2005 (Tacon and Halwart, 2007). Vietnam (126,000 t), Indonesia (67,700 t) and the Philippines (61,000 t) also have significant freshwater cage culture production, with other countries producing substantially less (Fig. 1.3a). While about 30 species are cultured

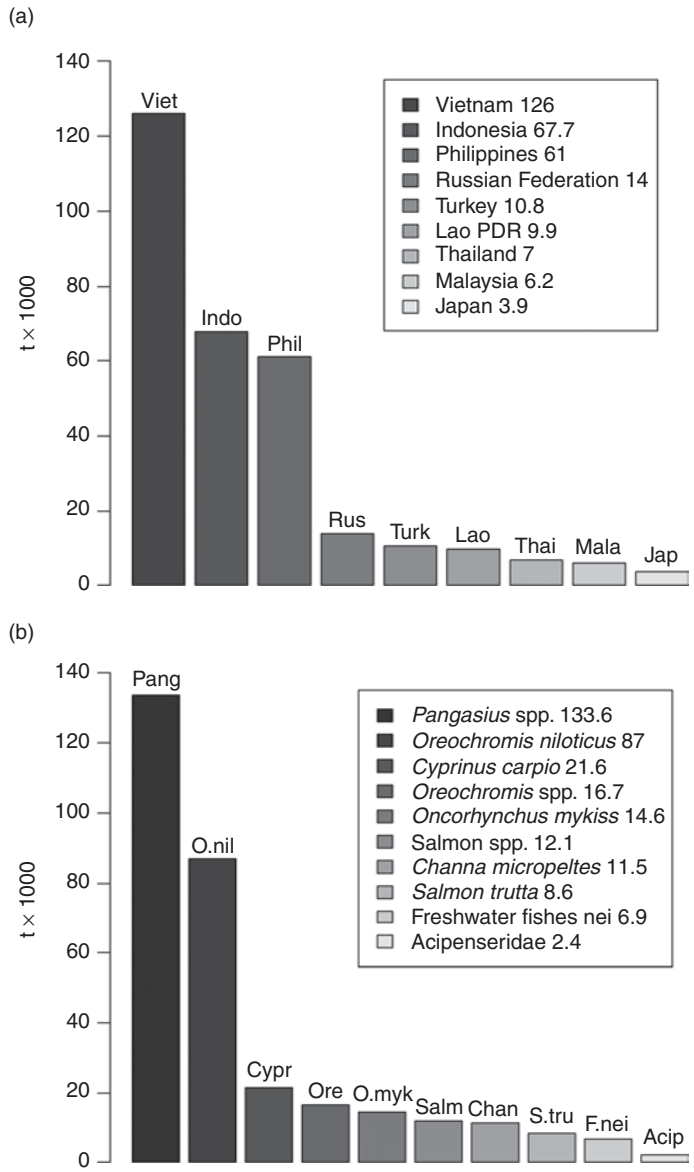


Fig. 1.3. (a) Excluding China, freshwater cage culture production (t × 1,000) for the top nine countries in 2005. Freshwater cage production in China was about 704,000 t in 2005. (b) Excluding China, the top ten species grown in freshwater cage culture in 2005. The data for China's freshwater cage culture is not specific enough to provide a breakdown by species (Tacon and Halwart, 2007). Source: FAO, 2007.

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in freshwater cages in China, the data are unfortunately not detailed enough to provide a breakdown by species. Excluding China, *Pangasius* spp. (a genus of shark catfish native to Asia) and tilapia (*Oreochromis niloticus* and *Oreochromis* spp.) are the most common species cultured in freshwater cages (Fig. 1.3b) and while these fish are consumed locally they also support important export markets. Norway and Chile are the top countries producing either marine or brackish water cage-reared fish with a combined production well in excess of 1 million t annually (Fig. 1.4a). China and Japan also have significant marine

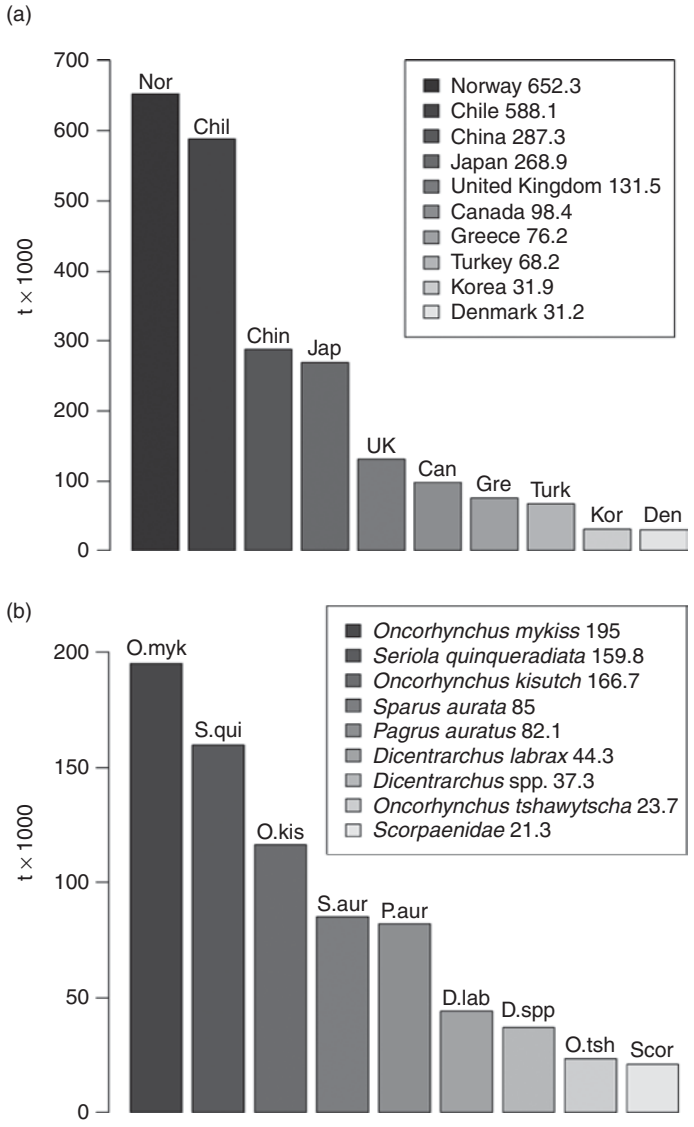


Fig. 1.4. (a) Top ten countries for cage culture in marine or brackish waters in 2005 (production is t × 1000). (b) Excluding Atlantic salmon, production of the top nine species cultured in cages in marine and brackish water in 2005. The production of Atlantic salmon in 2005 was approximately 1.2 million t. Source: FAO, 2007.

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and brackish water cage culture industries, each with production in the range of 250,000 to 300,000 t per year (Tacon and Halwart, 2007). Atlantic salmon is the most important species raised in marine net pens with more than 1 million t produced annually primarily in Norway, Chile, the United Kingdom and Canada (Tacon and Halwart, 2007). Two other important species, rainbow trout and coho salmon (*Oncorhynchus kisutch*), are raised in cages with a combined annual production of approximately 312,000 t (Fig. 1.4b). Sea bream (*Sparus aurata* and *Pagrus auratus*) and sea bass (*Dicentrarchus labrax* and *Dicentrarchus* spp.) each contribute about 85,000 t of product annually with the remaining species reared in marine or brackish water cages, providing 50,000 t or less annually (Tacon and Halwart, 2007; and Fig. 1.4b in this chapter).

While the quantity of finfish raised in cages is relatively small in comparison to the total aquaculture production, these are typically high-value species and they contribute substantially to the value of this sector (Table 1.1). Cage-reared fish may also contribute substantially on a species level when comparing commercial fisheries and the aquaculture sector. For instance, farmed salmon production (now in excess of 2 million t annually) is now double the commercial harvest of wild salmon (Noakes and Beamish, 2011). Also, a large portion of the wild harvest is low-value pink (*Oncohynchus gorbuscha*) and chum (*O. keta*) salmon. While pink and chum salmon are eaten fresh, more often pink and chum salmon are sold as frozen, canned or otherwise processed for human consumption or used for pet food or other purposes. In contrast, cage-raised farmed salmon are almost exclusively used to service the fresh fish market and if processed they tend to undergo value added processing to supply a niche fresh fish market. Thus, while there is some overlap in the markets for sockeye (*Oncohynchus nerka*), Chinook (*O. tshawytscha*), coho salmon and farmed salmon (Atlantic, coho and Chinook salmon) as well as rainbow trout, there are to a large degree distinct markets for salmon (and trout in the case of aquaculture) from these two different sources (farmed versus commercial fishery). That said, farm salmon production has had a significant negative effect on the prices paid for wild salmon to the point where some commercial salmon fisheries provide a very low economic return on investment or are in fact no longer economically viable (Knapp *et al*., 2007; Noakes and Beamish, 2011). This has been a source of significant conflict between the supporters of these two different sectors and it will continue to be a problem area in the foreseeable future. Consumer demand for salmon has not abated and there is every reason to believe that net pen farmed salmon production will increase substantially in the future.

Economic and Social Benefits

While providing high quality food for people is in its own right important, there are other noteworthy socio-economic benefits associated with aquaculture. For example, in 2008 there were about 44.4 million people directly engaged in fisheries and aquaculture work. About 12% of these 44.4 million workers were women who were primarily employed in the aquaculture sector (FAO, 2012a). By comparison, in 1980 there were 16.7 million people working in the fisheries and aquaculture sector so there was a net increase of 167% in employment in this sector between 1980 and 2008 (FAO, 2012a). While the increase in the number of fishers was about 42.5% (from 24.0 to 34.2 million, an increase of 10.2 million) between 1990 and 2008, the number of people employed in the aquaculture sector grew by 7.0 million (or 185%) during the same period (Table 1.3). Also, most of the growth in aquaculture jobs occurred in developing countries (Asia and Africa) in rural areas where these aquaculture operations are based – this is of enormous social and economic importance.

Interestingly, all of the growth in the fishing sector took place in the 1990s (there was actually a net decrease in jobs in the fishing sector between 2000 and 2008) with all of the increase in fisheries and aquaculture jobs since 2000 being attributed to growth in the aquaculture sector (Table 1.3). This is not surprising since catches in commercial fisheries have been relatively stable since about 1990. With 80% of world fish stocks being fully or over exploited, there is also little or no

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Table 1.3. The number (thousands) of fishermen and fish farmers in Asia and globally (including Asia).

		1990	1995	2000	2005	2008
Fishers and fish farmers	Asia	23,736	28,096	35,242	36,860	38,439
	World	27,737	32,043	41,287	42,868	44,946
Fish farmers	Asia	3,698	6,692	6,647	9,828	10,143
	World	3,783	6,803	6,983	10,467	10,793

prospect for significant increased employment in the commercial fishing sector in the foreseeable future. Conversely, the potential for future growth (and employment) in the aquaculture sector is significant given past and recent performance and the increasing demand for high quality fish products. While recent annual growth rates in the aquaculture sector of 5% or 6% may temper slightly in the future, it would not be unrealistic to expect 100,000 or more net new aquaculture jobs created on an annual basis for the foreseeable future (Table 1.3). Also, although estimates may vary, evidence suggests that for every person directly employed in fisheries and aquaculture about three others are indirectly employed (FAO, 2012a). Thus the total number of people working in the broader fisheries and aquaculture sector is likely in the order of 180 million people (directly or indirectly employed) with about 45 million associated with the aquaculture sector (FAO, 2012a). Again, many of these new jobs (perhaps as many as 100,000 direct jobs and 300,000 indirect jobs) will be in rural communities in developing countries where they will be of enormous social and economic importance.

The majority of fishers and fish farmers are in developing countries (Table 1.3), mainly in Asia (85.5%), Africa (9.3%) and Latin America (2.9%), and that's unlikely to change in the foreseeable future given the nature of the fisheries and aquaculture operations in the various regions (FAO, 2012a). As noted, the only growth in employment in this sector since 2000 has been associated with aquaculture and that's likely to continue to be the main source of growth in employment in this industry in the future. There has also, not surprisingly, been a difference in employment by region and fishery. For instance, direct employment in capital intensive fisheries and aquaculture ventures (such as in Europe, North America and Japan) was about 1.3 million in 2008, which represents an 11% decrease compared to employment levels in 1990 (FAO, 2012a). The reasons for this decrease in employment may include, among other factors, declining fish stocks (for a variety of reasons) and thus opportunities to fish as well as programmes to reduce fishing capacity (buyback schemes, license retirement, etc.) aimed at reducing overcapitalization in the industry. Whatever the reasons for the decline, it is unlikely the trend towards lower employment in the fishing industry will be reversed any time soon given that the vast majority (80%) of world fish stocks are currently fully or over exploited. This problem is further compounded by climate change (global warming) which is expected to adversely affect fisheries and aquaculture (De Silva and Soto, 2009), so it is likely that there will be at best shifts in fishing effort to match shifts in fish abundance and at worst real declines in employment and economic activity as stocks decline (IPCC, 2007).

There are also significant differences in the production efficiencies between and among regions. For instance, production per person in the fisheries and aquaculture sector in Asia and Africa is approximately 2 t per year while production per person in Europe and North America is at about 20 t per year (FAO, 2012a). This reflects in large part the differences in and reliance on technology such as the size of fishing vessels and gear used and other factors. Differences are particularly evident in the aquaculture sector where production in Norway is approximately 1720 t per person while Chile is about 72 t per person and China is roughly 6 t per person per year (FAO, 2012b). The primary species in Norway and Chile is Atlantic salmon that are grown in cages at high densities, whereas in Asia and Africa the main

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species grown are carp and tilapia that are primarily in ponds and fields. In general, cage culture typically has relatively high capital costs but require fewer people to work them efficiently. Operating costs depend in large part on the species being raised but are also typically higher for operations using cage culture. On the flip side, high-value species are typically grown in cage culture and the product is sold fresh and thus there is generally a larger return on investment. Conversely, while some of the fish caught in commercial fisheries is sold fresh, a large proportion (approximately 40% in 2008) is sold as frozen or processed (such as canned or cured). Japan, United States and Europe are currently the major import markets for fish, accounting for about 70% of the total imports (FAO, 2012a). Trade in fish is significant and important representing about 10% of the total agricultural exports in 2008 and about 1% of world merchandise in terms of value (FAO, 2012a). Not surprisingly, China is the leading fish exporter (~10% of total) and the continued and growing demand for high quality food fish will provide significant opportunities for growth and expansion of the aquaculture sector (FAO, 2012a). This is particularly true as new opportunities to expand production and develop export markets emerge. The development of technologies to culture species that are currently not grown commercially and to improve efficiencies for existing cultured species will help stimulate growth in this sector and in particular cage culture in order to maximize opportunities.

Aquaculture Sustainability in the Future

While aquaculture has contributed significantly to overall global fish production and food security, it has also attracted criticism with respect to its environmental performance and other impacts real or perceived (Homer *et al*., 2008; Subasinghe *et al*., 2009; Smith *et al*., 2010). Environmental and governance issues are two broad areas that must be addressed if this sector is to fully realize its growth potential in the future. This applies to all aquaculture ventures but particularly so for those involving cage culture since those systems often share the same aquatic environment as wild fish whether they are the same species being cultured or different species that occupy the same ecological niche. Fish health and disease, waste discharge, and escapement of cultured fish are all issues of significant concern to the public, and transparent proactive action is required to address these issues and to maintain the social licence from the public perspective and for this important industry. Of course, it is important to put all of the real and perceived problems in context by taking into account not only the problems, but the real and potential risk. For instance, for a variety of reasons including a long history of failed attempts to establish or re-establish feral populations of Atlantic salmon both within and outside their native range, the evidence suggests that escapes of Atlantic salmon from net cages in the west coast of North America pose a low ecological risk to the native Pacific salmon (Noakes. 2011). Conversely, escapes of a more invasive species such as Asian or snakehead carp being cultured outside their native range are likely to pose a much higher risk to native species over a broader range of ecosystems (Herborg *et al*., 2007). Thus, the safeguards required to minimize any negative consequences of higher risk scenarios (such as escapes of Asian or snakehead carp) need to be much more rigorous in order to minimize the impact of unintentional and unwanted ecological changes. In any event, systems should be put in place to minimize any undesirable impacts. While technology may help resolve the specific issue at hand, sound governance will also be required to restore and maintain public confidence.

With respect to aquaculture, governance needs to balance and encourage meaningful stakeholder participation in processes while not unduly preventing growth and improvement in the industry. To that end, the governance system must incorporate accountability, effectiveness, efficiency, predictability and fairness. While governance systems may include a host of regulatory and compliance issues, they should at a minimum create a regulatory framework for the management and control of fish health and infectious

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diseases, a variety of environmental issues as well as secure access to stock and sites for use by the aquaculture sector. While emphasis has typically been placed on the first two, access to stock and good quality sites (which should also minimize environmental and ecological impacts) are essential components of this system that will enhance the overall performance of the sector while protecting the public's interests. However, considerable care should be taken to avoid over-governance where several levels of government bureaucracy and redundancies actually inhibit or prevent progress and impede participation in the process. While good governance is important for all types of aquaculture and differences between countries and regions are expected, regulation and control are more critical for intensive aquaculture (cage culture) where fish are raised at higher densities. The potential for negative impacts is greater for intensive aquaculture operations (including cage culture) and good governance is important both for industry and to ensure public confidence and support. Technology can certainly help resolve or manage issues of concern but good governance is absolutely essential for sustainability.

An important topic that will require more consideration in the future is the broad range of interactions between aquaculture and commercial fisheries. There have already been considerable discussions about real and potential ecological interactions, but discussions on economic linkages have occurred to a much lesser degree even though they may be one of the root causes for conflicts between the two sectors (Pan and Leung, 2012). This may include links with wild fishery for the same or related species (for instance, farmed versus wild salmon) or competition through replacement of the same or related species in the global fish market. Without question, the rapid development of world salmon aquaculture has negatively impacted traditional markets for commercially caught salmon both from a quantity and quality perspective (Asche *et al*., 2005; Asche and Bjørndal, 2011). The economic viability of many wild salmon fisheries is questionable given the availability of fresh farmed salmon year round and this will not change in the foreseeable future (Knapp *et al*., 2007; Valderrama and Anderson, 2010). There is also some evidence that different farmed species of fish may replace other farmed species in the marketplace although it is difficult to get a precise answer given the multitude of choice consumers have with respect to animal and/or fish protein (Norman-López and Asche, 2008; Norman-López, 2009). These are the kind of discussions and analyses that need to take place when decisions are made by farmers (or strategic decisions by governments) about the viability of aquaculture ventures in the future (Asche *et al*., 2009). If they are to be sustainable, aquaculture ventures must be both ecologically and economically viable, recognizing they are competing in the global fish market and more broadly in the global food market. It may even be realistic or desirable to develop or use integrated modelling approaches for ecosystem and economic issues (Jin, 2012). This is particularly true for cage culture operations where the capital investment is significant.

So what does the future hold with respect to finfish cage aquaculture? The financial viability of some enterprises (such as salmon farming) is highly dependent on the price of fish feed which accounts for 50% to 70% of production costs. Significant advances in feed formulation such as the substitution of plant material for a portion of the protein used for the farmed salmon has certainly reduced costs and the dependency on fishmeal and fish oil and that is certainly encouraging. For some species such as tilapia, carp and catfish, the percentage of fishmeal and fish oil may be only in the 3% to 8% range so an increase in the cost of fishmeal and fish oil may have little impact whereas increases for cereal and cereal by-products can increase costs (FAO, 2011). For other species such as salmon and trout, fishmeal is and will continue to be an important component of the diet (feed) and increases in the cost of fishmeal and fish oil will affect the profitability of this sector. Also, increases in farming efficiencies continue to be made, particularly improvements in fish health and disease monitoring, prevention and treatment with the development of new vaccines, feed management systems, environmental monitoring and practices, as well as

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improvements in human health and socio-economic conditions. These, combined with improved governance systems, have and will allow aquaculture and particularly cage culture to successfully expand in the future. Significant problems such as major disease outbreaks (i.e. the infectious salmon anaemia (ISA) outbreak in the salmon farming industry in Chile) occur when there are breakdowns in oversight or control and this needs to be prevented. While a governance system based on industry self-monitoring and self-reporting can work effectively (Canada and Norway are good examples), there must be a commitment to performance excellence and open and transparent reporting for the industry to be sustainable. Again, care must be taken to ensure a balance between environmental protection and development as well as avoiding counter-productive systems of over-governance.

The use of introduced species (outside their native range) and hybrids has also played a significant role in the recent expansion of aquaculture and these species will continue to be important in the future. Tilapia and white legged shrimp (*Litopenaeus vannamei*), for example, are both important species and significant quantities are now being raised outside their native range. Hybrid tilapia (Nile tilapia (*Oreochromis nilotica*) and blue tilapia (*O. aureus*)) are also being cultured extensively and now represent approximately 25% of the tilapia production in China (Lui and Li, 2010). Thailand now produces hybrid catfish (*Clarias gariepinus* and *C. macrocephalus*), and a portion of the snakehead carp produced in China are a hybrid between *Channa argus* and *C. maculate*. The United States has also farmed a hybrid striped bass (*Morone chrysops* and *M. saxatilis*) for two decades. More crosses will be developed in the future and there is also interest in developing transgenic strains of fish for culture. Each of these have ecological issues associated with their use and these must be addressed before they gain approval and public acceptance. Closing the life cycle for new species has and will also provide opportunities for cage culture especially for high-value species like Alaskan blackcod (*Anoplopoma fimbria*) and bluefin tuna (*Thunnus maccoyii*). While the production (weight) of these species is likely to be relatively low, the economic value is expected to be significant for these cage-reared species.

The aquaculture sector will without question enjoy considerable growth in the future and by association cage culture will also expand albeit at perhaps a slightly lower rate. While cage culture does provide food locally, the economic benefits are likely greater based on the export markets they support and the local employment they create. The research and development required to support cage culture will also benefit non-cage culture aquaculture in the process. All in all the future is bright.

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2 Infectious Diseases of Coldwater Fish in Marine and Brackish Waters

Eva Jansson[1]* and Pia Vennerström[2]
[1]*National Veterinary Institute (SVA), Uppsala, Sweden;*
[2]*Finnish Food Safety Authority Evira, Helsinki, Finland*

Introduction

Cage culture of fish in cold water is a well-established industry dominated with the production of Atlantic salmon (*Salmo salar*). Production has rapidly increased during the last 15 years and according to FAO Fishery Statistics (2012, 2013) the global aquaculture production of salmon for 2011 has reached more than 1.7 million t, to a value of US$ 9.7 billion, (Fig 2.1). Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss),* brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) are also important species for cold-water aquaculture. More recent species are Arctic char (*Salvelinus alpinus*), Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), halibut (*Hippoglossus hippoglossus*), turbot (*Psetta maxima*) and striped bass (*Morone saxatilis*). Hake (*Merluccius* spp.) and ling (*Molva molva*) are other gadoid species that may be introduced in the future after their nutritional and environmental requirements have been investigated. Fish in cage culture live in the open water, and thereby interact with wild-living fish which often swim close to the net pens looking for excess feed. The close contact with water and wild living fish increases the risks for horizontal transmission of marine, infectious organisms; for example *Vibrio* spp., which naturally occur in the marine environment. Salmonids, from fertilized eggs up to smolts, live in fresh water and freshwater pathogens may be transmitted in connection with the transportation to the marine environment. The transport of the young fish to the marine environment, with increased handling and new surroundings is stressful and stress is a well-known trigger for infection and development of disease. Treatment against diseases by bathing fish with therapeutics is difficult in net pens, due to the open space cages that allow free passage of water. Several efficient vaccines are available that reduce the risks for infection if performed in sufficient time for the immune system to respond before the vaccinated fish are introduced to sea water. Vaccines are generally divalent or trivalent containing immunogens against important pathogens. These vaccines induce a stronger immune response when compared with the monovalent vaccines. In Norway and Scotland, a Code of Practice has been written for farmers on how to avoid infectious diseases in salmonids (Aunsmo *et al.*, 2005; Anon., 2011). Freshwater pathogens

* E-mail: eva.jansson@sva.se

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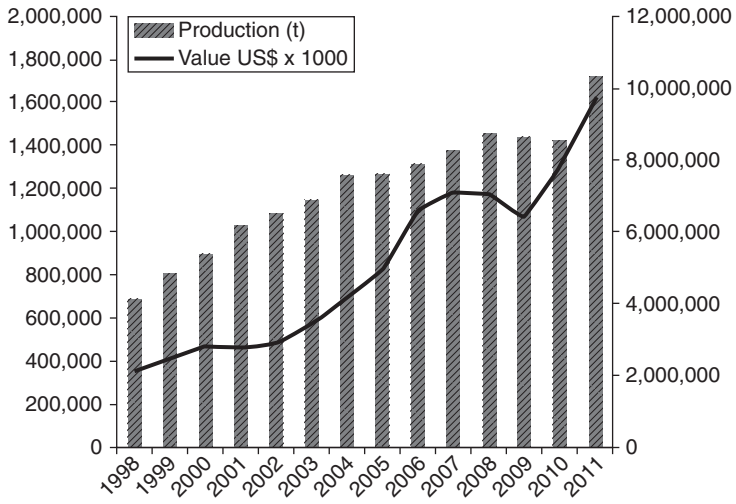


Fig. 2.1. The world production of salmon in aquaculture has rapidly increased during the latest decades and for 2011 appreciated to more than 1.7 million t. Source: FAO Fishery Statistics.

will be briefly covered in the present review but in more detail in Chapter 3, by Cain and Polinski. The present chapter focuses on the main marine pathogens: bacteria, fungi oomycetes, virus and parasites with impact on the production of coldwater fish in marine and brackish water.

Viral Diseases

Several viral diseases cause severe infections in cold-water fish, and mortalities are usually high in young fish such as fingerlings, but can also be significant in fish of any size depending on their health status. The economic losses in market-sized fish are significantly higher due to investment in feed and labour costs. Viral diseases are spread to new areas by movement of live fish, equipment or personnel. Movement of eggs are considered a lower risk, if disinfection of eggs has been carried out. There is evidence that viruses such as infectious pancreas necrosis virus (IPNV) and infectious haematopoietic necrosis virus (IHNV) can avoid disinfectants as they may enter the egg at the time of ovulation or during fertilization (Bovo *et al*., 2005). Therefore, live eggs and fish should only be moved from disease free areas with a known disease history that is based on screening programmes. The main viral diseases that have been reported to cause problems to cage culture in marine or brackish water are presented in this chapter: IHN, IPN, infections with salmonid alphaviruses (SAV), infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), cardiomyopathy syndrome (CMS), heart and skeletal muscle inflammation (HSMI) and viral encephalopathy and retinopathy (VER). In this chapter IHN, VHS and IPN are described with respect to sea water and a review from the freshwater perspective can be found in Chapter 3. VER is largely covered in Chapter 5 as generally a disease agent in warm water, but is also considered a threat to Atlantic halibut and cod farming (Bergh *et al*., 2002; Hellberg *et al*., 2007; Patel *et al*., 2007).

Infectious haematopoietic necrosis (IHN)

Infectious haematopoietic necrosis virus (IHNV) is a rhabdovirus that causes severe losses in the salmon industry. The main fish species affected are Pacific salmon (*Oncorhynchus* spp.), Atlantic salmon and rainbow trout. Epizootics caused by IHN have also been reported in other salmonids such as brown trout, brook trout and Japanese char (*Salvelinus leucomaensis*) (Bootland and Leong, 2011).

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A wide range of fish species, other than salmonids, can be infected and function as carriers of the virus. The susceptibility of non-salmonids to IHN is not clear (Bootland and Leong, 2011). In the marine environment, the disease was first reported in 1992 in cage-reared Atlantic salmon in British Columbia, Canada (Armstrong *et al*., 1993; Traxler *et al*., 1993). After this first report, IHN became a major disease problem in the area and the virus was also found to be endemic in wild fish, e.g. herring (*Clupea harengus*) that act as reservoirs and vectors (Armstrong *et al*., 1993; Traxler *et al*., 1993; Kent *et al*., 1998). Saksida (2006) reported that the virus is mainly spread between farms in the infected area because of farming practices, but also through a natural waterborne transmission route between farms in close proximity to each other.

Impact on fish production

IHNV is an important disease in marine net-pen farming. In an epidemic in British Columbia between 2001 and 2003, 12 million Atlantic salmon died or were culled. The average cumulative mortality on the infected farms was 58% (Saksida, 2006).

Diagnosis

IHN is diagnosed by virus isolation from organ samples of diseased fish, in cell culture followed by the identification of the virus by immunological or molecular techniques (OIE, 2013). The same method is used for screening clinically healthy fish for the presence of IHNV.

Clinical signs

The clinical signs of IHN for fish in the marine environment is similar as IHN in fresh water (see Chapter 3). Affected fish get lethargic, dark in coloration, with fin haemorrhage, pale gills, exophthalmia, distended abdomen and trailing casts. Internally, the abdominal cavity contains ascitic fluid and petechiae may occur in several tissues including visceral adipose tissue. Liver, kidneys and spleen are often pale. Necrosis of the haematopoietic tissue in the anterior kidney as well as liver and pancreas are the main histological findings.

Prevention and control

There is no cure for IHN and the best way to manage the disease is avoidance of contacts to farms with unknown disease history that may be infected. In sea areas where IHNV is endemic, the disease is controlled by keeping different year classes and fish species apart, reducing fish movement between farming sites, advancing harvest in infected sites and fallowing before restocking (Saksida, 2006). In a review made by Bovo *et al*. (2005) IHNV is described to survive outside the host in lake water for 7 weeks at 10°C, but survival time decreases rapidly with increasing temperatures. The survival decreases with increasing temperatures where a total inactivation was recorded after 20 min in 40°C and 10 min in 45°C. The virus is destroyed in less than 1 min above 55°C (Bovo *et al*., 2005). IHNV is sensitive to virucidal disinfectants.

Viral haemorrhagic septicaemia (VHS)

Viral haemorrhagic septicaemia virus (VHSV) is a rhabdovirus that infects a wide range of different wild and farmed fish species in fresh water and sea water. At least 63 different fish species have been shown to be susceptible to the virus. The isolated VHSV strains are divided into four genotypes (GI-IV). The genotypes appear to be associated to certain geographical locations and to some extent to certain fish species (Smail and Snow, 2011). Five subgroups have been described for Genotype I (GIa, GIb, GIc, GId and GIe) of which GIb and GId are marine isolates (Smail and Snow, 2011). Genotype Ib is isolated from the southern Baltic Sea, Skagerrak and Kattegat, the North Sea and the English Channel. Both genotypes GIb and GId have been isolated from net-pen reared rainbow trout, but only GIb has also been isolated from wild fish such as cod, herring, sprat (*Sprattus sprattus*), dab (*Limanda limanda*) and plaice (*Pleuronectes platessus*) (Einer-Jensen *et al*., 2004; Snow *et al*., 2004). Genotype Id strains are isolated from farmed rainbow trout in the brackish waters on the south coast of Finland (Raja-Halli *et al*., 2006) and from rainbow trout in Norway in 1969 (Einer-Jensen *et al*., 2004). Genotype II has been found in wild herring,

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sprat and lamprey (*Lampetra fluviatilis*) in the Baltic Sea (Skall *et al*., 2005a; Gadd *et al*., 2010; Gadd *et al*., 2011). Genotype III (GIII) is also isolated from a wide range of wild fish species in the marine environment in Skagerrak and Kattegat and the North Sea (Skall *et al*., 2005b; Duesund *et al*., 2010). Turbot and Atlantic halibut have, in laboratory challenge tests, been sensitive to GIII. A new variant of GIII was isolated from diseased sea-reared rainbow trout on the Norwegian west coast in 2007 (Dale *et al*., 2009; Duesund *et al*., 2010). Genotypes III have not been pathogenic to rainbow trout in experimental challenges, but the outbreak in Norway suggested that genotypes of VHSV might mutate and become pathogenic to some fish species. One reason for this suspicion was that cod, that are known to be carriers of GIII, were farmed in the same area as the rainbow trout that became infected. This may have given the virus an opportunity to transform and become pathogenic to rainbow trout. Genotype IV has been reported from marine fish on both the north-west and east coast of North America (Meyers *et al*., 1992; Smail and Snow, 2011).

Impact on fish production

Mortality due to VHSV in marine rainbow trout farms has been reported from France, Sweden, Finland and Norway. In France the mortality exceeded 85% after transfer to sea (Castric and de Kinkelin, 1980). VHS-virus (G1b) was isolated for the first time in Sweden in 1998, in rainbow trout from a small west-coast farm. The water temperature was just above 5°C. The fish (hatched 1997) showed exophthalmia, darkening, ascites and multiple haemorrhages in muscles and inner organs, but with a very low accumulated mortality, estimated at 2%. Older fish (1995, 1996) in the same farm showed no signs and were tested negative of infection. Contact farms were checked and tested negative for the disease. Although the farm was cleaned, disinfected and fallowed for 11 months, the virus was re-isolated two years later. Again the site was disinfected with a fallow period of 12 months and sentinel fish introduced before a restart. In addition a survey of the presence of the virus in wild fish populations in the area was initiated. Despite all sanitation, the virus could be detected in the farm for a third time (2002), and was eradicated in a similar manner to earlier occasions. At that time, it was established that the virus was transported into the farm by herring, in conjunction with spawning activity. It was recommended to the farmers to abstain from having fish in the cages at the time of the spawning of the herring (Nordblom and Norell, 2000; A. Hellström, Uppsala, 2013, personal communication). In Norway the mortality described for the infection with Genotype III was 10%. In Finland, Genotype Id has caused mortalities up to 50% in spring when water temperatures were rising from 2°C to 14°C. Significant mortalities are also recorded in slaughter-sized fish during autumn and winter, probably because of stress in relation to transport of fish to processing plants (P. Vennerström, Helsinki, 2013, personal communication).

Diagnosis

VHS is diagnosed by virus isolation from organ samples in cell culture, followed by the identification of the virus by immunological or molecular techniques (OIE, 2013). The same methods are also used in screening of clinically healthy fish for the presence of VHSV.

Clinical signs

The clinical signs of VHS for fish in the marine environment are similar to VHS in fresh water (see Chapter 3). The most typical signs in the acute phase of the disease are the widespread petechial haemorrhage that can be seen throughout the internal organs, serosa, muscle and eye (Figs 2.2 and 2.3). In survivors haemorrhage is reduced, but anaemia is severe and nervous signs are common. In the marine environment, where the conditions are optimal for the fish, the signs of the infection can be mild and mortality within normal range.

Prevention and control

There is no treatment for VHS and therefore prevention by avoiding infection is crucial. It is important to have no contact with farms of unknown disease history that may be infected. In areas where VHSV is endemic,

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Fig. 2.2. Viral haemorrhagic septicaemia in rainbow trout (*Oncorhynchus mykiss*). Note the numerous petechiae in the peritoneum wall.

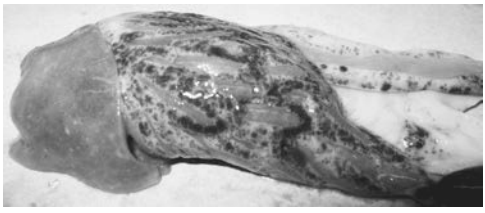


Fig. 2.3. Viral haemorrhagic septicaemia in rainbow trout (*Oncorhynchus mykiss*). Necrosis in the fat surrounding pyloric caeca.

disease may be controlled by keeping different year classes and fish species apart, reducing fish movement between farming sites and fallowing before restocking. The farming procedures should be planned in such a way that no contact from the processing plants to the farming sites should occur before slaughter. VHSV is described to survive outside the host in tap water for 49 days at 10°C, but survival time decreases to 10 days in suspended mud. The survival decreases in increasing temperatures where a 99.9% reduction in titre was recorded after 24 hours in 30°C and 10 min in 50°C. The virus is destroyed in 1 min above 70°C. VHSV is also sensitive to most virucidal disinfectants and UV-light.

Infectious pancreatic necrosis (IPN)

Infectious pancreatic necrosis virus (IPNV) is an aquabirnavirus belonging to the family *Birnaviridae*. Several aquabirnaviruses have been isolated from a wide range of aquatic organisms including fish, molluscs and crustaceans worldwide (Reno, 1999). Just a few of these are pathogenic to fish. According to Munro and Midtlyng (2011) only aquabirnaviruses isolated from salmonids or causing disease to salmonids should be called IPNV to avoid inconsistency concerning apathogenic aquabirnavirus-strains. IPNV is divided into seven genogroups (Blake *et al*., 2001; Nishizawa *et al*., 2005). IPNV is one of the most resistant viruses to physiological and chemical disinfection procedures in aquaculture and used as the test virus to determine the effect of disinfectants used in aquaculture.

Impact on fish production

IPN causes highest mortality to recently hatched salmonid fry at the time of first feeding. Mortality decreases with age and is usually not recorded in yearlings or older fish (Reno, 1999). Atlantic salmon, however, are an exception, as mortalities between 4–12 % are common in post-smolts, during the first months after transfer to sea. Due to the post-smolt mortality, IPN became one of the most important diseases in the Atlantic salmon industry; however, commercial vaccines have reduced this impact. Aquabirnaviruses are also found to be pathogenic for European and Japanese eel (*Anguilla anguilla* and *Anguilla japonica*) and flatfish species such as Atlantic halibut, turbot and Senegalese sole (*Solea senegalensis*) (Munro and Midtlyng, 2011).

Diagnosis

IPN is diagnosed by virus isolation from organ samples, in cell culture followed by the identification of the virus by immunological or molecular techniques (OIE, 2013). The same method is also used in screening of clinically healthy fish for the presence of IPNV.

Clinical signs

Disease signs of IPN in fry and fingerlings are covered in detail in Chapter 4. Briefly, the first signs of IPN in post-smolts are a change in swimming behaviour (corkscrew swimming),

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darkening of skin, loss of appetite and elevated mortality. The stomach and intestine are empty except for a loose mucoid exudate and the liver is pale yellowish. Petechiae may be seen in the visceral fat between the pyloric caeca where the pancreas is situated. Survivors may become cachectic with a thin body and apparently big head. Histopathological lesions in salmonids are principally seen in the pancreas and the intestinal mucosa, including pancreatic acinar cell necrosis, acute catarrhal enteritis and intraluminal inflammatory exudate in the pyloric caeca (Fig. 2.4).

Prevention and control

Injectable vaccines against IPNV in Atlantic salmon post-smolts are available and have been reported to give good but not complete protection (Munro and Midtlyng, 2011). It is important to avoid contact with farms with an unknown disease history that may be infected. In areas where IPNV is endemic, disease may be controlled by reducing stress, keeping different year classes and fish species apart, reducing fish movement between farming sites and a routine to fallow before restocking. Eradication of IPN is difficult because of several factors, including resistance to chemical and physical disinfection. IPNV can survive outside the host for almost a year at 4°C and 2 months at 15°C. The virus is very resistant to acids, e.g. it can survive in pH 2 for 5 weeks and is not inactivated in fish silage pH 3.8–4.3 at 22°C (Whipple and Rohovec, 1994; Bovo *et al*., 2005). IPNV is also highly resistant to UV-light. A 3-log10 reduction of IPNV requires a UV-dose of 246 mJ per cm^2, which is twice the dose that is reported for the inactivation of Nodavirus and more than 30 times what is required to inactivate ISA virus (Munro and Midtlyng, 2011). Fish surviving from an IPN outbreak may become lifelong carriers of IPNV, transmitting the virus via their eggs, milt, urine and faeces. The carrier state depends on factors such as age, fish species, water temperature and virus strain (Rodriguez *et al*., 2003). IPNV has also several vectors, including wild fish, molluscs and crustaceans that can sustain the infection.

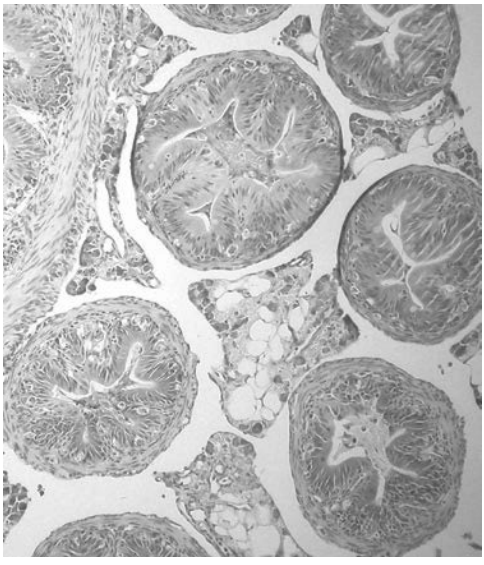


Fig. 2.4. Infectious pancreas necrosis (IPN) in rainbow trout (*Oncorhynchus mykiss*) fry with necrosis of the exocrine pancreas. Necrosis of epithelial cells in the mucus of the pyloric caeca is seen in acute IPN.

Infectious salmon anaemia (ISA)

Infectious salmon anaemia virus (ISAV) is an Orthomyxovirus causing infection in seareared Atlantic salmon. No other fish species have been reported to have ISA disease because of a natural infection. ISA is reported annually from Norway, where the disease has caused severe losses to the salmon industry (Rimstad *et al*., 2011; EFSA, 2012). In 1990 more than 80 fish farms were infected, but this situation has improved significantly because of large scale biosecurity. In recent years ISA disease reports have decreased to 3–20 affected farms annually (Rimstad *et al*., 2011). ISA has also been reported from farmed Atlantic salmon on the east coast of Canada (1996, 2000, 2012) and USA (2001), Scotland (1998, 2011), the Faroe Islands (2000) and Chile (2007) (Mullins *et al*., 1998; Rodger *et al*., 1998; Bouchard *et al*., 2001; Godoy *et al*., 2008; Murray *et al*., 2010; Christiansen *et al*., 2011). In 2002, ISAV was isolated from clinically healthy rainbow trout in Ireland (Geoghegan, 2002). ISAV isolates are divided into two

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major groups, one European and one North-American group. Certain ISAV strains are avirulent, as they have only been detected in clinically healthy wild or farmed Atlantic salmon. These apathogenic strains are genetically different from the virulent strains as they have a complete haemagglutinin gene, when the virulent strains have nucleotid gaps in certain parts of this gene (Rimstad *et al*., 2011). The part of the gene that has these deletions is called a highly polymorphic region (HPRΔ). The apathogenic strains, which have no deletion in this region are called HPR0 and are considered an ancient variant of ISAV from which pathogenic ISA strains may arise because of mutation in an HPR. It is stated that other genes are important for virulence (Rimstad *et al*., 2011; EFSA, 2012). ISAV strains with HPR0 are common in farmed Atlantic salmon in the Faroe Islands and have been reported from Scotland and Norway and usually detected from gill samples of clinically healthy fish. HPR0 strains are difficult to isolate on cell-culture and they are detected by RT-PCR from samples. ISAV has been isolated from wild sea trout in Norway that may represent a natural vector of the virus. Sea trout live close to fish farms and migrate regularly, which could explain how ISAV has spread to farms situated some distance from infected farms. It is not clear if trout are the original source of the virus or if it becomes infected from ISA positive fish farms. The infection is most likely transferred via infected water through gills, wounds in the skin, eyes or by ingestion (Rimstad *et al*., 1999). There is little evidence for vertical transmission, and if it occurs, it is considered an uncommon event (Rimstad *et al*., 2011). Sea lice are also suspected to be vectors of ISAV. Researchers have successfully managed to infect ISAV to brown trout, rainbow trout, Arctic char, chum salmon, coho salmon, herring and Atlantic cod but no clinical signs of ISA have developed in these species (Rimstad *et al*., 2011). ISAV was not detected by RT-PCR from experimentally infected alewine (*Alosa pseudoharengus*), American eel (*Anguilla rostrata*), Atlantic herring, Atlantic mackerel (*Scomber scombrus*), Atlantic cod, haddock, Atlantic halibut, pollock (*Pollachius virens*), American shad (*Alosa sapidissima*) or from winter flounder (*Pseudopleuronectus americanus*) (Rimstad *et al*., 2011). Blue mussel (*Mytilus edulis*) and scallops (*Pecten maximus*) have also been tested for ISAV but they have not been shown to be a reservoir for the virus (Skar and Mortensen, 2007).

Impact on fish production

The daily mortality of ISA is usually not more than 0.05–0.1% in affected populations, but mortality can continue for months and cumulate as high as 90%. Mortality may increase because of stress. Smoltification has been suspected to be a stress factor, as higher mortality is often seen during this physiological change (Rimstad *et al*., 2011).

Diagnosis

ISA is diagnosed by detection of clinical and pathological signs together with the demonstration of a systemic ISAV infection by virus isolation in cell culture followed by the identification of the virus by immunological or molecular techniques. Cell cultures recommended are ASK or SHK-1 cells but due to a sometimes weak cytopathogenic effect molecular techniques such as direct RT-PCR or real-time RT-PCR from organ homogenates of diseased fish are also used (OIE, 2013).

Clinical signs

Diseased fish are lethargic and listless and may sink to the bottom of the net pen. A severe anaemia with almost watery blood is common. Target cells for ISAV are endothelial cells, blood vessels of all organs including sinusoids, endocardium and endothelial macrophages (Rimstad *et al*., 2011). Mortality can be high but usually only for a short period, but may continue as low for months. Macroscopic findings include pale gills, exophthalmia and haemorrhage in eyes, scale oedema, petechiae in the skin, peritoneal surfaces and ascites. Liver and spleen are enlarged and dark due to severe necrosis and haemorrhage. In the early stage of the disease the histological findings are usually single cell necrosis, congestion and dilatation of the hepatic sinusoids. Later, degeneration of hepatocytes and haemorrhage, appearing to join

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and give the liver a bridging appearance, are common findings. The lamina propria of the foregut is congested and haemorrhage common. The clinical signs usually start in one net pen, but it may take weeks or months before fish in the other net pens on the farm get diseased (Rimstad *et al*., 2011).

Prevention and control

The best way to manage the ISAV is the avoidance of infection. It is important to avoid contact with farms without a known disease history. In endemic areas disease outbreaks and mortalities can be decreased by implementing high standard hygiene procedures that aim to reduce horizontal spread and infection pressure. These are gained by putting restrictions on infected farms, enforcing sanitary slaughtering, practising an 'all in all out' system so different age classes do not mix, cleaning farm equipment and fallowing the site before restocking. Waste water from the fish processing plants should be disinfected. The farming procedures should be planned in such a way that no contact from the processing plants to the farming sites should occur before slaughter when the site is emptied. It is also important to keep a health monitoring system that can detect the disease quickly to prevent spread (Rimstad *et al*., 2011). A vaccine against ISA is available and used in the USA, Canada, Faroe Islands and in some parts of Norway. Vaccination is not preventing infection and vaccinated fish can become carriers (Rimstad *et al*., 2011). ISAV is easily destroyed by several disinfectants used in aquaculture, e.g. iodophores, chloramine and peroxygen-based disinfectants (Bovo *et al*., 2005). ISAV is readily destroyed by UV (Øye and Rimstad, 2001).

Salmonid alphavirus (SAV) infections (pancreas disease PD and sleeping disease SD)

Salmonid alphaviruses (SAV) belong to the family *Togaviridae* and six different subtypes of SAV have been isolated from Atlantic salmon or rainbow trout (Fringuelli *et al*., 2008). Subtypes 1 and subtypes 3–6 cause pancreas disease (PD). SAV-1 and SAV-4 are primarily isolated from marine Atlantic salmon in Ireland and Scotland (Nelson *et al*., 1995; Weston *et al*., 2005; Fringuelli *et al*., 2008). SAV-2 is the cause of sleeping disease (SD) in rainbow trout in fresh water in France, UK, Spain, Italy and Germany but also occurring in sea reared fish. SAV-3 and recently SAV-2 have been detected in Norway from Atlantic salmon (Hjortaas *et al*., 2013). SAV-3 has also caused PD signs in marine-reared rainbow trout in Norway. In Scotland SAV-5 has been detected in Atlantic salmon and SAV-6 in Ireland (Fringuelli *et al*., 2008). The route for the transmission of SAV is horizontal.

Impact on fish production

Infections caused by SAVs have a complex epizootiology where fish age, fish species and strain, farming site, water temperature and stress influence the outcome. PD has caused severe economic losses in Norway. In one site of 500,000 fish, the losses were 14.4 million NOK (Aunsmo *et al*., 2009). Economic losses in Ireland, during 2003–2004, were estimated to be €35 million, with a €12 million loss of profit (Ruane *et al*., 2008). Mortality rates have varied in different countries. In Ireland a mortality of 10–50% in yearly smolt input is reported (McLoughlin *et al*., 2002). According to Munro *et al*. (1984), 15% of the survivors of a PD outbreak failed to grow and became runts.

Diagnosis

PD and SD are diagnosed by detection of clinical and pathological signs, light microscopy together with the demonstration of infection by virus isolation in cell culture followed by the identification of the virus by immunological or molecular techniques. Direct RT-PCR or real-time RT-PCR from organ homogenates of diseased fish are also used (Graham *et al*., 2006; Christie *et al*., 2007).

Clinical signs

The first signs of PD can be a sudden inappetence, lethargy and high morbidity. Increased amount of faecal casts can be seen in the

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water in the cages and mortality is increased. Fish may have problems staying in the right position or are listless near the edges of the net pens. Few gross pathological changes are prevalent in the early stages of the disease; major findings consist of an empty stomach and yellow casts in the gut. In some cases petechial haemorrhage can be seen in the fat tissue surrounding the pyloric caeca. Later the body fat decreases and after several weeks the body becomes long and thin with little fat surrounding the pyloric caeca. Microscopical changes are mainly found in pancreas, heart and skeletal muscle. In the early stage of the disease, only 2–5% of fish show acute pancreatitis which makes early diagnosis of PD and virus isolation difficult (McLoughlin *et al*., 2002). Most of the pancreatic acinar tissue of affected fish is destroyed with variable inflammatory response consisting of mononuclear cell infiltration and occasional fibrosis. Regenerative changes may appear as early as 4 weeks after infection or chronic inflammatory lesions may persist for a longer period (McLoughlin *et al*., 2002; Taksdal *et al*., 2007). Heart lesions may occur in parallel with the pancreatic necrosis but generally they appear later. Lesions consist of multifocal cardiomyocyte necrosis in both compact and spongy ventricular and atrial muscle to varying degrees from small focal lesions to severe lesions including entire heart musculature. The pathogenesis in younger fish that still have the ability to replace damaged heart tissue by cell division may vary from older fish (Graham and McLoughlin, 2011). Skeletal muscle lesions appear 3–4 weeks after pancreatic and heart muscle lesions and it is possible that only skeletal lesions can be found in fish that are in a late stage of the disease. Skeletal lesions consist of hyaline degeneration, with swollen fragmented eosinophilic sarcoplasm, central migration of myocytic nuclei and invasion of phagocytic macrophages. Fibrosis may be present depending on the severity of muscle lesions. Red muscle fibres tend to be more affected than white muscle (Graham and McLoughlin, 2011). Fish with SD lie on their side on the bottom of the cage as if they are sleeping. This is a result of necrosis of skeletal red muscle in the late stage of the infection. Similar pancreatic and heart muscle lesions as described for PD can be seen in the early stage of the disease. Differential diagnoses for alphavirus infections are IPN, cardiomyopathy syndrome (CMS), heart and skeletal muscle inflammation (HSMI) and myopathies with nutritional background.

Prevention and control

The best way to manage the disease is the avoidance of infection. It is important to avoid contact with farms without a known disease history. In endemic areas, disease outbreaks and mortalities can be decreased by implementing high standard general hygiene procedures that aim to reduce horizontal spread and infection pressure. Good sea lice control can decrease the onset of disease as sea lice may be reservoirs or vectors of the disease (Weston *et al*., 2002; Karlsen *et al*., 2006). Losses may be decreased in the onset of an infection by withholding of feed for 5–10 days in the net pens with clinical signs. It may take 2–3 months for all net pens on a farm to become infected (Crockford *et al*., 1999). Fish should not be kept too long without feed as hunger will enhance cardiac and skeletal muscle damage and mortality (Rodger *et al*., 1991). Commercial vaccines against PD are available.

Cardiomyopathy syndrome (CMS)

Cardiomyopathy syndrome is a disease caused by piscine myocarditis virus (PMCV) that belongs to the family *Totiviridae* (Løvoll *et al*., 2010). The disease has been described in Norway since the mid-1980s causing losses to Atlantic salmon in marine fish farming. A viral aetiology was suspected for several years, but experimental transmission and identification of the causative agent for the disease was not confirmed until 2011 (Bruno and Noguera, 2009; Løvoll *et al*., 2010; Haugland *et al*., 2011). CMS is also described in Scotland, the Faroe Islands and Canada in farmed Atlantic salmon (Rodger and Turnbull, 2000; Brun *et al*., 2003). The disease affects adult salmon 12–15 months after transfer to sea water until slaughter (Brun *et al*., 2003).

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The disease is found throughout the year, but more cases can be seen during spring and autumn (Aunsmo *et al*., 2005). Lesions that resemble CMS have also been described in wild Atlantic salmon (Poppe and Seierstad, 2003). Transmission of the virus is horizontal (Haugland *et al*., 2011).

Impact on fish production

The economic impact of CMS to fish farming in Norway is estimated to be 4.5–8.8 million Euros per year (Brun *et al*., 2003). Mortalities vary from a few per cent up to as high as 60% (Rodger and Turnbull, 2000; Brun *et al*., 2003).

Diagnosis

Diagnosis is based on characteristic clinical and histopathological lesions. PMCV can also be demonstrated with real-time RT-PCR (Haugland *et al*., 2011).

Clinical signs

Affected fish are usually in good body condition and show few or no clinical signs before death. Diseased fish show poor appetite, they are lethargic and develop severe ascites and exophthalmia, haemorrhage and scale oedema in the skin. Sinus venosus and atrium of the heart is usually enlarged and the pericardial cavity may be filled with blood or blood clots because of a ruptured atrium or sinus venosus. Fibrinous casts may be seen on the surface of a mottled liver. Microscopic lesions are found in the myocardial trabeculae of the atrium and ventricle of the heart. The heart lesions include proliferation of endocardium and subendocardial cells and myocardial degeneration with infiltration of lymphocytes and macrophages (Ferguson *et al*., 1990; Rodger and Turnbull, 2000; Bruno *et al*., 2013).

Prevention and control

There is no treatment for CMS and little is known on how to prevent and control the disease. General instructions on how to prevent viral diseases should be followed. In endemic areas disease outbreaks and mortalities can be decreased by implementing high standard general hygiene procedures and reducing stress. Complementing an 'all in all out' system with a short production period may prevent CMS. Control of IPN may prevent CMS as earlier IPN 'outbreaks' have increased the risk of CMS. Enforced slaughtering should be considered if the disease affects fish close to market size (Aunsmo *et al*., 2005).

Heart and skeletal muscle inflammation (HSMI)

Heart and skeletal muscle inflammation (HSMI) is an emerging disease of farmed Atlantic salmon. The causative agent is suspected to be a piscine reovirus (PRV) (Palacios *et al*., 2010). HSMI was diagnosed for the first time in Norway in 1999 and has spread widely in the last decade along the Norwegian coast. The disease is also described in Scotland and Ireland (Ferguson *et al*., 2005). It is a differential diagnosis for CMS with prominent heart lesions similar to those described for CMS.

Impact on fish production

The difference between CMS and HSMI is that fish with HSMI tend to recover when CMS fish die of the disease. Another difference is that HSMI causes disease outbreaks in smaller fish (0.3–1 kg) and sooner (5 to 9 months) after transfer to sea compared with CMS. Mortality may vary from negligible up to 20% (Kongtorp, 2008).

Diagnosis

Diagnosis is made based on clinical signs and histopathological lesions.

Clinical signs

Fish with HSMI are lethargic, gather close to the edges of the net pen and mortality is elevated. At necropsy the haemopericardium and a pale heart can be observed. The liver is often pale and yellowish or dark and congested. A fibrinous coat may be seen on the surface of the liver of some fish (Kongtorp, 2008). The histopathological changes include moderate to severe myocarditis with inflammation-associated

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necrosis of both spongy and compact myocardium in the ventricle. A varying degree of epi- and endocarditis can also be detected. Myositis can be seen in the red skeletal muscle (Kongtorp *et al*., 2004). Multifocal liver necrosis has also been reported in some cases.

Prevention and control

There is no treatment for HSMI. The best way to manage the disease is the avoidance of infection. It is important to avoid contact with farms with unknown disease history. In endemic areas disease outbreaks and mortalities can be decreased by implementing high standard hygiene procedures that aim to reduce horizontal spread and infection pressure. Losses can be decreased by implementing the 'all in all out' system so different age classes do not meet, farming equipment can be cleaned and the farming site kept empty before restocking (Aunsmo *et al*., 2005).

Viral encephalopathy and retinopathy (VER)

Viral encephalopathy and retinopathy (VER) is a disease caused by a nodavirus. VER is described in over 30 fish species in the marine environment in different parts of the world (Munday and Nakai, 1997). (See also Chapter 5 on diseases in marine warm water.) In the northern cool marine environment it causes severe disease in Atlantic halibut in Norway and Atlantic cod in Scotland, Canada and Norway, and turbot in Norway (Starkey *et al*., 2001; Bergh *et al*., 2002; Johnson *et al*., 2002; Patel *et al*., 2007).

Impact on fish production

VER causes high mortality that often may reach 100% in affected larvae and juveniles. The mortality for Atlantic cod has been reported by Patel *et al*. (2007) to be 10–15% in 5–24 g sized fish. A significant portion of surviving fish become carriers and their growth may be retarded (Johansen *et al*., 2004).

Diagnosis

See Chapter 5.

Clinical signs

The main clinical signs include dark pigmentation and abnormal swimming behaviour. Signs produced by these nodaviruses in the cold marine environment resemble those in the warm water environment. See Chapter 5 for detailed information.

Prevention and control

There is no treatment for VER. The best way to manage the disease is the avoidance of infection. It is important to avoid contact with farms with unknown disease history. If brood stock material is collected from wild fish they should be tested free of nodavirus before transfer to farming facilities. Implementing high standard general hygiene procedures, which aim to reduce horizontal spread and infection pressure are important. Waste water from the fish processing plants should not get in contact with live fish on farms. If several fish species are farmed in close vicinity, nodaviruses should be considered in the planning as several fish species are susceptible (Aunsmo *et al*., 2005).

Bacterial Diseases

Bacterial infections cause extended economic losses for the aquaculture industry. The most important bacterial pathogens in marine and brackish waters include infections with *Vibrio*, *Francisella*, *Piscirickettsia*, *Aeromonas*, *Pseudomonas* and the recently proposed Candidatus *Branchiomonas cysticola* as the aetiological agent of epitheliocystis in the marine environment. A significant economical, as well as ethical, concern in the cold marine environment is also the occurrence of 'winter ulcer disease' with isolations of *Moritella viscosa* and *Tenacibaculum* spp. A few reports describe infections with *Mycobacterium* spp. in farmed fish. There is a risk for transmission of these bacteria from wild-living fish species and the difficulties for treatment, as well as the zoonotic potential, make these infections obvious for consideration in the marine environment.

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Salmonids hatched and reared in freshwater environments are, after smoltification, transported to brackish or marine localities for further growth for the food industry. Several pathogens in fresh water also cause morbidity and mortalities in the brackish or marine environment. *Renibacterium salmoninarum* causing bacterial kidney disease (BKD) and *Yersinia ruckeri* causing enteric redmouth disease are examples of such infections, briefly described here and in depth in Chapter 3.

Good water exchange rates on fish farms, general improvement of hygiene standards and adequate husbandry are precautions that cannot be undervalued in order to avoid infection and mortalities. In case of disease it is important to remove dead and moribund fish from the cages, and to keep diseased and healthy fish separate. Treatment with antibiotics should be kept as a last measure and only used when an outbreak has been diagnosed. Sensitivity to antimicrobials is a global issue and all precautions should be taken to limit the use of these compounds, to minimize the risks of development of antibiotic resistance and to reduce the exposure of animals, humans and the environment. The development of functional vaccines have definitely improved fish health, as well as reduced the use of antibiotics in aquaculture (NORM/NORM-VET, 2011 (Fig. 2.5)). The introduction of new fish species in aquaculture, among those several marine species, requires continuous effort to apply adequate diagnostic procedures and to produce new efficient vaccines especially adapted for these new species and their 'new' pathogens.

Vibriosis

Vibriosis is the most significant disease affecting fish and invertebrates in brackish and marine environments, for cultured and wild-living species (Frerichs and Roberts, 1989; Frans *et al*., 2011). Infection with *Vibrio anguillarum* (synonym *Listonella anguillarum* (Thompson *et al*., 2011) is the main pathogen in salmonid aquaculture during their first summer at sea. Pacific salmon, Atlantic salmon and rainbow trout are affected but also cod, haddock and several marine living species mainly in shallow waters. *V. anguillarum* is a Gram-negative, curved rod. A total of 23 serotypes of *V. anguillarum* have been described, based on the bacterium's O-antigens (O1–O23). Serotypes O1 and O2 are the most significant for salmonid aquaculture but mortalities are, to a lesser extent, also reported to be caused

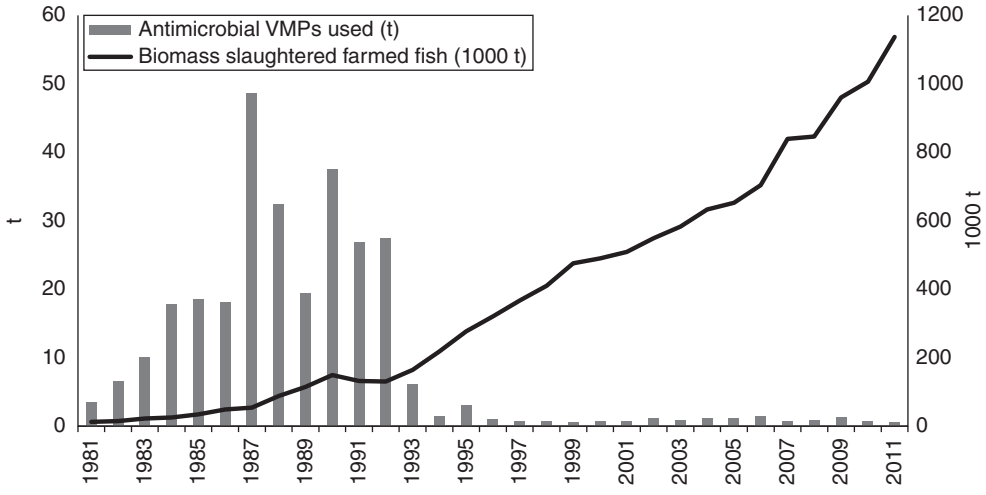


Fig. 2.5. Total sales, in tonnes of active substance, of antimicrobial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 1981–2011 versus produced biomass (slaughtered) farmed fish. Source: NORM/NORM-VET, 2011.

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by serotype O3 (Sørensen and Larsen, 1986; Silva-Rubio *et al*., 2008a). *V. anguillarum* serovars O4, O6 and O8 have been isolated from cod larvae and also from diseased cod (Buchmann *et al*., 1993). The main bacterial disease for cod and haddock farming is caused by *V. anguillarum* serotype O2a and O2b. *V. ordalii*, formerly named *V. anguillarum* biotype 2, was initially isolated from diseased Pacific salmon in mariculture on the Pacific coast of North America (Harrel *et al*., 1976). Since then *V. ordalii* has also been reported in Japan, Australia, New Zealand, Europe and in South America, causing a haemorrhagic sepsis in several salmonid species. Immunological characterization has established an antigenically homogenous species even if some cross reactivity is observed with *V. anguillarum* serotype 2 (Toranzo *et al*., 2005; Silva-Rubio *et al*., 2008b). Cold-water vibriosis (Hitra disease, haemorrhagic syndrome) caused by *Vibrio salmonicida* was reported for the first time from Atlantic salmon in northern Norway (Egidius *et al*., 1981). Based on combined analysis of selected genes (recA, rpoA, pyrH, gyrB and 16S rRNA) and phenotypic traits, this pathogen was reclassified as belonging to a new linage within the *Vibrionaceae* in the new genus, *Aliivibrio* gen. nov., and renamed *Aliivibrio salmonicida* (Urbanczyk *et al*., 2007). The abbreviation of this new name to *A. salmonicida* requires attention due to the risk of confusion with the furunculosis-causing bacterium, *Aeromonas salmonicida*. Most serious losses are in Atlantic salmon but this *Aliivibrio* is also isolated from rainbow trout and Atlantic cod (Egidius *et al*., 1981; Sørum *et al*., 1990). Cold-water vibriosis occurs, as the name implies, below 10°C, in fish-farming areas surrounding the North Atlantic, in eastern Canada and in the USA.

Impact on fish production

Vibriosis caused by *V. anguillarum* has caused major economic consequences in salmonid aquaculture before the establishment of the commercial vaccination programmes (Toranzo *et al*., 2005). Several reports describe mortalities caused by *V. anguillarum* also in cod farming (Egidius, 1987; Samuelsen *et al*., 2006; Rodger and Colquhoun, 2008). Mortalities of up to 8%, recorded in Atlantic salmon in Chile after transfer to sea water at a temperature of 9°C, was found to be caused by *V. ordalii* (Colquhoun *et al*., 2004). Serious taps due to cold-water vibriosis have been sporadically reported since the 1970s from salmonid producing countries. Experimental challenge of salmon with *A. salmonicida* demonstrated a rapid accumulation of the bacterium in the blood, after just a few hours, and it was suggested to be the place for proliferation in the host. The bacteria also colonize the intestine and this might be a main source for release of the pathogen to the environment and spread in the population through these 'healthy carriers' (Bjelland *et al*., 2012). Commercial vaccines give excellent protection and losses due to vibriosis generally have a low impact on fish production, if vaccination is carried out according to recommended instructions.

Diagnosis

Diagnosis of vibriosis caused by *V. anguillarum* or *V. ordalii* is based on clinical signs and isolation of the bacterium from the kidney/spleen on blood agar or on tryptic soy agar (TSA) after 1–2 days at 20–30°C. Circular growing cream-coloured colonies are sensitive to the vibriostatic agent O/129 and their biochemical profile can be verified by standard biochemical tests or by the API20E test (Austin *et al*., 1997; Austin and Austin, 1999). Differentiation between *V. anguillarum* and *V. ordalii* is possible by comparison of selected phenotypic characteristics or by ribotyping and plasmid analysis (Schiewe *et al*., 1981; Tiainen *et al*., 1995). The actual serovar of *V. anguillarum* is decided by slide-agglutination using verified and specific antiserum. Several PCR-based diagnostic methods for detection of nucleic acid from *V. anguillarum* and *V. ordalii* are available (Hong *et al*., 2007). The halophilic and psychrophilic A. *salmonicida* requires supplementation with NaCl to a final concentration of 2% and incubation at 15°C for growth of the small (0.5 mm), circular and translucent colonies after 3 days. Isolated bacteria can be identified by biochemical tests and also by PCR (Bruno *et al*., 1986; Bjelland *et al*., 2012). Motility is important for the bacterium's attachment to its host and this curved rod can carry up to nine polar flagella.

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Clinical signs

V. anguillarum cause rapid and high mortalities in unvaccinated fish. Initial signs include raised scales and darkening of the body. The infection proceeds with haemorrhage at the base of the fins, in the skin, in the musculature to a generalized septicaemia. Fish show indications of anaemia and splenomegaly. Large multiple haematomas can be seen in the liver. Formations of micro-colonies in the skeletal and heart muscle, gill tissue and in the gastro-intestinal tract by *V. ordalii* in Pacific salmon have been observed. The bacteraemia appear later, compared with infection by *V. anguillarum* (Ransom *et al*., 1984). Adult salmon, affected by cold-water vibriosis, are usually lethargic, dark coloured, showing a reduced appetite and extensive petechial haemorrhage along the belly and at the fin bases. Anaemia, petechial haemorrhage, in connection with the pyloric caeca, abdominal fat, the surface of the peritoneum, the liver and swimbladder are also described. The peritoneal cavity or the swimbladder might contain a blood-containing fluid. Histopathological lesions consists mainly of sloughing and necrosis of intestinal mucosa, haemorrhaging and an increase of eosinophilic granular cells (Bruno *et al*., 1986). Pathological findings in cod are more diffuse but are described to include lesions in the eye in form of keratitis, cranial haemorrhages and enlargement of the spleen (Sørum *et al*., 1990). Grossly swollen and necrotic fins with a pale grey coloration was reported in moribund cod in Scotland. Muscle haemorrhage and petechiae in the pancreatic fat were observed internally (Jones *et al*., 2000).

Prevention and control

Early detection and removal of moribund and dead fish will reduce the infection pressure. Studies have demonstrated lower mortality rates in farms managed by experienced farmers (Thorburn, 1987). There are efficient vaccination programmes against vibriosis caused by *V. anguillarum*, *V. ordalii* and *Aliivibrio salmonicida* that generally eliminated mortalities and reduced the need for antimicrobial treatments. There is a risk of new problems with vibriosis, when new fish species are introduced for mariculture as these species might be susceptible to different serotypes, which today are not included in commercial vaccines.

Franciselliosis

Infections with *Francisella* have been reported from fish in fresh, brackish and marine environments. It causes a granulomatous disease with varying mortality (Birkbeck *et al*., 2011). In cold water, cod are especially affected but francisellosis can also cause mortality in freshwater Atlantic salmon parr, reported from Chile (Birkbeck *et al*., 2007). Francisellosis is also reported from warm-water species such as tilapia *Oreochromis* spp. (Hsieh *et al*., 2006) and the marine living three-lined grunt, *Parapristipoma trilineatum* (Kamaishi *et al*., 2005). Molecular characterizations have classified isolates from coldwater fish as *Francisella noatunensis* subsp. *notunensis* (*F. piscicida*) and isolates from warmwater fish as *Francisella noatunensis* subsp. *orientalis* (Colquhoun and Duodu, 2011). *Francisella notunensis* is a facultative intracellular, Gram-negative coccoid/short rod that can be isolated on cysteine and blood containing agar media. The present chapter will focus on infections in cold water species caused by *F. noatunensis* subsp. *noatunensis*.

Impact on fish production

Francelliosis has a serious impact on the production of cod in aquaculture, especially as adult fish is affected with mortalities up to 40% (Olsen *et al*., 2006). Muscle lesions result in rejection at slaughter. Similarly, franciselliosis has also been reported from Atlantic salmon parr held in freshwater cages in Chile (Bohle *et al*., 2009).

Diagnosis

Diagnosis is based on clinical signs and histopathology that can be verified by immunohistochemistry or by molecular based techniques (Duodu *et al*., 2012). Histopathological findings include a massive granuloma formation

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in vital organs. Cultivation is possible on cysteine heart agar with 5% sheep blood showing greyish-white colonies after 3–4 days cultivation at 20–22°C.

Clinical signs

Cod become emaciated, dark in colour, swim with a sluggish behaviour and raised haemorrhagic nodules or ulceration can be observed in the skin. Extensive gross lesions in the form of moderate to massive occurrence of white nodules of various sizes in heart, kidney, liver and in the spleen (Fig. 2.6) are observed. The kidney and spleen are swollen and occasionally ascites is present. There is extensive granulomatous inflammation in the kidney and spleen interstitium, in the parenchyma of the liver and in the lamina propria of the intestine (Alfjorden *et al*., 2006; Olsen *et al*., 2006). The progression of the disease is temperature dependent with increasing lesions up to 20°C. *Francisella* can also be isolated from cod at a few degrees above 0°C (Colquhoun and Duodu, 2011).

Prevention and control

Infection is through the horizontal route (Ellingsen *et al*., 2011); however, vertical transmission cannot be excluded. Regular screening programmes and avoidance are the only possible actions for controls as antimicrobial-based therapy has temporary effects and there are no commercial vaccines. Clinical disease caused by *F. noatunensis* has been demonstrated in wild-living cod in the Skagerrak Sea (Alfjorden *et al*., 2006); this indicates the obvious risk for transmission from wild-living fish to farmed gadoid species. *F. noatunensis* can survive for up to 60 days in water, but can after a period enter a viable but non-culturable and avirulent stage (Duodu and Colquhoun, 2010). Cohabitation experiments with blue mussels (*Mytilus edulis*) did not indicate direct transfer from infected mussels to Atlantic cod but *F. noatnenesis* was found to survive through the mussel's digestive system in an infective state (Wangen *et al*., 2012).

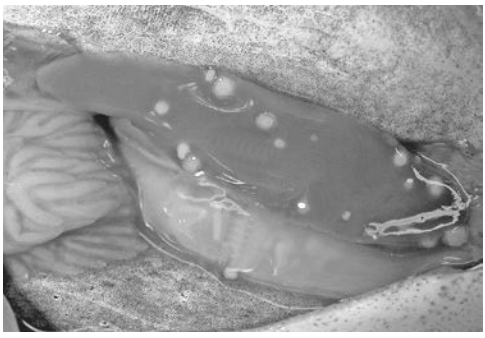


Fig. 2.6. *Francisella noatunensis* infection in wild-living Atlantic cod (*Gadus morhua*), caught outside the Swedish west coast. Note the circular white nodules in spleen. Image courtesy of Anders Alfjorden, National Veterinary Institute, Sweden.

Salmonid rickettsial septicaemia

Piscirickettsia salmonis is a facultative intracellular Gram-negative bacterium causing salmonid rickettsial septicaemia (piscirickettsiosis) in the marine environment. It was also reported from fish in brackish waters (Almendras and Fuentealba, 1997). The first outbreak was reported from Chile in coho salmon (Bravo and Campos, 1989), this was followed with outbreaks in pink salmon and in Atlantic salmon, in rainbow trout and in Chinook salmon (Cvitanich *et al*., 1991). The severity of the infection is varied and it depends on species and localization but the economic consequences have been most significant in salmonid farming in Chile. Infections with *P. salmonis* have also been detected in Europe (Olsen *et al*., 1997), in North America and also a rickettsia-like organism in Tasmania (Corbeil *et al*., 2005). Smolts in 51 fish farms along the Norwegian west-coast were considered to be infected with *P. salmonis* during 1988–1992 with a low cumulative mortality (Olsen *et al*., 1997). The most obvious signs are the white, circular, sometimes haemorrhagic foci observed in the liver. Sporadic cases have followed except for 2002 when 18 farms were affected (Karatas *et al*., 2008). This indicates that a less virulent strain of *P. salmonis* circulates along the Norwegian coast compared with isolates from Chile. Arkush *et al*. (2006) injected *P. salmonis* in white sea bass and induced a cumulative mortality of 29% and the persistence of the organism in the tissues for up to four months,

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indicating sea bass to be a potential reservoir for maintenance of this organism in the marine environment (Arkush *et al*., 2006).

Impact on fish production

Piscirickettsiosis has caused extensive losses of coho salmon in Chile during the 1980s with mortalities up to 90%. Clinical disease appeared 6 to 8 weeks after transfer to the marine environment. The disease is mainly a problem in the marine environment, particularly for smolts up to market size, however the pathogen has been detected from salmon in fresh water (Gaggero *et al*., 1995) despite a short survival time in fresh water.

Diagnosis

Distinctive crater-like lesions and nodules in the liver are the first indications of piscirickettsiosis. Diagnosis of piscirickettsiosis has been based on histological investigations confirmed by immunohistochemistry. Imprints or sections from internal tissues, stained with Giemsa or Gram, demonstrate pleomorphic bacteria, occasionally containing eosinophilic cytoplasmic granules. *P. salmonis* can be isolated in CHSE-214 cell culture at an optimal temperature of 15–18°C giving a cytopathogenic effect (Fryer *et al*., 1990). Isolation is possible on cysteine and blood enriched agar at temperatures of 16–22°C (Mauel *et al*., 2008; Mikalsen *et al*., 2008). Confirmation of isolated bacteria can be made by IFAT or ELISA enzyme-linked immunosorbent assay (Aguayo *et al*., 2002) or by PCR (Mauel *et al*., 1996). *P. salmonis* can also be detected in formalin-fixed material by real time PCR (Karatas *et al*., 2008).

Clinical signs

Affected fish are lethargic, show a lack of appetite and may swim close to the surface. Gills become pale and the body gets a dark coloration. Internal lesions consist of splenomegaly and swollen kidney especially in the posterior region. The liver of affected fish often have large whitish or yellow multifocal nodules, occasionally ruptured resulting in crater-like lesions. The clinical signs vary between the affected species. The nervous system seems often to be affected in Atlantic and coho salmon visible as an irregular swimming behaviour. Ulcerations and skin lesions in the form of raised nodules and white spots are also described.

Prevention and control

Horizontal transmission is demonstrated and there are indications for transmission through vectors and by vertical transmission (Larenas *et al*., 2003). The intracellular survival of *P. salmonis* in host tissue may explain the poor effectiveness of antibiotics used for therapy.

The widespread occurrence of *P. salmonis* in the marine environment has been recognised as the main reason for heavy losses in Chile. Promising results for a vaccine against piscirickettsiosis has been demonstrated by use of a mixture of recombinant proteins triggering the humoral response, as well as protection after challenge (Wilhelm *et al*., 2006). Recent studies also demonstrated protection by use of an oral vaccine that can be administered through the daily feeding with promising effects during the seawater stage (Tobar *et al*., 2011)

Winter ulcer disease

Moritella viscosa, formerly *Vibrio viscosus*, can be isolated from open ulcers on the flanks of Atlantic salmon during the winter season, a condition named 'winter ulcer disease' (Benediktsdóttir *et al*., 2000). Winter ulcer disease was initially described from Norway, Iceland and Scotland (Lunder *et al*., 1995; Benediktsdóttir *et al*., 1998; Bruno *et al*., 1998a). Shallow skin lesions and diffuse or petechial haemorrhages in internal tissue characterize the disease. Mortalities are often low but the open ulcers facilitate the entry for other pathogens.

Impact on fish production

'Winter ulcers' have an economic impact in aquaculture as the large open muscle tissue cause mortalities and rejections at slaughter. The economic consequences of winter ulcer disease have been serious for the salmon

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aquaculture in Norway and in Iceland. An important issue of this disease relates to the ethical questions raised. Atlantic salmon, rainbow trout and cod are the most affected species but the pathological lesions are also seen in experimentally infected turbot (Björnsdóttir *et al*., 2004).

Diagnosis

M. viscosa can be isolated on blood agar containing 2% NaCl at 15–20°C. The bacterium is sensitive to the vibriostat O/129 and can be confirmed by biochemical tests or by PCR (Grove *et al*., 2008).

Clinical signs

Winter ulcers are characterized by open ulcers, up to several square cm on the flanks, with degeneration in the underlying muscles. Macroscopic lesions include paleness of the gills and fin rot. Internal haemorrhage in the liver, pyloric caeca and in the perivisceral fat are described together with congestion, and kidney and spleen necrosis (Bruno *et al*. 1998a). The disease can induce high mortalities at low temperatures, in the marine environment often at temperatures below 10°C, but can also develop to a more chronic condition.

Prevention and control

Antibiotic treatment is usually not used as infected fish generally stop feeding. A commercial vaccine is available against winter ulcers caused by *M. viscosa*, however winter ulcer disease is also reported in vaccinated salmon (Gudmundsdóttir and Björnsdóttir, 2007).

Bacteria related to *Tenacibaculum* spp. are also isolated from similar ulcers at low temperatures. Specialty agar media are required for the isolation of *Tenacibaculum* spp., it is therefore possible that the influence of *Tenacibaculum* spp. in these lesions is underestimated.

Tenacibaculosis

Tenacibaculosis is an important infectious disease in marine fish caused by *Tenacibaculum maritimum* formerly named *Flexibacter maritimus* (Wakabayashi *et al*., 1986; Suzuki *et al*., 2001). The disease was already reported to cause serious losses in Dover sole (*Solea solea*) in the 1970s. After that outbreaks have been reported in mariculture of sea bass (*Dicentrarchus labrax*), turbot, Atlantic salmon, rainbow trout, Chinook salmon and several species of wild-living fish, indicating a lack of strict host specificity (Avendaño-Herrera *et al*., 2006). The disease affects mainly young fish but adults are also affected. An increased prevalence and severity has been reported at temperatures above 15°C and the condition of the skin and stress also affects the susceptibility (Magariños *et al*., 1995; Toranzo *et al*., 2005).

Impact on fish production

Mortalities of up to 10% per day were reported in Dover sole. The first signs of disease appeared 60 to 100 days after hatching and are more frequent during summer than in winter (McVicar and White, 1979).

Diagnosis

Microscopic investigation of gills and skin of affected fish demonstrates abundant long, thin Gram-negative rods. Species of *Tenacibaculum* can be isolated on special agar media such as Marine agar or Anacker and Ordal agar supplemented with NaCl. *T. maritimum* show phenotypic similarities with *Flavobacterium columnare* but is strictly dependent on increased levels of NaCl for growth, in contrast to *F. columnare* (Bernardet *et al*., 1990). Three major serotypes of *T. maritimum* have been identified based on the O-antigens (Avendaño-Herrera *et al*., 2005). Characterization is possible by API ZYM tests as most strains are auto-agglutinating and inactive in standard biochemical tests. Recently a new real-time PCR has been developed for the specific identification of *T. maritimum* (Fringuelli *et al*., 2012a).

Clinical signs

In Dover sole the infection is characterized initially by slight blisters or dark areas of the skin surface followed by loss of epithelial surfaces exposing haemorrhagic dermal tissues.

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Invasion with saprophytic organisms result in development of necrotic ulcers and the condition was named 'Black patch necrosis' (McVicar and White, 1979). Infections caused by *T. maritimum* occasionally involve lesions in the eye, mouth and jaw in the form of necrotizing stomatitis and gill lesions. A yellow pigmentation at the edges of the lesions is often seen due to an accumulation of *T. maritimum* (Avendaño-Herrera *et al*., 2006).

Prevention and control

Antibiotic therapy is possible after determination of the sensitivity to actual antibiotics. Antibiotic therapy is, however, often hampered by loss of appetite in affected fish.

Different kinds of bath treatments have been used. Hydrogen peroxide has been recommended as a general disinfectant for treatment of tanks and water culture before introduction of fish (Avendaño-Herrera *et al*., 2006). A vaccine has been developed against tenacibaculosis in turbot, both for bath administration in juveniles and for i.p. injection in fish at a weight of 20–30 g (Toranzo *et al*., 2005). As different serotypes of T. *maritimum* occur, further characterizations of different isolates is going on to select suitable strains for new vaccines and for establishment of a reproducible infection model (Van Gelderen *et al*., 2010a; Van Gelderen *et al*., 2010b).

Infections with *Aeromonas salmonicida* in the marine environment

Aeromonas salmonicida subsp. *salmonicida,* a Gram-negative small rod, causes furunculosis; a serious disease mainly for salmonid fish in fresh water (see Chapter 3) but it also causes disease in brackish water. A heterogeneous group of atypical *A. salmonicida* occur, different in morphology, reaction in biochemical tests, as well as in a variety of surface antigens, including the A-layer protein and lipoploysaccharides (LPS) in freshwater, brackish and marine environments. Among those, *A. salmonicida* subsp. *achromogenes* is a common reason for atypical furunculosis in the marine environment. *A. salmonicida* subsp. *achromogenes* is a significant pathogen for salmonid fish and has been reported from various marine fish species, including cod, halibut, wrasse (*Ctenolabrus* spp.), wolf-fish (*Anarhichas lupus*), turbot, whiting (*Merlangius merlangus*) and haddock (Treasurer and Laidler, 1994; Treasurer *et al*., 2007). Atypical *A. salmonicida* infection is a disease problem for cod in aquaculture and cod may be a possible carrier of *A. salmonicida* to salmonid net pens. The strong host-response with an accumulation of fibroblasts to limit the spread of the infection is probably a reason for the low mortality rates due to the infection. Stress may possibly induce a more acute infection.

Impact on fish production

Epizootiological experiences from Sweden regarding furunculosis and atypical furunculosis demonstrates a different distribution and transmission of the two diseases. Distinct epidemics due to infection with *A. salmonicida* subsp. *salmonicida* have been documented and opposite to infection with *A. salmonicida* subsp. *achromogenes* that are frequently isolated from feral fish. Acute disease due to *A. salmonicida* subsp. *achromogenes* have been more frequent among Arctic char and brown trout compared with in salmon and rainbow trout in Swedish waters (Wichardt *et al*., 1989).

Diagnosis

A. salmonicida subsp. are cultured on traditional agar as blood or TSA agar at 20°C after 2–3 days of incubation and further identification is possible by biochemical tests. PCR techniques are also available for the detection of *A. salmonicida* in which several subsp. can be identified (Beaz-Hidalgo *et al*., 2008; Keeling *et al*., 2013). Several questions exist about the classification of the atypical *A. salmonicida* subsp. and cultivation on agar is therefore still important to avoid false negative PCR results.

Clinical signs

Skin ulcers of varying size are a common sign in infections with *A. salmonicida* subsp. *achromogenes*. The infection is often seen as an acute sepsis. Pathological lesions in cod and haddock include haemorrhage, necrosis and granuloma

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formation in vital tissues. Cod showed extensive superficial necrotic areas on the skin surface and several deep well-defined ulcers. Naturally infected haddock had extensive endocarditis in the spongy myocardium. Histopathological investigation showed a well-developed host reaction in cod, characterized by encystment of the bacteria in spleen and kidney. Salmon injected with the isolated bacteria showed no signs of encystment (Cornick *et al*., 1984; Morrison *et al*., 1984). Ulceration and extended granuloma formation was also described from cod in Iceland infected with a *A. salmonicida* subsp. *achromogenes* related bacterium (Magnadóttir *et al*., 2002).

Prevention and control

Commercial vaccines against furunculosis exist and provide protection against several atypical *A. salmonicida* subsp. (Gudmundsdóttir and Björnsdóttir, 2007). Stocking of cod and haddock in areas of salmon fish farms, surrounded by different wild marine fish, has been suggested to be a reason for vaccination of cod and haddock with bivalent vaccines against furunculosis and vibriosis to obtain maximum protection for transmission (Treasurer *et al*., 2007). Vaccines for non-salmonid fish are currently being tested (Shoemaker *et al*., 2013).

Epitheliocystis

The first description of the condition, now named epitheliocystis, is from 1920 in common carp (*Cyprius carpio*). Epitheliocystis affects mainly the gills and the skin epithelium by an enlargement of the epithelial cells to spherical cysts surrounded by a hyaline capsule. These spherical cysts might reach a diameter of 400 µm. The involvement of bacteria in this condition was initially suggested by Hoffman *et al*., after investigation of affected farmed bluegill (*Lepomis macrochirus*) in the USA (1969). Infected cysts contain pleomorphic Gram-negative cocci or coccobacilli with an ultrastructural similarity to Chlamydiaceae or Rickettsiaceae (Nylund *et al*., 1998). Recent studies demonstrate high amounts of a novel bacterium dominating the cysts in salmon, in the marine environment, tentatively classified as *Candidatus Branchiomonas cysticola* (Toenshoff *et al*., 2012). The intracellular inclusions in the gill epithelial cells of farmed salmon in fresh water were earlier found to be associated with *Candidatus Clavochlamydia salmonicola* (Mitchell *et al*., 2010). Koch's postulates has not been fulfilled for epitheliocystis, as the bacterium(s) involved have not been cultured. Epitheliocystis is observed in healthy fish but severe cases are also described, with extended mortalities, currently from more than 50 species of fish in fresh water as well as in marine environments (Nowak and LaPatra, 2006).

Clinical signs

Affected fish show lethargy, respiratory failures with an increased respiratory frequency with flared opercula. Transparent, white to yellow cysts, up to 1 mm, can be observed on the gill filaments. Generally the mortality is low but in case of respiratory failures, mortalities up to 100% have been recorded (Nowak and LaPatra, 2006).

Impact on fish production

Mortalities of 4–100% have been reported in association with epitheliocystis, with the highest mortalities in young fish, often without any proliferative host response. It is possible that environmental conditions affect the mortality as 10% mortality was reported in smolts of Atlantic salmon in Norway (Nylund *et al*., 1998), but there was no mortality in salmon in Tasmania, although the condition was reported to be present in 20% of the salmon and affected fish show reduced growth (Nowak and LaPatra, 2006). An outbreak of epitheliocystis was reported from large-mouth bass (*Micropterus salmonides*) cultured in raceways at 15°C with a total mortality of 10% (Goodwin *et al*., 2005).

Diagnosis

A preliminary identification of epitheliocystis can be based on the macroscopically observed white to yellow cysts on the gills or sometimes in the skin of affected fish. Gill histopathology

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is recommended for diagnosis of epitheliocystis, but also the pseudobranch can be useful for investigation (Crespo *et al*., 1999; Nowak and LaPatra, 2006). Cysts in gill lamella, at a diameter of up to 400 µm, sometimes even larger, surrounded by a thick cellular epithelial, eosinophilic capsule, containing inclusion bodies with fine basophilic granules of 0.5–2.0 µm characterize epitheliocystis, Fig. 2.7 (Groff *et al*., 1996; Nylund *et al*., 1998; Mitchell *et al*., 2010). The granular basophilic inclusion contains pleomorphic, coccoid or coccobacillary cells. The condition is due to a proliferative hyperplasia of the branchial epithelium of the gills. Hypertrophy of chloride and goblet cells and inflammation in gill lamella with necrosis are described. PCR is not recommended for diagnosis before the aetiological agent(s) involved in epitheliocystis have been carefully investigated.

Prevention and control

Antibiotic treatment has been shown to give effect (Goodwin *et al*., 2005) as well as the use of UV to disinfect the water, or a reduction in infectious pressure. Bluegill showed increased epitheliocysts at 12°C compared with temperatures above 20°C (Hoffman *et al*., 1969). A vaccine is not expected in the near future. Prevention is therefore best accomplished by careful husbandry. High stocking densities and nutrient levels in the water should be avoided, as these are important risk factors.

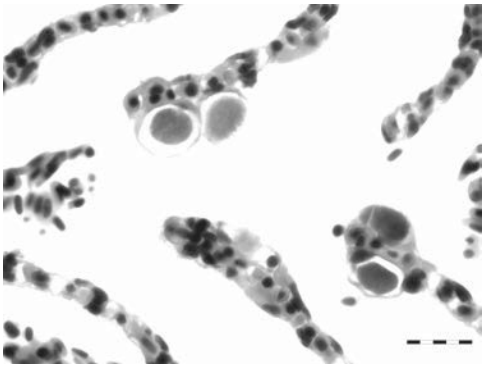


Fig. 2.7. Epitheliocystis in Atlantic salmon (*Salmo salar*). Observe the cysts in gill lamella surrounded by an epithelial capsule containing inclusion bodies with granulas. Image courtesy of David Bruno, Marine Scotland, Aberdeen.

Mycobacteriosis

Mycobacterium spp. have been reported in wild and ornamental fish, and from farmed species (Bruno *et al*., 1998b; dos Santos *et al*., 2002). The species that are considered significant for marine water aquaculture are *M. marinum* and *M. salmoniphilum* (*M. chelonae*) (Whipps *et al*., 2007; Jacobs *et al*., 2009). Mixed cultures of *M. marinum*, the *M. fortuitum* complex and *M. gordonae* have been isolated from wild-caught Atlantic menhaden (*Brevoortia tyrannus* (Clupeidae)), with ulcers and granuloma formation in liver, spleen, kidney and heart (Stine *et al*., 2005). *M. shottsi* was among several *Mycobacterium* spp. frequently recovered from striped bass along the Atlantic coast of the United States (Rhodes *et al*., 2004). The zoonotic potential of several *Mycobacterium* spp. is a risk for people handling fish (Gauthier and Rhodes, 2009) and probably under diagnosed as these bacteria might grow at 30°C, a temperature possible in hands and fingers, and therefore could go undetected in routine bacterial incubations at 37°C.

Impact on fish production

Increased mortalities of market size Atlantic salmon have been reported in Scotland (Bruno *et al*., 1998b) and in smolts in sea-cages in British Columbia (Brocklebank *et al*., 2003). Even if mortality rates were low, it is possible that a chronic infection with mycobacteria lowers the feed conversion rate and increases the susceptibility to other fish pathogens.

Diagnosis

Mycobacteriosis is mostly diagnosed through staining of smears or formalin fixed histological sections with Ziehl-Neelsen, demonstrating acid-fast bacteria dispersed or close together forming nodules in tissues. These small coccoid bacteria stain positive in Gram. Isolation of the bacteria on agar is necessary for identification of species. This can be complicated

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due to the fastidious requirements of several species in this genus. Löwenstein-Jensen agar and Stonebrink's agar are used for some species; however, others can be cultured on standard agar media such as blood agar or tryptic soy agar (TSA). Slow growing *Mycobacterium* spp. can take up to several months to grow, and therefore this increases the risk for contamination of more rapidly dividing contaminants.

Clinical signs

Skin ulcers and granuloma formation in the form of greyish-white nodules in kidney, spleen and liver, with haemorrhage in musculature (Bruno *et al*., 1998b).

Prevention and control

Feeding of untreated fish is considered a serious risk for transmission of mycobacteriosis to fish in aquaculture. The use of controlled fish feed has significantly reduced this risk. The occurrence of wild-living fish, feeding close to net pens is a more realistic route for horizontal transmission today. Mycobacteriosis is generally a chronic condition and antibiotic treatment in case of manifested disease is not a solution, as these bacteria have a high mutation rate and thereby an increased risk for development of resistance.

Infections with Pseudomonas spp. in the marine environment

Several *Pseudomonas* species can be isolated from diseased fish. For example, *Pseudomonas anguilliseptica* has been a serious pathogen in eel farming, but is now regarded as a significant pathogen for other fish species in brackish water and the marine environment. *P. anguilliseptica* has been isolated from Atlantic salmon, rainbow trout, sea trout, whitefish (*Coregonus* spp.), cod and wild herring in northern Europe (Wiklund and Bylund, 1990; Ferguson *et al*., 2004), in turbot, black spot sea bream (*Pagellus bogaraveo*) and gilthead sea bream (*Sparus aurata*) in Spain. The optimum temperature for disease outbreaks is below 16°C and outbreaks in the Mediterranean area consequently occur during the winter months (Toranzo *et al*., 2005).

Impact on fish production

Mortality is variable depending on affected fish species. A low mortality of 2% is reported from cod farming but mortalities up to 50% are reported from salmonid fish (Wiklund and Bylund, 1990).

Diagnosis

P. anguilliseptica, a long slender Gram-negative rod is slow growing on blood agar, preferentially at temperatures between 15–20°C on this medium or on TSA agar. The small (1 mm in diameter) semi-transparent colonies appear after 3–4 days of incubation and are easily overgrown by more rapidly dividing bacteria, hence the pathogen can be readily undetected. The bacterium is inactive in biochemical tests but serological as well as molecular-based diagnostic methods are available (Blanco *et al*., 2002; Romalde *et al*., 2004).

Clinical signs

Salmonid fish infected with *P. anguilliseptica* show petechial haemorrhage in skin peritoneum and in the liver. Affected cod get lethargic and show a reduced fright response. Gross lesions consist mainly of eye lesions and fin erosion. Histopathologically, a granulomatous inflammation of connective tissues surrounding the skeleton/cartilage of the head region is observed (Ferguson *et al*., 2004).

Prevention and control

No vaccines are available and antibiotic treatment has given a weak response (Wiklund and Bylund, 1990).

Bacterial kidney disease (BKD)

BKD causes morbidity and mortality in salmonid fish and is reported from Europe, North and South America as well as from Asia. Mortalities can be significant at most life stages in both fresh and marine waters, even if the disease mostly has a chronic character. There is a different susceptibility to BKD between salmonid species and Pacific salmon,

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as Chinook, coho and sockeye salmon are regarded as more susceptible compared with Atlantic salmon and rainbow trout. Transmission is possible horizontally by contaminated water but also vertically from infected female brood fish to the egg (Bruno and Munro, 1986). The slow development of the disease and the possibility of latent carrier fish transmitting the disease underline the importance of efficient and sensitive diagnostic assays for screening and control. High prevalence of *R. salmoninarum* in feral salmonid stocks indicates these are important reservoirs for maintenance of the infection locally (Meyers *et al*., 1993a, 1993b; Jónsdóttir *et al*., 1998). Two separate incidences of BKD have been suggested to be the reason for new epizootic outbreaks in Iceland during 2003–2007. Systematic screening, culling and stamping out of millions of fish with serious economic consequences was necessary to get the situation under control (Árnason, 2010).

Impact on fish production

Extended mortalities are reported from sensitive Pacific salmon species. Mortalities can also be high in, for example, grayling, salmon and Arctic char during stressful conditions as during rapid temperature rises, transfers or during sexual maturation. Inefficient food conversion rate and fillets condemned due to muscle lesions with significant economic consequences also demonstrate the importance of continuous control and restrictions on movements of affected populations, especially since there is no cure or commercial vaccine available.

Diagnosis

Clinical indications of BKD are the enlarged greyish kidney and white nodules in internal organs such as kidney, heart, liver and spleen (Fig. 2.8). Superficial haemorrhages can be visible externally (Fig. 2.9). This can be verified by culture, immunological- or molecular-based assays. Various stages of BKD are described, as well as more diffuse lesions in different species of fish that require highly sensitive and specific assays for tracing infected populations or individuals. Isolation of *R. salmoninarum* on agar has been used in screening programmes but the slow growth for at least 12 weeks, hamper its practical use in diagnostics (Benediktsdóttir *et al*., 1991). Immunofluorescent antibody techniques, ELISA and several PCR-based methods are described with good sensitivity that is more useful in routine diagnostics. A soluble heat stable antigen (p57) is released by *R. salmoninarum* in to the tissue during infection (Turaga *et al*., 1987; Daly and Stevenson, 1987). This antigen is the target in several ELISA techniques (Pascho *et al*., 1987; Gudmundsdóttir *et al*., 1993; Jansson *et al*., 1996). Several PCR methods exist with different target genes of *R. salmoninarum* (msa gene, 16S rRNA, abc transporter permease) (Chase and Pascho, 1998; Pascho *et al*., 1998; Jansson *et al*., 2008; Elliott *et al*., 2013). The comprehensive validation of several commonly used assays for detection of *R. salmoninarum* states that there is no single assay that demonstrates the highest analytical and diagnostic performance characteristics for determination of the prevalence of BKD for all possible occasions (Elliott *et al*., 2013).

Clinical signs

External lesions include petechiae and haemorrhagic areas around the fins and along the lateral line and superficial blebs or blisters of the skin, containing a white, yellowish or haemorrhagic fluid, sometimes forming cavitations in the musculature. Exophthalmia, pale gills, indicative of anaemia,

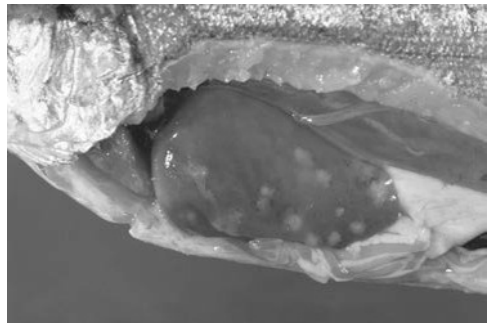


Fig. 2.8. Bacterial kidney disease (BKD) in Atlantic salmon (*Salmo salar*). White circular nodules in liver.

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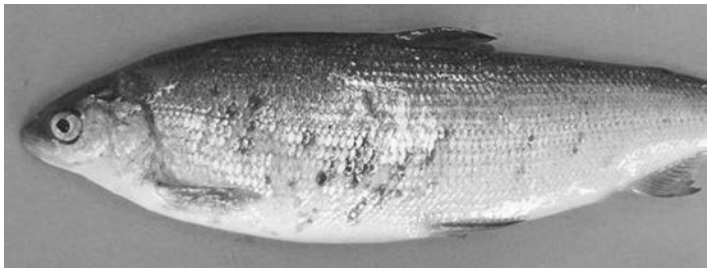


Fig. 2.9. Bacterial kidney disease (BKD) in whitefish (*Coregonus lavaretus*).

and distended abdomen are described. Most obvious is the swollen kidney with greyish-white nodules of varying sizes. Similar nodules can be observed in spleen, heart and in the liver and occasionally ascitic fluid accumulated in the peritoneum.

Prevention and control

Antibiotic treatment has a temporary effect, possibly due to the intracellular survival of the bacteria, and is not recommended for therapy. Vaccination experiments have so far had limited progress and today no commercial vaccines are available. Regular screening programmes to prevent horizontal and vertical transmission of *R. salmoninarum* are important aspects for control of BKD. Undetected, subclinical infections are always a risk for further spread, and high sensitivity in screening methods are crucial. Wild broodstock are in some countries used for production of eggs in aquaculture or in national programmes. Broodstock culling has been demonstrated to reduce the prevalence of BKD in endemic areas (Gudmundsdóttir *et al*., 2000). The culling programme requires that female broodstock are individually tested for BKD and that the eggs are kept separate until the result of the tests are available. All eggs from females testing positive for *R. salmoninarum* have to be discarded. This requires rigorous logistical planning at the farm level. Complete disinfection of facilities is an important step in the eradication of the disease. Sodium hypochlorite effectively reduces the numbers of *R. salmoninarum* but a small number of bacteria were found to remain alive after treatment with 200 mg per l free chlorine (Hirvelä-Koski, 2004).

Yersiniosis

Enteric redmouth (ERM) disease, infection with *Yersinia ruckeri,* is a well-known haemorrhagic sepsis in the freshwater environment worldwide (see Chapter 3). Recent reports describe outbreaks of *Y. ruckeri,* in previously vaccinated fish, caused by *Y. ruckeri* biotype 2, a different clonal group compared with the classical *Y. ruckeri* biotype 1 (Wheeler *et al*., 2009). Biotype 2 is reported from Europe as well as from United States to be responsible for a new more aggressive form of ERM causing disease in brackish water (Ström-Bestor *et al*., 2010; Welch *et al*., 2011). Please refer to Chapter 3 for further information.

Fungal-Oomycetes Diseases

Selected fungal or oomycete pathogens are described from the marine environment.

Exophiala spp.

Infections of fungi of the genus *Exophiala* spp. have been reported from more than 20 species of fish including channel catfish (*Ictalurus punctatus*), cut throat trout (*Oncorhynchus clarkii*), salmon, dogfish (*Mustelis canis*), King George whiting (*Sullaginodes punctata*) and Japanese flounder (*Paralichthys olivaceus*) (Fijan, 1969; Otis *et al*., 1985; de Hoog *et al*., 2011). Infections in the hatchery phase reached 40% in salmon with cerebral lesions at temperatures of 12–14°C in Canada (Carmichael, 1966). *Exophiala* infections have also been the reason for visceral infections in Atlantic salmon in the marine

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environment (Otis *et al*., 1985; Pedersen and Langvad, 1989). Infections described in fish have mainly been attributed to infections with *E. salmonis*, *E. psychrophila* or *E. pisciphila* (Fijan, 1969; Richards *et al*., 1978; Pedersen and Langvad, 1989).

Impact on fish production

For several years, long-lasting epizootics in salmon caused by infection of fungi belonging to *Exophiala* have been reported from Norway (Pedersen and Langvad, 1989). Several reports describe the occurrence of black yeast-like fungi in farmed fish. Unfortunately isolates have not all been characterized by molecular techniques that are necessary today for a correct identification of *Exophiala* spp. (Zeng and De Hoog, 2008). It is therefore possible that the impact of these infections on fish production is underestimated. Compromised fish, for different reasons, are probably more susceptible to infection by *Exophiala* spp.

Diagnosis

Histopathology is used for the diagnosis of *Exophiala* spp. after staining with PAS and Grocott's silver stain, showing a positive reaction of branching septate hyphae of the fungus, especially in kidney and liver. *Exophiala* spp. can be cultivated on Corn Meal Agar or Sabouraud dextrose agar with grey to black colonies with up to 10 days incubation at 25°C. Molecular identification is necessary for determination of the actual species.

Clinical signs

Clinically, the fish become dark and lethargic, with erratic and whirling swimming behaviour. Occasionally dermal nodules are present. Numerous round yellow to white granulomas are present in visceral organs (liver, kidney, spleen) with prominent enlargement of the posterior kidney, containing branched, irregular, wide, septate hyphae. Infections of *Exophiala* spp. have been described as chronic granulomatous lesions but *E. salmonis* have also caused an acute form characterized by abscesses in kidney and liver containing polymorphonuclear leucocytes (Otis *et al*., 1985).

Prevention and control

Exophiala salmonis has been isolated from soil but the mechanism of transmission to the fish is unknown. No vaccine is available and treatment with antimicrobial substances is ineffective. A recent report describes *E. salmonis* isolated from a cystic mass from an immunocompromised man, showing the fungus also has a zoonotic potential (Yoon *et al*., 2012).

Epizootic ulcerative syndrome

Epizootic ulcerative syndrome (EUS) (red spot disease, mycotic granulomatosis, ulcerative mycosis) was described for the first time in Japan in 1971 (Egusa and Masuda, 1971). EUS is described from more than 60 species of fish from Asia, Australia, North America and Africa, in fresh and brackish waters, but is so far not described from Europe or South America. The recent finding of EUS in brown bullhead in Lake Ontario, Canada demonstrates that EUS has the potential to cause disease in the northern hemisphere. Clinical disease is described over a wide temperature range from 10–33°C (Lilley *et al*., 1998; Hawke *et al*., 2003; Sosa *et al*., 2007). EUS is caused by the filamentous oomycete *Aphanomyces invadans*, classified into *Stramenopiles* or *Chromista* together with diatoms and brown algae. The broad range of fish species affected highlights the need for awareness of this epizootic disease as a result of increased global trade of ornamental and garden fish. Flooding and heavy rainfall have triggered the occurrence of the ulcerative lesions which are optimal for sporulation of the oomycete, increases the stress level of fish and possibly also to mix the sediments containing infective spores (Sosa *et al*., 2007). See Chapter 6 for more detail.

Diagnosis

Histopathology shows an extensive granulomatous myositis with penetrating hyphae from the oomycete into muscles and vital organs after staining with H&E and Grocott's methenamine-silver nitrate. The oomycete can be isolated on GP-POX agar, PG-1 agar producing a white mycelium (Fig. 2.10).

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To avoid the contamination of bacteria when cultured on agar it is recommended to include antibiotics in the agar. Growing hyphae can be stimulated to sporulation (Fig. 2.11) as a further step for confirmation, and PCR assays are available (Vandersea *et al*., 2006; Oidtmann *et al*., 2008).

Clinical signs

Clinical signs are varying between the affected fish species and the stage of infection. Susceptible fish species show, in the early stages of disease, reduced appetite and become lethargic followed by haemorrhage, petechiae and open ulcers in the skin. Often there is a single superficial lesion observed on the flank. Lesions in advanced stages increase to large necrotic open ulcers on the body, often located to the head region. There is limited information about the susceptibility of European fish species.

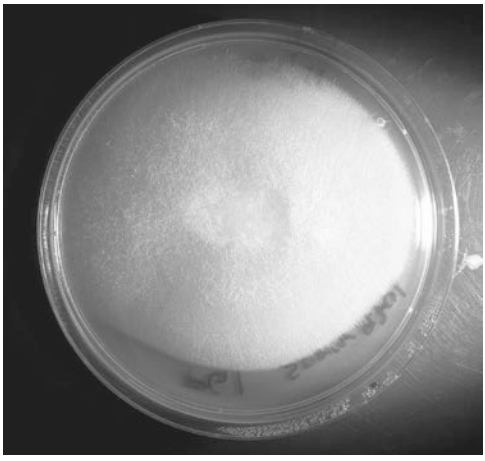


Fig. 2.10. Epizootic ulcerative syndrome, white mycelium of *Aphanomyces invadans* growing on Peptone glucose (PG-1) agar.

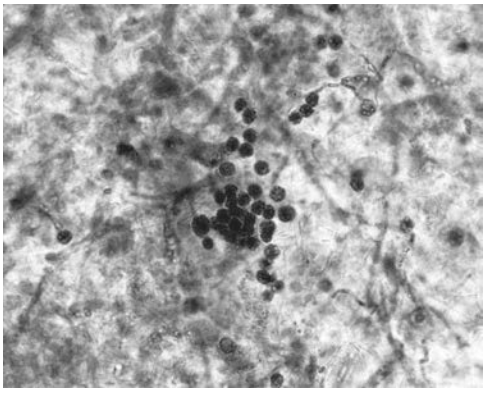


Fig. 2.11. Induced sporulation of *Aphanomyces invadans,* spores with a diameter of 6–10 µm. Cotton blue × 400.

Prevention and control

No vaccines are available and no effective curative treatments can be recommended. In non-endemic areas prevention is recommended to avoid the introduction.

Parasitic Diseases

Protista and Metazoa

Among the Protista and Metazoa, there are some important pathogens for net pen-reared fish. *Paramoeba perurans* (basionym *Neoparamoeba perurans*), causing amoebic gill disease, has become one of the most serious threats to the marine aquaculture. *Ichthyobodo* spp. and trichodinia ciliates infect the gill surface of salmonids and some marine fish, but are most common in the freshwater environment (see further in Chapter 3). Outbreaks of cryptobiosis caused by *Cryptobia salmositica* have been reported from sea cages on the west coast of North America. There are no reports of outbreaks in freshwater cages although outbreaks occur in freshwater streams and hatcheries. Another diplomonad, *Spironucleus salmonis* (formerly *Hexamita salmonis, Spironucleus salmonicida*) has caused extra-intestinal infections in Atlantic salmon in Norway. Four myxosporean (*Parvicapsula* spp., *Myxobolus aeglefini*, *Kudoa thyrsites* and *Chloromyxum truttae*) and three microsporidians (*Loma salmonae, Nucleospora salmonis* and *Microsporidium cerebralis*) infect internal organs or gills. In this chapter, some of these important marine Protista and Metazoan parasites affecting coldwater marine net pen culture are discussed.

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Protista

AMOEBIC GILL DISEASE. Amoebic gill disease (AGD) caused by infections with *Paramoeba perurans* (basionym *Neoparamoeba perurans*) (Young *et al*., 2007; Feehan *et al*., 2013) cause devastating losses of farmed fish in the marine environment. AGD was for the first time reported from coho salmon *Oncorhynchus kisutch* reared in net pens in Washington state, USA, and also in land-based tanks in California (Kent *et al*., 1988). AGD has also been reported from Atlantic salmon, rainbow trout, Chinook salmon, turbot, sea bass and ayu (*Plecoglossus altivelis*). The widespread distribution of AGD includes the west coast of United states, Australia, Chile, New Zealand, Japan, South Africa, Ireland, Scotland and Norway (Nowak, 2012). The name *Paramoeba pemaquidensis* was first proposed for the causative agent of AGD. This was later revised to *N. perurans* after morphological studies of the trophozoites of the amoeba (Dyková *et al*., 2000). Phylogenetic analysis of nuclear SSU rDNA sequences shows, however, that *Neoparamoeba* and *Paramoeba* are phylogenetically inseparable and *P. perurans* comb. nov. is the recently proposed name of this pathogen (Feehan *et al*., 2013). The amoeba colonizes the gills and as low as 10 amoebae per l water is enough to induce AGD in Atlantic salmon. *P. branchiphila* and *P. pemaquidensis* have been cultured from gills of fish with AGD but it has not been possible to induce the disease experimentally (Morrison *et al*., 2005; Vincent *et al*., 2007). *In situ* hybridization studies on gill tissue samples from Australia, USA and Europe with presumed cases of AGD further supported *N. perurans* to be the only agent to induce AGD (Young *et al*., 2008a). The environmental distribution of *N. perurans* is not clarified but *N. perurans* has been detected by PCR in water from cages with AGD-infected salmon and also in fresh water that previously had been used for treatment of affected fish (Bridle *et al*., 2010). *N. perurans* was, however, not detected in wild fishes in- and outside net pens affected with AGD, which indicates a high host specificity of the amoeba (Nowak *et al*., 2004).

Clinical signs. Affected fish swim at the water surface of the net pens, are lethargic and show respiratory failures, often with flared opercula and extensive mucus production. Whitish gross gill lesions indicate hyperplastic lesion of gill filaments at varying degrees (Adams *et al*., 2004). Gills are the main tissue affected in all descriptions of AGD; however, cardiac dysfunction was observed in Atlantic salmon, possibly contributing to the higher susceptibility in this species compared with brown trout and rainbow trout (Leef *et al*., 2005).

Impact on fish production. AGD has been recognised as a significant problem in mariculture since the mid 1980s due to increased mortalities and production costs, depending on labour-intensive treatments and impaired growth (Mitchell and Rodger, 2011). Varying mortalities of 12–82% are reported due to AGD (Kent *et al*., 1988; Steinum *et al*., 2008; Mitchell and Rodger, 2011). A number of viral, bacterial and parasitic pathogens are associated with gill lesions in the marine environment and affected fish may be more susceptible to concurrent infections, probably increasing the mortality rates (Mitchell and Rodger, 2011).

Diagnosis. Histopathology of AGD-affected gill filaments demonstrates an epithelial hyperplasia, causing a thickening of the blood–water diffusion distance that impairs the diffusion of respiratory gases. Large numbers of the amoeba, 15–40 µm in diameter, can be observed on the gills and are also easily seen in fresh wet-mount preparations. *P. perurans* has been cultivated *in vitro* (Crosbie *et al*., 2012). Identification involves examining stained sections and molecular methodology as *in situ* hybridization or PCR (Young *et al*., 2008a, 2008b). Real-time PCR was found to be useful for semi-quantitative identification of 18S rRNA of P. *perurans* in gill swabs from farmed salmon (Bridle *et al*., 2010). Real-time PCR was also possible for detection of P. *perurans* in formalin-fixed, paraffin-embedded blocks of gill tissues (Fringuelli *et al*., 2012b).

Prevention and control. Environmental factors such as the concentration of free-living

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amoebae in the water, low rainfall, salinity, increasing water temperature and actual fish species affect the severity of the outbreak (Rozas *et al*., 2012). Freshwater bathing has been routinely used and found effective to reduce the numbers of infective amoebae (Clark *et al*., 2003). The technique is time demanding and does not completely eliminate all amoebae, which after a couple of weeks, reintroduce the disease. Different disinfectants have been tested as hydrogen peroxide, chlorine dioxide, chloramines T, potassium permanganate, levamisol, bithionol but with questionable success in farm situations (Embar-Gopinath *et al*., 2005; Nowak, 2012). No commercial vaccines are available, restrictions in movement of affected fish and overall good hygienic standards are therefore important to reduce the risk of spread. Dead fish can be a reservoir of *P. perurans* since the amoebae survive on fish gills post-mortem (Douglas-Helders *et al*., 2000).

ICHTHYOPHONUS HOFERI. The parasite *Ichthyophonus hoferi* is enzootic in many marine fish populations in the northern Atlantic and Pacific Oceans. Natural infections in Atlantic herring, in sprat and in flounder (*Pleuronectes flesus*) along the Swedish west coast demonstrated the nature of the granulomatous inflammation to be host and tissue dependent, with the occurrence of creamy white nodules on the heart as the most predominant lesions in herring and flounder (Rahimian, 1998). Significant mortalities are reported in wild-living species such as in herring with impact on fisheries, as well as the proportion of different life-stages in the oceans. Feeding fish with untreated fish is a main route for transmission, but feeding with the commercial controlled heated feeds used in salmonid aquaculture, gives a minimal risk for infection (McVicar, 2011).

Impact on fish production. All species of fish can be affected although these infections occur more frequently in the marine environment.

Diagnosis. Diagnosis is based on histopathology after H&E staining. Microscopically the lesion consists of granulomas containing encysted large PAS positive spores. Spores are large, round or oval at 10–250 µm, surrounded by a thick double contoured wall. Occasionally, large irregular shaped hyphae without septa are observed (Roberts, 2012).

Clinical signs. Fish are emaciated with visible cysts in the muscles, beneath the skin, each at a size of about 1mm^2 in diameter, making a rough appearance of the skin and indicative of ichthyosporidosis. Rupture of these cysts gives crater-like, dark coloured ulcers and scoliosis is occasionally observed. If the infection has reached the brain, fish show irregular swimming behaviour. Internally, numerous granulomas are observed as white nodules in organs such as heart, liver, spleen, kidney and in the luminal wall of the intestine. The clinical signs vary between fish species and life stages (McVicar, 2011).

Prevention and control. Feeding with controlled sterilized feed will minimize the risks for transmission of spores. Horizontal transmission of *I. hoferi* has been demonstrated in freshwater rainbow trout fish farms. This water-borne infective route for transmission has not been demonstrated from the marine environment (Gregg *et al*., 2012). Environmental factors may facilitate horizontal transmission and possibly vector organisms such as zooplankton are suggested for further study. Strict sanitary measurements are necessary to limit infection at a fish farm which has to be completely emptied of fish before disinfection.

Microsporidia

LOMA SALMONAE. *L. salmonae* is an intracellular microsporean that primarily infects gills of salmonid species including farmed rainbow trout and sockeye salmon in fresh water in North America, Japan, France, Scotland and Norway. Fish are infected directly by ingesting spores (Shaw *et al*., 1998).

Impact on fish farming. Loma is an economically important parasite for hatchery reared salmonids. Mortalities can vary from 2.4%

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up to 70% (Speare and Lovy, 2012). The parasite infects gills and other vascularized tissues. Damaged gills, infected by other pathogens, may raise the impact of the disease.

Diagnosis. Xenomas formed by the parasite are easy to detect on gills either by eye or by light microscopy from wet mount preparations. Histology is also a good tool for diagnostics (Speare and Lovy, 2012).

Clinical signs. The parasite forms whitish cysts (xenomas) up to 0.5 mm in diameter on the gills, which eventually rupture, and cause severe gill inflammation. Affected fish show respiratory distress and impaired swimming, exophthalmia, ascites and small haemorrhage on the opercula. Xenomas can even be found in other tissues and severe inflammation as a result of ruptured cysts (Speare and Lovy, 2012).

Prevention and control. Fish held in untreated natural water with wild salmonids are likely to become infected if the infection is endemic in the water source. The most effective way of managing *L. salmonae* is to treat the fish with oral drugs such as monensin, fumagillin or albedazole. It is crucial that the treatment is administered at the right time of the infection, namely when the first xenomas are expected to form as this is the period when parasites are spreading. Xenomas start to develop when 260–304 thermal units (TU) have been reached and the treatment window is around 280 TUs (Speare and Lovy, 2012).

DESMOZOON LEPEOPHTERII (PARANUCLEOSPORA THERIDION). *Desmozoon lepeophterii*, also called *Paranucleospora theridion* (Nylund *et al*., 2010), is a microsporidia that was first described as a parasite of salmon louse (*Lepeophtheirus salmonis*) in Scotland (Freeman, 2002). Later, it was also reported from Norway and the Pacific coast of North America. The parasite is transmitted between fish via the salmon and sea louse, and has also been found in Atlantic salmon, sea trout and rainbow trout. Salmon louse and sea louse (*Caligus elongatus*) is the main host and Atlantic salmon is an intermediate host of *D. lepeophterii*. Infective spores develop under the cuticle of salmon louse from where they are released into the water and infect salmon through their gills (Nylund *et al*., 2010).

Impact on fish farming. The impact of the parasite on fish farming is not clear. The parasite seems to be opportunistic causing acute disease when the host is immunocompromised (Freeman and Sommerville, 2011). *D. lepeophterii* has been suspected to have associations with the development of other diseases like pancreas disease (PD), heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and proliferative gill inflammation (PGI). Nerland Hamadi (2011) found association only to PGI.

Diagnosis. The parasite can be shown by calcofluor white stain of histological sections or real-time PCR (Nylund *et al*., 2010; Nerland Hamadi, 2011).

Clinical signs. *D. lepeophterii* cause few visible signs. Occasionally mild gill pallor is recorded and heavily infected fish may have slightly enlarged and greenish-grey kidney with white flecks. In histological sections hyperplasia of the renal interstitium with mitotically active immature white blood cells can be observed. Heart tissue may show endocardial activation and hyperplasia. In the spleen reticular cell proliferation of ellipsoids can sometimes be observed (Freeman and Sommerville, 2011).

Prevention and control. Heavy lice burden and temperatures around 15°C are suggested to be a reason for heavier *D. lepeophterii* infections (Nerland Hamadi, 2011; Nylund *et al.*, 2011).

Flagellates

CRYPTOBIA SALMOSITICA. *Cryptobia salmositica* is a parasitic flagellate that causes cryptobiosis in salmonids. It has been reported from all Pacific *Oncorhynchus* species and has been causing high mortalities in freshwater hatcheries and in sea farms on the west coast of North America. There are no reports of

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outbreaks in freshwater cages, although outbreaks occur in freshwater streams and hatcheries. The parasite can be transmitted by a bloodsucking leech (*Piscicola salmositica*) or directly between fish in fish farms. Infection occurs in fresh water prior to transfer to sea cages – subsequent outbreaks are likely due to stress in surviving infected fish and also through direct transmission (in the absence of leeches) between fish in sea water. Direct transfer between fish occurs in both fresh and sea waters but transmission is much more efficient in sea water, as shown in experimental studies (Woo, 2012).

Impact on fish farming. The mortalities have been varying between genetically different fish stocks and parasite isolates from negligible up to 100% (Woo, 2012).

Diagnosis. The diagnosis can be made by microscope examination of blood or ascitic fluid in wet mount preparations where the parasite is easily detected during the acute phase. Another technique which is also suitable for testing before and after the acute phase is the haematocrit centrifuge technique (Woo, 2012). Briefly, freshly collected blood in haematocrit tubes is centrifuged cold (5–10°C) for 5 min at 13,000 g. The junction of the plasma and packed red blood cells are examined under microscope for parasites. There are several immunological techniques (e.g. ELISA) that can be used to diagnose *C. salmositica* infections (Woo, 2012).

Clinical signs. The severity of the disease is dependent on the number of parasites infecting the fish, fish size and genetics of the fish. The signs start approximately 3.5 weeks post infection with anorexia. The acute stage of the disease peaks about 4–8 weeks post infection when the fish excrete a lot of mucus on the skin; they are anaemic, lethargic and lie on the bottom of the tank. Other signs are exophthalmia, general oedema and abdominal distension due to ascites (Woo, 2012).

Prevention and control. Infection can be prevented by controlling the number of leeches, vectors for the parasite, on the farm. Leeches are sensitive to drying and freezing and can therefore be controlled by draining areas in hatcheries. Adult leeches are also sensitive to chlorine. In certain cases, e.g. protection of broodfish, *C. salmositica* can be treated with chemicals during disease outbreaks. Fish surviving an infection acquire immunity to future infection. Experimental vaccines have been developed but are not commercially available (Woo, 2012).

Diplomonad flagellates

SPIRONUCLEUS SALMONIS. Parasites of the genus *Spironucleus* occur as opportunistic diplomonad flagellates of the upper intestine, pyloric caeca and gall bladder of several freshwater species worldwide (Bruno *et al*., 2013). In the late 1980s *S. barkhanus* (formerly *S. salmonis* or *Hexamita salmonis*) was causing severe infections in farmed Chinook salmon in British Columbia and in Atlantic salmon in Norway (Kent *et al*., 1992; Poppe *et al*., 1993). Ultrastructural studies have resulted in a reclassification of *Spironucleus* spp., and today *S. salmonicida* is recognized as the reason for systemic infections including extending mortalities of salmonids in the marine environment (Williams *et al*., 2011). The parasite has two phases: a blood and an intestinal phase (Guo and Woo, 2004a). Infection is gained via inlet water or from infected fish that excrete the parasite into the water via their faeces.

Impact on fish farming. The parasite causes significant losses in the fish farming industry and has caused near or complete loss of all stocks in some fish farms in Norway (Williams *et al*., 2011).

Diagnosis. Diagnosis of *Spironucleus* is based on identification of the trophozoites from smears of intestinal mucus, blood and affected organs of freshly killed fish (Fig. 2.12). Trophozoites should be observed in wet mounts with an oval or pyriform, flattened body, 10–12 µm in length with three pairs of anterior flagella and one pair posteriorly. A granulomatous inflammation and necrosis in vital tissues characterize the systemic inflammation in the marine environment (Bruno *et al*., 2013). The haematocrit centrifuge technique is a rapid

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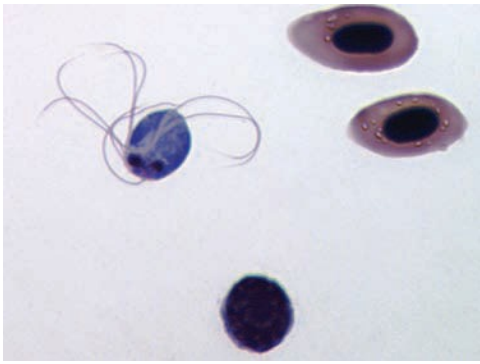


Fig. 2.12. Trophozoite of *Spironucleus* from the blood of an experimentally infected Chinook salmon (*Oncorhynchus tshawytscha;* × 1000) (Woo, 2006). Image courtesy of CAB International, UK.

and sensitive diagnostic tool for detecting trophozoites during the blood phase of the disease (Guo and Woo, 2004a), while the ELISA is very useful for epidemiological studies where large numbers of fish have to be examined (Guo and Woo, 2004b; Woo, 2006).

Clinical signs. In adult fish the parasite does not cause severe disease, but in juveniles it can cause abnormal behaviour and increased mortality with catarrhal enteritis, distension of the abdomen, cholecystitis and exophthalmia. In Atlantic salmon in Norway affected fish were generally in good condition, but were usually smaller than healthy fish (Mo *et al*., 1990) In British Columbia Chinook salmon seem more normal or have distended abdomen and pale gills (Kent *et al*., 1992). Fish with intestinal infection are anaemic and show ascites, enteritis with yellow and runny intestinal content with excess of mucus (Woo and Poynton, 1995).

Prevention and control. Introduction of *Spironucleus* can be minimized by keeping new fish stock in quarantine for 14–21 days. With good husbandry and nutrition the amount and impact of infections can be reduced (Woo and Poynton, 1995). Metronidazole has been used in feed for treatment of infections (Gratzek, 1993), but their use on food fish farms are mostly forbidden. Other effective drugs tested are non-nitroimidazoles like albendazole, aminosidine, diethylcarbamazine and nitroscanate, all at doses of 40 g per kg fish feed (Williams *et al*., 2011). *Allium sativum* (garlic) has recently been investigated as an alternative for treatment of spironuleosis (Millet *et al*., 2010).

Metazoa

KUDOA THYRSITES. *Kudoa thyrsites* is a myxosporean that infects muscle of several marine fish. Atlantic salmon in the Pacific Northwest, Spain and Ireland have been reported to be infected (Kent and Poppe, 2002).

Impact on fish farming. The flesh of the infected fish becomes soft when stored on ice for 3–6 days or if it is cold smoked. The condition is not noticed during processing. The parasite is more common in sexually mature fish (Kent and Poppe, 2002).

Diagnosis. The characteristic spores can be shown by light microscopy of fluid collected from a cut surface of the muscle or by crushing a piece of muscle. The parasite can also be shown with Giemsa staining in histological sections from muscle tissue (Dawson-Coates *et al*., 2003). Detection of the parasite in early stages of infection can also be made by PCR from tissues (Funk *et al*., 2007).

Clinical signs. The muscle of the infected fish gets very soft, almost liquefied, during storage (Dawson-Coates *et al*., 2003).

Prevention and control. The life-cycle of the parasite and how it is transmitted to fish is unknown. Fish become infected in sea water and therefore exposure is inevitable. As sexually mature fish are more prone to the infection, the removal of them before harvest could minimize the problem (Kent and Poppe, 2002).

Crustacean parasites

Sea lice, Lepeophtheirus salmonis *and* Caligus *spp.*

Sea lice, in marine and brackish water environments consist of several species of ectoparasitic copepods of the genera *Lepeophtheirus* and *Caligus* that belong to the family

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Caligidae. *Lepeophtheirus salmonis* and *Caligus elongatus* are mostly detected in sea-caged Atlantic salmon in the northern hemisphere. *C. lacustris* is common in rainbow trout on brackish water farms in the Baltic Sea and *C. rogercresseyi* the predominant parasitic in the salmonid industry in Chile (Bravo *et al*., 2013). The caligid-parasites have eight to ten life stages, of which three (nauplius I, II and copepodid) are free living in water and are important for the spread of the parasite. The copepods attach to the fish skin and stay attached during the following four stages (chalimus I-IV) and after this the first preadult stage is reached and the parasite becomes a free-living individual that can move on the surface of the fish (Boxaspen, 2006; Revie *et al*., 2009).

IMPACT ON FISH FARMING. Sea lice are the most economically important parasites of salmon cage culture. According to the Institute of Marine Research in Norway the parasite is estimated to cost industry NOK 500 million a year. They cause skin erosion usually in the head region and heavily infested fish die, especially smolts, after their transfer to sea water (Lester and Roubal, 1995).

DIAGNOSIS. Diagnosis is made by detecting the parasites on the skin of affected fish.

CLINICAL SIGNS. Parasite infestation causes small white-grey patches on the head region, base of dorsal fins and perianal area. Circular lesions become more prominent when the epidermis is damaged, and are followed by inflammation with infiltration of macrophages and fibrosis in the affected area. The lesions can lead to severe erosion that extends deep into the underlying tissue. The larval stage of the parasites feeds on mucus and cells around the area of attachment, but the free moving preadults and adults can cause the most serious damage. Fish with skin damages are exposed to other infections and may have difficulties in keeping the osmotic balance (Boxaspen, 2006).

PREVENTION AND CONTROL. Several chemotherapeutics have been used to treat sea lice infections, including organophosphates, pyrethroids, emamectin benzoate and hydrogen peroxide. Sea lice develop resistance if the drugs are used repeatedly (Denholm *et al*., 2002; Boxaspen, 2006). Resistant parasites/larvae can also be transported via water current to other farms. Today fish farmers and fish health enterprises follow the infection rates of sea lice on farms by counting different parasite stages on fish to plan when treatment will be most effective. Alternative approaches to deal with the problem have been developed, including the successful use of small fish (wrasse) that feed on sea lice from farmed fish, although not always easy to implement in practice (Torrissen *et al*., 2013). The number of sea lice and outbreaks can also be regulated by other factors, like keeping age classes separate, keeping distance between farms and taking into account direction of water current. Diseased and dead fish should also be collected to decrease infection pressure and good hygiene in the net pen.

Pennellidae

Ceratothoa gaudichaudii

Ceratothoa gaudichaudii is an isopod that has been reported to cause economic impact on farmed coho and Atlantic salmon in Chile (Kent and Poppe, 2002). This parasite attaches to the inner surface of the fish mouth or gills where it feeds on blood and causes erosion of gill lamellae, and ulcers on the gills and mouth.

Digenetic trematodes

Cryptocotyle lingua

Fish are an intermediate host to *Cryptocotyle lingua,* a trematode that penetrates fish skin and causes small black spots on the surface of the fish. The first intermediate host for this parasite is a snail from which cercaria are released and infect fish. During the spring the shallow water close to shore can contain high concentrations of cercaria (Mellergaard, 1996). If smolts are transferred to these sites they become heavily infected with this parasite which causes stress and osmoregulatory problems and may die. Overall this is rare in farmed fish.

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Nematodes

Anisakidae (Anisakis simplex, Pseudoterranova)

Anisakidae are roundworms that are found in marine mammals and fish. *Anisakis simplex* and *Pseudoterranova decipiens* are the most frequently reported species in human anisakiasis. Anisakidae have a complex life cycle, and at least three hosts are involved. Adult worms are found in the intestine of marine mammals such as dolphins, sea lions and whales. Eggs are released via faeces to the water where they embryonate and are eaten by small crustaceans. The parasite develops into third stage larvae that encyst in fish that eat infected crustaceans (Dick and Choudhury, 1995). Humans get anisakiasis when these encysted larvae are eaten, e.g. in poorly cooked fish meat. *A. simplex* is invasive and therefore can penetrate host tissue. *A. simplex* is usually found in humans in the mucosa or submucosa of the stomach or intestine and migrates to other tissues such as omentum, pancreas, liver and even lung. *Pseudoterranova* is usually not invasive and therefore does not cause serious signs. However they can remain alive in the stomach and crawl back up into the oesophagus and up to the pharynx and cause a tingling feeling in the throat and patients often cough up these parasites (Sakanari and McKerrow, 1989).

IMPACT ON FISH FARMING. *A. simplex* has not been reported in farmed Atlantic salmon before 2012 when it was detected for the first time in Norway in Atlantic salmon 'runts' that had been discarded from processing because of poor performance (Mo *et al*., 2013). *A. simplex* is reported in wild Atlantic salmon and has in recent years been reported as the cause of a disease called Red Vent Syndrome (RVS), e.g. in Scotland. RVS is a condition where wild salmon have inflamed, swollen bleeding vents with *A. simplex* embedded in the dermal, sub-dermal and muscle tissues in the inflamed area (Noguera *et al*., 2009).

DIAGNOSIS. Diagnosis of *Anisakidae* is made by determining the species using light microscopy to study the morphology structures of the parasites. *A. simplex* are small white worms 9–36 mm in length, with a straight anterior gut structure consisting of oesophagus, ventriculus and intestine. *P. decipiens* are typically white to reddish brown in colour, 9–58 mm in length, and have an anteriorly projecting intestinal caecum (Olson *et al*., 1983). A multiplex PCR method has been reported for the rapid identification of *Anisakis simplex* sensu stricto (Umehara *et al*., 2008).

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3 Infectious Diseases of Coldwater Fish in Fresh Water

Kenneth D. Cain[1]* and Mark P. Polinski[2]
[1]*Department of Fish and Wildlife Science, University of Idaho, Moscow, USA;* [2]*National Centre of Marine Conservation and Resource Sustainability, University of Tasmania, Launceston, Australia*

Introduction

The culture of coldwater fish in fresh water environments is practised globally and primarily involves salmonids. Cage culture of coldwater species in fresh water occurs in many areas, but is limited in terms of capacity and revenue when compared with marine net pen farming. Freshwater cage culture generally occurs in lakes, reservoirs, large rivers or manmade canals (Fig. 3.1 a, b). Disease concerns are related to the life stages, but would mimic those experienced at other freshwater aquaculture sites that rear fish in earthen ponds, tanks or raceways.

Commercial culture of coldwater fish species is widespread, but is often associated with resource enhancement and mitigation aquaculture programmes. In such cases, fish (usually anadromous salmonids) hatched and reared in fresh water are released at smoltification into the environment where they 'imprint' prior to migrating to the ocean or other large bodies of water such as the Great Lakes of the United States and Canada. In the United States (particularly Washington, Oregon, California and Idaho), government and tribal hatcheries produce over 300 million salmon annually for supplementation and enhancement programmes (Paquet *et al*., 2011). The level of understanding we have today in the areas of fish diseases and health management is often linked directly to research initiated as part of these programmes. Even net pen salmon farming in the ocean relies on a freshwater rearing stage for Atlantic salmon (*Salmo salar*) and other salmonids. In this chapter, the primary disease problems that affect different life stages of freshwater fish reared in a variety of water sources (ranging from ground water and springs to rivers, lakes and reservoirs) will be highlighted. This includes diseases that are current problems in freshwater cage culture of coldwater species or that could be potential problems in the future. Disease impacts and risks can be assumed to translate directly to cage and pen culture in a freshwater environment.

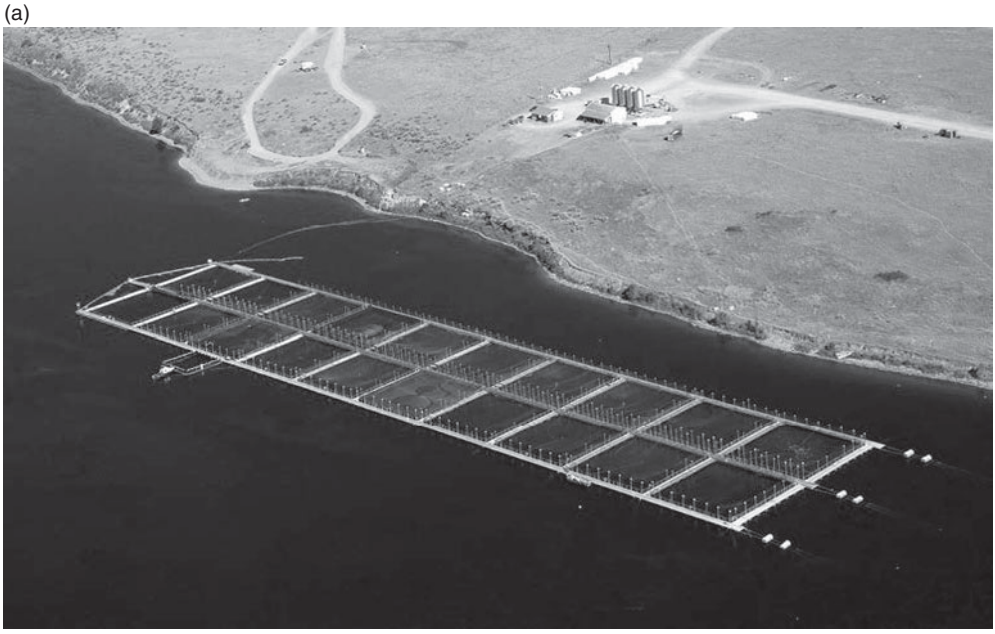
Diseases discussed in this chapter occur regularly in coldwater species at approximately 15°C or less and manifest primarily in a freshwater environment. In some cases, the pathogen may be contracted in fresh water but becomes problematic and disease occurs once fish are transferred or migrate to sea water. If the majority of cases are observed in sea water then the reader is referred to the Chapter 2 or other appropriate chapters.

E-mail: kcain@uidaho.edu

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(a)



(b)



Fig. 3.1. Freshwater rainbow trout cage culture operation located on the Columbia River in Washington State, USA (a), and freshwater cage culture of Chinook salmon on manmade glacial fed canal, New Zealand (b). Columbia River image courtesy of J. Bielka, Pacific Seafoods.

Examples of disease agents that may occur or be transmitted in fresh water but continue to cause problems in sea water are *Aeromonas salmonicida* (causative agent of furunculosis), *Renibacterium salmoninarum* (causing Bacterial Kidney Disease) and *Henneguya salminicola* (a Myxozoa that affects flesh quality). In some cases a disease may have few reports in fresh

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water (e.g. salmonid rickettsial septicaemia) or impact coldwater fish to a lesser extent than warmwater species (e.g. columnaris disease), and these will only be briefly discussed in this chapter.

Diseases Caused by Viral Pathogens

Viral haemorrhagic septicaemia

Introduction

Viral haemorrhagic septicaemia (VHS or Egtved disease) continues to be a serious disease in both fresh water and marine fish culture facilities. VHSV is known to affect more than 60 fish species and is distributed widely across the northern hemisphere (Smail and Snow, 2011). The high virulence, wide range of susceptible host species, and ability for rapid spread of this pathogen is of significant concern for many global fisheries and the disease prevalence is closely monitored by the Office International des Epizooties (OIE). Although the VHS virus has negative implications for culturing marine fish (Chapter 2), freshwater isolates often manifest with severe pathology and associated mortality, thus making the disease of primary concern in fresh water.

Characterization

VHSV is an enveloped, negative sense, single-stranded RNA virus in the *Rabdoviridae* family, similar to the infectious haematopoietic necrosis virus (IHNV) discussed later in this chapter. Viral replication occurs in the cytoplasm, ultimately producing bullet shaped virions of approximately 180 × 60 nm in size. As with most Rabdoviruses of vertebrates, the viral genome codes for five proteins which include a large matrix protein (M), nucleocapsid protein (N), polymerase-associated phosphoprotein (P), surface glycoprotein (G) and virus polymerase (L). Additionally, the VHSV genome codes for a sixth non-virion protein (Nv) which is unique to a specific set of the aquatic Rhabdoviruses known as Norirhabdoviruses (Betts and Stone, 2000). Phylogenetic analyses based on N, P, G and Nv genes has grouped VHSV into four major genogoups that roughly correlate to original geographic location: European freshwater and north European marine isolates (genogroup I), marine isolates from the Baltic Sea (genogroup II), isolates of the North Sea (genogroup III) and all North American isolates (genogroup IV) (Skall *et al*., 2005). Genogroup IV has further been subdivided by sequence analysis to delineate marine (IVa) from freshwater isolates (IVb) (Elsayed *et al*., 2006). It is currently believed that all freshwater isolates have originated from marine ancestors, most of which have arisen in the past 50 years in multiple adaptive events (Einer-Jensen *et al*., 2004). This may help to at least partially explain the juxtaposition between the rather stable relationship and relative low virulence of marine viral isolates with their hosts and the highly pathogenic and lethal effects caused by the recently evolved freshwater strains, as the host–pathogen relationship has had more time to equilibrate in the marine environment. These factors may at least partially be explained in host entry by the virus, as Brudeseth *et al*. (2008) demonstrated gill epithelium of rainbow trout (*Onchorynchus mykiss*) to be far more susceptible to a freshwater genogroup I virus relative to its marine counterpart, which also helps in explaining the refractivity of rainbow trout to the marine strain of this virus by immersion that would otherwise cause mortality if administered by intra-peritoneal injection (Skall *et al*., 2004). As such, it would appear that virus from the marine environment will continue to be a threat to the freshwater culture of coldwater fish and the adaptive pressure presented by fresh water will be a target for virulence studies in the future.

Impact

Although a wide range of species have been shown to be susceptible to VHS (Skall *et al*., 2005), the most serious effects have occurred in cultured rainbow trout in Europe. It is difficult to put a value on the costs incurred as a result of disease outbreaks, preventative procedures and scientific investigations into understanding and treating this disease;

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however, VHS has been regarded as a major monetary concern in affected areas since the 1950s and has caused significant economic loss in multiple European countries (Olesen and Korsholm, 1997). The strain most responsible for the impacts in European aquaculture has been genotype Ia, which has long been considered to be the most virulent and pathogenic form of this virus where mortalities reaching 100% are often observed. However, a severe epidemic in the Great Lakes region of the United States caused by the IVb genotype resulted in massive losses of primarily wild fish species throughout the region. Although the majority of North American fish mortality has occurred in wild populations, there is a well-founded concern for its potential impact in aquaculture, particularly with regard to cage culture in areas where the virus now appears to be endemic or is likely to spread. In Asia, the disease has primarily been restricted to marine culture species (Skall *et al*., 2005), but given the likely marine origins for the current virulent freshwater European and American strains (Einer-Jensen *et al*., 2004) and the growing aquaculture in the region, the possibility for novel VHS identification in freshwater Asian countries must be considered.

Clinical signs

VHS can manifest as both an acute or chronic disease in freshwater fish, and both juvenile and adult fish may become infected and present clinical signs. General non-specific symptoms in chronic infections include lethargy, dark coloration and moderate exophthalmia. Fish may also appear anaemic and internal organs may show some haemorrhaging and oedema. Chronic infections apparent in stressful environments may develop into latent carrier infections with no abnormal presentation apart from potential hyperactivity. Acute infections with heavy mortality can approach 100% in susceptible species such as rainbow trout. The characteristic signs of severe infections include haemorrhaging in the ocular tissue, skin and fin bases. In some cases, intermittent spiral swimming may be observed due to infection of brain and nervous tissue (Olesen and Skall, 2009). Internal signs will often include swollen, anaemic kidneys and spleen, as well as pin-point haemorrhaging throughout the viscera and occasionally in the skeletal muscle. If fish survive an initial infection with VHSV, they are typically immune to further outbreaks.

Diagnostic techniques

As with most viral detection methods, the 'gold standard' in diagnostics is laboratory culture on a susceptible cell line followed by specific nucleotide or protein identification. Bluegill fry (BF-2) and rainbow trout gonad (RTG-2) cell lines have been shown to be the most sensitive for detection of freshwater isolates (Lorenzen *et al*., 1999). However, other cell lines such as epithelioma papulosum cyprini (EPC) and Chinook salmon embryo (CHSE-214) are also susceptible and have frequently been used for routine diagnostics (Wolf, 1988) as a result of their availability in diagnostic facilities. Following cell culture isolation, confirmation of VHSV protein can be accomplished using an enzyme-linked immunosorbent assay (Way and Dixon, 1988) or nucleic acid identity can be confirmed using viral specific reverse transcriptase polymerase chain reaction (Miller *et al*., 1998). A quantitative RT-PCR method has also been developed which has shown higher sensitivity for detection of viral RNA in tissue than conventional cell culture techniques and shortens the time required to identify pathogen presence (Hope *et al*., 2010). However, this method (as with all PCR-based detection methods) identifies viral specific RNA and not necessarily infective virions and/or the definitive cause of a disease.

Control

Prevention or eradication continues to be the most appropriate method in combating this disease in farmed fish. Eradication of infected stocks has been used to effectively eliminate this pathogen from several European countries (Stone *et al*., 2008; Dale *et al*., 2009; Olesen and Skall, 2009). The disease rarely manifests above 18°C, and the enveloped nature of the virus results in VHSV having relatively poor environmental stability. Virions have been

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shown to lose infectivity after approximately two weeks at 15°C in fresh water (Hawley and Garver, 2008); although it should also be noted that virus held in purified water at 4°C remained infective for more than a year. Ultraviolet radiation (UV), hypochlorite and iodophor-based disinfection are all effective at eliminating pathogenic virus from water and equipment (Enzmann, 1983; Yoshimizu *et al*., 1986). There is currently no commercial vaccine or therapy for the treatment of VHS. Experimental DNA vaccination against VHSV has been demonstrated to be extremely effective (Lorenzen *et al*., 1998, 2001; Byon *et al*., 2006); however, their injection-based delivery has not been deemed economically feasible for large-scale fish production. A potentially more cost effective oral delivery method for administering a DNA vaccine has since been developed with promising experimental results (Adelmann *et al*., 2008), although it has yet to become commercially available. Additionally, increasing water temperature to 21°C during a VHSV immersion challenge of Japanese flounder (*Paralichthys olivaceus*) precluded mortality and provided strong protection in subsequent re-challenge of fish at 15°C (Nishizawa *et al*., 2011). More studies will be needed; however, this data may rekindle the possibility for live vaccination to combat this disease.

Infectious haematopoietic necrosis

Introduction

Infectious haematopoietic necrosis (IHN) caused by the IHN virus (IHNV) is the second of three serious diseases caused by Rabdoviruses discussed in this chapter (the others are VHS and SVC). Like VHS, IHN can cause severe mortality in naïve freshwater salmonid fish populations and it has been a major concern for Pacific salmon aquaculture in North America for more than 60 years. Most salmonid species are known to be highly susceptible, and endemic populations are widespread including North America, Europe and Asia (Bootland and Leong, 1999). Large losses of revenue and fish have occurred in salmonid aquaculture as a result of this virus (Congleton, 1988), and IHNV is of specific concern to the OIE due to its high pathogenicity and possibility for transmission within global salmon fisheries.

Characterization

To date, natural epizootics have exclusively been observed in salmonid species. Nevertheless, non-salmonids have been infected in experimental studies and some wild species are known to harbour viable pathogen with no clinical disease (Castric and Jeffroy, 1991; LaPatra *et al*., 1995). Like VHSV, the virus genome consists of a single-stranded negative-sense RNA with L, G, N, P, M and Nv coding regions as previously described for VHSV. It is believed that IHNV originated in North America, and phylogenetic analysis based on the G-coding nucleic acid sequence has led to the description of three distinct genogroups which correspond roughly to original endemic geographic distribution: the upper (U) genogroup found in the Columbia River basin of Washington state extending north through Canada to Alaska, the middle (M) genogroup found in the north-western United States Columbia River basin inland to the Snake River of Idaho, and the lower (L) genogroup found in northern California and southern Oregon (Kurath *et al*., 2003). Subsequent spread of the virus to Europe in the 1980s has led to the evolution of a distinct European genotype, shown to be originally derived from the North American M genogroup (Enzmann *et al*., 2005). Similarly in Japan, a JRt genotype has been identified with ancestral ties to the North American U genogroup introduced in the 1970s (Nishizawa *et al*., 2006).

Impact

IHN has been a problem to the freshwater culture of salmonid fish in North America since the 1950s. By the 1980s the disease had spread to Europe and Asia through the transportation of fish and eggs and continues to impact salmon aquaculture in both regions (Bootland and Leong, 2011). The disease has been known to cause high mortality in naïve fish, and spinal deformities can occur among surviving fish potentially limiting growth

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and subsequent value. All salmonids are believed to be susceptible to the virus; however, the ability of IHNV to cause disease among different species is often genotype specific (LaPatra *et al*., 1990a, b, 1993; Garver *et al*., 2006). For example, LaPatra *et al*. (1990b) showed an isolate from the M genogroup incurred 64% cumulative mortality in rainbow trout following immersion challenge, yet isolates from either the U or L genogroups only produced 4% and 6% mortality, respectively, under similar conditions. In general, isolates of the U genogroup have the highest virulence in sockeye salmon (*Oncorhynchus nerka*), M isolates in rainbow trout and L isolates in Chinook salmon (*Oncorhynchus tshawytscha*) (LaPatra, 1998; Garver *et al*., 2006). However, pathogenic virulence between viral isolates is also known to vary between stocks of fish within a species (Garver *et al*., 2006). Following acute infection with the disease, survivors are generally immune to re-infection (Lorenzen and LaPatra, 1999).

Clinical signs

IHNV targets the endothelial cells of blood capillaries and haematopoietic tissues for replication. As a result, clinical signs typically include oedema and haemorrhaging in the visceral organs, particularly in the kidney, spleen and liver. General anaemia may be observable by pale gills and a low haematocrit. Nonspecific signs of acute infection are similar to VHSV and include lethargy interspersed with increased activity, darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia and in some cases external petechial haemorrhaging. Fish will go off feed, and a trailing faecal cast may become evident.

Diagnostic techniques

Traditional detection of IHNV is based on virus isolation in cell culture. Both EPC and CHSE cell lines are known to be susceptible and are typically used for screening of this virus. Confirmatory identification may be achieved by use of immunological based methods such as plaque neutralization (Jorgensen *et al*., 1991), indirect fluorescent antibody test (Arnzen *et al*., 1991), and an enzyme-linked immunosorbent assay (Dixon and Hill, 1984; LaPatra *et al*., 1989). Molecular methods such as polymerase chain reaction (Arakawa *et al*., 1990; Purcell *et al*., 2006) or use of a DNA probe (Deering *et al*., 1991) can also be used for confirmatory testing following cell culture, and alternatively applied to directly analyzing tissue. Further, a reverse transcription loop-mediated isothermal amplification (RT-LAMP) protocol has been developed for detection of IHNV which has been shown to be more sensitive for identifying viral RNA than RT or nested PCR (Gunimaladevi *et al*., 2005). This detection method may also provide a useful 'on-site' screening and monitoring tool for cage-culture facilities, as reaction conditions require only a single-temperature heat block and UV light source to amplify and identify targeted nucleic acid sequence (Notomi *et al*., 2000).

Control

Avoidance is currently the best control method for this disease. Iodophor disinfection of eggs is highly effective in stopping egg-associated transmission, and other typical disinfectants including hypochlorite and UV are effective for sanitizing equipment (Winton, 1991). Both whole-inactivated cell and DNA vaccines for IHN have been shown to be efficacious in preventing disease (Lorenzen and LaPatra, 2005) and are currently licensed for commercial use in Atlantic salmon net pen aquaculture on the west coast of North America (Kurath, 2008). Unfortunately, the delivery methods for the currently licensed vaccines are injection-based, and thus can be costly and time intensive, especially for the vaccination of large numbers of fish. Selective breeding has shown promise in producing resistance to disease in rainbow trout (Purcell *et al*., 2010) and a potential means of control in areas where IHNV is known to occur.

Spring viraemia of carp

Introduction

The aetiological agent for spring viraemia of carp (SVC) is the third serious Rhabdovirus

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to infect fish in cold water. SVC can affect a number of host species, none of which are directly associated with typical coldwater cage culture. Nevertheless, outbreaks of SVC are associated with cold environmental conditions (10–17°C) in the culture of cool or warmwater species, which can occur in natural or large artificial aquatic environments due to seasonal changes. The virus is thought to enter through the gill and has reportedly been isolated from rainbow trout (Khiabanian Asl *et al*., 2008), but disease and/or mortality has (as yet) not been attributed to this pathogen in cultured or wild populations of salmonids.

Characterization

The virus genome is a non-segmented, negative-sense, single strand of RNA that encodes the five characteristic N, P, V, G and L proteins of the rhabdoviral family, but lacks the non-virion (Nv) gene present in the previously discussed Norirhabdoviruses VHSV and IHNV (Ahne *et al*., 2002). Four genogroups have been phylogenetically characterized for SVCV, which possess some nucleotide similarity to pike fry rhabdovirus (PFRV) which is considered to be within the same genogroup. Additionally, cross reactivity for antibodies between these two pathogens are also known, and thus sequencing analysis or other species specific identification methods are required to diagnose this disease (Stone *et al*., 2003). The virus can remain infective for up to 6 weeks outside the host in an aquatic environment (Ahne, 1982).

Impact

SVCV is a highly virulent, often deadly disease in multiple species of cultured carp and is of specific concern to the OIE. Susceptible species include common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*), crucian carp (*Carassius carassius*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), orfe (*Leuciscus idus*) and tench (*Tinca tinca*). In general, a prudent assumption is that all cyprinid fish in cool climates may be susceptible to SVCV. The virus has also been detected in sheatfish (*Silurus glanis*), pike (*Esox lucius*) and reportedly isolated from rainbow trout. Disease outbreaks manifest when the environmental temperature is between 10–17°C, which often only occurs in the winter and spring months for susceptible cool- to warm-water species. Young fish up to 1 year of age are most susceptible to clinical disease, but all age groups may become infected. SVC has long been associated with the culture of carp in Europe where up to 40% mortality was routinely observed in the aquaculture environment (Ahne *et al*., 2002). The disease has also been more recently identified in both Asia (Liu *et al*., 2004) and North America (Goodwin, 2002).

Clinical signs

General signs of disease include exophthalmia, anaemia resulting in pale gills, and haemorrhaging of the skin and at the base of the fins or vent opening. The virus has some propensity for causing inflammation of the swim bladder, which often results in abdominal distension, lethargy and loss of equilibrium resulting in side-swimming or sinking. Mucoid faecal casts may also be present. In some cases, a sudden onset of mortality can occur without clinical manifestation.

Diagnostic techniques

SVCV identification may be achieved by the isolation of the virus in cell culture, IFAT (Faisal and Ahne, 1984) or ELISA (Way, 1991) from infected tissues. Direct diagnosis, should be confirmed by a virus neutralization (VN) test or RT-PCR and sequence analysis, as antibodies used in both IFAT and ELISA are known to cross-react with PFRV (Jorgensen *et al*., 1989). A loop-mediated isothermal amplification method has also been developed for identifying this virus for potential application by culture facilities without elaborate diagnostic equipment (Shivappa *et al*., 2008).

Control

As with the other Rabdoviruses, the best control is prevention. There is no commercial vaccine or chemical therapeutic available,

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although both component and live attenuated vaccines have shown promise in experimental trials (Leong and Fryer, 1993; Kanellos *et al*., 2006). Typical disinfectants are effective at inactivating infective virions from equipment, and it is known that vertical transmission from mother to egg does not occur for this virus. Increasing water temperature (if possible) to above 18°C is known to decrease the severity of an outbreak (Sano *et al*., 2011), and reducing stocking densities and minimizing over-wintering stressors such as low oxygen, parasites or other poor water quality parameters may aid in reducing the possibility and severity of disease in infected populations. Resistant breeding has also been employed as a method for limiting disease impact in areas where this virus is endemic (Kirpichnikov *et al*., 2009).

Infectious pancreatic necrosis

Introduction

Infectious pancreatic necrosis virus (IPNV) is a member of the ubiquitous birnavirus family which causes pancreatic necrosis in salmonid fish worldwide (Reno, 1999). The virus infects a wide range of host species; however, the major problem continues to revolve around salmonids. Once considered a freshwater disease, in recent decades IPN has also become a significant disease in the salt water culture of salmonids (Chapter 2). In this chapter, we will focus on the fresh water aspect of the disease.

Characterization

IPNV has a single-shelled, non-enveloped, icosahedral virion of approximately 60 nm diameter. The genome consists of a double-stranded RNA with two segments – one coding for the RNA-directed RNA polymerase (VP1), the other coding for structural associated proteins (VP2-VP4) as well as an anti-apoptosis protein (VP5). The structural VP2 has been shown to be highly immunogenic (Heppell *et al*., 1995), the diversity and replication efficiency of which has been directly correlated to virulence and the ability to cause disease (Coulibaly *et al*., 2010; Skjesol *et al*., 2011). Historically, isolates causing IPN have been classified into two major serogroups, A and B, containing 10 distinct serotypes (nine from A and one from B) (Hill and Way, 1995). Phylogenetic analysis has alternatively categorized isolates into seven genogroups (Blake *et al*., 2001; Nishizawa *et al*., 2005), which roughly correspond to the previously described serotypes and original geographic distributions with some modification. Isolates from both A and B serogroups (all seven genogroups) infect fish that live in or migrate to freshwater environments. The virus itself is extremely environmentally stable and is known to remain infective for months in aqueous conditions (Smail *et al*., 1993).

Impact

Rainbow trout and brook trout are most susceptible, although all salmonid species can be infected. Outbreaks of IPN in fresh water are typically only observed in young fish less than 6 months of age (LaPatra *et al*., 2000), although chronic low levels of infection have been observed in fish during all life stages. Typically acute infection becomes apparent within a week following exposure and the disease outbreak is concluded within another week (Reno *et al*., 1978), during which time severe mortality occurs. The most severe outbreaks occur between 10–14°C. Due to its environmental stability and ability to chronically persist in adult fish, eradication is extremely difficult once an outbreak has occurred.

Clinical signs

Acute infection of IPN in young fish is rapid. Typically within a week of becoming infected fish go off feed and become darker in colour. Often fish loose equilibrium and swim with abnormal body rotation. Trailing faecal casts are common and petechial haemorrhaging on the ventral surface and exophthalmia are also common nonspecific signs. A pale and anaemic liver is a hallmark of this disease, although general anaemia throughout the viscera is also common.

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Diagnostic techniques

Primary isolation from tissues has typically relied on cell culture, and many teleost cell lines have been shown to be susceptible (Reno, 1999), including BF-2, EPC and CHSE-214. Confirmation or direct testing of tissue can be conducted by a FAT (Swanson and Gillespie, 1981), real-time RT-PCR (Orpetveit *et al*., 2010) or RT-LAMP (Soliman *et al*., 2009).

Control

Strict bio-security measures will aid in avoidance of this pathogen. This should include measures to prevent both horizontal and vertical transmission such as egg disinfection, screening and separation of new stocks, and disinfection of all potentially contaminated equipment. The virus is environmentally stable and is also considered one of the most resistant viruses to disinfection (Munro and Midtlyng, 2011). For example, the UV-C dose required to inactivate IPNV (>100 mWs per cm^2) is 50 times higher than what is required to inactivate aquatic rhabdoviruses such as VHSV, IHNV and SVCV (Skall and Olesen, 2011). The development of at least some resistance to IPNV in rainbow trout has been shown by selective breeding (Guy *et al*., 2009), although the process requires multiple generations. Injection-based commercial vaccines are available against IPNV and have successfully been applied in various aspects of aquaculture for both adult and juvenile fish (Ramstad *et al*., 2007). Unfortunately, their use as a preventative treatment for salmonid fry typically infected in fresh water is virtually impossible due to their small size and fragility. De las Heras *et al*. (2010) recently showed promising results from oral administration of a DNA vaccine during early feeding of young (1 gram) rainbow trout by encapsulating viral DNA within an alginate particle to allow better uptake through the intestine without exposing the antigen to digestive degradation. These findings, coupled with the recent identification and immunogenic role of the crystalline capsid structure of IPNV (Coulibaly *et al*., 2010), may lead to more effective vaccination strategies against this virus.

Sleeping disease

Introduction

Salmonid alphaviruses (SAVs) are a serious danger to Atlantic salmon and rainbow trout culture in Europe and cause significant economic loss to the European aquaculture industry. Two diseases are attributed to SAVs: pancreas disease (PD) in Atlantic salmon and sleeping disease (SD) in rainbow trout. The occurrence of PD in salmon is associated with the marine environment and is covered in Chapter 2. Sleeping disease is an infection of farmed rainbow trout in fresh water and will be the focus in this chapter. Nevertheless, both PD and SD are caused by closely related alphavirus subtypes and there are many overlaps in aetiology, detection and control for these two diseases.

Characterization

Sleeping disease is caused by salmon alphavirus-2 (SAV-2); a single-stranded, positive-sense RNA virus with a spherical enveloped capsid of approximately 65 nm diameter. There are two other subgroups of salmon alphavirus, SAV-1 and SAV-3, of which both cause disease in marine Atlantic salmon (see Pancreas Disease; Chapter 2) (McLoughlin and Graham, 2007). Amino acid identity for both structural and nonstructural proteins between these subgroups is over 93% (Weston *et al*., 2002), suggesting high similarity between both freshwater and marine isolates regarding replication and host interactions. For all terrestrial aphaviruses, an arthropod vector (such as a mosquito) is involved in the transmission of disease. Currently no such vector has been definitively identified for SAV. Sea lice have been suggested as a possible vector for the marine SAVs (Petterson *et al*., 2009), although viral replication within sea-lice has yet to be achieved. A freshwater associated vector is unknown; however, horizontal fish-to-fish transmission has been demonstrated (Boucher *et al*., 1995), thus precluding the necessity of a vector.

Impact

Sleeping disease was first described in 1994 in cultured rainbow trout in France (Boucher

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and Baudin Laurencin, 1994), but has since spread to many areas in Europe (McLoughlin and Graham, 2007). Atlantic salmon, rainbow trout and brown trout (*Salmo trutta*) are susceptible (Boucher *et al*., 1995), although rainbow trout are most affected by this disease in fresh water. The disease can cause variable mortality, but losses up to 43% have been recorded in cage culture of rainbow trout in the UK (Graham *et al*., 2007).

Clinical signs

Both SD and PD cause sequential necrosis in the pancreatic tissue, cardiac muscle and skeletal muscle. Characteristic 'sleeping' behaviour in rainbow trout experiencing a severe disease outbreak is lying motionless on their sides along the bottom of the holding area as a consequence of damage to skeletal red muscle fibres (Castric *et al*., 1997). Other nonspecific signs may include exophthalmia, bloated abdomen, faecal casting and lack of appetite. Internally, lesions in the cardiac and skeletal muscles may be visible. In cage culture, clinical signs and mortalities have been observed 8 to 15 weeks following infection which resolved after an additional 10–20 days (Graham *et al*., 2007).

Diagnostic techniques

Following preliminary diagnosis based on clinical signs and histopathology, a variety of confirmatory techniques are available including virus isolation, serology and RT-PCR which are reviewed elsewhere (McLoughlin and Graham, 2007). Specifically, real-time PCR protocols using SYBR green chemistry (Graham *et al*., 2006) or Taqman probes (Christie *et al*., 2007) have been shown to be sensitive and specific for identifying SAV.

Control

There is no commercial vaccine or therapeutic available to combat SAV. However, clinical disease is often associated with stress conditions, so in areas where the virus is endemic, good management and hygienic practices can aid in minimizing the impacts of disease. Temperature is known to play a significant role in the infectivity of this virus (Metz *et al*., 2011) and warm (>15°C) water temperatures may also aid in minimizing the severity of disease and fish mortality. An injection-based inactivated virus vaccine has also shown promise in experimental trials (Karlsen *et al*., 2012).

Salmonid herpes virus infections

Introduction

Three herpes viruses have been identified to cause disease in cultured salmonid fish: herpes-virus salmonis (HPV or SalHV-1), *Oncorhynchus masou* virus (OMV or SalHV-2), and epizootic epitheliotropic disease virus (EEDV or SalHV-3). Although these viruses have a limited host and geographic distributions with virtually no severe disease outbreaks in recent years, the general latent nature of herpes virus makes identification of possible reservoirs difficult and disease re-emergence must be considered. Both SalHV-2 and -3 have been known to cause mortality in cultured fish, and are believed to persist in endemic populations in Japan and the Great Lakes region, respectively.

Characterization

Salmon herpes viruses are enveloped double-stranded DNA viruses of approximately 200 nm diameter. For all three genotypes, infectivity is optimal around 10°C. Taxonomic and genetic classification of herpes-like viruses has been somewhat unsettled in the past decade. The discovery of herpes viruses in fish and molluscs which are distantly related to those in birds and mammals required a reordering of herpes viruses and the formation of two new families – the *Alloherpesviridae* family, which incorporates bony fish and frog viruses, and the *Malacoherpesviridae* family, which contains those of molluscs (Davison, 2010). The *Alloherpesviridae* has been further phylogenetically subdivided into two clades, with the three salmon herpes viruses branching together (approximately 80% amino acid similarity of viral DNA polymerase between genotypes) within the clade containing Salmonid, Ictalurid, Acipenserid and Ranid viruses (Waltzek *et al*., 2009). Although genetically similar, each SalHV

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genotype has rather stringent host specificity. SalHV-1 has only been known to infect Pacific-run rainbow trout (Wolf *et al*., 1978; Hedrick *et al*., 1986) and SalHV-3 has exclusively caused disease in lake trout (*Salvelinus namaycush*) of the Great Lakes Region, USA (Bradley *et al*., 1989). SalHV-2 has somewhat wider prevalence and distribution, but still only appears to (as yet) cause disease in Pacific (*Oncorhynchus* spp.) salmon in Japan (Yoshimizu *et al*., 1995).

Impact

SalHV-1 was originally isolated from rainbow trout from Washington state in 1978 (Wolf *et al*., 1978), and again in 1986 in northern California from anadromous populations of the same species (Hedrick *et al*., 1986). This genotype has been shown to cause cytopathic effect in cell culture (Wolf *et al*., 1978); however, mortality events in susceptible cultured species has never been directly attributed to this virus. SalHV-2 has been documented in Japanese aquaculture since the late 1970s (Kimura *et al*., 1981), but somewhat surprisingly has not spread to any other global region. The disease particularly affects young fish in freshwater environments, and although recent outbreaks have been limited, severe mortality has previously been attributed to this disease where mortality events have reached in excess of 80% of cultured stocks (Furihata *et al*., 2003). Multiple species including rainbow trout, masou, sockeye, coho and chum salmon are known to be susceptible to SalHV-2, which has been observed in both wild and cage-cultured fish in Japan (Furihata *et al*., 2003). The endemic nature of this virus in Japan and known infectivity of ocean-run populations suggests that this virus is also present in other coastal areas of Asia where natural runs of Pacific salmon occur, but is relatively unconfirmed. SalHV-3 caused severe mortality in stocks of lake trout in the Great Lakes Region in the late 1980s resulting in over 15 million mortalities of cultured fish in a 5 year span (Bradley *et al*., 1989). Disease outbreaks are currently limited; however, SalHV-1 was confirmed to persist in spawning adult lake trout as late as 2009 in Lake Superior (Kurobe *et al*., 2009).

Clinical signs

Infection with SalHV-1 has only been identified in adults with no clinical signs. Both SalHV-2 and SalHV-3 are known to cause acute disease and mortality in young (<1 year-old) fish. Onset of acute disease is rapid with mortality occurring within 1 to 2 weeks following infection and is associated with systemic haemorrhaging. Pathology may be observed in haematopoietic tissues and nonspecific signs may include lethargy, dark body colour, and abnormal corkscrew or hyperactive swimming. The most definitive macroscopic signs include oncogenic and ulcerative conditions of epithelial tissues of the mouth and jaw as the infection becomes chronic. Lesions may also develop on the skin and fin bases, although to a lesser extent than Buccal-associated tissue. These ulcerative conditions have been known to persist for up to a year following acute infection. Gross internal signs during latent infection are minimal.

Diagnostic techniques

Historically, virus was identified by cell culture isolation using RTG-2 or CHSE-214 cell lines followed by microscopy or immunological identification (Wolf *et al*., 1978; Kimura *et al*., 1981; Hedrick *et al*., 1986). Unfortunately, inoculation of infected fish tissues onto established cell lines has not always resulted in the isolation of virus (McAllister, 1993), making false negatives a concern. Currently, viral DNA can be specifically detected using PCR-based techniques for both SalHV-2 (Aso *et al*., 2001) and SalHV-3 (Kurobe *et al*., 2009).

Control

Salmonid herpes viruses are most likely vertically transmitted, and thus disinfection of eggs can aid to minimize the potential threat of this disease. Virus can also be transmitted horizontally, and thus separation (if possible) of young fish from potential reservoir populations is also advisable. SalHV-2 is known to be inactivated by UV and iodophor treatment (Hisae *et al*., 2002). A formalin-killed SalHV-2 vaccine has been used successfully to reduce viral titres in ovarian fluid of infected fish

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(Yoshimizu, 2009), and selective breeding has also been successful in eliminating severe disease outbreaks in rainbow trout following four to five generations. An antiviral agent, Acyclovir, was also shown to be effective in inhibiting replication of SalHV-2 *in vitro* as well as *in vivo* during experimental infections of chum salmon fry (Kimura *et al.*, 1983a, b).

Diseases Caused by Bacterial Pathogens

Furunculosis

Introduction

Furunculosis is one of the oldest known fish diseases and is caused by the Gram-negative bacterium *Aeromonas salmonicida*. It has been studied extensively and its name is derived from its clinical sign – large boils (furuncles) under the skin of infected fish. It has a wide distribution and has been detected in fish from many different countries worldwide. Early reports were from cultured and wild fish in Europe (Emmerich and Weibel, 1894). The host range of *A. salmonicida* is extensive and it occurs in both fresh water and marine environments and affects fish (salmonid and non-salmonid) of all ages (Bernoth, 1997; Wiklund and Dalsgaard, 1998). It is infective to almost all fish species and many may serve as carriers, making eradication of the disease difficult (Herman, 1968). Furunculosis causes significant problems in salmonids. Brook trout (*Salvelinus fontinalis*), Atlantic salmon and brown trout are particularly susceptible. Rainbow trout are less affected and somewhat resistant (McCarthy, 1977). For additional background on furunculosis, there are a number of reviews available (Bernoth, 1997; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999).

Characterization

A. salmonicida is readily isolated from internal organs and most isolates produce a distinct brown diffusing melanin-like, water-soluble pigment when cultured on agar plates containing appropriate media. Early literature referred to the bacterium causing furunculosis as *Bacterium* or *Bacillus salmonicida* (McCraw, 1952), but later this was named *Aeromonas salmonicida* (Griffin *et al*., 1953). It is generally accepted that there are four subspecies of *A. salmonicida* in fish. The subspecies *salmonicida*, which is considered the 'typical' strain that causes furunculosis, is focused on in this section. It is characterized as a non-motile, non-sporulating, fermentative, Gram-negative, aerobic bacillus which reduces nitrate, liquefies gelatin, hydrolyses starch and produces cytrochrome oxidase. Other subspecies are *masoucida*, *achromogenes* and *smithia* and these are often referred to as 'atypical' strains (Plumb and Hanson, 2011). The taxonomy of *A. salmonicida* is not always clear and discrepancies exist in the literature regarding subspecies classification. For example, another subspecies, *nova*, is included in a classification scheme summarized by Munro and Hastings (1993). This classification placed subspecies into three groups:

- Group 1 – Includes *A. salmonicida salmonicida* as the 'typical' strain derived from salmonid fishes.
- Group 2 – Includes *A. salmonicida achromogenes* as an 'atypical' strain from salmonids that shows variation in some biochemical properties and includes former species *masoucida*.
- Group – Includes *A. salmonicida* nova as an 'atypical' strain that is associated with disease in non-salmonid fishes.

This classification has not been widely accepted and there is a lack of reliable traits for subspecies discrimination. According to Wiklund and Dalsgaard (1998) further studies have to be based on a larger number of strains and use techniques such as polynucleotide sequencing and DNA-DNA or RNA-DNA hybridization.

Impact

A. salmonicida salmonicida is widespread and furunculosis can occur in all life stages of salmonids. It is thought that most disease outbreaks are a result of movement of infected stocks or introduction from wild carrier fish. The primary mode of transmission is horizontal and evidence suggests that ingestion of bacteria and subsequent transfer across

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the intestinal wall are the most common routes of infection (Jutfelt *et al*., 2006). The organism can be shed in reproductive fluids but typical egg disinfection procedures should eliminate vertical transmission risks due to egg surface-associated bacteria. As mentioned above, *A. salmonicida* can affect salmonid and non-salmonid stocks; however, focus here is primarily on the 'typical' strains and their impacts on salmonid species.

Serious losses due to furunculosis have been reported in farmed and wild fish stocks (Roberts, 2012), and this disease causes major impacts to both commercial salmonid aquaculture and public resource hatcheries aimed at stocking of public waters. Originally, furunculosis occurred almost exclusively in fresh water, but intensive culture of species such as Atlantic salmon resulted in severe outbreaks at the smolt stage when these fish were moved to sea water. Roberts (2012) reported a more acute and highly contagious form of the disease which came about due to increasing intensive salmon culture. This was a particular problem in Atlantic salmon smolts in their first year at sea. Although many life stages are susceptible, Munro and Hastings (1993) suggested that young fry are less commonly infected.

Clinical signs

The classic boil-like furuncle is observed in some fish, but often fish die from an acute infection without any obvious clinical features. Furuncles may be present on the sides or dorsum of fish and appear as raised or haemorrhagic areas (Fig. 3.2) and can ulcerate and release necrotic cells and tissue along with bacteria. This is thought to increase the risk of horizontal transmission by increasing bacteria in the water column. Depending on the nature of the infection, fish can become dark, lethargic and have petechial haemorrhaging at the base of fins (Munro and Hastings, 1993). If the disease occurs in very young fish, high mortality may be observed, but otherwise limited signs may be apparent beyond some dark fish, anorexia and congregation near outlets.

Internal signs associated with furunculosis can include ascites and an empty intestine filled with mucus and cellular debris. Haematocrit values are often very low and blood vessels around the pyloric caeca and intestine can become inflamed. Toxins are often released by *A. salmonicida* and will cause liquefaction of tissues and severe inflammation.

Diagnostic techniques

There are a number of effective ways to diagnose furunculosis through culture of *A. salmonicida* or identification histological sections. Typically a definitive diagnosis would be based on a combination of clinical signs and associated isolation of *A. salmonicida* from affected fish organs/tissues. The bacterium is most often cultured on tryptic soy agar (TSA), but will grow on Brain heart



Fig. 3.2. Rainbow trout showing furuncle type lesions under the skin attributed to infection with *Aeromonas salmonicida*.

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infusion agar (BHI). Within 48 hours of culture at 22–25°C, typical small raised circular colonies appear, that are nonmotile, oxidase positive and fermentive (Roberts, 2012). The brown pigment associated with the typical strain will usually develop within 10 days, but atypical strains generally lack this pigment. Colonies can easily be collected on a loop and bacteria examined on a slide following a variety of staining procedures. The appearance of short Gram-negative rods measuring 0.8–1.3 by 1.3–2.0 μm that often occur in pairs, chains or clumps is characteristic (Roberts, 2012). Confirmatory diagnosis of *A. salmonicida* can be accomplished rapidly by serological assays such as the fluorescent antibody test (FAT) or a range of other immunological tests (Thoesen, 1994). Molecular methods based on PCR have been developed and could be used to confirm isolates or for identification of carrier fish in a population (Hiney *et al*., 1992; Oakey *et al*., 1998).

Control

Furunculosis is often controlled by either treatment of infected fish using feed-delivered antibiotics, or by prevention through vaccination. Proper fish culture methods and strict attention to biosecurity measures are also essential. Fish infected with *A. salmonicida* may pose a risk to other cultured or wild fish stocks and movement of such fish may be restricted through various regulatory authorities. The main method of controlling furunculosis is to prevent or eliminate *A. salmonicida* from water sources. This is much more feasible in facilities where spring or well water sources are available, but becomes more difficult with wild fish that inhabit water supplies. Most cage culture operations would be at risk. If exposure of fish is anticipated, the best approach to controlling disease is through the implementation of a vaccination programme.

Some of the very first reports on the development of fish vaccines were against furunculosis (Duff, 1942). Today, there are a range of vaccines commercially available for this disease and the use of oil-adjuvanted vaccines in the Atlantic salmon industry is now almost universal. This, combined with improved husbandry and a variety of other precautions, has dramatically reduced problems and limited the need for antibiotic treatments. If fish become infected, losses can be minimized by removing fish showing clinical signs such as furuncles, improvement of environmental conditions to reduce stress and/or by treatment with antibiotics. Treatment should be considered a last resort as many *A. salmonicida* isolates have developed resistance to certain antibiotics including oxytetracycline (OT), oxolinic acid, trimethoprimsulphadiazine and amoxicillin (Richards *et al*., 1992). Antibiotic sensitivity testing should be completed and is essential prior to treatment, especially due to the potential of resistant bacterial strains. Oxytetracycline has commonly been applied and is one of the most used antibiotics in aquaculture (Austin and Austin, 1993). However, in many regions, OT is no longer routinely used for treatment of furunculosis due to the development of resistant strains of *A. salmonicida* (Smith *et al*., 1994). Another antibiotic known as Romet™, which contains Ormetoprimsulfadimethoxine, is the present antibiotic of choice for furunculosis. Additionally, recent approval of florfenicol (AQUAFLOR®) in the USA for use in food-fish under a veterinary feed directive has provided further treatment options.

Other potential control options include the use of various immunostimulants which usually contain some form of β-1,3 glucan from yeast or bacteria added to the feed. Such immunostimulants have also been tested as adjuvants within vaccines and in some cases shown to enhance antibody formation and protection (Midtlyng *et al*., 1996). Another prevention strategy includes immunization of broodstock in a way to passively transfer immunity to salmonid fry (Kawahara *et al*., 1993), but the practical application and benefit of such methods are not known. Recent efforts have increased to find alternative approaches that would aid in disease control and promote overall health benefits in fish. This has included the use of naturally occurring bacteria from the gut of fish (probiotics) that exhibit antagonistic activity to select fish pathogens. Such probiotics have shown promise for control of furunculosis (Irianto and Austin, 2002).

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Motile aeromonad septicaemia

Introduction

Motile aeromonad septicaemia (MAS) is often associated with *Aeromonas hydrophila*, but can be caused by other ubiquitous species including *A. caviae* and *A. sobria*. *A. hydrophila* is a common environmental bacterium that is found in clean and polluted freshwater systems as well as some marine systems (Roberts, 2012). It is considered the cause of many severe disease outbreaks in wild freshwater and pond cultured fish. Infections and associated disease result from the opportunistic nature of this bacterium, and are exacerbated by stress and water temperature. Both cold-water and warmwater species are susceptible and if conditions are favourable a bacterial haemorrhagic septicaemia can occur. Due to the ubiquitous nature of the bacterium, it is often found on the skin and gills of fish and can be part of the normal intestinal flora of healthy fish (Newman, 1982; Holmes *et al*., 1996). Fish may be at risk of disease due to *A. hydrophila* at any time of the year due to this ubiquitous nature; however, epizootics are more frequent in the spring in species such as carp due to increasing temperatures and poor condition as fish recover from winter (Bullock *et al*., 1971). Salmonids are susceptible and acute outbreaks can occur when handling or crowding stresses are combined with elevated water temperatures. Interestingly, *A. hydrophila* can infect other species besides fish and has been isolated from frogs, alligators, turtles, shrimp and humans (Newman, 1982).

Characterization

A. hydrophila is a short motile Gram-negative rod (0.4–1.0 μm in length). It produces circular, convex pale white to cream coloured colonies on TSA agar at 15°C (Brown and Bruno, 2002). It may be cultured at warmer temperatures and Roberts (2012) suggested isolation on selective Rimler-Shotts (R-S) agar media containing novobiocin, which has been found useful for putative identification from potentially contaminated material. It can be identified based on biochemical profiles (Newman, 1982; Popoff, 1984), is isolated from organs such as the kidney, and may be found in the blood of infected fish (Roberts, 2012). It is aerobic and oxidase, catalase and aesculin positive.

Impact

Most fish in fresh water are susceptible to *A. hydrophila* including tilapia (*Oreochromis* spp.), brown trout, striped bass (*Morone saxatilis*) and carp (Fijan, 1972; Thune *et al*., 1982). When fish are under stress, *A. hydrophila* and other motile aeromonads can cause MAS which may also be referred to as red sore or red pest disease. Tail and fin rot may be common and, if stressful conditions persist, followed by a rapid rise in mortality in susceptible fish (Fijan, 1972). Since *A. hydrophila* is a common ubiquitous bacterium found naturally in soil and freshwater environments, it is considered opportunistic and outbreaks can occur seasonally when temperatures increase. It is widespread and found in Europe, the Americas and Asia where acute losses and greater than 80% mortality have been reported between 20–22°C (Brown and Bruno, 2002). Handling, transfer, crowding, low dissolved oxygen and nutritional status are all stressors that can affect susceptibility of fish species to *A. hydrophila*. Internally, it appears that *A. hydrophila* can multiply in the epithelium of the intestine, resulting in heavy shedding in the faeces (Brown and Bruno, 2002).

Clinical signs

The most apparent clinical sign of MAS is the development of a widespread haemorrhaging that is irregular in shape and may be present from the gills to the vent. Fish will often be dark and lethargic and exhibit tail rot, haemorrhage or ulcerated shallow necrotic lesions on the skin. Since toxins and other extracellular products are linked to virulence, internal organs can be congested and haemorrhage often observed on the viscera. Internally, clear to blood-stained ascites fluid can be found along with anaemic conditions and a swollen kidney or spleen. This bacterium was previously referred to as *A. liquefaciens* most likely due to the leakage of fluid from affected internal organs such as the kidney or spleen following incision (Roberts, 2012).

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Diagnostic techniques

Diagnosis of MAS due to *A. hydrophila* will often be presumptive based on clinical signs and previous occurrence. However, definitive diagnosis typically requires culture and confirmation through biochemical and other tests. Media that will support growth of *A. hydrophila* include TSA, BHI and R-S agar. Serological and molecular methods such as PCR are available for identification of *A. hydrophila* (Cascón *et al*., 1996), but these should be used only to confirm culture results. It should be emphasized that due to the ubiquitous nature of *A. hydrophila* and the other aeromonads, they can often be found as contaminants in culture due to their natural presence on skin or other internal organs. Therefore, pure cultures from affected fish and their organs are typically needed to confirm the diagnosis.

Control

The best approach to control MAS will often include improvements in husbandry or changes in rearing practices to eliminate specific stressors. However, antibiotics can be effective if sensitivity is properly determined prior to treatment. The development of antibiotic resistant strains is widespread and in many cases has limited the usefulness of some treatments in the past (Mitchell and Plumb, 1980). Vaccination is a possible preventative strategy that could limit MAS in specific populations. Experimental vaccines have been developed and early formulations met with varying levels of success due to antigenic diversity of *A. hydrophila* (Ramadan *et al*., 1994; Yin *et al*., 1996). However, recent development of attenuated live strains (Pridgeon and Klesius, 2011) may have greater promise for aquaculture than earlier vaccines.

Enteric redmouth disease/yersiniosis

Introduction

Enteric redmouth (ERM) is an important disease that has resulted in large economic impacts in aquaculture worldwide. ERM or yersiniosis is caused by the bacterium, *Yersinia ruckeri*. This bacterium was originally isolated in the Hagerman Valley of Idaho, USA from diseased rainbow trout (Bullock *et al*., 1971; Tobback *et al*., 2007). The disease affects primarily rainbow trout but other salmonids can be impacted and outbreaks can lead to high losses. There are a variety of *Y. ruckeri* strains or biotypes and the severity of disease may depend on this and the salmonid host (Brown and Bruno, 2002). Although this disease can be devastating to an operation and fish can become carriers of the pathogen, much success in the control of ERM has been realized through the use of commercial vaccines.

Characterization

Y. ruckeri is a Gram-negative bacterium, and is characterized as a short motile rod from 0.5–0.8 × 1.0–3.0 μm (Roberts, 2012). Optimum temperatures for growth range from 22–25°C and white to cream coloured colonies 1–2 mm in diameter should form within 24–48 hours (Plumb and Hanson, 2011). There are five most commonly recognized serovars; however, Stevenson *et al*. (1993) described six serotypes based on whole cell analysis. Serovar Type I (Hagerman strain) is the most commonly isolated and is often considered the most virulent. Vaccine efficacy has been linked to biotype and much work has been done on typing various strains of *Y. ruckeri*. At least two clones may have emerged separately in Europe and North America (Wheeler *et al*., 2009). Furthermore, atypical biotypes have been associated with mass mortality and vaccine failure in Atlantic salmon in Australia (Bridle *et al*., 2012).

Impact

Y. *ruckeri* was considered a major disease and severely impacted the commercial trout industry in Idaho, USA (Ross *et al*., 1966). In the past, ERM resulted in losses of up to 35% for the US trout industry with an estimated annual impact of $2.5 million in the Hagerman Valley of Idaho (Busch, 1978). Following initial isolation, the bacterium was reported from other areas in north-west US and Canada. It was reported in Europe in the mid-1980s and

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can now be found in Norway, Denmark, UK, France Germany, Italy, South Africa and Australia (Llewellyn, 1980; Bragg and Henton, 1986). It continues to cause problems in many areas and preventative strategies to limit disease outbreaks are often implemented.

Y. ruckeri is considered an obligate pathogen and rainbow trout are the species that is most often impacted from ERM/yersiniosis, but *Y. ruckeri* appears to be able to infect all salmonids reared in fresh water. Fish can readily become carriers and it has been isolated from wild salmonids and non-salmonid fish including Walleye (*Stizostedion vitreum vitreum*), sturgeon (A*cipenser baeri*), carp and goldfish (*Carassius auratus*) (McArdle and Dooley-Martin, 1985; Michel *et al*., 1986; Enriquez and Zamora, 1987; Vuillaume *et al*., 1987). Sources of infection have been linked to carrier fish in water sources, but interestingly, the bacterium has been isolated from non-fish hosts such as crawfish, mammals (muskrat), human, sea gulls and even from sewage and river water (Michel *et al*., 1986; Stevenson *et al*., 1993). It has been found that infected fish with no clinical signs may not necessarily transmit the infection; however, Hunter *et al*. (1980) found that if carrier steelhead with no clinical signs were stressed at 25°C, *Y. ruckeri* was transmitted.

Clinical signs

In small fry, acute infections can result in heavy losses. *Y.ruckeri* causes a septicaemic infection and the disease can manifest in a range of forms from subclinical to acute infections. Chronic forms of the disease may linger in larger fish where clinical signs include dark coloration, lethargy and blindness. Affected fish will appear weak and may congregate near the surface or edges of ponds or at the downstream end of raceways. As the disease name suggests, a common clinical sign of ERM that is often associated with subacute infections involves ulceration and haemorrhage in the oral cavity leading to reddening in the mouth, jaw and on the head. In many cases however, such signs never develop and fish may just appear dark and die without other apparent external clinical pathology. *Y. ruckeri* infections in Atlantic salmon, as described by Frerichs *et al*. (1985), did not exhibit classic reddening of the mouth or opercula. Bloody ascites, splenomegaly, inflammation in the vent area and yellowish fluid in the intestine may be common. Venous and capillary congestion of brain and blood vessels is often observed along with intestinal haemorrhaging and petechial haemorrhage in the musculature (Brown and Bruno, 2002). Bacteria may spread from the gills to other tissues, such as muscle and liver, leading to tissue oedema and focal areas of necrosis. Exophthalmia and haemorrhage of the ocular cavity may lead to rupture of the eye.

Diagnostic techniques

Diagnosis of ERM is usually made based on clinical signs and confirmed through histology and/or culture of *Y. ruckeri* from infected tissues of moribund fish. Typical isolation is on general purpose media such as TSA or BHI and colonies appear circular and non-pigmented. *Y. ruckeri* is fermentative, citrate and catalase positive, oxidase and cytochrome oxidase negative, and does not produce indole in tryptone broth (Brown and Bruno, 2002). It should be noted that variation in some biochemical tests have been reported for some isolates leading to false positives (Hastings and Bruno, 1985). Therefore, immunodiagnostic procedures utilizing specific antibodies or molecular techniques such as PCR can be incorporated for confirmatory diagnosis. It is possible to detect *Y. ruckeri* infected fish using PCR and sampling kidney tissue directly (Argenton *et al*., 1996). This can be useful for detecting carrier fish, and can even be applied non-lethally using blood samples (Altinok *et al*., 2001).

Control

Control of ERM is primarily achieved through prevention methods utilizing commercial vaccines. Vaccination is viewed as highly effective and the single most important tool for limiting impact to the industry. In areas where salmonids are impacted by ERM or yersiniosis, correct implementation of a vaccination programme should limit major disease problems. Generally, healthy fish are

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immersion vaccinated in a suspension of killed bacteria at a size of 4 g or larger and if necessary a booster immunization may follow (Larsen and Pedersen, 1997). The efficacy of such vaccines is affected by bacterium serotype, host species, weight and temperature. In some cases, immunity is longer lasting and more effective if fish can be vaccinated by injection. As with any vaccine, proper administration and fish culture practices that eliminate environmental or other infections will greatly affect efficacy.

When ERM is diagnosed and treatment considered necessary, antibiotics such as oxytetracycline, ciprofloxacin (oxolinic acid) and amoxicillin (Busch, 1983) have been shown to be effective against *Y. ruckeri*. In general, antibiotic use for treatment of ERM is not widespread (presumably due to the success of commercial vaccines) and antibiotic-resistant strains of *Y. ruckeri* have decreased over the years (Brown and Bruno, 2002).

Bacterial coldwater disease

Introduction

Rainbow trout fry syndrome (RTFS) or bacterial coldwater disease (CWD) is caused by the Gram-negative bacterium *Flavobacterium psychrophilum*. The disease was first referred to as 'peduncle disease' and described in rainbow trout displaying lesions on the caudal peduncle (Davis, 1946). The bacterium causing disease, however, was not isolated and identified until 1948 from coho salmon displaying similar lesions (Borg, 1948). The disease affects fish ranging in size from early sac fry to fingerling and production size salmonids. *F. psychrophilum* is capable of infecting many species of fish, but generally salmonids are considered the most susceptible. RTFS is often used to describe this disease when early hatched alevins are affected by *F. psychrophilum*. When fish are impacted at these early stages, mortality may be more than 50% (Holt *et al*., 1993).

There has been considerable debate about the sources of infection due to *F. psychrophilum*, especially when early life stages are affected. Although it is a common bacterium considered ubiquitous in the environment, there is growing evidence that *F. psychrophilum* can be transmitted vertically and is capable of surviving within the egg, which may influence early life stage outbreaks. *F. psychrophilum* has been isolated from salmonid milt (Kumagai and Nawata, 2011), ovarian fluid (Madsen *et al*., 2005; Chen *et al*., 2008), egg surfaces (Vatsos *et al*., 2001, 2006), and the contents of both unfertilized and eyed eggs (Brown *et al*., 1997). Although true (intra-ovum) vertical transmission of the bacterium in salmonids has not been conclusively demonstrated, many factors that affect *F. psychrophilum* survival within eggs have been investigated. In fact, Ekman *et al*. (2003) demonstrated through nanoinjection that *F. psychrophilum* can survive within the yolk of fertilized rainbow trout eggs. Furthermore, it is known that the bacterium can survive when exposed to high levels of lysozyme and there is evidence that iodophor concentrations routinely used for egg surface disinfection do not effectively kill *F. psychrophilum* (Brown *et al*., 1997).

Characterization

The pathogen causing RTFS has undergone several taxonomic changes. It was initially referred to as *Flexibacter psychrophilus* and *Cytophaga psychrophila*, but DNA–RNA hybridization studies have resulted in reclassification as *Flavobacterium psychrophilum* (Bernardet *et al*., 1996; Bader and Shotts, 1998). *F. psychrophilum* is a rod-shaped Gram-negative bacterium that does not produce fruiting bodies. Cell morphology is typical of other *Flavobacterium* sp. and cells range from 0.2–0.75 × 2–7 μm depending on the age and growth environment of the culture (Pacha, 1968; Lorenzen *et al*., 1997; Kondo *et al*., 2001; Vatsos *et al*., 2003). *F. psychrophilum* lacks pili and flagella but moves by gliding motility, which has been implicated as a potential virulence factor. LaFrentz *et al*. (2011) demonstrated that at least one *F. psychrophilum* gliding motility protein, GldN, can be highly immunogenic and hypothesized that gliding motility could aid the bacterium in gaining entry to cells or fin tissue.

The outer membrane of *F. psychrophilum* consists of proteins, LPS and a glycocalyx

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layer of varying thickness that appears to be loosely associated with the cell, expressed differentially between strains, and is immunogenic (LaFrentz *et al*., 2007). *F. psychrophilum* forms yellow-pigmented colonies, and colony morphology is often described as 'fried egg' meaning that it is a round, convex colony with a thin spreading margin. However, a number of strains do not have such colony morphology and form a convex colony with smooth edges. *F. psychrophilum* strains commonly secrete enzymes that can degrade collagen, chondroitin sulphate, gelatin and casein (Bertolini *et al*., 1994; Ostland *et al*., 2000). *F. psychrophilum* is unable to hydrolyse starch or break down simple and complex carbohydrates, and all strains appear to be catalase positive but variation in cytochrome oxidase utilization has been noted (Nematollahi, *et al*., 2003). Biochemically, *F. psychrophilum* is relatively homogenous, but many different strains or serotypes have been characterized. In general, serological characteristics have suggested linkages to different geographic regions or different host species, and strains range from highly virulent to non-virulent. Genetic characterization of isolates, however, suggests greater heterogeneity and less correlation between *F. psychrophilum* and host specificity making it difficult to develop a standardized classification system.

Impact

Most salmonid-producing areas are affected by RTFS. *F. psychrophilum* has been isolated from fish in the United States and Canada, Chile, Japan, Korea, Australia, and is widespread throughout Europe from a wide range of salmonid and non-salmonid species. However, disease outbreaks most notably affect salmonids. Important species such as coho salmon along with rainbow and steelhead trout are considered most susceptibile, but many other important species, including Atlantic salmon (Valdebenito and Avendaño-Herrera, 2009), are impacted. Mortality in susceptible species can range from 20% to 90% (Bruno, 1992), and over the past two decades, reports of RTFS impacts have increased. In the USA this may reflect adaptations to higher temperatures (> 10°C) for some *F. psychrophilum* strains and increased resistance to commonly applied antibiotics. RTFS occurs in freshwater cage culture of salmonids, but the greatest impacts have been noted in commercial trout culture utilizing flow through raceway systems. Impacts result not only from mortalities during an outbreak but from poor performance and increased deformities in survivors that reduce product market value of food fish. Another aquaculture sector that is heavily impacted is the public steelhead and salmon mitigation hatcheries in the northwest USA where RTFS causes greater overall losses than any other fish disease (J. Varney, personal communication).

Clinical signs

Clinical signs of *F. psychrophilum* infections can vary. In general, if RTFS manifests as an acute septicaemic infection, mortality may be high with limited external clinical signs. If coho salmon are infected during the fry stage, erosion of the epithelial layer covering the yolk sac may be evident due to septicaemia (Holt, 1988). As fish increase to fingerling size and older, erosion of the caudal peduncle region may be observed, but this clinical sign may not be apparent. Infected fish may exhibit spiral swimming behaviour, go off feed, have frayed fins, pale gills, exophthalmia, haemorrhage and dark pigmentation in the caudal region. Internally, splenomegaly is common along with ascites and general septicaemia as bacteria infiltrate into internal organs. Fish that survive outbreaks of RTFS often show poor performance and may have a range of deformities including lordosis, scoliosis and posterior spinal compression (Fig. 3.3) affecting the caudal region (Conrad and DeCew, 1967; Madsen *et al*., 2001). In Japan, ayu (*Plecoglossus altivelis*) are affected with clinical signs including ulcerative lesions in the caudal region and lower jaw, anaemia and haemorrhaging (Miwa and Nakayasu, 2005).

Microscopic examination can reveal long, thin Gram-negative bacteria from externally affected areas. Following sampling of moribund fish, imprints and/or histological sections from the spleen and other organs including the liver and kidney, often show presence of long filamentous rods (Fig. 3.4).

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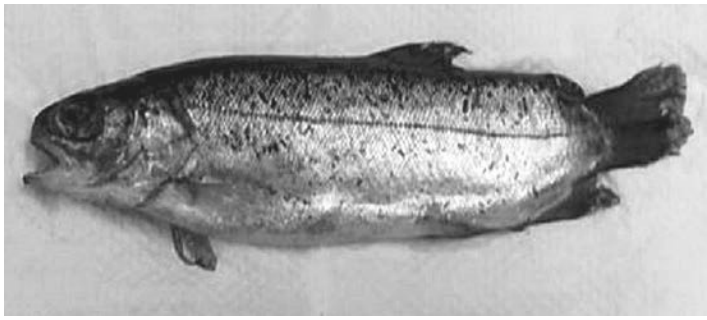


Fig. 3.3. Posterior spinal compression present in rainbow trout surviving outbreak of RTFS. Image courtesy of S.L. LaPatra, Spring Clear Foods.



Fig. 3.4. Long filamentous rod of *Flavobacterium psychrophilum*.

The liver can show vascular degeneration and necrotic hepatocytes, but *F. psychrophilum* can be easily observed in spleen or kidney imprints from heavily infected fish.

Diagnostic techniques

Diagnosis is often based on clinical signs followed by isolation of yellow-pigmented bacteria characteristic of *F. psychrophilum*. However, yellow-pigmented bacteria are common and it is important to implement confirmatory tests to identify *F. psychrophilum* as the causative agent of RTFS. This is usually accomplished through culture combined with other biochemical, molecular or serological methods to specifically identify *F. psychrophilum*. Spleen, kidney or other organs such as the brain are sampled and plated on the appropriate agar media. *F. psychrophilum* is a slow growing aerobic bacterium that is generally incubated at 15–18°C. Yellow-pigmented colonies usually appear within 4–7 days. Similar to other *Flavobacterium* species, *F. psychrophilum* requires low-nutrient media for successful culture. One of the most common is tryptone-yeast extract-salts (TYES) media, but others such as Cytophaga media, Shjieh agar, Anacker & Ordal, and Hsu-Shotts have been used (Cain and LaFrentz, 2007).

Biochemical confirmation of *F. psychrophilum* is not always reliable. Therefore, definitive diagnosis should utilize serological or PCR based assays. *F. psychrophilum* can be confirmed from culture or from infected fish tissues such as the kidney, using an enzyme-linked immunosorbent assay or fluorescent antibody test. Lindstrom *et al*. (2009) recently developed a highly specific monoclonal antibody (FL43) against the outer membrane fraction of the *F. psychrophilum* for routine detection or broodstock screening using an ELISA and FAT. Assay protocols are available and FL43 has been produced for commercial availability. Molecular assays based on PCR are also available, and a nested PCR that can be used on culture, tissues or reproductive fluids is highly sensitive (Taylor, 2004).

Control

Control of RTFS is achieved through proper culture and management techniques aimed at reducing stress, promoting strict biosecurity and maintaining high water quality. If an outbreak occurs, several options are available. Removing all dead fish is important to limit the spread of *F. psychrophilum* as it is shed into

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the water column. Immersion bath treatments using salt, antibiotics (water-soluble oxytetracycline) or potassium permanganate have been shown to be effective in some cases, but must be administered before fin or caudal erosion is evident (Cipriano and Holt, 2005). Once an outbreak is confirmed, the most effective treatment for RTFS involves antibiotic administration through the feed. In the USA, there are currently two antibiotics licensed for use against *F. psychrophilum* in freshwater reared salmonids: oxytetracycline dihydrate and florfenicol. Florfenicol was recently approved and requires veterinary approval prior to feed incorporation and sale. Although antibiotic treatment is effective, there is always concern that resistance strains of *F. psychrophilum* may develop. The occurrence of strains resistance to oxytetracycline is well documented in a number of countries. Cases of *F. psychrophilum* strains acquiring resistance to florfenicol have not been reported; however, minimum inhibitory concentration of florfenicol has been documented to be higher than average for isolates at some farms (del Cerro *et al*., 2010; Hesami *et al*., 2010; Henríquez-Núñez *et al*., 2012).

Ideally, preventative methods such as vaccination would be preferred for RTFS and would limit the risk of antibiotic resistant strains. Early work focused on killed whole cell vaccine formulations, but limited success was achieved in the absence of an adjuvant (LaFrentz *et al*., 2002; Rahman *et al*., 2003). Recent work has led to the development of live-attenuated *F. psychrophilum* strains that when used as vaccines are capable of conferring protection in fish. LaFrentz *et al*. (2008) developed such a strain using a rifampicin resistance strategy and showed that fish immunized by injection or immersion were significantly protected from *F. psychrophilum* infection. Field trials on rainbow trout (1 g initial weight) immersion immunized have shown the vaccine to be safe and provide protection from natural outbreaks of RTFS (Cain, unpublished data).

Promising alternative control methods have been suggested, and one option may be to incorporate naturally occurring gut bacteria as probiotics in the feed. Burbank *et al*. (2011) isolated two *Enterobacter* strains from the intestinal tract of healthy fish that could inhibit *F. psychrophilum* growth *in vitro*. When mixed into a commercial feed and fed to rainbow trout they were capable of significantly reducing mortality following disease challenge. Other management-based approaches to control RTFS may need to focus on reducing overall bacterial prevalence at facilities and reducing the risk of vertical transmission by screening broodstock and culling eggs originating from heavily infected broodstock.

Bacterial kidney disease

Introduction

Bacterial kidney disease (BKD) is a systemic disease that originates in fresh water, but can affect salmonids during both freshwater and seawater phases of their lifecycle. It is caused by the Gram-positive bacterium *Renibacterium salmoninarum*, and was first described in Scotland in 1930, where it was documented in Atlantic salmon from the Dee and Spey rivers (Mackie *et al*., 1933). With the exception of a few salmonid producing countries such as Australia and New Zealand, BKD is widespread. It is considered an obligate pathogen of fish in the family *salmonidae*. It is generally found in trout and salmon (subfamily *Salmoninae*); however, Faisal *et al*. (2010a) isolated *R. salmoninarum* from wild populations of whitefish, *Coregonus* spp. (subfamily *Coregoninae*) in the Great Lakes, USA. It often occurs as a slow chronic infection that results in serious losses at times of physiological stress, such as smoltification. Although *R. salmoninarum* can be transmitted horizontally from fish to fish, intra-ovum vertical transmission plays a major role and is important to consider for disease management of an exposed stock (Bruno and Munro, 1986; Evelyn *et al*., 1990).

Characterization

R. salmoninarum, was first characterized as a coryneform bacterium that was a strongly Gram-positive, non-motile rod measuring 0.3–1.0 × 1.0–1.5 μm that might occur in pairs or V formations (Sanders and Fryer, 1980). It is extremely fastidious with a strict requirement for L–cysteine in growth media (Evelyn *et al*., 1990).

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It is acid-fast, non-sporulating, and periodic acid Schiffs (PAS) positive. Growth in culture is slow and primary isolation may take from 8–12 weeks to produce white to yellowish circular colonies at 15°C (Brown and Bruno, 2002). Improved methods of culturing *R. salmoninarum* have been developed (Evelyn *et al*., 1990; McIntosh *et al*., 1997), and Faisal *et al*. (2010b) developed an improved culture method that results in colony growth within 5–7 days. In general, serologic (ELISA and FAT) and PCR-based molecular assays have become important for detection and quantification of infection levels in fish, and are widely utilized for diagnostic and management purposes (Roberts, 2012).

Impact

Juvenile salmonids are severely impacted by BKD, but in many cases significant mortality can occur in adults. BKD is a concern in freshwater and marine cage culture. The disease has been documented as causing significant losses in wild Pacific salmonids (Kent *et al*., 1998), linked to large epizootics of Chinook salmon in the Great Lakes, USA (Holey *et al*., 1998), and has been identified in wild Atlantic salmon in north-eastern USA (Smith, 1964). In North America, losses in Pacific salmon stocks have reached as high as 80% historically (Evenden *et al*., 1993). Prevalence of BKD in such stocks has been attributed to the mode of transmission and the persistence of *R. salmoninarum* in the egg following iodophor disinfection. This emphasizes the critical need to establish *R. salmoninarum*-free broodstock whenever possible. If *R. salmoninarum* is endemic in a region, epizootics may occur seasonally when stressors such as increasing or declining water temperatures occur.

Disease severity may also be influenced by water quality, and Warren (1963) suggested that hatcheries with soft water had greater incidences of BKD when compared to hatcheries with high total hardness. Smolts transferred from fresh to sea water are particularly susceptible to BKD; for example, *R. salmoninarum* infected coho salmon smolts held in sea water experienced 17% mortality over 150 days compared to 4% for siblings held in fresh water (Fryer and Sanders, 1981).

Clinical signs

A range of clinical signs have been reported for BKD which include darkening of fish, exophthalmia and lethargy. Spawning salmon may exhibit haemorrhaging at the base of fins, and in farmed trout, petechial haemorrhaging or raised vesicles may be found on the side or lateral line of fish. Upon necropsy, classic signs that include white-grey granulomatous lesions may be observed in the kidney of affected fish. Other internal signs include bloody ascites and enlargement of the kidney. Granulomatous lesions are common for BKD and may be found in organs such as the heart, liver, spleen, kidney and even the gill and muscle of affected fish (Bruno, 1986).

Histological characterization of BKD often focuses on lesion development in the kidney and other organs. Necrosis of tissues occurs and extends to areas between kidney tubules where granulomas containing *R. salmoninarum* may be observed along with leucocytes and other cellular debris. Bacteria can often be observed in organs and tissues and macrophage proliferation may be evident. *R. salmoninarum* is able to survive in macrophages, which represents a potential mechanism to avoid the immune response (Bruno, 1986; Grayson *et al*., 2002). In the kidney, the bacterium may also be observed within endothelial cells lining the glomerular blood vessels as well as the lumen of collecting ducts, but is typically not observed within the proximal tubules (Brown and Bruno, 2002). Bruno (1986) noted that the nuclei of endothelial cells are diffusely stained, slightly cloudy and may contain bacteria.

Diagnostic techniques

For most bacterial pathogens culture is the primary method for identification and diagnostics. However, this is not typically the case for *R. salmoninarum*. Although culture is sensitive, it has not been a primary diagnostic tool due to the slow growth and impractical application when treatment or management decisions need to be implemented. This may change with improvements of culture methods; however, histology, immnoassays such as FATs and ELISAs will continue to be important tools for

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diagnosing *R. salmoninarum*. In North America, broodstock populations of farmed and wild fish (returning for stock enhancement or mitigation programmes) are screened using a commercially available ELISA and procedures have been standardized among many diagnostic laboratories. Until recently, the ELISA was the most sensitive assay available to screen tissue samples for *R. salmoninarum*; however, quantitative PCR assays have been developed (Powell *et al*., 2005) and their use may become routine in the future. Each assay has limitations when confirmatory diagnosis is required. For example, the ELISA and FAT are most often carried out on kidney and ovarian fluid, respectively. These assays rely on polyclonal (and sometimes monoclonal) antibodies that are most often directed against a soluble antigen (p57) of the bacterium. Such antibodies have been shown to cross-react with other bacterial species (Brown *et al*., 1995) and, due to the soluble nature of p57, may react to antigen in the tissue even in the absence of an active infection. PCR methods developed to the gene encoding p57 or other genes also have potential problems in that they are detecting bacterial DNA and again may not effectively report the presence of viable bacteria.

Control

The ideal method of controlling BKD would be to limit exposure of fish to *R. salmoninarum*. Care should be taken if utilizing water sources containing wild fish stocks, or whenever fish are introduced into a farm or hatchery. In areas where the pathogen is endemic, outbreaks may occur. Antibiotic therapy can, in some cases, limit the severity of an outbreak but is typically not satisfactory and may require long term treatment.

Interestingly, prophylactic feeding of erythromycin to juvenile fish along with antibiotic injection in broodstock has been reported in some hatchery programmes as a management strategy for BKD in Pacific salmon. However, this strategy does not effectively limit outbreaks of BKD, and only when management strategies incorporated a culling and segregation programme based on screening broodstock for high levels of *R. salmoninarum* antigen did such a programme achieve success (Munson, *et al*., 2010). Such screening programmes utilize a polyclonal ELISA against the heat-stable, soluble p57 antigen of *R. salmoninarum* (Pascho and Mulcahy, 1987) to evaluate antigen levels in kidney tissue samples. The great success of such programmes is due to the ability to quantify infection levels in broodstock based on ELISA optical density (OD) values. By standardizing infection levels based on 'high', 'medium' or 'low' OD values, managers can cull eggs from heavily infected fish or segregate progeny from infected adults if stocks are highly valuable. Such a strategy could be applied to any population where broodstock are regularly detected with *R. salmoninarum*. Indeed, all fish or eggs entering a facility should be tested for the presence of *R. salmoninarum* and if detected those stock should not be used unless absolutely necessary.

Development of a BKD vaccine has only met with modest success. There is, however, at least one commercial vaccine. This vaccine 'Renogen' is a live formulation that consists of a closely related soil bacterium, *Arthrobacter davidanieli*, which elicits the production of cross-reactive antibodies to *R. salmoninarum* following immunization (Griffiths *et al*., 1998). In field trials, this vaccine elicited significant protection against BKD in Atlantic salmon and is most effective when administered by injection (Salonius *et al*., 2005). However, Alcorn *et al*. (2005) reported a lack of protective immunity in Chinook salmon following administration of this vaccine along with five experimental vaccines. Such results suggest that further work is required in the area of BKD vaccines and species specific responses must be considered.

Bacterial diseases with secondary impact on coldwater fish reared in fresh water

Salmonid rickettsial septicaemia

Salmonid rickettsial septicaemia (piscirickettsiosis), caused by *Piscirickettsia salmonis* is primarily a problem in fish reared in sea water, but it has been reported in fresh water and is briefly mentioned here. Lannan and Fryer (1994) showed that *P. salmonis* lost its viability immediately when exposed to fresh water,

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which likely explains the lack of occurrence in fresh water. However, coho salmon and rainbow trout developed piscirickettsiosis when held in fresh water between 60–90 days of age and it was noted that mortality occurred between 6–12 weeks of age following transfer to seawater cages (Gaggero *et al*., 1995). Further aspects of this disease are covered in Chapter 2.

Columnaris

Although columnaris, caused by *Flavobacterium columnare* is covered in Chapter 6, it should be recognized that this disease is a major and potentially emerging problem in salmonid aquaculture. In Finland, *F. columnare* impacts rainbow trout production and is considered one of the most important bacterial pathogens (Suomalainen *et al*., 2005). In Chile, columnaris outbreaks have occurred and it appears to be emerging in coho salmon and rainbow trout aquaculture as the industry expands (Alvendño-Herrera *et al*., 2011). In the USA, incidences of *F. columnare* infections have recently increased in commercial rainbow trout production facilities (LaFrentz *et al*., 2012). Furthermore, columnaris is known to affect adult wild and hatchery reared salmon migrating through large rivers such as the Columbia and Fraser River in North America. The risk is especially relevant to freshwater cage culture operations if established on these systems or similar large rivers with seasonal changes in water temperatures. In such cage culture and other intensive operations, columnaris may become an external problem creating a situation leading to severe gill disease and secondary problems (Fig. 3.5), or may manifest systemically as evidenced by other classical clinical signs such as 'saddleback' lesions (Declercq *et al*., 2013).



Fig. 3.5. Columnaris associated gill disease of cage-cultured rainbow trout with secondary oomycete involvement. Image courtesy of J. Bielka, Pacific Seafoods.

Diseases Caused by Fungal/ Psuedofungal Pathogens

Saprolegniasis

Introduction

Saprolegnia is a genus of freshwater mould that is a ubiquitous part of most aquatic environments. Although *Saprolegnia* can tolerate a wide temperature range (0°C to 35°C), cold (3–15°C) conditions are preferred and where disease manifestations become most apparent in aquaculture (van West, 2006). Generally, *Saprolegnia* are saprotrophic organisms, feeding on dead and decaying organic matter. However, they can often become opportunistic pathogens when organic debris or necrotic tissue provides a foundation from which growing filaments or zoospores can easily spread to living tissue. In some instances, specific strains or species are also believed to directly target living hosts during sporulation (Willoughby and Pickering, 1977). Given the right environmental conditions, it is likely that any fish species would be susceptible to infection by this pathogen at any life stage; however, the most severe infections in culture environment often have occurred during egg incubation, larval rearing and in post spawning adult salmon broodstock. Saprolegniosis is ultimately fatal for both eggs and fish if left untreated, and is a serious and widespread concern in freshwater fish culture.

Characterization

Saprolegnia belong to a class (Oomycota) of 'fungal-like' organisms that exhibit many characteristics, such as filamentous hyphae, sporulation and utilization of saprotrophic nutrition by extracellular digestion, that are similar to that of many fungi. Indeed, the 'cotton mould' appearance of growing *Saprolegnia* filaments is visually quite similar to that of many terrestrial fungal moulds. However, a diploid life stage, cellulose cell-walls and

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non-septate filaments taxonomically separate *Saprolegnia* from true fungi and classify them more closely with other heterokonts such as diatoms and brown algae. Phylogenetic comparisons also support this classification with protest heterokonts rather than with fungi (Guerriero *et al*., 2010). The life cycle includes both sexual and asexual reproductive phases. In the asexual phase, a spore or sporangium is formed at the end of hyphae which release motile zoospores (Bruno *et al*., 2011). These primary zoospores swim (via an apical flagellum) for a short time before they encyst (become dormant), and then release a secondary zoospore. Secondary zoospores are motile (via a lateral flagellum) for a longer period than primary zoospores and are considered the main dispersion and infective form of *Saprolegnia* (Willoughby, 1994). The secondary spore can also release new zoospores and the repeated cycles of encystment and motile zoospores (called polyplanetism) can allow for the organism to survive and persist in the environment for long periods (Beakes, 1982). The secondary zoospores of some *Saprolegnia* species also possess hairs, and with many species (or strains within a species) the hairs are hooked to, presumably, facilitate attachment to a living host (Beakes, 1982; Burr and Beakes, 1994; Grandes *et al*., 2000).

Impact

A practical assumption is that any freshwater fish may be opportunistically parasitized by *Saprolegnia* within its tolerated temperature rage (approximately 2–35°C). At least three species of *Saprolegnia* are known to infect either fish or fish eggs (*S. parasitica*, S. *diclina* and *S. ferax*) and infectivity by additional species is probable. *S. parasitica* is often viewed to be the species of most concern and estimated costs during the aquaculture of salmon and catfish is in the tens of millions of dollars annually (van West, 2006). However, this appears to be a matter of context as both *S. diclina* and *S. ferax* have been shown to be more pathogenic during egg incubation of Atlantic salmon eggs than *S. parasitica* (Thoen *et al*., 2011), which more likely targets adult organisms rather than eggs. A financial value is harder to define regarding loss during eggs and larvae production, but in developing culture of burbot (*Lota lota*), mortality of both eggs and larvae has approached 100% without administration of chemical prophylactics (Polinski *et al*., 2010).

Clinical signs

Infection is characterized by filamentous 'cotton-like' tufts that appear on the external surface of fish and eggs. For fish, infection will often initiate around the site of previous injury and radiate out in a circular, crescent or whorled pattern. Environmental stresses and previous disease infection are predisposing factors that enhance the likelihood of infection with *Saprolegnia*.

Diagnostic techniques

Visualization of cotton-like tufts can be followed by microscopic observation to identify non-septate filamentous hyphae for presumptive diagnosis (Fig. 3.6). Low-nutrient culture media can also be used for continued observation of newly emerging hyphae for sexual differentiation and definitive diagnosis (Stueland *et al*., 2005). Species confirmation by microscopy is somewhat difficult, as it relies on morphological identification of sexual structures (Wood and Willoughby, 1986). Phylogenetic sequencing is becoming increasingly available for species and strains which can be utilized for PCR identification (Thoen *et al*., 2011), but as yet not all species can be differentiated

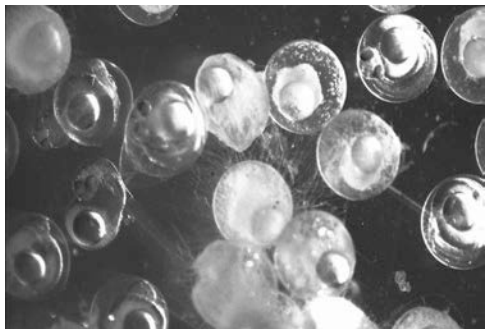


Fig. 3.6. *Saprolegnia* colonizing the eggs of burbot during late stage embryo development. Hyphae can be seen extending from dead eggs to engulf adjacent live embryos.

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by this method. Nevertheless, with specific regard to the culture of fish, *Saprolegnia* species identification is fairly inconsequential as both treatment and preventative measures are not species-specific.

Control

The ubiquitous nature of *Saprolegnia* generally precludes the ability for complete avoidance in coldwater fish culture. As previously stated, minimizing environmental stress, physical injury and external pathogenic diseases can aid in preventing initial infection of fish. During egg and larval rearing it is important to promptly remove detritus such as dead egg casings, faeces and excess feed so as to eliminate the preferred food source of this opportunistic pathogen. If infection occurs, or if pre-emptive measures are desired, chemical therapeutics can be used to effectively eliminate or prevent infection. Malachite green was historically used for treatment of this disease with excellent success but is currently banned in most of the world due to its carcinogenic and toxicological effects (van West, 2006). Other compounds found to inhibit the growth of *Saprolegnia* include sodium chloride, formalin and hydrogen peroxide, of which hydrogen peroxide has become the chemical of choice for most situations due to its lower environmental and human handling effects relative to formalin, and the large quantities (> 30 g per l) of sodium chloride required for effective control (Marking *et al*., 1994). Hydrogen peroxide has been applied successfully at 250–500 mg per l as 15 minute daily treatments during egg incubation of multiple coldwater species (Barnes and Stephenson, 2003; Barnes and Soupir, 2006; Soupir and Barnes, 2006; Polinski *et al*., 2010), and shows continued effectiveness when administered during fry and larval development at 50–250 mg per l by 1 h immersion every other day (Rach *et al*., 1997; Gaikowski *et al*., 1998; Gaikowski *et al*., 1999; Polinski *et al*., 2012). For adult salmonids, 50–100 mg per l for 60 min exposure may be used (FDA, 2007), although treatment below 100 mg per l may not be sufficient to adequately control an established infection (Marking *et al*., 1994).

Microsporidiosis

Introduction

Microsporidia are obligate intracellular parasites. They have previously been classified as Protists but are now considered to be a unique phylum of fungi. Currently, approximately 1200 species are known to parasitize a wide variety of host species including humans and Protists (Lom, 2002). The majority of microsporidians infect arthropods; however, more than 150 species are known to parasitize fish in freshwater, marine and euryhaline environments (Lom, 2002; Lom and Nilsen, 2003). With regard to the freshwater culture of coldwater fish, three species of microsporidia (*Nucleospora salmonis*, *Loma salmonae* and *Kabatana takedai*) infect, and cause disease and mortality in salmonid hosts. Nevertheless, new species of microsporidium continue to be identified, and as more fish species are farmed, there is an increased likelihood of novel and/or previously inconsequential microsporidium involved in disease outbreaks. Here we describe some general characteristics of microsporidium infection, and its known consequences regarding freshwater salmonid culture.

Characterization

Microsporidia are now considered to be a unique phylum of eukaryotic fungi which appear to have lost several genes in the evolutionary process and have undergone considerable gene compaction which has been attributed to their growing adaptation to intracellular parasitism (Keeling and Fast, 2002). Lower order classification continues to be tenuous due to the great diversity in morphology and life cycle strategies within this taxon, even within phylogenetically similar species (Franzen, 2008). Still, phylogenetic characterization is proving to be the most consistent form of classification and its importance in ordering and identifying these organisms will likely continue to expand in future (Issi *et al*., 2012). All microsporidia are unified by a few encompassing characteristics; namely, that they exist only as spores outside a host cell and invasion of

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a host involves inoculation of the infective sporoplasm directly into the cytoplasm of a host cell through the use of a polar tubule. Once inside a host cell, the parasite either causes serious degradation of the cytoplasm (e.g. *K. takedai*), and/or elicits host cell hypertrophy, leading to the production of a parasite-hypertrophic host cell complex known as a xenoma (e.g. *L. salmonae*) (Lom and Nilsen, 2003; Lom, 2005). In the unique case of the *Nucleospora* (including *N. salmonis*), the merogonic (or asexual proliferative phase) is carried out in the host cell nucleus, whereas all other known microsporidia of fish conduct this phase in or adjacent to the cytoplasm (Lom and Nilsen, 2003). Comprehensive overviews of the life cycle, taxonomy and host cell interactions concerning microsporidium in fish have been reviewed elsewhere (Lom, 2002; Lom and Nilsen, 2003; Lom, 2005; Franzen, 2008).

Impact

The microsporidian species *N. salmonis*, *L. salmonae* and *K. takedai* have long been known to cause severe disease in salmonids (Putz *et al*., 1965; Awakura, 1974; Hedrick *et al*., 1990) with high fish mortality. Additionally, *L. fontinalis* causes xeonma formation in brook trout (Morrison and Sprague, 1983), although its distribution, host preference and ability to cause mortality appear to be limited. The susceptible host species, geographic distribution and tissue tropism of these organisms are summarized in Table 3.1.

Clinical signs

The pathology in fish is species dependent. For *L. salmonae* and *L. fontinalis*, large white xenoma cysts may be visible on gill tissue (Bruno *et al*., 1995). *K. takedia* caused spindle-like cysts to form in trunk and heart muscle which lack xenoma walls or other distinct boundaries. A loss in condition may be observed in infected fish, and mortality can occur in severe cases. *N. salmonis* infection of haematopoeitic cells of the kidney induces anaemia and a leukemia-like state with generalized signs including darkening of the skin, anaemia and sluggish behaviour typical of many systemic pathogens.

Diagnostic techniques

Both electron and light microscopy of fixed tissues have been used to confirm the presence of microsporidia (Lom, 2002; Lom and Nilsen, 2003; Franzen, 2008). However, without excessive pathology or enlarged xenomas microscopic identification can be difficult. Currently molecular techniques have been developed to identify microsporidial-specific DNA (Issi *et al*., 2010; Abdel-Ghaffar *et al*., 2012). Additionally, species-specific PCR (Barlough *et al*., 1995; Docker *et al*., 1997) and LAMP

Table 3.1. Distribution, host specificity and tissue tropism concerning microsporidians *Nucleospora salmonis*, *Loma fontinalis*, *Loma salmonae* and *Kabatana takedia*. Data sourced from Lom (2002).

Species	Host specificity	Distribution	Tissue tropism
Kabatana takedai	Rainbow trout (*Oncorhynchus mykiss*); *O. masou*; *O. keta*; *O. nerka*; *O. tschawytscha*; *O. gorbuscha*; *Salvelinus leucomaenis*; *S. malma*; *Salmo trutta*	Hokkaido Island, Japan; Sakhalin Peninsula, Russia	Heart muscle; trunk muscle
Loma fontinalis	*Salvelinus fontinalis*	Nova Scotia, Canada	Gill lamellae
Loma salmonae	*Oncorhynchus mykiss*; *O. gorbuscha*; *O. kisutch*; *O. masou*; *O. nerka*; *O. tshawytscha*; *Salmo trutta*	North America; Japan; Europe	Endothelial cells in gill filaments
Nucleospora salmonis	*Oncorhynchus tschawytscha*; *O. mykiss*	Pacific coast of North America	Nuclei of haematopoetic cells in kidney

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(Sakai *et al*., 2009) nucleic acid amplification protocols have also been developed.

Control

There are no chemical therapeutants available for treatment of a microsporidium infection. Multiple chemical therapeutics have been tried, but have met with marginal success (Dykova, 2006). However, indirect enhancement of the host immune response through the use of β-glucans has some benefits in preventing and reducing the spread of infection of *L. salmonae* (Guselle *et al*., 2007).

Diseases Caused by Parasitic Pathogens

Proliferative kidney disease

Introduction

Proliferative kidney disease (PKD) is caused by the extrasporogonic stage of a Myxozoa parasite, *Tetracapsuloides bryosalmonae* (Canning *et al*., 2000) and affects salmonids in fresh water. The disease was named by Roberts and Shepherd (1974) due to the clinical characteristics of the disease in the kidney and spleen. It is a primary problem in rainbow trout and the disease-causing organism was originally referred to as PKX (Kent and Hedrick, 1985). The life cycle of this parasite was described by Canning *et al*. (1999), who confirmed the alternate host and was able to successfully transmit *T. bryosalmonae* from infected bryozoans to rainbow trout. Anderson *et al*. (1999) also confirmed that 18S rDNA sequences from PKX databanks were similar to those identified from freshwater bryozoans.

Characterization

Spores of *T. bryosalmonae* released by freshwater bryozoans penetrate the skin of fish to cause infection. Once in the fish host, these spores are thought to proliferate rapidly and primarily migrate to the kidney and spleen but also reach other internal organs (Roberts, 2012). The extrasporogonic stage is often identified in the kidney, appears as large cells (≥ 20 μm), and may be seen in stained tissue sections or imprints (Brown and Bruno, 2002). The disease causes diffuse and chronic inflammation of the haematopoietic tissue, and the organism is surrounded by inflammatory cells. Later stages of the infection result in formulation of granulomatous tissue and sporoblasts of the parasite in the lumen and walls of kidney tubules. PKX cells are often observed in affected tissue and organs, and have even been found in the gills of fish.

Impact

PKD is often associated with seasonal temperature changes in hatchery-reared salmonids, particularly rainbow trout. PKD in salmonid was first described and recognized in North America following a disease outbreak at the Hagerman State Fish Hatchery in Idaho in 1981 (Smith *et al*., 1984). In Europe and the British Isles it has been present for many years and is a major disease that affects rainbow trout production. France and Italy have been impacted heavily (Ferguson and Ball, 1979), and on some farms, 100% of the fish have been affected. Both wild and farmed salmonids, including grayling (*Thymallus thymallus*) (Wahli *et al*., 2002) and Arctic char (*Salvelinus alpinus*), (Kent and Hedrick, 1985) can be infected, and if water sources containing infected bryozoans are used for fish culture, then such operations would be at risk. Although PKD is a problem in Europe and North America, evidence based on phlyogeographic studies suggests that spread of the parasite is not typical of other Myxozoa and it appears that fish may be dead-end hosts for this parasite (Henderson and Okamura, 2004).

Clinical signs

Clinical signs of fish with PKD vary but, typically, they have distended abdomens accompanied by longitudinal swelling at the lateral line, dark coloration, exophthalmia, pale gills and apparent respiratory distress in moribund fish. Fish may be anaemic and show nervous disorders and loss of equilibrium in the water column. Swelling of the kidney is common and may be accompanied by grey bulbous ridges most often near the posterior of this organ. The spleen and liver may be

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affected and abdominal swelling due to excess ascites fluid in the peritoneum is often apparent (Roberts, 2012).

Diagnostic techniques

Clinical and gross pathological signs can aid in diagnosis of PKD if disease history is well documented on farms; however, definitive diagnosis requires recognition of lesions along with examination of kidney and/or other organs to identify *T. bryosalmonae* spores in tissue sections or organ imprints. A variety of stains can enhance identification and monoclonal antibodies specific for *T. bryosalmonae* are available for immunological confirmation (Adams *et al*., 1992). Additionally, Castagnaro *et al*. (1991) discovered a lectin that could enhance diagnostics by effectively binding to the PKX organism. Histological examination may reveal spores surrounded by macrophages or other phagocytes. However, definitive confirmation of PKD may require molecular techniques such as PCR or serological assays incorporating specific antibody based reagents.

Control

Effective control methods for PKD are limited and no commercial vaccine is available. It is known that water temperature can change the dynamics of the infection in both the bryozoan and the fish host. Although decreasing water temperatures can limit the effects of PKD, Ferguson (1981) found that prolonged holding of juvenile fish at temperatures higher than 15°C could also minimize the disease. Even after recovery from PKD a portion of the population may still remain chronically infected and show clinical signs of disease. Attempts have been made to control PKD using malachite green, fumagillin and its synthetic analogue TNP-470 (Morris *et al*., 2003). Some efficacy has been suggested but toxicity and potential environmental risks can be a concern and have limited use of such treatments. Vaccine development may be feasible but limited information or success has been reported in the literature.

Proper fish culture practices that limit stress and maintain good water quality are important to limit effects of PKD. If possible, lowering summer water temperatures or keeping fish on pathogen-free water sources until they are fully immunocompetent is effective and should be considered in areas where *T. bryosalmonae* is present. A preferred option would be to control bryozoans or limit spore release from them; however, this has not been successful due to the number of spores that can be released from only small colonies of bryozoans.

Whirling Disease

Introduction

Whirling disease is caused by *Myxobolus cerebralis* and is a widespread parasitic infection originating in Europe where it was first reported in 1893 (Hofer, 1903). The parasite has a complex life cycle and infects fish primarily during early life stages. In the USA, whirling disease was first diagnosed in 1958 (Hoffman, 1990) and was suspected to be introduced via movement of fish from Europe. Cultured fish can be impacted if the infectious triactinomyxon (TAM) stage is present in the rearing water. Early incidences in cultured fish were associated with earthen pond rearing where the intermediate host (an oligochaete worm *Tubifex tubifex*) was present and allowed the pathogen to perpetuate. Such observations spurred a move away from earthen pond culture to concrete raceways, which interrupted the life cycle of the parasite. Concern over the disease in cultured fish declined, but in the 1980s it was quickly realized that the parasite could infect and cause disease in wild fish (Nehring and Walker, 1996) and once established could not be eliminated from a water body. Dramatic population declines were documented in wild rainbow trout populations in the Madison River in Montana and the Colorado River in Colorado, USA (Walker and Nehring, 1995). Currently, *M. cerebralis* is well-established in many river systems and is widespread across the western USA.

M. cerebralis has an affinity for cartilage in the head and spores develop to cause skeletal lesions. Spores may impact the nerves

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resulting in blackening of the caudal region in fish (Fig. 3.7), and can cause improper development of the spine and cranium leading to a range of deformities. The quantity of TAMs during exposure and the life stage of fish often determine the severity of disease. In alevins, exposure to high doses of the infectious stage can result in 100% mortality, whereas fish exposed at later stages may show limited infection rates and no mortality (Markiw, 1991). This is thought to relate primarily to the level of bone ossification as fish get older, which impacts the parasite's ability to destroy developing cartilage.

Characterization

M. cerebralis has a two-host life cycle (Fig. 3.8) involving an aquatic oligochaete worm that ingests mature spores following death of the salmonid host. The life cycle is initiated with release of TAMs into the water column from an infected *T. tubifex* and they find a susceptible host (El-Matbouli *et al*., 1999). TAMs are infective and each TAM has four appendages. Three of these appendages measure between 170–200 μm (Markiw, 1992) and the fourth measures approximately 140 μm. The fourth appendage contains the epispore that has a minimum of 64 sporoplasms within it (El-Matbouli *et al*., 1995). Once the TAMs contact fins, skin, gills, oesophagus or the digestive tract in fish, they penetrate and release sporoplasms into the epidermis (Markiw, 1989; El-Matbouli *et al*., 1995). These sporoplasms have a tropism for cartilage and upon release they migrate via peripheral nerves and the central nervous system to host cartilage (El-Matbouli *et al*., 1995, 1999; MacConnell and Vincent, 2002). It takes time for myxospores to develop in the cartilage, and at approximately 860 degree days (measured as Celsius temperature units (CTUs)) they can be observed in tissue of infected fish (Hedrick and El-Matbouli, 2002). Myxospores are 8–10 μm in diameter and have two polar capsules (Markiw, 1992). Once the fish host dies and decays or is consumed and excreted by pisciverous predators, myxospores settle out to the sediment and are ingested by *T. tubifex* where they then multiply within the intestine. Prior to ingestion by *T. tubifex*, spores are extremely tough and can remain viable in the environment for many years. Following ingestion, TAMs are released by death of the worm or intermittent egestion, which can occur for greater than 12 months (El-Matbouli *et al*., 1995; El-Matbouli and Hoffmann, 1998).

Impact

Water source for a cage culture operation is of great concern and cages must not be located where *M. cerebralis* infected fish are present. Movement of infected fish is often highly restricted. If infected fish are detected in a facility, quarantine and depopulation may be required. If an outbreak of whirling disease occurs, heavy impacts resulting in high mortalities or deformed unmarketable survivors can result. In most cases, whirling disease is considered a chronic infection in fry and fingerlings (El-Matbouli *et al*., 1995)



Fig. 3.7. Characteristic black tail often associated with clinical signs of *Myxobolus cerebralis* infection.

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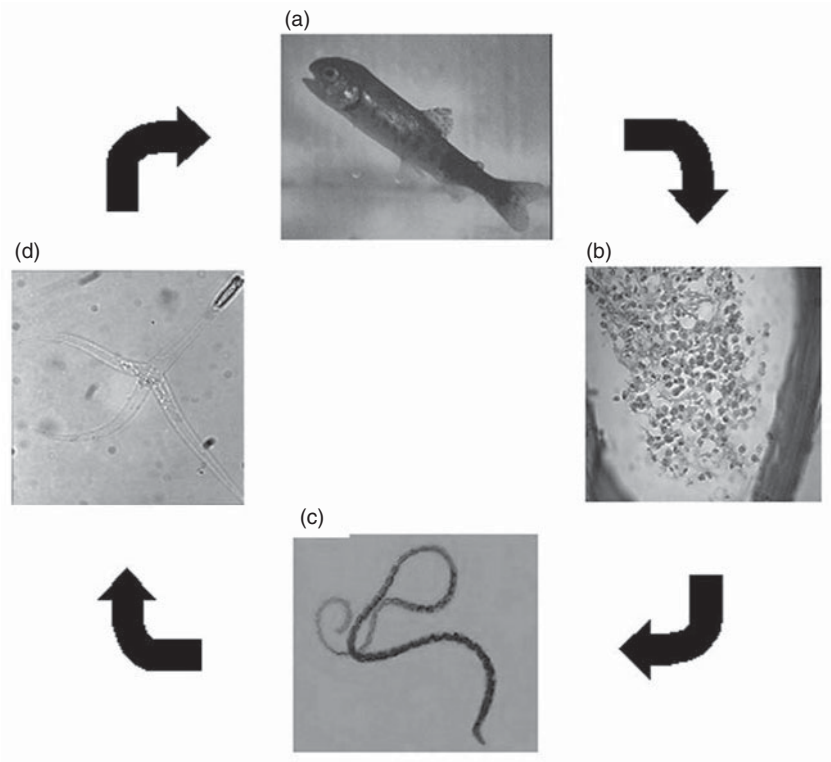


Fig. 3.8. Life cycle of *Myxobolus cerebralis*. (a) Susceptible salmonid host becomes infected by TAM spores. (b) Myxospores form in the head cartilage of infected salmonids. (c) *Tubifex tubifex* ingest myxospores and TAMs develop in the digestive tract. (d) TAMs are released into the environment or consumed by a susceptible host.

and hatchery-released or wild fish populations may experience mortality (Markiw and Wolf, 1974; Hedrick, 1998). Although all salmonids are susceptible, infection onset and intensity depends on many factors including fish species (Hedrick *et al*., 1999, 2001; Baldwin *et al*., 2000; Vincent, 2002), size, age, genetics, water temperature and concentration of TAMs during exposure (Hoffman and Byrne, 1974; Vincent, 2002). Rainbow trout are the most susceptible while coho salmon, brown trout, Arctic grayling and lake trout are considered relatively refractory (Hedrick *et al*., 1999).

Clinical signs

The classic signs of whirling disease include tail chasing (whirling), skeletal deformities (primarily in the head and vertebrae of fish), blackening of the tail and mortality. It is hypothesized that whirling behaviour is linked to damage to cartilage surrounding the organ of equilibrium (Markiw, 1992). While discoloration of the caudal region of the tail may be due to inflammation and compression that impair nerves that influence pigmentation (Rose *et al*., 2000). Infected fish can often exhibit no clinical signs of disease or, depending on factors described above, mortality can reach 100%. Infected fish may cease feeding and will in almost all cases become lifelong carriers of *M. cerebralis*. Internal examination may reveal normal tissues and organs, and only upon histological examination will tissue damage characteristic of *M. cerebralis* infection be recognized. Staining of cranial sections will often show characteristic areas of tissue damage including inflammation, lysis and digestion of cartilage.

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Diagnostic techniques

Although disease history and clinical signs may provide a presumptive diagnosis, techniques to diagnose whirling disease and detect *M. cerebralis* most often focus on identification of spores within cartilage of fish. Methods to accomplish this include pepsin-trypsin-dextrose (PTD) digestion of the cartilage of the head (Lorz and Amandi, 1994), histopathology and PCR (Andree *et. al*., 1998).

The PTD digest is aimed at extraction of myxospores from the cartilage of infected fish and involves removal or de-fleshing of soft tissue in the head of fish. The cartilage can then be dissolved and mature myxospores isolated and identified based on shape, size and presence of polar capsules. Although typically considered a 'gold standard', it is possible that other myxospore species similar in size could be identified as *M. cerebralis*. An advantage of PTD is that myxospores can be easily enumerated and provide a relative indication of infection intensity. However, the length of time it takes to perform the assay is a consideration and has led to greater use of molecular PCR-based detection methods for confirmation of *M. cerebralis*.

In younger fish where spores may not have developed, histopathology can identify damage to the cartilage tissue in fish. It can also be useful to demonstrate the presence and severity of infection based on degree of inflammation and numbers of granulomatous lesions within the cartilage of the head (Baldwin *et al*., 2000), but is considered less sensitive than PTD.

PCR-based methods are now widely used to detect *M. cerebralis* infections with follow-up methods to confirm the parasite as *M. cerebralis*. *M. cerebralis* detection using PCR has advantages over other methods in that it can be applied to both hosts at all life stages. PCR can also detect the pathogen within environmental samples in some cases and is more sensitive than other diagnostic methods (Andree *et al*., 1998). PCR assays for *M. cerebralis* originally targeted ribosomal sequences (Andree *et al*., 1998), but additional assays utilizing a segment of the heat shock protein 70 (Hsp70) gene have been developed (Epp *et al*., 2002). PTD and histology have the advantage of providing relative severity of infections; however, a quantitative PCR assay based on Hsp70 has been shown to relate directly to the histology score of *M. cerebralis* infected fish (Cavender *et al*., 2004).

Control

The only way to control whirling disease is to prevent exposure of fish to the infectious stage of *M. cerebralis*. Due to the risk associated with carrier fish, they are often required to be destroyed or their movement is severely restricted if a facility is detected as positive for this parasite. To prevent exposure, an *M. cerebralis* free water source must be used for rearing of fish, especially during early susceptible life stages. Although various treatments have been investigated to control the effects of whirling disease, none have been satisfactory. A vaccine could theoretically be developed and antibodies can be produced to TAMs following exposure or immunization of rainbow trout (Adkinson *et al*., 1997). However, considering the mode of infection and the use of the nervous system by the parasite to avoid the immune response, it is doubtful that vaccination would be an option for prevention of this disease. Facilities that have been diagnosed as positive for *M. cerebralis* have had some success by depopulating and completely disinfecting if they have a closed water source.

Ceratomyxosis (*Ceratomyxa shasta*)

Introduction

Ceratomyxosis is caused by the myxosporean parasite *Ceratomyxa shasta*. It is primarily a freshwater problem and can cause high mortalities on wild and hatchery reared salmonids. It has been documented in the western USA, particularly within the Columbia River basin: British Columbia, Canada; and in the Yukon River of Canada and Alaska (Hoffmaster *et al*., 1988; Follett *et al*., 1994). The disease was first noted following an epizootic in hatchery rainbow trout in Shasta County, California, USA (Noble, 1950). *C. shasta* has an affinity for the intestine but

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can infiltrate and destroy other tissues. It is considered an important pathogen in North America.

Characterization

Similar to other Myxospores, *C. shasta* has two spore stages, one associated with the terminal stages of infection in fish, and an actinospore type infection stage associated with the intermediate host. The mature myxospores from fish measure 14–17 μm in length and 6–8 μm in width at the suture line (Yamamoto and Sanders, 1979). They can be distinguished as 'kidney shaped' due to their elongated and crescent-shaped appearance (Fig. 3.9). The actinospores can be identified as being approximately 10 × 8 μm and have three valve cells encapsulating three polar capsule cells and one binucleated sporoplasm (Bartholomew *et al*., 1997). Several strains of *C. shasta* have been identified based on internal transcribed spacer region 1 (ITS1) genotypes (Atkinson and Bartholomew, 2010a, b).

The life cycle of *C. shasta* involves a freshwater filter feeding polychaete worm (*Manayunkia speciosa*) as an intermediate host that when infected will release the infectious actinospores, and the salmonid host, where myxospore stage develops (Bartholomew *et al*., 1997). The parasite cannot be transmitted vertically or horizontally and myxospores must be ingested by the polychaetes where they release sporoplasms in the gut (Meaders and Hendrickson, 2009). The actinospores develop in the worm for approximately 7 weeks at 17°C followed by release of pansporocysts, each containing eight actinospores (Bjork, 2010).

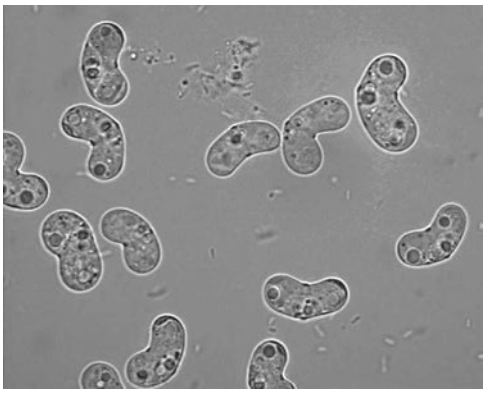


Fig. 3.9. Typical crescent shaped spores of *Ceratomyxa shasta*. Image courtesy of J.L. Bartholomew, Oregon State University.

Impact

C. shasta has only been found in fish within the Pacific Northwest region of USA and Canada, where it is considered endemic. It has been found as far north as Alaska, where infected adult salmon have been identified in a number of drainages including the Yukon (Meyers *et al*., 2008). The economic importance of ceratomyxosis can be significant and *C. shasta* is one of the most virulent Myxozoan parasites. Heavy losses of hatchery and wild salmonids occur, and in juvenile fish mortality can reach 100%. It is also of concern in adult fish where pre-spawning mortality has been documented. Chapman (1986) reported disease incidences as high as 94%. Variability on disease severity has been reported between strains of fish, but outbreaks have occurred in rainbow and cutthroat trout and Chinook, chum and coho salmon (Ibarra *et a1*., 1994). Once fish become infected the parasite persists and mortality can continue for some time.

To minimize impacts on aquaculture, utilizing resistant strains of fish or species with minimal susceptibility would be beneficial. Interestingly, fish populations that have evolved in areas where *C. shasta* is endemic have shown high levels of resistance (Zinn *et al*., 1977). Hatcheries have been forced to alter water sources or treat the water supply if ceratomyxosis outbreaks regularly occur.

Clinical signs

Depending on infection level, age and species of fish, typical signs include darkening, lethargy, anorexia and exophthalmia. The abdomen may be distended and in some cases haemorrhaging and swelling may be evident around the vent of fish (Fig. 3.10). Juvenile fish are often emaciated and the gastrointestinal tract may be swollen, haemorrhagic and contain heavy mucus. Other organs including the liver, spleen, kidney, heart, gills, gall bladder and skeletal muscle may be affected

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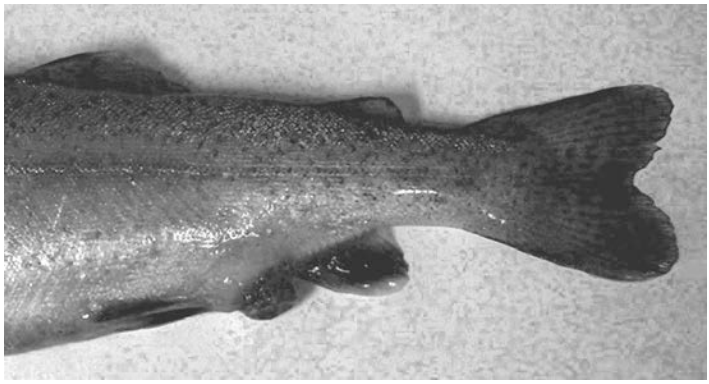


Fig. 3.10. Rainbow trout showing swelling and haemorrhaging of anus due to Ceratomyxosis. Image courtesy of J.L. Bartholomew, Oregon State University.

by the parasite and appear necrotic or haemorrhagic (Wales and Wolf, 1955). In adult fish the pyloric caeca may be thickened and haemorrhagic. The wall of the intestine may be perforated due to the development of nodular lesions leading to damage and increased mortality (Bartholomew *et al*., 1989).

C. shasta causes an acute inflammatory response in the intestine and associated cell types such as leukocytes; macrophages and fibroblasts may be evident upon microscopic examination. The intestine is often heavily infected and inflammation may result from the presence of spores within multiple layers of the intestine. Once the spores penetrate the intestine they may spread and enter the bloodstream and other organs. The epithelial lining of the intestine may show extensive necrosis and become replaced by fibrous connective tissue containing parasites and host cells (Bartholomew *et al*., 2004).

Diagnostic techniques

Presumptive diagnosis of ceratomyxosis may be based on clinical signs, intestinal inflammation and examination of wet mounts. To identify spores from wet mounts, samples should be taken by scraping the intestinal wall (Zinn *et al*., 1977) or from ascites. Tissue lesions should be examined and wet mounts viewed under phase contrast or brightfield microscopy allowing identification of multicellular presporogonic trophozoites (Bartholomew, 2003). Histological examination of grossly infected tissues or intestinal sections may be effective in identifying spores. By staining with Giemsa or haematoxylin and eosin, the multicellular trophozoites can be identified and will appear light blue with dark staining nuclei surrounded by a clear halo (Hallett and Bartholomew, 2012).

To confirm presence of *C. shasta*, visual examination should show the characteristic kidney-bean-shaped myxospores in wet mounts or histological sections. Serological methods to confirm early stage infection have been developed based on specific monoclonal antibodies (Bartholomew *et al*., 1989), but these have been largely replaced by molecular confirmation using PCR (Palenzuela *et al*., 1999; Palenzuela and Bartholomew, 2002; Bartholomew, 2003).

Control

There is no direct control or prevention for *C. shasta* other than avoidance of the parasite. The parasite can only be transmitted by contact with the infectious stage and since the invertebrate host of this parasite has a more limited range than that of other myxospores such as *M. cerebralis*, it is less likely to establish in a typical hatchery environment. However, for cage culture in areas where this polychaete may occur, or where *C. shasta* is endemic, there are greater risks. Intake water treatments in hatcheries with ozone, UV or sand filtration have had varying degrees of success but would not be an option for cage culture. Testing of therapeutants to control

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ceratomyxosis has been minimal, but fumagillin and its analogue TNP-470 were administered to fish prophylactically or following infection, but minimal effect was observed (Ibarra *et al*., 1990; Whipple *et al*., 2002). Feeding of glucan immunostimulants also provided no protection (Whipple *et al*., 2002). In areas where the disease occurs, the use of non-susceptible species or resistant strains of fish would be the most beneficial. Work with resistant fish has shown that the parasite could invade gills and become established in blood vessels, but was cleared from the blood within 2 weeks (Bjork, 2010).

Henneguya salminicola

Introduction

This parasite has also been referred to as *Henneguya zschokkei* and is an internal myxosporean parasite that is known to affect salmonids. *H. salminicola* is of concern due to its impact on the marketability of wild or culture fish. It can become a problem in adult fish following transfer or migration to sea water and is covered further in Chapter 2. Infection with this parasite results in an unpleasant appearance of the flesh due to localization and cyst development in the musculature, which can significantly affect product value (Kent *et al*., 1994).

Characterization

Although a number of *Henneguya* species have been described from freshwater fish, only *H. salminicola* appears to be a significant problem and risk to freshwater cage culture. Its oovid spores are approximately 11 × 9 μm in size, and have two polar capsules along with two posterior processes up to 40 μm in length. Little is known about the life cycle of this parasite, but it is assumed that an intermediate host is involved similar to other Myxozoa parasites such as *M. cerebralis*. Kallert *et al*. (2005) recently discovered that the life cycle of another *Henneguya* sp., *H. nuesslini* that impacts salmonids, also involves production of an infectious triactinomyxon in the oligochaete worm *T. tubifex*.

Impact

Fish infected with *H. salminicola* do not typically result in mortality. Spores produce a protease that often affects fish that are transferred or migrate to sea water. The protease results in hydrolysis and liquefaction of the flesh (Bilinski *et al*., 1984; Boyce *et al*., 1985), which in turn limits the commercial value of affected fish. It has been found to affect five species of salmon in Asia and North America, most notably along the Pacific coast into British Columbia (Boyce *et al*., 1985).

Clinical signs

The ovoid cysts are white with cream coloured content in the musculature and other organs. The cysts may eventually rupture through the integument and release spores into the water column. Following release, open ulcers may be present and represent a risk to secondary infection by other pathogens. Species susceptibility risks are not well established, but Boyce *et al*. (1985) reported high prevalence in coho and sockeye salmon that appeared to be linked to length of fresh water residence of juvenile fish.

Diagnostic techniques

Diagnosis of *H. salminicola* would be based on a combination of clinical signs and microscopic identification of the characteristic spores containing two polar capsules and two tail-like projections. Spores would be detected upon examination of musculature containing cysts, or sampling of whole fish using ultrasonic equipment (Brown and Bruno, 2002). Molecular and antigenic characterization has been described and would possibly provide a more definitive diagnosis (Clouthier *et al*., 1997).

Control

There is no method of prevention or control of this parasite. However, it is assumed that if it has a life cycle similar to other myxospreans then a water source free of the infective stage of the parasite should be used.

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Gyrodactylosis

Introduction

Gyrodactylus is a genus of ectoparasite known to infect more than 150 species of fish ranging from salt to fresh water under many different temperatures and environmental conditions (Bakke *et al*., 2002, 2007). However, with regard to coldwater aquaculture and cage culture, a single species, *Gyrodactylus salaris*, has undoubtedly had the most significant impact by causing high mortality in both wild and cultured freshwater salmonids in Europe, specifically Atlantic salmon in Norway. Although most *Gyrodactylus* infect only a single host species (Bakke *et al*., 2002), *G. salaris* is known to infect multiple salmonid hosts, of which Atlantic salmon appear to be highly susceptible.

Characterization

G. salaris is a small monogenean ectoparasite (flatworm) of about 0.5 mm in length. The parasite attaches to the fish by a large specialized posterior attachment organ, the haptor, which has 16 hooks around its margin. Initial infections are usually found around fins and the head of the host fish, which can spread to the entire body surface. Although *Gyrodactylus* species are known to inhabit both fresh and salt water, most species are not considered eurohaline and *G. salaris*, for example, only survives in fresh water. All *Gyrodactylus* have an unusual mode of reproduction in that adult worms contain several generations of embryos boxed one inside another akin to a 'Russian-doll' (Fig. 3.11). Each parasite gives birth to a fully grown worm which attaches to the host alongside its parent. Although most species of *Gyrodactylus* currently identified (71%) are specific for a single host species (Bakke *et al*., 2002), *G. salaris* is known to infect multiple hosts including Atlantic salmon (Fig. 3.12), rainbow trout, Arctic char, brook trout, grayling, lake trout and brown trout (listed in order of presumed susceptibility). The biology, reproduction and host specificity of *Gyrodactylus* has been discussed in detail in recent comprehensive reviews (Bakke *et al*., 2002; Cable and Harris, 2002; Bakke *et al*., 2007).

Impact

G. salaris has been reported from at least 13 European countries and will likely spread further via the trade of infected salmonids, particularly rainbow trout (Bakke *et al*., 2007). Norway has been the hardest hit by this pathogen, and it has been estimated that the direct cost associated with *G. salaris* in that

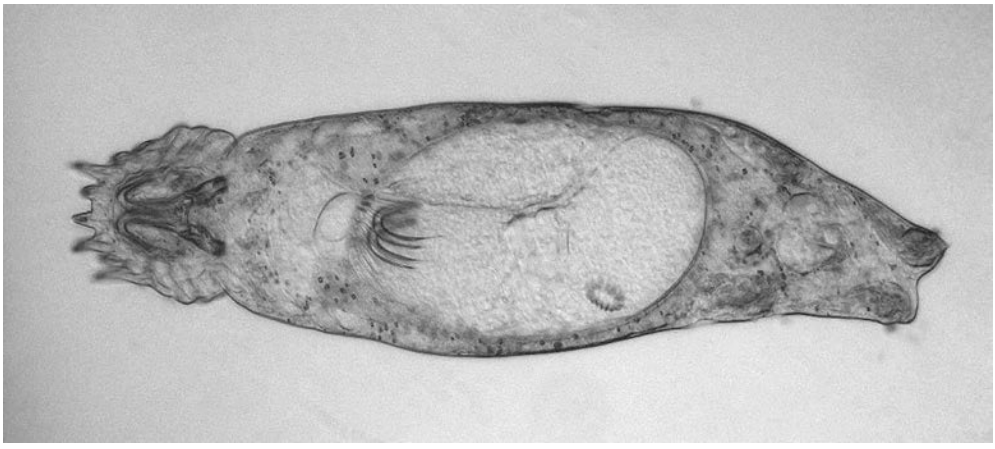


Fig. 3.11. The image presents an adult *Gyrodactylus salaris* and two developing embryos in a 'russian-doll' formation. The second embryo, located inside the first, is visible only on the basis of a ring of marginal hooks. Image courtesy of T.A. Bakke, University of Oslo.

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Fig. 3.12. Electron micrograph of *Gyrodactylus salaris* attached to the skin of an Atlantic salmon. Image courtesy of T.A. Bakke, University of Oslo.

country has been over US$600 million during the past 35 years (Bakke *et al*., 2007). Natural salmonid populations have been decimated by nearly 90% in many Norwegian rivers (Johnsen *et al*., 1999), and the annual loss in production during the culture of salmon associated with this pathogen is thought be between 15–20% (Bakke *et al*., 2007).

Clinical signs

Usually there are no clinical signs in fish with a low (< 50–100 parasites) level of infection. As infection increases, flashing is typically observed and fish may also become greyish due to increased mucus production. Heavily parasitized fish become lethargic and are usually found in slower-moving water (Mo, 2009). During heavy infection, the dorsal and pectoral fins may become whitish as a result of increased thickening of the epidermis. Secondary fungal infections (*Saprolegnia* spp.) are also commonly observed due to tissue destruction and necrosis caused by *G. salaris* (Johnsen, 1978; Bakke *et al*., 2007).

Diagnostic techniques

The parasite is extremely difficult to see with the naked eye, but can be seen with a hand held lens *in situ* or from fin clippings using a dissection microscope. Scrapings (wet mounts) from skin or fins are used to detect *Gyrodactylus* specimens on fish by compound microscopy; however, preparations of wet mounts are usually not suitable for identification to the species level and low levels of infection are often missed (Mo, 2009). For surveillance monitoring or a suspected low-level infection, fin examination by dissection microscopy is the most straightforward solution, as even in low-level infections *Gyrodactylus* can usually be observed on fins if at all present on the organism (Mo, 2009). If species confirmation is required, it has historically been obtained based on morphology and morphometry of hamuli (anchoring hooks) and bars in the opisthaptor attachment organ (Harris *et al*., 1999). Additionally, several strains of *G. salaris* have been identified on the basis of genotyping with the mitochondrial cytochrome oxidase 1 (CO1) marker (Meinila *et al*., 2002; Hansen *et al*., 2003; Meinila *et al*., 2004; Hansen *et al*., 2007) and PCR sequencing for species confirmation will likely become a more prevalent definitive diagnostic tool in future.

Control

G. salaris is sensitive to most commonly used disinfection chemicals (i.e. compounds containing hypochlorite or iodine), which should be used to disinfect equipment associated with infected or potentially infected stock. Acidified aluminium sulphate has been used to effectively eliminate *G. salaris* from fish in laboratory trials (Poleo *et al*., 2004), and attempts have been made to use this chemical for eradication of the parasite in river systems in Norway, but its overall effectiveness on such a large scale is uncertain at best (Soleng *et al*., 2005; Bakke *et al*., 2007). Rotenone has also been used to eradicate infected stocks; however, as this chemical can indiscriminately kill all gilled aquatic animals and invertebrates, including fish at the concentrations needed to kill *G. salaris*, its use in natural environments has caused controversy (Bakke *et al*., 2007). Avoidance or separation of infected stocks, if possible, remains the best control practice with regard to minimizing the impact of this aquatic pathogen.

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Ichthyobodosis (Costiasis)

Introduction

The genus *Ichthyobodo* (formerly *Costia*) encompasses a number of species that externally parasitize fish. Two species (*I. necator* and *I. salmonis*) have had significant impact on the freshwater culture of coldwater fish. Additionally, *I. salmonis* is considered to be eurohaline and can infect fish in both the freshwater and marine environment.

Characterization

Ichthyobodo are ectoparasitic flagellates that infect skin or gill tissues of fish. For many years the genus *Ichthyobodo* contained a single variable species, *I. necator*, identified from fish worldwide (Robertson, *et al*. 1985; Urawa, 1998). Initial work by Lamas and Bruno (1992) suggested more than one species was involved and recently, small subunit ribosomal RNA gene (SSU rDNA) sequences of flagellates from different hosts and environments have shown that *I. necator* represented multiple species (Todal *et al*., 2004; Isaksen *et al*., 2011). Of these, two have been identified from farmed salmonids in fresh water: *I. necator* which has been re-described to a species well characterized by its SSU rDNA sequence which appears to be fresh water specific (Isaksen *et al*., 2007), as well as the euryhaline *I. salmonis* from farmed Atlantic salmon (Isaksen *et al*., 2011).

Impact

I. necator and *I. salmonis* are common in aquaculture and both species have been associated with ichthyobodosis and mortality (Isaksen *et al*., 2010, 2011). Severe infections can often cause moderate to severe mortality in salmonid fry, and lower-level chronic infections are associated with decreased growth, condition factor and an increased likelihood of secondary infection.

Clinical signs

Moderate to severe ichthyobodosis is generally easily identified by a greyish coating on the dorsal surface of fish and flashing behaviour; this is less obvious in fish with low infections.

Diagnostic techniques

Parasites are readily detected using light microscopy of fresh smears of skin or gills from heavily infected fish (Fig. 3.13, 3.14), but low-level infections may be missed using this technique (Isaksen *et al*., 2010). Molecular methods have been developed for sensitive detection of *Ichthyobodo* spp. from skin or gill tissue, from which species-specific information can be obtained following sequence analysis (Isaksen *et al*., 2011, 2012).

Control

Chemical treatments are generally impractical in cage culture environments and largely ineffective. The transfer or bathing of *I. salmonis* infected fish in salt water is not effective at eliminating the parasite due to its euryhaline tolerance. As *I. necator* appears to be exclusive to fresh water, saltwater treatment may provide some benefit; however, such a treatment has not been substantiated and species-specific identification would be necessary to ensure any potential efficacy. Moving fish to colder temperatures has also been suggested to prevent parasite development (Dykova, 1995), but is unlikely to completely free a population of this parasite. The best way of avoiding the disease is through good fisheries management and reducing stress by minimizing high stock densities and improving water quality.

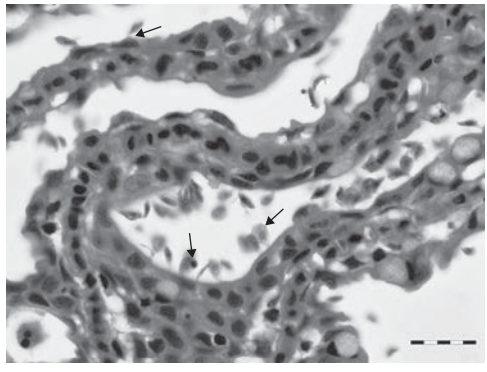


Fig. 3.13. Light micrograph of *Ichthyobodo spp*. (arrows) parasitizing the gills of an Atlantic salmon. Bar = 20 m. Image courtesy of D.W. Bruno, Marine Scotland Science.

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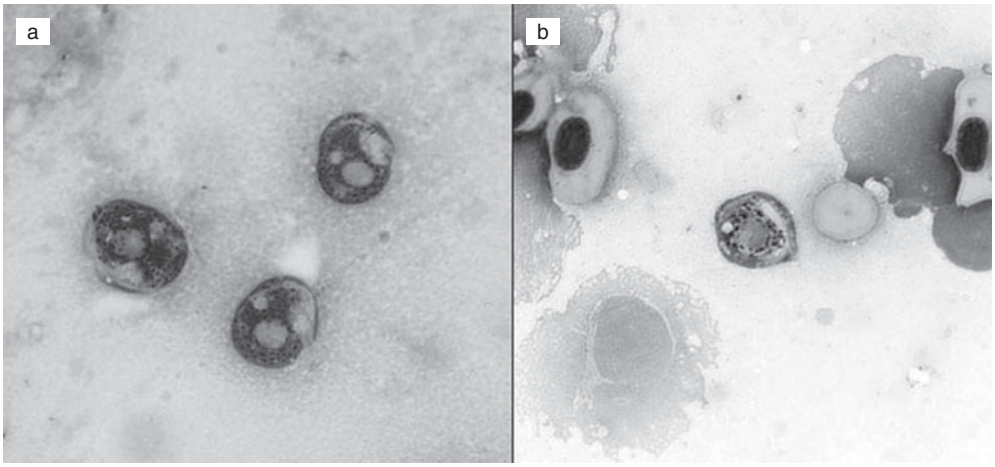


Fig. 3.14. Light micrographs of *Ichthyobodo necator* (a) and *Ichthyobodo salmonis* (b) following Diff-Quick stained smear from infected skin (a) and gill (b) of Atlantic salmon. Image courtesy of T.E. Isaksen, University of Bergen.

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4 Non-infectious Disorders of Coldwater Fish

Heike Schmidt-Posthaus[1]* and Mar Marcos-López[2]
[1]*Centre for Fish and Wildlife Health, University of Berne, Switzerland;* [2]*Marine Laboratory, Marine Scotland Science, Aberdeen, United Kingdom*

Introduction

This chapter is on non-infectious disorders of coldwater finfish (marine and fresh water). In the marine environment, coldwater finfish aquaculture is dominated by the production of salmonids, mainly comprising Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhynchus tshawytscha*) and sea trout (*Salmo trutta trutta*). Other fish species such as Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Psetta maxima*) are gaining in importance. The on-growing of these newer aquaculture species takes place in either on-shore tanks (most common technique for flatfish) or off-shore cages (method most commonly used for Atlantic cod). Juvenile production has been a major bottleneck for these species due to high mortalities during weaning. Other biological and physiological difficulties faced by industry include skin malpigmentation, low quality and discontinuous spawning, and high incidence of skeletal deformities (Fernández and Gisbert, 2011). These examples highlight the importance of understanding the environmental needs and nutritional requirements of any proposed new aquaculture species, which are especially vital for larvae stages. Coldwater freshwater fish are reared in water that originates from rivers, streams or ground-water. Culture systems include open or recirculation systems and fish (mainly salmonids) are kept in tanks or cages. Our focus is on diseases and disorders of rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*) and Arctic char (*Salvelinus alpinus*). Compared to infectious diseases, non-infectious disorders are often regarded as economically less devastating. However, infectious diseases are often promoted either directly or indirectly by a weakened immune response due to adverse environmental conditions (Barton, 1997). Therefore, knowledge of adverse physical, chemical or biological factors, and imbalances in nutrition, are crucial for good fish husbandry.

Production Problems

Smolt failure in salmonids

Salmonid species with fresh and seawater life cycle phases undergo a physiological, morphological and behavioural transformation known as smoltification, which allows them to adapt from fresh water to marine conditions.

* E-mail: heike.schmidt@vetsuisse.unibe.ch

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This is a period of high risk in which fish must prepare for a significant osmoregulatory challenge. Under natural conditions, the main triggers for smoltification are parr size, internal nervous and endocrine signals, and external photoperiod and water temperature (Maugars, 2007). Wild salmon normally smoltify in spring after having spent one or more winters in fresh water. Intensification of salmon aquaculture and the increasing market demand for a continuous fish supply have led to production changes in order to decrease economic costs and increase throughput all year round. These changes have mainly focused on varying the duration of the freshwater stage. In captivity, where manipulations of day length, temperature and food supply are possible, these mechanisms are used to produce different age classes and season-related smolts; allowing transfers to sea in their first autumn (S0.5), in spring after their first winter (S1), in their second autumn (S1.5) or in spring after their second winter (S2). Smolts transferred in the autumn are known as out-of-season smolts (Speare, 2002). Overall, growth rate and required time at sea to reach market size are comparable for both smolt types (Duncan *et al*., 1998). However, the growing cycles differ and both smolt types show the highest growth in summer/autumn, which relates to temperature and photoperiod conditions (Duncan *et al*., 2002).

Clinical signs, gross and histopathological lesions

The time period in which smolts are ready to be moved to sea water (smolt window) is finite, and therefore early or late transfers may have detrimental consequences such as osmotic perturbations and mortalities (Stead and Laird, 2002). Even under good transfer conditions, the acclimation period and the stress caused by the transfer are likely to influence the fish feeding response and immune system. During the first days at sea, low levels of mortality, decreased growth and increased susceptibility to infections can occur (Speare, 2002; Toften *et al*., 2003). Failed smolts are smaller and retain the characteristic parr marks (vertical dark stripes) instead of adopting the silver coloration shown by smolts due to deposition of guanine. In addition, failed smolts have fewer chloride cells in the gills (McCormick and Saunders, 1987).

Control and prevention

Monitoring of smoltification is essential for a successful transfer. As a routine, levels of Na^+/K^+-ATPase activity are measured to assess osmoregulatory competence and adaptability to sea water (Borgatti *et al*., 1992).

Stress

Farmed fish are susceptible to a wide range of stressors, and different species display a wide variation of physiological and behavioural responses. Capture, transport, grading, treatment, crowding, malnutrition, poor water quality, changes in environmental conditions (light, temperature, salinity), contaminant exposure, inadequate housing, disease, physical trauma, noise and predators can all be a cause of stress (Ashley, 2007; Harper and Wolf, 2009). Transportation includes capture, loading, transport and unloading; stressful events that can cause negative effects over a long period of time. During transport, on- and unloading are the most stressful procedures, while the actual transport can act as a calming recovery period between individual stress events (Iversen *et al*., 2005). In Atlantic salmon, a high proportion of disease outbreaks occur during the first months after sea transfer (Harris and Bird, 2000; Speare, 2002).

Clinical signs, gross and histopathological lesions

The primary stress response involves the secretion of adrenergic and glucocorticoid hormones by the chromaffin and interrenal cells, respectively. This increases alertness, respiration rate, blood pressure, hepatic glycogen catabolism and decreases the immune response (Harper and Wolf, 2009). These responses have an initial beneficial effect on the individual. However, if the stress stimuli persist, the initial adaptive mechanisms become overcompensated and negative effects occur, such as chronic immune suppression,

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depletion of energy reserves and osmoregulatory impairment (Harper and Wolf, 2009). Stress levels and gross and microscopic morphological changes due to stress are difficult to assess in fish. Changes are unspecific and can have a multifactorial origin. Stress can decrease growth rate and increase susceptibility to infection. Externally, fish may show increased mucus production, darker coloration and skin erosion (mainly on the fins). Histological changes may be observed in organs such as gills, liver, kidney and skeletal muscle, although these will depend on the nature of the stressor (Harper and Wolf, 2009). Some of the chronic changes include skeletal muscle atrophy, hepatocyte atrophy due to glycogen catabolism (Wolf and Wolfe, 2005), gill lamellar epithelium hypertrophy, hyperplasia and/or oedema, and increased numbers of renal melanomacrophages (Agius and Roberts, 1981; Wolke, 1992).

Control and prevention

When handling fish, care must be taken to avoid abrasions, removal of scales and skin mucus, and excessive air exposure. A 24 h recovery period between handlings is recommended (Gatica *et al*., 2010). In well boats used for transfers or treatments it is extremely important to avoid overcrowding, keep oxygen levels high and minimize the build up of metabolic wastes. Temporary starvation (24 h) is recommended before certain management procedures to reduce stress. This reduces metabolism, oxygen demand and waste production (Ashley, 2007).

Behaviour related problems

Behavioural indicators can be used to assess welfare in cultured fish (Martins *et al*., 2012). Normal behaviour (regarding feeding, respiration, aggression and swimming) and behavioural responses to stress, pain and fear are relatively species specific. Freedom to express normal behaviour by providing sufficient space and proper facilities is an important element of fish welfare. When held at high densities, individual fish tend to respond to the behaviour of others and the group demonstrates a schooling swimming behaviour, which minimizes the risk of collisions (Føre *et al*., 2009). This is seen in captive Atlantic salmon (Oppedal *et al*., 2011), while schooling behaviour is not as well developed in Atlantic cod, and this species tends to swim along the net walls and bottom (NMFCA, 2009). Cod also show a more curious behaviour towards the external environment and a tendency to bite at nets, which make them more prone to escapes (Damsgård *et al*., 2012). Housing conditions should try to imitate the natural environment. Some species like wrasse (in increasing demand as biological control against sea lice on salmon) need hiding structures (environmental enrichment) which, besides allowing wrasse to express their normal behaviour, will also serve as a refuge against salmon attack (Ashley, 2007). For flatfish culture, flat bottom cages or sand substrate should be used to allow for normal behaviour, avoid abrasions and provide camouflage opportunities (Daniels and Watanabe, 2010). In the culture of dover sole, the use of a sand substrate in the rearing tanks also prevents the occurrence of black patch necrosis, an ulcerative condition caused by *Tenacibaculum marinum* (McVicar and White, 1982).

Aggression and cannibalistic behaviour occur in some species (e.g. tilapia, Atlantic cod) and are promoted under certain culture conditions. Species, stocking densities and feeding methods have a strong influence on the levels of social interactions and dominance hierarchies (Ashley, 2007). In species showing strong social hierarchies (e.g. salmonids), dominance can lead to aggression, chronic stress and reduced feeding and growth in submissive individuals (Ejike and Schreck, 1980; Alanara and Brannas, 1996). Aggression can result in fin, skin and eye damage (Speare, 2002), facilitating the entrance of secondary infectious organisms. Aggression problems occur more frequently in Chinook than in Atlantic salmon (Speare, 2002), and in Atlantic salmon diploids than in triploids (H. Migaud, Inverness, 2012, personal communication). Fin rot, a common problem in farmed salmon and rainbow trout, can result from abrasions with containment structures (e.g. nets, cages) or due to aggressive interactions. High stocking densities

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may increase competition for resources and therefore aggression levels, but very low stocking densities have also proven to induce aggressive behaviour and poor feeding in trout (Ellis *et al*., 2002) and Atlantic salmon (Speare, 2002). Greaves and Tuene (2001) observed that the majority of damage through contact among Atlantic halibut occurs during feeding. Hand feeding where food is spatially dispersed but concentrated in time, or feeding to satiation using automatic feeders throughout the day decreased the level of aggression. Food availability and feeding methods, stocking densities and appropriate housing conditions are, therefore, key factors to prevent behavioural problems.

Predators

Aquatic mammals and birds are the main predators of cage-reared fish. Direct or indirect losses due to predators can be significant if preventive measures are not taken. Control methods include cage nettings and visual and acoustic scaring devices. Predators can kill the fish (Fig. 4.1) or cause wounds facilitating entry for pathogens. Besides health and welfare considerations, damaged fish will have a lower market value. Predators such as seals can also cause net damages allowing escapes, which will have economic, environmental and legal consequences. The presence of predators in the surroundings of the cages can also induce a fear and stress response in the fish.

Environmental Problems

Algal blooms

Harmful algal blooms (HABs), commonly known as red tides, are mass accumulations of one or more phytoplankton species causing negative effects to other organisms, such as fish, shellfish or humans that have consumed intoxicated shellfish. Around 200 phytoplankton species have been identified as potential HABs, from which between 60–80 species are known to produce toxins (Rodger *et al*., 2011). The phytoplankton species involved include, among others, diatoms, dinoflagellates, dictyochophytes, raphidophytes and cyanobacteria. In finfish cage aquaculture, HABs are responsible for high mortalities and economic losses. HABs are pathogenic to fish directly or indirectly by different mechanisms, including oxygen depletion in the water due to phytoplankton respiration and decomposition, mechanical damage to the gills, production of toxins (e.g. neurotoxins, hepatotoxins) or production of noxious substances (e.g. haemolysins, reactive oxygen species) which affect the permeability and/or integrity of the gill epithelium (Rodger *et al*., 2011). Gas-bubble trauma due to extreme oxygen saturation from algal photosynthesis has also been described (Kent *et al*., 1995).



Fig. 4.1. Farmed Atlantic salmon (*Salmo salar*) with severe lesion due to seal attack. Image courtesy of Sonia Duguid, Marine Scotland Science.

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Clinical signs, gross and histopathological lesions

Affected fish can show abnormal behaviour such as lethargy, disorientation, loss of equilibrium, swimming near the surface, respiratory distress and bursts of hyperactivity. At sub-lethal doses effects on fish may include loss of appetite and growth. During necropsy, excessive mucus and petechiae in gills can be observed. Histopathological changes in gills include acute necrosis and sloughing of epithelial cells, lamellar oedema, lamellar hyperplasia and fusion, branchitis and/or haemorrhage. Causative algae can occasionally be observed in fresh and histology gill preparations. Liver and intestinal mucosa damage can occur, although this will depend on the type of phytoplankton involved and therefore its mechanism of action (Jones *et al*., 1982; Treasurer *et al*., 2003).

Diatoms

Unlike most phytoplankton, diatoms have a rigid silica shell (frustule) that aid to induce disease as the siliceous spines (setae) penetrate into the gill epithelium causing damage, haemorrhaging, irritation, hyperplasia and excess mucus production. A pronounced neutrophilic infiltration and giant cells surrounding spine remnants have also been observed (Ferguson, 2006). The chain-forming diatoms *Chaetoceros wighami* and *C. debile* were responsible for high mortalities of Atlantic salmon in Scotland (Bruno *et al*., 1989), while *Chaetoceros concavicornis* and *C. convolutes* are known to cause mortality in rainbow trout and salmon in Canada and the United States (Yang and Albright, 1992). Speare *et al*. (1989) and Albright *et al*. (1993) reported salmonids exposed to sublethal doses of *Chaetoceros* spp. suffered from gill damage and were more prone to bacterial diseases such as vibriosis and bacterial kidney disease. Certain diatoms (e.g. *Pseudo-nitzschia* sp.) produce the neurotoxin, domoic acid.

Dinoflagellates

Dinoflagellates include neurotoxin producing species such as *Karenia* spp. and *Alexandrium* spp., which produce brevetoxins and saxitoxins respectively. Fish kills by *K. brevis* are common in the warmer waters of the Gulf of Mexico (Magaña *et al*., 2003). *K. mikimotoi* (formerly *Gyrodinium aureolum*), first described in Japan but believed to have spread through the discharge of ballast waters, is now one of the most important species affecting North West Europe and causes fish kills in Norway, Ireland and Scotland (Dahl and Tangen, 1993; Silke *et al*., 2005; Davidson *et al*., 2009). Reports of finfish mortalities associated with *Alexandrium* spp. are rare, but the potent neurotoxin produced by *A. tamarense* is thought to have been responsible for high losses in salmon cages in southeast Nova Scotia in 2000 (Cembella *et al*., 2002) and in Faroe Islands in 1984 (Mortensen, 1985).

Dictyochophytes

Mass mortalities due to species of the dictyochophyte *Pseudochattonella* have been reported in sea caged fish from Europe, Japan and New Zealand. Blooms of *P. farcimen* have occurred in Scandinavian countries causing high deaths of farmed Atlantic salmon in Denmark, Norway and Sweden (Jakobsen *et al*., 2012). In 2010, *P. verruculosa* severely affected Chinook salmon culture in New Zealand (MacKenzie *et al*., 2011).

Rraphidophytes

Blooms of the raphidophyte *Heterosigma akashiwo* have been associated with massive fish kills worldwide (Lewitus *et al*., 2012). The mechanism by which *H. akashiwo* kills fish is not completely understood, but it is suggested to involve production of reactive oxygen species (ROS), ichthyotoxins and/or haemolysing compounds. The presence of HAB-associated bacteria has been reported from several phytoplankton species, including *H. akashiwo*. These bacteria are believed to play an important role in algal growth and toxin production (Carrasquero-Verde, 1999; Landsberg, 2002).

Cyanobacterias

Cyanobacteria species produce different types of toxins, which include hepatotoxins (microcystins and nodularins). An algal microcystin toxin has been suggested as the cause of the

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net pen liver disease affecting Atlantic salmon in British Columbia. Affected fish showed cytomegaly, nuclear enlargement, vacuolation and/or necrosis of the hepatocytes (Kent, 1990; Andersen *et al*., 1993; Stephen *et al*., 1993).

Risk factors

Algal blooms are naturally occurring events, but their frequency and distribution seem to have increased in recent years. Environmental factors favouring algal blooms are complex and difficult to predict, but eutrophication of costal waters due to anthropogenic activities, special environmental conditions (e.g. coastal upwelling, climatic oscillations) and climate change have been raised as predisposing factors. The increased awareness of both the public and scientific community, and the improvement of detection and prediction methods are also likely to have influenced the increased reporting of HABs worldwide. In addition, particular phytoplankton species have now been reported in new and distant locations. Potential pathways of introduction of non-indigenous species are anthropogenic movements through, e.g. ballast waters and commercial shellfish movements (Heil *et al*., 2005). Fish and phytoplankton species involved, exposure time, algal concentration in the water, environmental conditions, site location and presence of other diseases or stress factors will affect the severity and impact of an algal bloom. Farmed fish are more susceptible to HABs since they cannot swim away from the blooms and intensive rearing conditions are more oxygen demanding.

Control and prevention

The speed at which blooms occur makes control difficult. However, potential preventive and control measures are routine monitoring for early detection, reduction of stressful and high oxygen demanding activities (e.g. feeding), supply of additional oxygenation, movement of cages if possible, control of nutrients load, mucolytic agents and biological or chemical algaecides (Boesch *et al*., 1996; Kim, 2006; Rodger *et al*., 2011). Short term submergence of pens has also been suggested (Dempster *et al*., 2009). Site location is important; deep waters, vertical water mixing and strong tidal currents reduce the likelihood of HABs (Noga, 2010). Phytoplankton species identification is important in order to use appropriate control measures, since per example extra oxygenation can be detrimental in cases of toxin producing blooms. Water samples must be collected as soon as a bloom is suspected. Algal concentration and identification are required to associate fish morbidity/mortality with a bloom.

Harmful zooplankton – jellyfish

Jellyfish cause significant fish losses worldwide. Small jellyfish (e.g. class Hydrozoa) can pass through the nets and reach the fish inside the cages, while bigger species (e.g. class Scyphozoa) tend to break in contact with the nets, and the freed tentacles sting fish. In jellyfish blooms, fish can die from suffocation due to anoxia or gill damage and/or from toxicity via nematocysts discharge and release of haemolytic, cytotoxic and/or neurotoxic chemicals (Lotan *et al*., 1996; Ferguson *et al*., 2010). Jellyfish can sting skin, eyes or gills, but if ingested they can also induce damage in the gastrointestinal tract (Bruno and Ellis, 1985). In sub-lethal cases, stinging may result in gill and skin damage or ulceration which can lead to secondary bacterial infections. Jellyfish have been suggested as vectors for certain bacterial diseases; i.e. *Tenacibaculum maritimum* affecting farmed Atlantic salmon (Tørud and Håstein, 2008; Ferguson *et al*., 2010).

Jellyfish damage is usually underestimated and only severe events are reported. Small jellyfish can be difficult to observe and, in the absence of water samples or jellyfish evidence in fresh or histology preparations, low levels of mortality/morbidity can be diagnosed as unspecific gill and/or skin pathology. In 2007, *Pelagia noctiluca* caused massive losses to the Atlantic salmon industry in Northern Ireland, killing all the stock (~ 250,000 salmon) from the only Northern Irish salmon farm (Doyle *et al*., 2008). During that time, *P. noctiluca* swarms were also reported from the Scottish coast (Doyle *et al*., 2008; Hay and Murray, 2008). *P. noctiluca* occurs worldwide in both warm and temperate

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waters, but global warming may allow its expansion to northern waters. *Muggiaea atlantica* caused high losses (>100,000 salmon) in Norway (Fosså *et al*., 2003) and was also suggested as the cause of a catastrophic event (>1,000,000 salmon) in Ireland in 2003 (Cronin *et al*., 2004). Other jellyfish species associated with fish kills in cold waters are *Phialella quadrata* in Scotland (Bruno and Ellis, 1985; Ferguson *et al*., 2010), *Apolemia uvaria* in Norway (Bamstedt *et al*., 1998) and *Aurelia aurita* in Scotland and Ireland (Bruno and Poppe, 1996; Mitchell *et al*., 2011).

Clinical signs, gross and histopathological lesions

Affected fish show abnormal behaviour including decreased feeding, lethargy, jumping, gasping, head shaking and/or collision with cage walls. After a bloom, residual effects (i.e. decreased growth and performance) may also occur. At inspection, gill haemorrhage, necrosis and excess mucus production, eye damage and darker or burn-like marks in the skin can be observed (Rodger, 2007; Tørud and Håstein, 2008; Rodger *et al*., 2011). Histopathology can reveal ballooning degeneration, spongiosis, hydropic degeneration of the affected epitheliums. Haemorrhage, focal dermal necrosis can also be noted in the skin (Ferguson *et al*., 2010; Rodger *et al*., 2011). On occasion, remains or whole jellyfish can be observed between filaments in the gills (Fig. 4.2).

Risk factors

Jellyfish abundance naturally fluctuates with climate cycles. Jellyfish swarms may occur year round, but blooms are most common from spring to autumn. Purcell *et al*. (2007) suggested an increase in jellyfish blooms in recent years, but the lack of long term data make it difficult to draw definitive conclusions. However, climate change, increased eutrophication due to anthropogenic activities and other human activities (e.g. overfishing) may favour jellyfish multiplication. Floating aquaculture structures also provide a suitable surface for polyp settlement (Purcell *et al*., 2007).

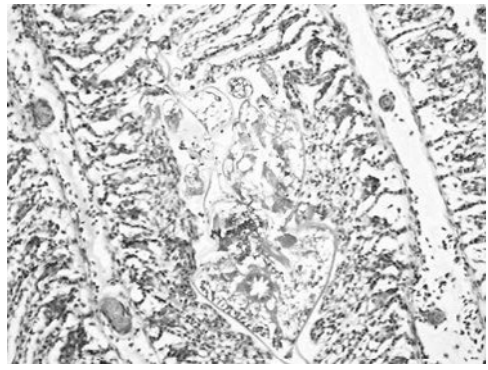


Fig. 4.2. Farmed Atlantic salmon (*Salmo salar*) with small jellyfish (species not identified) between gill filaments. Note localised lamellar epithelium necrosis, sloughing and focal thrombosis. H&E stain (x40). Image courtesy of Hamish Rodger, Vet-Aqua International.

Control and prevention

Blooms are difficult to predict and therefore control measures are difficult to put in place. As with algal blooms, site location, routine monitoring and reduction of eutrophication causes, when possible, are important factors to consider. Mitigation measures such as oxygenation and stop feeding can be helpful. Other protective devices, e.g. bubble curtains, have been proposed but they are still experimental (Rodger *et al*., 2011).

Physicochemical parameters

Gas bubble disease

Gas bubble disease is seen in a wide range of fish species and under various circumstances (Harvey and Cooper, 1962; Rucker, 1975; Saeed and Al-Thobaiti, 1997). Influencing factors include age, species, metabolic rate, water temperature, management and water source. Salmonids, especially larval stages, are very sensitive (Wood, 1974; Smith, 1988; Noga, 2010). The disease occurs when the total pressure of dissolved gases (oxygen or nitrogen) in the water is higher than their atmospheric pressure. Under this circumstance, the excess of aqueous gas tends to leave the solution to equilibrate the gas concentration in both phases. If this occurs in the

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fish blood vessels or tissues, gas bubble disease results (Noga, 2010). Most gas emboli are produced by excess nitrogen (Marking, 1987), since oxygen is metabolically assimilated and it is less likely to form persistent bubbles. The acute form occurs at gas saturations of 110% to 115% and the chronic form at 103% to 105% (Hoffmann, 2005). Even small changes in temperature and pressure can induce the diffusion of liquid gas into gas nuclei, as solubility is decreased with increasing water temperature (Weiss and Price, 1980).

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. The acute form is often characterized by increased mortality without any overt clinical signs (Fig. 4.3a). If they occur, unspecific signs like agitation, darkening of the skin and increased breathing rates can be observed. In larval fish, gas bubbles are most common in the subcutis and the yolk sac (Fig. 4.3b), but in larval flatfish the edges of the body fins seem to be particularly predisposed (Noga, 2010). In older fish, pinhead-sized gas bubbles can be visible under the skin, mouth epithelium, gills, swim bladder, peritoneum, and in all chambers of the eye, where damage can lead to blindness and phthisis (Noga, 2010; Fig. 4.3c–e).

Oedema of the gill lamellae with degeneration of overlaying epithelium, oedema and bullous degeneration of buccal and intes-

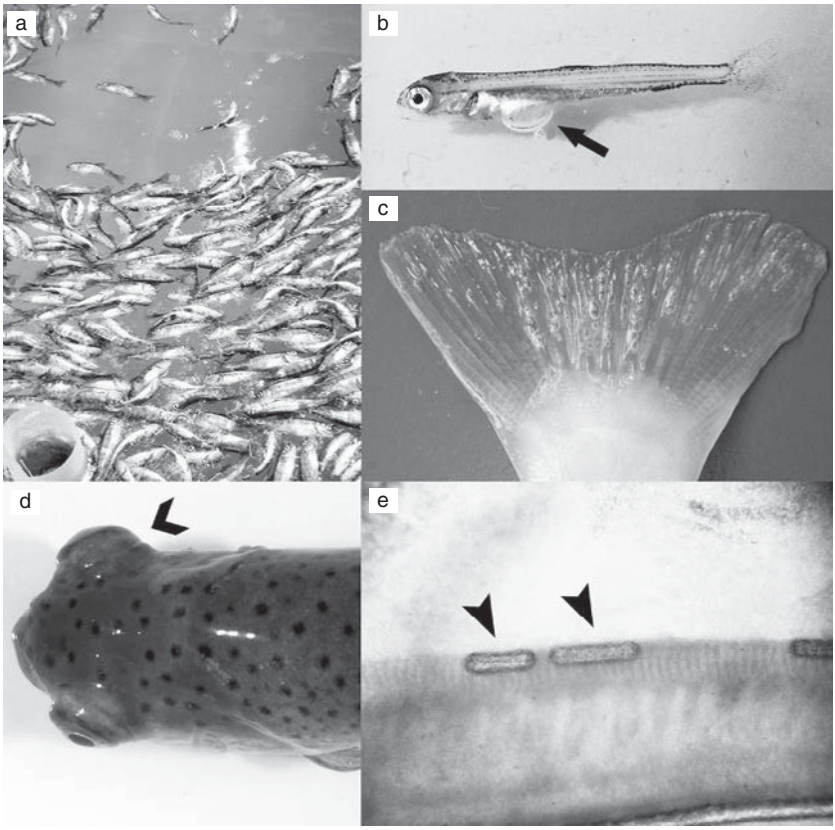


Fig. 4.3. (a) Gas bubble disease, acute massive mortality of rainbow trout (*Onchorhynchus mykiss*) due to hypersaturation of water, acute form. (b) Gas-bubble disease in the yolk sac of a fry (arrow). (c) Gas filled dermal vesicles on the tail fin in a rainbow trout (*Onchorhynchus mykiss*). (d) Rainbow trout (*Onchorhynchus mykiss*) showing exophthalmos because of gas filled vesicles in the eyes (open arowhead). (e) Gas emboli in filament vessels of a rainbow trout (*Onchorhynchus mykiss*) (closed arrowheads), H&E stain.

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tinal mucosa, and vacuolar degeneration of the renal tubular epithelium have been recorded (Pauley and Nakatani, 1967; Smith, 1988; Roberts, 2012a). Gas bubbles can also be found in other parenchyma and in the central nervous system (Hoffmann, 2005). During recovery, secondary infectious diseases of the gills are common (Speare, 2010). Intravascular gas emboli and occlusion of large branchial vessels are one of the major causes of death (Edsall and Smith, 1991; Fig. 4.3e) with up to 100% mortality (Colt, 1986).

Clinical signs of the chronic form are mainly seen in salmonid larvae (Hoffmann, 2005). They are also unspecific, including chronic low mortalities (<5%), hyperinflation of the swim bladder and extravascular emboli in the gastrointestinal tract and mouth (Noga, 2010). Secondary effects, like increased sensitivity to infectious diseases and decreased growth are also reported (Baath *et al*., 1989).

DIAGNOSIS. The diagnosis is carried out on the farm because gas bubbles can disappear during transport. The presence of gas emboli is pathognomonic for gas bubble disease. Determination of gas supersaturation is based on the measurement of the total concentration of dissolved gas in the water.

PREVENTION AND CONTROL. A direct therapy of gas bubble disease is not possible. Factors leading to hypersaturation of the water and the excess of dissolved gases have to be eliminated by using packed column degasser.

Oxygen deficiency

INTRODUCTION. Oxygen demand depends on fish species, age and metabolic rate. Salmonids have higher oxygen requirements, young stages are more sensitive than adults and oxygen demand increases with metabolic rate. The latter is influenced by, e.g. feeding and water temperature. Warmer temperatures increase metabolism and reduce dissolved oxygen levels. In cage culture, anoxic conditions can result from eutrophication, algal and zooplankton blooms, high stocking densities or when fish are shipped (for transfer or treatment) in insufficiently aerated containers. The decrease in oxygen availability to tissues can lead to necrotic or apoptotic lesions, although adaptive responses have been reported (Geng, 2003; van der Meer *et al*., 2005).

CLINICAL SIGNS AND GROSS LESIONS. Clinical signs of oxygen deficiency are increased breathing rate and accumulation of the affected fish near the surface or the water inflow. At necropsy, fish show abducted opercula and open mouth. In chronic cases, low oxygen levels can lead to stress with subsequent decreased performance and increased susceptibility to diseases (Hoffmann, 2005; Noga, 2010).

PREVENTION AND CONTROL. In case of low oxygen concentrations, additional aeration of the water is necessary; however, pure oxygen should not be used for aeration because of the high possibility of hypersaturation. Additional handling of fish (e.g. grading) should be avoided and feeding reduced.

Water-borne irritants

AMMONIA. Although ammonia is the primary metabolic waste product of fish it also originates from the bacterial decay of nitrogenous compounds from faeces or uneaten food. Ammonia is present in two forms; the unionized form (NH_3) is much more toxic to fish than the ionized form (NH_4) (Meade, 1985; Russo, 1985). The concentration of each form depends on temperature, pH and salinity. High pH, high water temperature and low salinity favour the formation of NH_3 and H_2O, while low pH, low water temperature and high salinity accelerate the formation of NH_4 and OH (Emerson *et al*., 1975; Meade, 1985). High ammonia levels can also originate from agricultural and mining operation runoffs or other human activities, excessive biological waste accumulation due to high stocking densities and/or excessive feeding, or insufficient water aeration (Randall and Tsui, 2002; Spencer *et al*., 2008; Noga, 2010). However, in cage culture at appropriate stocking densities and site locations, ammonia toxicity should not occur. Sensitivity is different among fish species. In salmonids, unionized ammonia levels should not increase above 0.002 mg per l H_2O, whereas marine species could tolerate up to 0.05 mg

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per l H_2O (Hoffmann, 2005). Ammonia toxicity may be exacerbated by increased pH or temperature, excessive exercise, starvation and stress (Randall and Tsui, 2002; Spencer *et al*., 2008). The precise pathogenesis of ammonia poisoning is still unresolved; however, high ammonia levels in water will increase ammonia concentration in blood and tissue, causing elevated blood pH, osmoregulatory disturbance, increased tissue oxygen consumption and decreased blood oxygen levels (Arillo *et al*., 1981; Schwedler *et al*., 1985).

Clinical signs, gross and histopathological lesions. Acute ammonia toxicity can cause behavioural abnormalities, such as hyperexcitability and appetite suppression (Daoust and Ferguson, 1983; Ortega *et al*., 2005). In the most severe cases, convulsions, coma and death are reported (Randall and Tsui, 2002). Chronic toxicity has been associated with gill epithelial hyperplasia and hypertrophy (Frances *et al*., 2000; Lease *et al*., 2003; Benli *et al*., 2008; Spencer *et al*., 2008). However, it is unclear if these changes are directly related to ammonia or induced by other poor water quality parameters that usually accompany high ammonia levels. In a study carried out by Daoust and Ferguson (1983), chronic ammonia toxicity in rainbow trout did not produce histological gill changes despite obvious neurological signs. Ammonia related lesions have also been reported in kidney, liver, intestine and ovary (Dey and Bhattacharya, 1989; Banerjee and Bhattacharya, 1994, 1995; Benli *et al*., 2008). Sub-lethal levels can be associated with poor performance.

Diagnosis, prevention and control. Ammonia concentration can be determined using commercial kits. Ammonia levels can be reduced over the short term by addition of fresh water. However, in cultured fish prevention of ammonia toxicity is preferable to therapy. High quality feed with highly digestible protein will reduce ammonia production. Reducing stocking densities or feeding rates and increasing water flow are also effective. Treating water with zeolite is another possibility (Noga, 2010). Supplementation of vitamin C has been reported to reduce the stress induced by chronic high ammonia levels (Liu *et al*., 2008).

NITRITE. In the presence of oxygen, ammonia is converted into nitrite by *Nitrosomas* bacteria and into nitrate by *Nitrobacter* bacteria. Nitrite is toxic to fish while nitrate, which can be used by plants or converted into nitrogen, is mostly unproblematic. Nitrite intoxication usually follows an increase in ammonia, especially in the autumn when low water temperature inhibits the activity of *Nitrobacter* bacteria. In flow-through systems and cage cultures with high water interchange nitrite poisoning does not occur as there is no significant conversion from ammonia to nitrite during the short time the water is present in the system (Noga, 2010).

Clinical signs, gross and histopathological lesions. Nitrite is transported through the gills into the bloodstream where it causes oxidation of haemoglobin (Hb) to methaemoglobin (MetHb) (Lewis and Morris, 1986). MetHb cannot bind oxygen and therefore oxygen uptake is restricted even when water oxygen levels are normal. Affected animals show respiratory distress, lethargy and jump to increase oxygen content in the water. MetHb is brown and at concentrations around 40%, gills can show a pale-brownish discoloration.

Diagnosis. A complete diagnosis includes measurement of nitrite in the water and detection of methaemoglobinaemia, although the practicalities of testing should be assessed for each particular case. MetHb concentrations in the blood above 25% are considered abnormal, but susceptibility to toxicity varies among species.

Prevention and control. The most obvious way to prevent nitrite intoxication is to avoid high ammonia levels. Chloride inhibits the uptake of nitrite by competing with nitrite for uptake by the chloride cells in the gills. This can be used for treatment by adding NaCl into the water in case of high nitrite levels. Calcium also reduces toxicity of nitrite but much less than chloride, as well as bicarbonate (Lewis and Morris, 1986). Different species show varying sensitivity and some fish are resistant (Tomasso, 1986). Salmonids in fresh water are among the more sensitive

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organisms with 96-hour LC50 values ranging from 0.2 to 12 mg per l (Lewis and Morris, 1986). In theory, saltwater fish are also sensitive, but extremely high levels are required, which will not occur under culture conditions (Daniels and Boyd, 1987).

Toxicity

A wide range of toxins can affect fish (Di Giulio and Hinton, 2008). Fish are exposed, either intentionally or unintentionally, to a vast array of chemical and particulate contaminants, both of natural or man-made origin (Harper and Wolf, 2009). Examples include pharmaceuticals and agricultural chemicals, manufacturing by-products, animal or human waste materials and mining effluents (Fig. 4.4). In some exposures, the toxin is a mixture of known and unknown contaminants, in which case it is impossible to differentiate specific responses (Dutta *et al*., 2005). Components are usually taken up by gills and skin, whereas oral exposure is less important. In the gills, components are normally taken up because of the osmotic activity via chloride cells. On the other hand, chloride cells are also responsible for excretion of metabolic end products, especially nitrogenous products. The osmotic activity is influenced by the water chemistry: temperature, water hardness, pH, conductivity and salinity. The sensitivity of fish varies between species but also between age classes, larvae being more sensitive than older fish. In adults, spawning season and translocations from freshwater to marine cultures are critical time points. When salmonids are moved from their freshwater rearing site to the marine cage culture on-growing site is especially a period of high risk (Specker and Schreck, 1980; Harris and Bird, 2000). Because of the large variety of potential toxic components in the water, we will only discuss the general reactions of fish to toxicity and the different presentations: acute and sub-acute to chronic changes.



Fig. 4.4. Slurry intoxication in a common rudd (*Scardinius erythropthalmus*), arrowheads show remnants of slurry in the gill cavity.

ACUTE INTOXICATIONS.

Clinical signs and gross lesions. Acute intoxications are characterized by high mortality, usually in all age classes and different species. Direct intoxications lead to direct changes in different organs, while indirect intoxications are usually caused by oxygen deprivation, e.g. caused by slurry rain-wash in the water supply.

Diagnosis. In case of suspected intoxication, recording the history of the case is a central point for diagnosis. Samples of water and affected fish have to be taken and analysed. Toxic substances can usually be found in gills and skin and in target organs which depend on the chemical properties of the toxic substance, e.g. lipophilic substances in CNS and/or liver, chlorinated hydrocarbon in spleen, heavy metals in kidney and liver (Schlenk and Benson, 2001; Noga, 2010).

CHRONIC INTOXICATIONS.

Clinical signs and gross lesions. Chronic intoxications are usually difficult to diagnose as mortality is low. Some substances can lead to immune depression predisposing fish to secondary parasitic or bacterial infections, which in turn mask the original/primary problem. Histologically, degeneration and atrophy of lymphatic tissue, and increased melanomacrophage centres in spleen, kidney and liver can be visible (Wolke, 1992; Hoffmann, 2005).

Diagnosis. Analysis of fish tissue is usually the only way. Depending on the properties of the substances they can be found in different target organs, like lipophilic substances in

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fat tissue, gonads and CNS. Concentrations in the gills are usually low or absent. One example of a chronic intoxication is aflatoxicosis in rainbow trout (see the section 'Toxic organic components' below).

Nutritional and Feed Related Problems

Adequate nutrition is essential for animal maintenance and health, including stress and disease resistance (Trichet, 2010). Supplementation of special nutrients, like amino acids (AA), essential fatty acids (FA), vitamins or minerals, may have a positive effect on health and disease resistance (Trichet, 2010; Oliva-Teles, 2012). However, at very high levels, toxicity may occur and unbalanced diets may also induce negative interactions or antagonisms among nutrient components (Oliva-Teles, 2012). The presumptive diagnosis of feed related problems is often difficult and done by exclusion after ruling out infectious agents or other possible causes. In addition confirmation is often impossible, since the respective food is mostly not available anymore and additional factors (e.g. environmental) have to be considered. The water supply and the chemical composition of the water may also play a role, since besides the minerals and micronutrients out of the food, fish are capable of uptaking some of these elements via gills. Additionally, microorganisms in the water may serve as an additional source of nutrients. Therefore, in contrast to terrestrial animals, fish nutritional requirements can be difficult to determine; moreover considering the large existing differences between fish species. The most frequent clinical signs of nutritional imbalances are unspecific, like inappetence, skin darkening, lethargy, decreased growth and increased susceptibility to infectious diseases; a fact that makes diagnosis even more difficult.

Starvation

Starvation may be due to a complete deprivation of food or to behavioural or mechanical factors that prevent fish from feeding, although complete deprivation is rare. Behavioural starvation is frequent in reared wild fish or novel aquaculture species when they refuse artificial food. This is an important problem when attempting early weaning in certain larval fish species such as Atlantic halibut, Atlantic cod, walleye or turbot. Non-feeding larvae may survive no longer than a month. Once the degree of catabolic breakdown reaches a point of physical deterioration where they cannot recover, mortality is likely even if food is available (Theilacker, 1978). Other behavioural starvation factors include hierarchical activities, especially in salmonid cultures. This is mainly a problem when fish of different lengths/sizes are housed together or when the stocking density is low.

Diet is an important influence in the fish immune system (Trichet, 2010); consequently, starved fish or fish suffering from chronic malnutrition will be prone to secondary infections.

Clinical signs, gross and histopathological lesions

Starved larvae are called 'pin heads' since they have a very slender body with normal sized head. Fish show skin darkening and pale gills. At necropsy, no abdominal fat tissue is visible and the gall bladder is severely enlarged due to bile retention. Histologically, there is atrophy of muscle fibres (including reduction of sarcoplasmic content, myofiber vacuolation and nuclei centralization), fibroblast proliferation in the intestinal tract and shrinkage of the exocrine pancreas. Due to the catabolic breakdown and accumulation of metabolic end-products, like ceroid and lipofuscin, there is proliferation of the melanomacrophage centres (Agius and Roberts, 1981; Hur *et al*., 2006).

Deficiencies and imbalances

Proteins

Depending on the fish species, their physiological state and environmental conditions, 30% to 50% total protein is required for maximum growth and disease resistance (Halver, 2002). Protein requirements depend on the

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bioavailability of the protein source, the AA composition and the dietary energy level. Overfeeding protein promotes the excretion of nitrogenous compounds in the environment, which can lead to stress, increased disease vulnerability and direct changes to the gills (Hoffmann, 2005). The essential AAs (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), which cannot be synthesized in cells, are the same for all fish species (NRC, 2011). Beside these, cysteine and tyrosine can only be synthesized from their precursors methionine and phenylalanine, and are therefore regarded as semi-essential. Taurine is not classified as essential but seems to be necessary to larval and juvenile marine fish. Fishmeal is especially rich in taurine, whereas plant meal contains only low or no detectable levels (Gaylord *et al*., 2006; Spitze *et al*., 2003). This is important when fishmeal is substituted by vegetable meal. Alternative protein sources may have inadequate levels of essential AAs, lower digestibility, lower palatability and presence of anti-nutrients (Gatlin *et al*., 2007). Food processing may also play a role, as high temperature reduces the availability or induces decomposition of amino acids (Spitze *et al*., 2003). It was shown that direct adding of free AA does not have the same effect on growth and nitrogen utilization as diets containing whole proteins (Peres and Oliva-Teles, 2005).

CLINICAL SIGNS AND GROSS LESIONS. The most frequent indicator of AA deficiency is reduced growth and poor feed conversion. Furthermore, protein malnutrition leads to increased susceptibility to infectious diseases by affecting mainly the non-specific defence mechanisms, while the humoral immune response seems to be unaffected (Kiron *et al*., 1995a, b). More specifically, deficiencies in tryptophan, leucine, lysine, arginine or histidine lead to spinal deformities in salmonids (Kloppel and Post, 1975; Mazid, 1978; Walton *et al*., 1982; Tacon, 1992). Lenticular cataract in Atlantic salmon and rainbow trout is caused by methionine and tryptophan deficiencies, while histidine deficiency has been identified as the cause of cataracts in Atlantic salmon smolts after sea water transfer (Breck *et al*., 2003, 2005; Waagbø *et al*., 2010). In this last case, differences in the osmoregulation and buffering capacity of the lens were related to dietary histidine levels, but Breck *et al*. (2005) identified a strong genetic influence which was not associated with histidine levels.

Carbohydrates

Fish do not have special carbohydrate requirements and do not need carbohydrates for maintenance (Hemre *et al*., 1991; Sa *et al*., 2007). Salmonids have a limited capacity to metabolize dietary carbohydrates while herbivorous and omnivorous species utilize them more efficiently (Lovell, 1998).

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. Excessive dietary carbohydrates may affect disease resistance and stress tolerance (Oliva-Teles, 2012). In salmonids, increased carbohydrates results in glycogen deposition and hepatocyte degeneration (Hemre *et al*., 2002).

Lipids, including rancidity

Lipids are the main energy source, particularly in carnivorous fish as carbohydrates utilization is insufficient in these species (Oliva-Teles, 2012). FAs are the nutritionally active components of dietary fat. All fish require n-3 and n-6 polyunsaturated FAs, however marine and freshwater species differ in their specific requirements (Sargent *et al*., 2002). Additional requirements depend on developmental stage and source of essential FAs. FAs are essential for growth and weight gain in most fish, including reproduction and movement (Tocher, 2003). Additionally, essential FAs are precursors of eicosanoids, which include prostaglandin, prostacyclin and thromboxane, with a wide range of physiological functions, including immune responses (Sargent *et al*., 2002; Wall *et al*., 2010). However, FAs are particularly susceptible to oxidation. During food storage, lipoxidase, originating from soya and myo- and haemoglobin from meat meal, can peroxide by autoxidation. Exposure to sunlight and high temperatures increases the rate of food rancidity. Further iron (Fe) and copper (Cu)

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accelerate lipid oxidation (Castell, 1971; Schnitzer *et al*., 1995). During autoxidation, several components are formed including free radicals, hydroperoxide, peroxide aldehydes and ketones, which can destroy essential FAs, proteins and vitamins (namely vitamin A and C) which are essential for maintenance and biomembrane stability.

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. Essential FA deficiency results in increased mortality, retarded growth, decreased reproductive success and hepatomegaly due to lipoid liver disease (Tacon, 1992). Hepatocytes in fish with lipoid liver disease are completely filled with fat vacuoles, which eventually degenerate, followed by melanomacrophage infiltration which contain ceroid (Fig. 4.5a), a breakdown product of the phospholipid metabolism. Additionally, there is increased auxiliary haematopoiesis. The haematopoietic tissue in the spleen and kidney degenerates, which further increases the number of melanomacrophages filled with ceroid. Anaemia is evident in salmonids because of the failure to secrete haemopoietin (Castell *et al*., 1972; Takeuchi and Watanabe, 1982; Bell *et al*., 1985). If the disease is still mild, animals can recover completely but this is improbable in

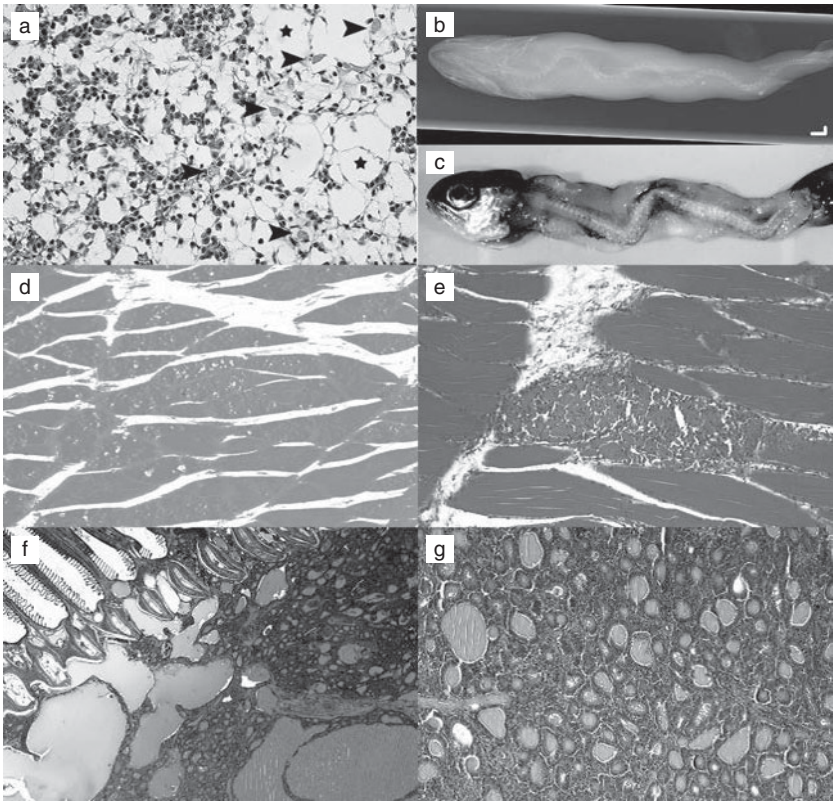


Fig. 4.5. (a) Lipoid liver degeneration in rainbow trout (*Onchorhynchus mykiss*); hepatocytes are severely enlarged and filled with fat vacuoles (stars); melanomacrophages filled with ceroid (arrowheads), H&E stain. (b), (c) Vitamin C deficiency in rainbow trout (*Onchorhynchus mykiss*). (b) Osteoid changes in the vertebrae are associated with a decrease in radio-opacity. (c) Skeletal deformities, including lordosis and scoliosis, stress fractures and accompanied haemorrhages. (d), (e) Vitamin E deficiency. Myofibrillar degeneration characterized by hypereosinophilia, loss of cross striation and fragmentation, H&E stain. (f), (g) Iodine deficiency. Thyroid follicular hyperplasia. Epithelial cells are often cuboidal, the size of follicles is variable and sometimes groups of epithelial cells without colloid filling can be observed, H&E stain.

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advanced disease. All salmonids are susceptible to lipoid liver degeneration; but rainbow trout are most often affected. Other conditions reported are steatitis, skeletal myopathy, as well as increased susceptibility to fin erosions in association with infection with *Flavobacterium* spp. (Tacon, 1992; Rainuzzo *et al*., 1997). Replacing fish oil with vegetable oils affects the dietary FA composition and the ratio of n-3/n-6 polyunsaturated FAs. In Atlantic salmon, low n-3/n-6 polyunsaturated FA ratios lead to decreased B cell numbers and antibody production after vaccination or experimental infection (Thompson *et al*., 1996) and cardiovascular disorders (Bell *et al*., 1991, 1993). In flatfish, an unbalanced FA composition may lead to malpigmentation and impaired eye migration during larval development (Hamre *et al*., 2007). Excessive levels of FA, on the other hand, can inhibit the immune functions. For example, in Atlantic salmon excessive essential FA led to increased mortality and decreased antibody production after bacterial challenge (Erdal *et al*., 1991).

PREVENTION AND CONTROL. Feed should be stored in a cool area and away from direct sunlight. Commercial diets contain surplus of vitamin E, an antioxidant which prevents lipid autoxidation. Also other antioxidants (e.g. vitamin C, carotene, glutathione, EDTA and synthetic antioxidants) can be added.

Vitamins

Vitamins are required in trace amounts for normal growth, reproduction and disease resistance (NRC, 2011). In times of high metabolic activity, like growth or spawning, requirement can increase. Supplementation of certain vitamins, particularly vitamin A, E and C, is supposed to have a positive effect on the immune system (Wahli *et al*., 1998; Hernandez *et al*., 2007; Koshio, 2007; Yang *et al*., 2008; Cerezuela *et al*., 2009; Lim *et al*., 2010); however, this is still debatable (e.g. Wang *et al*., 2006; Lim *et al*., 2010; Oliva-Teles, 2012). Vitamin requirements are very similar in fish (Woodward, 1994; Kaushik *et al*., 1998). Fat-soluble vitamins (e.g. vitamin A or E) are stored in the body and are only slowly metabolized, while water-soluble vitamins (e.g. vitamin C) are rapidly excreted. Diagnosis of a vitamin deficiency can be difficult, as clinical signs and histopathological features are often unspecific and diets are usually not deficient in only one micronutrient (Roberts, 2002). Hypervitaminoses are mainly reported in association with fat-soluble vitamins, although they are unlikely under practical conditions (Tacon, 1992; Halver, 2002).

VITAMIN A (RETINOL). Vitamin A (fat-soluble) is crucial for growth, including bone development, normal eye function, mucous membrane maintenance, cellular membrane permeability, corticosterone synthesis and disease resistance (Thompson *et al*., 1994; Cuesta *et al*., 2002; Hardy, 2012). Carotenoids are precursors of vitamin A and also enhance immune response (Amar *et al*., 2000, 2001, 2004).

Clinical signs, gross and histopathological lesions. Vitamin A deficiency leads to reduced growth, depigmentation and eye changes, including exophthalmia, clouding and thickening of corneal epithelium, keratomalacia and retinal degeneration (Tacon, 1992; Moren *et al*., 2004). In salmon, vitamin A and astaxanthin, a carotenoid, are essential during the first-feeding period. Undersupply induces poor growth and low survival rates (Christiansen *et al*., 1994). In salmonids, hypervitaminosis results in epithelial squamous metaplasia, hepatomegaly, splenomegaly, osteopathy and choroidal and corneal inflammation (Poston *et al*., 1966; Poston, 1971; Ørnsrud *et al*., 2002). Additionally, in flounder vitamin A toxicity is characterized by increased mortality, retarded growth, abnormal vertebral growth and changes in pigmentation (Dedi *et al*., 1995; Bolker and Hill, 2000; Martinez *et al*., 2007). Unless the condition is extreme, it is readily reversible. Exceptions are early life stages of Atlantic salmon, where vitamin A stress or toxicity occurs at relatively low levels (Ørnsrud *et al*., 2002).

VITAMIN E (TOCOPHEROL). The metabolism of vitamin E is closely related to selenium (Poston *et al*., 1976). Regeneration of oxidized tocopherol is catalysed by selenium. Therefore, high levels of either one would be

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sufficient to compensate for the other (Lin and Shiau, 2009). Vitamin E is the main antioxidant by donating a phenolic hydrogen atom to lipid free radicals – this stabilizes and terminates the lipid peroxidation chain reaction. Another mechanism to prevent FA peroxidation is by quenching single oxygen. Therefore, to prevent oxidative stress, vitamin E is usually supplemented in high amounts (Martinez-Alvarez *et al*., 2005). Vitamins in the diets usually contain a protected form (α-tocopherol acetate) to prevent oxidation during feed storage, although these forms cannot be used as natural antioxidants in the diet (Hardy, 2012). Feeding diets containing already oxidized oil can affect the liver antioxidant enzymes and dietary vitamin E can decelerate this process (Mourente *et al*., 2002; Tocher *et al*., 2002, 2003). Additionally, vitamin E may influence the biosynthesis of n-3 polyunsaturated FAs through alteration of the cellular oxidation potential or peroxide tone (Mourente *et al*., 2007). Other roles of vitamin E are modulation of biological membranes and immune functions, like enhancement of antibody production and macrophage phagocytic activity (Bendich, 1990). Vitamin E deficiency syndromes and associated pathology are more pronounced at lower water temperatures and stress.

Clinical signs, gross and histopathological lesions. Vitamin E deficiency is associated with increased mortality, reduced growth and egg hatching rate/spawning efficiency, muscular degeneration (Figure 4.5d, e), exophthalmia, ascites, pale gills with clubbed epithelial proliferation, epicarditis, lower antibody response and increased susceptibility to secondary infections (Cowey *et al*., 1984; Ndoye *et al*., 1989; Obach and Baudin Laurencin, 1992; Tacon, 1992; Wahli *et al*., 1998; Montero *et al*., 1999). Anaemia is caused by erythrocyte membrane fragility, which leads to increased erythrocyte degradation and splenic haemosiderosis.

VITAMIN C (ASCORBIC ACID). Vitamin C is a cofactor for prolyl-4-hydroxylase, which is responsible for hydroxylation of proline to hydroxyproline. Furthermore, vitamin C is involved in hydroxylation of L-lysine to hydrolysine. Both components are essential parts of collagen, which is present in connective tissue, bone matrix, and scar tissue in wound repair (NRC, 2011). The vitamin C is also involved in reproduction, immune response (Gabaudan and Verlhac, 2001) and stress reduction due to, e.g. hypoxia, low temperature, osmotic stress or ammonia (Dabrowski *et al*., 2004; Chagas and Val, 2006; Falcon *et al*., 2007; Liu *et al*., 2008). Another important function of vitamin C is its antioxidant capacity, especially in combination with vitamin E. The synergistic effects of vitamin C and vitamin E may be due to the ability of vitamin C to regenerate vitamin E and the ability of vitamin E to spare the requirements of vitamin C (Sealey and Gatlin, 2002; Lim *et al*., 2010). Imbalance is age-dependant, with young stages being usually more sensitive than adults (Frischknecht *et al*., 1994).

Clinical signs, gross and histopathological lesions. Clinical findings associated with vitamin C deficiency are often unspecific; they include reduced growth, darkening and decreased reproduction success (Tacon, 1992). In salmonids, skeletal deformities, including lordosis and scoliosis, stress fractures, opercular deformation and gill filament distortion (Fig. 4.5b, c) are not uncommon (Tacon, 1992). These changes are due to a deforming diathesis of the cartilage, with replacement of bony tissue by osteoid. Additionally, fish fed vitamin C deficient diets lose the capacity to initiate fibroplasia after wound induction, including repair of dermal fibres and revascularization and re-establishment of normal dermal and muscle structures (Halver *et al*., 1969; Wahli *et al*., 2003). The epidermis is not affected and epidermal cells immediately migrate over the damaged area independent of vitamin C levels. Vitamin C deficient Atlantic salmon show reduced unspecific immune responses due to decreased complement activity (Hardie *et al*., 1991). Especially in combination with low vitamin E levels, fish are more susceptible to infectious diseases with high mortality (Obach and Laurencin, 1992; Wahli *et al*., 1998; Montero *et al*., 1999). In marine species, like turbot, vitamin C deficient diets lead to blockage of tyrosine catabolism and to hypertyrosinaemia. Tyrosine

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crystals accumulate in different parenchymal organs, especially kidney, liver and spleen. The crystals induce a granulomatous reaction of foreign body type with epitheliod macrophages (Baudin-Laurencin *et al*., 1989).

Minerals

DEFICIENCIES. Fish may obtain minerals from both the diet and the aqueous environment, therefore deficiencies are less common than in terrestrial animals. Moreover, mineral supplementation is not expensive and consequently deficiency can be avoided. However, deficiencies of certain minerals may still occur in association with reduced bioavailability, i.e. because of antagonism with other minerals or other dietary elements, like vitamins, fibre or phytic acid (Hilton, 1989; Tacon, 1992; Sarker and Satoh, 2007). One exception represents phosphorus (P), of which concentrations are usually low, both in marine and fresh water. Therefore, high quantities are needed in the diet. Availability of P is dependent on the diet source with inorganic and animal P being more available than P from plant sources (Pimentel-Rodrigues and Oliva-Teles, 2007) because plant P is stored as phytate and animals lack the enzyme phytase (Tacon, 1992; Oliva-Teles *et al*., 1998). With the increasing need to replace fishmeal by plant meal as a protein source, the probability of P deficiency will increase. Additionally, P imbalance can occur due to dietary imbalance of calcium (Ca), because of the antagonistic effect of excess Ca on the absorption of P (Nakamura, 1982). Excess Ca may also inhibit absorption of other trace elements, like zinc (Zn), iron (Fe), manganese (Mn) and magnesium (Mg) (Ketola, 1978; Lall, 1979; Watanabe *et al*., 1980; Tacon, 1992; Sugiara *et al*., 2000). However, high amounts of P in the diet can have negative effects on growth and Zn availability (Tacon, 1992).

Selenium (Se) is a component of the Se-dependent glutathione peroxidase, which acts together with vitamin E as an antioxidant (NRC, 2011). In cases of chronic stress, Se utilization is increased and therefore diet supplementation may be necessary to meet the requirements (Rider *et al*., 2009).

Clinical signs, gross and histopathological lesions. With the exception of Fe and iodine (I), where deficiencies are characterized by specific syndromes, reduced growth and feed efficiency, anorexia and increased mortality are reported with deficiencies of all minerals and trace elements (Tacon, 1992). In the presence of low P levels, bone demineralization with deformed head, ribs and vertebrae is reported in salmonids (Ogino and Takeda, 1976; Skonberg *et al*., 1997). Combined deficiencies of P and Se in fast-growing strains of Atlantic salmon, particularly in post-smolts, can cause failure to ossify and resorption of P from the bones, which become deformed, especially at stress sites, like the jaw, giving the animals a characteristic appearance, (Bruno, 1990; Roberts *et al*., 2001). They also show spinal deformities, like kyphosis or scoliosis (Sullivan *et al*., 2007a). Inadequate P levels in fry or fingerlings result in twisted or curled dorsal vertebral spines, which are often encrusted with mineralized plaques (Sullivan *et al*., 2007b).

Cataracts are reported with Mg, Zn, Mn or Cu deficiencies (Tacon, 1992). Mg deficiency additionally can lead to nephrocalcinosis, vertebral curvature and degeneration of muscle fibres, epithelial cells of pyloric caeca and gill filaments. Zn deficiency is also associated with short-body dwarfism, fin erosion and poor egg hatchability (Tacon, 1992; Hardy, 2012). Additionally, Zn and Mn deficiencies lead to depressed activity of natural killer cells and therefore to reduced immune function (Inoue *et al*., 1998). Low Se levels can lead to muscular dystrophy, and depressed glutathione peroxidase activity, leading to cell membrane instability due to oxidative damage and reduced immune response (Tacon, 1992; NRC, 2011). Fe deficiency is characterized by hypochromic microcytic anaemia. However, low Fe levels protect against bacterial infection by limiting the available Fe amount needed by bacteria. I deficient salmonids exhibit thyroid follicular hyperplasia (Kawatsu, 1972; NRC, 2011) (Fig. 4.5f, g). I deficiency, in combination with energy limitation and unbalanced FA composition, has been suggested as a contributing factor for malpigmentation and impaired eye migration in flatfish larvae (Hamre *et al*., 2007). I supplementation was

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also shown to have a positive effect on the immune system in Atlantic salmon (Lall, 2002).

TOXICITY. Minerals like lead (Pb), cadmium (Cd), copper (Cu) or mercury (Hg) are often insufficiently regulated and can accumulate in the body with toxicity signs (NRC, 2011). However, most of the toxicities have been induced under laboratory conditions.

Clinical signs, gross and histopathological lesions. Clinical signs associated with mineral toxicity are summarized by Tacon (1992). In rainbow trout reduced growth and feed efficiency has been associated with Se, Cu and Cr toxicities. Se toxicity is characterized by nephrocalcinosis, increased susceptibility to infections and high mortality in rainbow trout (Hilton *et al*., 1980; Hilton and Hodson, 1983; Hicks *et al*., 1984). High Cu exposure can lead to increased intestinal cell proliferation and apoptosis (Berntssen *et al*., 1999) and higher susceptibility to bacterial infections, as Cu is often the limiting nutrient factor for bacterial growth and multiplication (Hilton and Hodson, 1983). Cd toxicity is associated with scoliosis, hyperactivity and decreased bone Ca content. High levels of Pb induce scoliosis, lordosis, black tail, anaemia and degeneration of caudal fin in rainbow trout (Tacon, 1992). For minerals, the level of toxicity can vary depending on the food uptake together with water temperature and metabolic activity, as well as the Ca content in the diet.

CALCIUM. Despite the high demand of Ca, deficiency is unlikely as Ca is supplied by the diet and additionally can be absorbed through the gills. Therefore, it is more important in terms of imbalances with other dietary components, which can lead to calcification, and because of its capacity to impair absorption of heavy metals. As in mammals, metastatic or dystrophic calcification also occurs in fish. Metastatic calcification is normally caused by hypercalcinosis, while dystrophic calcification occurs most frequently in degenerated areas, like chronic granuloma, due to changes in tissue pH. In salmonids, a specific syndrome, nephrocalcinosis, is associated with dystrophic calcification. The cause of this chronic condition often remains undetermined in specific cases, but high CO_2 levels, as well as Mg deficiency and Se toxicity, are implicated (Hilton and Hodson, 1983; Hicks *et al*., 1984). Associations to dietary Ca levels are not obvious. Mortality is usually low, and although growth may be retarded, the main problem is reduction of carcass quality at slaughter.

Clinical signs, gross and histopathological lesions. Nephrocalcinosis is characterized by widespread Ca deposits in renal tubules, collecting ducts and ureters, in the lumen and along the epithelial cells. This condition is especially common in intensively reared rainbow trout and brook trout. Macroscopically, chalky white material can be seen on the surface of the kidney. In the early stages, lesions are usually restricted to thick, white ureters (Fig. 4.6a). If the condition exacerbates the kidney becomes swollen, greyish and the ureters become more sinuous and thickened. In severe cases, the dorsal musculature may also be affected. Histologically, mineral deposits evoke a granulomatous inflammation affecting both excretory and interstitial parts of the kidney, followed by interstitial fibrosis (Fig. 4.6c). Obstruction of urine flow leads to distended tubuli and collecting ducts and tubular and glomerular degeneration (Harrison and Richards, 1979) (Fig. 4.6b). Often the lesions are associated with gastric submucosal granuloma.

Lithiasis is also described in the biliary system of Atlantic and Pacific salmon (Roberts and Shepherd, 1997). Similar to the mineral deposits in the kidney, there is no correlation with dietary Ca levels. The obstruction of bile ducts leads to retrograde distension and jaundice. Cholelithiasis is normally asymptomatic, but in severe cases hepatic cirrhosis can occur.

Toxic organic components

Mycotoxins

Aflatoxin is a mycotoxin produced by *Aspergillus flavus*, a blue-green mould which is a common contaminant on oil seeds under warm humid conditions, especially if environmental temperatures rise above 27°C,

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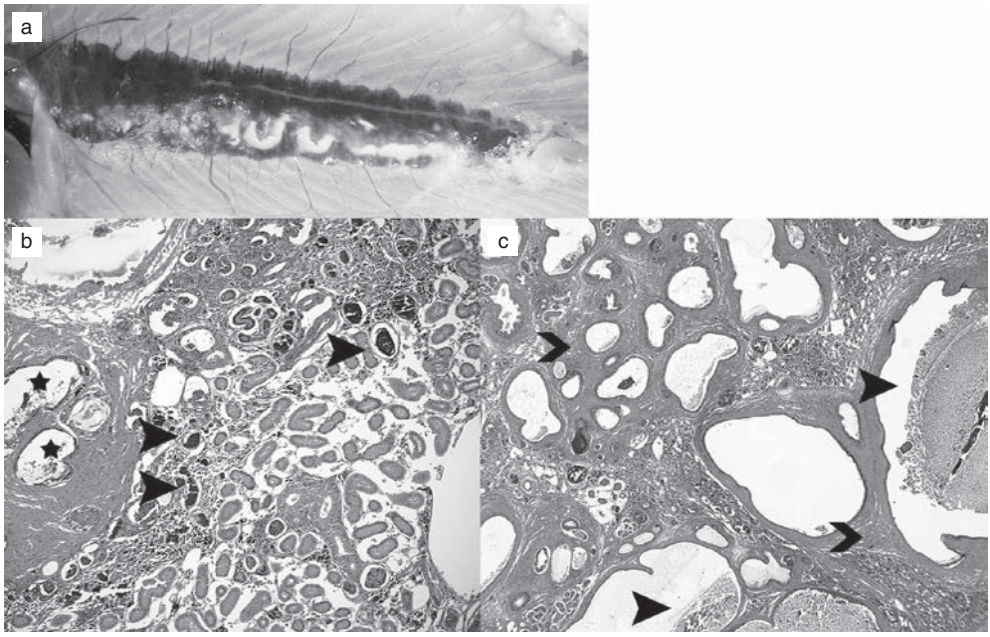


Fig. 4.6. Nephrocalcinosis in rainbow trout (*Onchorynchus mykiss*). (a) In the early stages, lesions are usually restricted to thick, white ureters on the surface of the kidney. (b) Obstruction of urine flow leads to extended tubules and collecting ducts and tubular and glomerular degeneration (stars); calcium deposits are found in renal tubules and collecting ducts. These deposits are located in the lumen and along the epithelial cells (closed arrowheads), H&E stain. (c) Mineral deposits (closed arrowheads) evoke a severe granulomatous inflammation affecting both tubules and interstitial parenchyma, followed by interstitial fibrosis (open arrowheads), H&E stain.

humidity levels above 62% and moisture levels in the feed above 14% (Payne *et al*., 1988; Motalebi *et al*., 2008). Four major aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) are identified. In rainbow trout, an extremely sensitive species, AFB_1 is a potent carcinogen. This condition is rare in temperate regions but in some developing countries where storage of grains involves exposure to humid and warm conditions, aflatoxicosis is still a serious problem (Motalebi *et al*., 2008).

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. Aflatoxin induces neoplastic changes after a few months in rainbow trout fed contaminated diet with levels as low as 0.01 ppb. Histologically, the lesion is characterized by a malignant invasive hepatocellular or bile duct carcinoma, often with a high amount of fibrovascular stroma. This is one of the few examples of neoplastic lesions in fish which regularly metastasize, often to kidney, spleen or gills (Majeed *et al*., 1984). At higher concentrations, the toxin induces acute toxicity with hepatic necrosis, branchial oedema and generalized haemorrhage (Bauer *et al*., 1969; Hardy, 2012). At extremely low levels, aflatoxin may induce retarded growth and reduced haemoglobin concentrations and erythrocyte numbers (Jantrarotai and Lovell, 1990). Nowadays, thanks to the increased awareness and controlled food storage conditions in developed countries, this condition is rare.

PREVENTION AND CONTROL. Improper storage is one of the most important factors favouring the growth of aflatoxin-producing moulds and it is the main element that can be controlled by the fish producer. Experimentally, dietary chlorophyllin was shown to be a potent dose-dependent inhibitor of AFB_1

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hepatocarcinogenesis in rainbow trout when fed together with the carcinogen (Breinholt *et al*., 1995).

Soybean induced enteritis

Fishmeal is traditionally used as the main protein source in fish feed for carnivorous species such as salmonids. However, the costs and availability of fishmeal can be a limiting factor, which has led to an increased research on alternative protein sources. The replacement of fishmeal with protein from plants has not been successful for carnivorous fish, which need high levels of good quality protein (Dersjant-Li, 2002). Soybean protein is of good quality and is highly digestive (Dersjant-Li, 2002), but certain fish species (e.g. Atlantic salmon, carp) have proven to be immunologically sensitive to some of its components (Urán, 2008; Urán *et al*., 2008). In sensitive species, the inclusion of excessive soybean meal induces an inflammatory response of the distal intestine known as soybean induced enteritis (Urán, 2008). Salmonids are highly susceptible, while Atlantic cod is unaffected (Roberts, 2012c). Nowadays it rarely occurs, but it can still be a problem in situations where access to fishmeal is restricted or in new aquaculture species when the status of sensitivity is not known. Both dietary and non-dietary factors in soyabean are believed to be involved (Ferguson, 2006; Knudsen *et al*., 2008; Urán, 2008; Lilleeng, 2009) and the severity of the inflammation depends on the fish species, soybean meal origin and processing, soybean meal levels and temperature. Negative effects are dose dependent and inclusion levels as low as 10% can induce enteritis in Atlantic salmon (Urán *et al*., 2009). Urán (2008) reported the onset of the clinical signs in Atlantic salmon was delayed at lower temperatures (8°C versus 12°C).

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. The induced enteritis affects the distal intestine, and can lead to a disturbed digestive process and a reduced health status (Lilleeng, 2009). There is widening and shortening of the intestinal folds, loss of apical epithelial vacuolation, proliferation of epithelial cells and infiltration of the lamina propria with a mixed population of inflammatory cells which include macrophages, neutrophils and eosinophilic granular cells (Baeverfjord and Krogdahl, 1996; Ferguson, 2006). Urán (2008) also observed blockage of endocytosis and a strong decrease of the microvilli length. The enteritis can be induced within a few days (2 to 7 days depending on the dose) and can resolve completely within 3 weeks after removal of soybean meal. Recovery changes can start as early as 2 days after feed change (Baeverfjord and Krogdahl, 1996; Ferguson, 2006; Urán *et al.*, 2009).

Vaccination and Treatment Related Problems

Vaccines

Vaccines against bacterial and viral diseases are widely used in intensive aquaculture. They can be administered orally (via feed), by immersion or by intraperitoneal (i.p.) injection. The type of vaccine used depends on the species and the size of the fish; e.g. i.p. vaccines are used in fish over 30 g.

Clinical signs, gross and histopathological lesions

The intraperitoneal administration of oil adjuvanted vaccines can cause side effects including retarded growth (Midtlyng and Lillehaug, 1998), granulomatous peritonitis, intra-abdominal adhesions and melanization (Ferguson, 2006). Numerous melanomacrophages and remnants of vaccine seen as clear vacuoles can be observed in the granulomatous response (Fig. 4.7). The extensive granulomatous inflammation is likely to impact the exocrine pancreas and subsequent production of digestive enzymes (Ferguson, 2006). In severe cases, melanized foci can penetrate into the abdominal wall, and occasionally into the skin (Koppang *et al*., 2005). Muscle necrosis, fibrosis and granulomatous inflammation can be seen in these lesions. Bjørge *et al*. (2011) observed that the severity of the peritonitis induced in Atlantic salmon was directly related to behavioural changes, including reduced feeding response and

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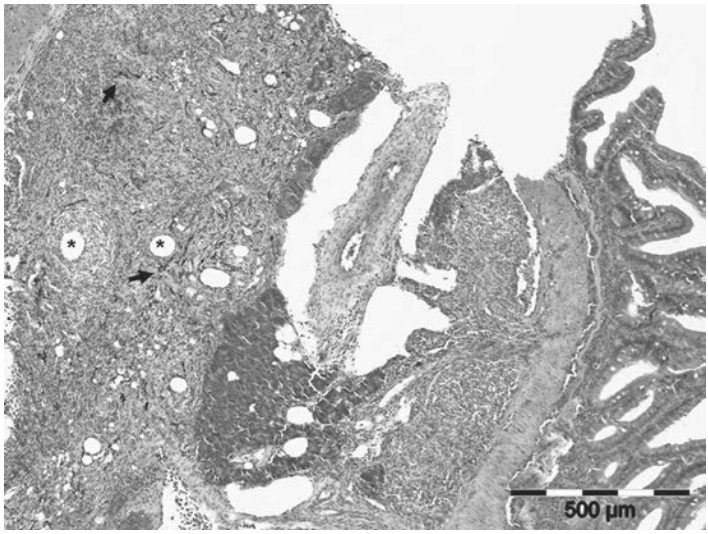


Fig. 4.7. Farmed Atlantic salmon (*Salmo salar*) showing severe granulomatous peritonitis around pancreas and pyloric caeca after i.p. vaccination. Note the presence of melanomacrophages and the clear vacuoles (*), remnants of vaccine. H&E stain.

decreased activity. The decreased growth observed in post vaccinated fish is likely related to both the poor feeding response and the chronic enzymatic insufficiency induced (Ferguson, 2006).

Chemical treatments

In cages, chemical bath treatments (such as hydrogen peroxide, formalin, chloramine T and commercial sea lice treatments) applied to combat fungal infections and external parasites can be administered within well boats or inside the cages by fitting tarpaulins around them. The margin between effective and toxic concentrations is narrow and will depend on the fish species, health status, stocking density, exposure time and water temperature. For example, temperatures higher than 13°C increase hydrogen peroxide (H_2O_2) toxicity (Rodger *et al*., 2011). Bruno and Raynard (1994) observed 35% mortalities at 13.5°C but none at 10°C in Atlantic salmon. Currently H_2O_2 doses used for treating Atlantic salmon against sea lice and amoebic gill disease are around 1700 mg per l and 1200 mg per l for 20 minutes respectively. Tides and currents can affect the final concentration and distribution of the chemical in the water. To avoid heterogeneous distribution of the chemical, several inlet pumps at different cage locations and water agitation systems should be installed. To decrease the volume of chemical needed, the nets of the cages can be raised in the tarpaulin system. This, however, increases the fish density leading to an increase in the oxygen consumption and demand. Oxygen levels should be monitored and extra oxygenation supplied when required.

Clinical signs, gross and histopathological lesions

Chemical bath treatments can be toxic for the fish. Treated fish can show a reduction in the amount of oxygen bound to haemoglobin and gill damage, including lamellar epithelial swelling, lifting, hyperplasia, necrosis and intercellular oedema (Johnson *et al*., 1993; Powell and Perry, 1997). Several adverse effects have been associated with the excessive use of antibiotics in fish, such as immunosuppression, nephrotoxicity, growth retardation, development of resistances, environmental problems (i.e. residues in fish farm sediments) and drug residues in fish products (Yonar, 2012). Antibiotics are normally administered

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within the diet. During treatment, in order to avoid decreased feeding and subsequent waste of antibiotics to the environment due to decreased appetite or palatability, the feeding times are reduced so fish appetite is increased at next feeding point. Different antibiotics are licensed in different countries. At high concentrations, some antibiotics have been reported to induce toxicity. Erythromycin, effective against Gram-positive bacteria, can impair kidney function by inducing vacuolar degeneration of proximal renal tubular epithelium (Roberts, 2012c). Sulphonamides can induce necrosis of the renal tubular cells and hepatocytes (Roberts, 2012c). Oxytetracycline, a broad spectrum antibiotic, has been associated with immunosupression in rainbow trout (Yonar *et al*., 2011) and liver damage (Bruno, 1989).

Neoplasia

Neoplasia is uncontrolled proliferation and dispersal of autologous cells throughout the host body. It is based on accumulated DNA mutations. Even in the absence of special mutagens, like chemicals, viruses or radiation, there will be an increasing chance of mutations with increasing age and therefore an increasing chance of spontaneous neoplasms. As fish in culture usually do not survive to old age, incidental tumours are rare in cultured fish. Therefore, neoplasms in farmed fish are indicators for possible dietary or environmental contamination or viral infection (Overstreet, 1988; Rafferty *et al*., 2009), although it is often difficult to distinguish between infectious and non-infectious causes of neoplasms. In general, metastases are far less common in fish neoplasms than in mammals, although the reason for this is still not clear (Martineau and Ferguson, 2006). Therefore, distinguishing between benign and malignant neoplasms in fish is often difficult. However, judging the stage of progression (from preneoplasic foci to anaplastic, invasive growing neoplasms) might be useful in respect of, e.g. estimating exposure duration to carcinogens. Two different mechanisms may be involved in neoplasia development. The first is inactivation of tumour suppressor genes and the second is activation of oncogenes. Tumour suppressor genes prevent the uncontrolled proliferation of a cell by triggering the arrest of the cell cycle or by programmed cell death (apoptosis). As these genes are recessive, all copies have to be non-functional for uncontrolled proliferation of a cell to occur. Oncogenes also play a role in tumour development. In their intact form, these genes are called 'proto-oncogenes', while after activation, they are called 'oncogenes'. Oncogene mutations can result in hyperactivation or overproduction of the encoded protein or in gene amplification, also resulting in protein overproduction (Meuten, 2002; Martineau and Ferguson, 2006).

Viruses

In fish, the only known tumour-inducing viruses belong to the retroviruses, mainly inducing haematopoietic and mesenchymal neoplasms, whereas epithelial cells seem to be resistant to tumour transformation by viruses (Martineau and Ferguson, 2006). In contrast, hepatic and epidermal neoplasms appear to be strongly influenced by environmental pollution (Harshbarger and Clark, 1990).

Chemical carcinogens

The long time between exposure to environmental chemical carcinogens and cancer development often makes it difficult to prove a causative relationship. Many man-made carcinogens, like polycyclic hydrocarbons (PAHs), are hydrophobic and rapidly associated to particulate matters (Knap and Williams, 1982; Chiou *et al*., 1998). Therefore, bottom-dwelling fish are especially exposed to these carcinogens. Fishmeal and fish oil are the most important sources of contaminations with anthropogenic carcinogens, like PAHs or polychlorinated biphenyls (PCBs), in commercial fish feed (EFSA, 2005). Chemical carcinogens can cause mutations in both tumour suppressor genes and oncogenes.

Classification of fish tumours is generally based on criteria developed for human

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medicine, which are based on phenomena (morphology, staining or immunohistochemical characteristics) that characterize the identity and behaviour of neoplastic cells (WHO, 2000; Meuten, 2002).

Common neoplasms in coldwater finfish

Skin

The most common skin tumours are *papillomas*, many are flat shaped and show a discrete expansive growth of altered epidermal cells. Specialized cells, such as goblet cells, are usually missing or rare by displacement or necrosis. The proliferated epithelium is often spongiotic and infiltrated with mainly lymphocytes. Hyperplastic plaques are seen in 'papillomatosis' of Atlantic salmon. This neoplastic lesion is most common in young fish in freshwater stocks during mid-summer months. Clinically, they appear as blue-grey raised plaques (Shchelkunov *et al*., 1992) and they usually regress spontaneously without lesions, although sometimes they become secondarily infected following ulceration (Carlisle and Roberts, 1977). *Squamous cell carcinomas* are less common, but in Atlantic salmon a progression from the papillomatous lesion has been recorded in individual animals (Roberts and Shepherd, 1997). The epidermal papillomas of Pacific flatfish are characterized by the presence of X-cells in addition to the malpighian cell proliferation. These lesions were classified as pseudoneoplasms (Grizzle and Goodwin, 2010). The X-cells lack desmosomes and tonofilaments and show signs of degeneration, like swollen mitochondria and a vesicular endoplasmic reticulum (Peters *et al*., 1981). The identity of these cells is still uncertain. In the beginning, a viral cause was suspected (Peters *et al*., 1983), but there are also indices that the X-cells are of parasitic, namely protist, origin (Miwa *et al*., 2004). Experimental transmission of the disease to uninfected animals has not been successful. Mainly young fish are affected at the upper pigmented side of the body. Atlantic species of flatfish, like Atlantic turbot and dab, occasionally show epizootic epithelial hyperplasia. These are usually homogenous, flat plaques of proliferating malpighian cells up to 15 mm in diameter.

Pigment cell tumours include melanomas, erythrophoromas, xanthophoromas, guanophoromas and iridophoromas. Melanomas are most common; however, they are mainly seen in aquarian fish. In H&E stain, pigments are visible in melanomas and iridophoromas. As in mammals, some melanomas can be amelanotic and show variation in their cellular morphology from round to fusiform or spindle-shaped (e.g. Gimenez-Conti *et al*., 2001). Iridophoromas are composed of spindle-shaped cells with a distinct olive-green pigment (Schmidt-Posthaus *et al*., 2005) (Fig. 4.8a, b).

Fibromas and *fibrosarcomas* are the most common mesenchymal tumours affecting a wide variety of different species. They are often loosely organized, sometimes also called myxoma, or hard, as found in the dermal sarcoma of walleye caused by a retrovirus (Martineau *et al*., 1990, 1992). Generally, they are enclosed by a covering layer of epithelium. The cut surface is usually firm and shiny, pale pink or whitish, and occasionally with central necrosis. Histologically, the appaerance is comparable to the criteria described for human medicine (WHO, 2000; Meuten, 2002). In fish, fibrosarcomas are characterized by their invasiveness rather than metastases which are rare. Environmetal and infectious agents are suggested as possible causes for fibromas or fibrosarcomas, which can occur in high prevalences in farmed and wild fish (Black *et al*., 1982; Martineau *et al*., 1990, 1992).

Peripheral nerve sheath tumours are also common neoplasms, especially in goldfish (Schlumberger, 1952, 1957; Marino *et al*., 2007), damselfish (Schmale *et al*., 1983, 1996), but also in coho salmon (Masahito *et al*., 1985). Generally these tumours are benign.

Lipomas are occasionally seen in the dermis as soft, greasy masses. Typically, they appear as well differentiated, rounded, and encapsulated masses. Liposarcomas are not described in fish.

Haemangiomas, benign proliferations of small blood vessels, are also sometimes seen in the dermis (Meyers and Hendricks, 1983).

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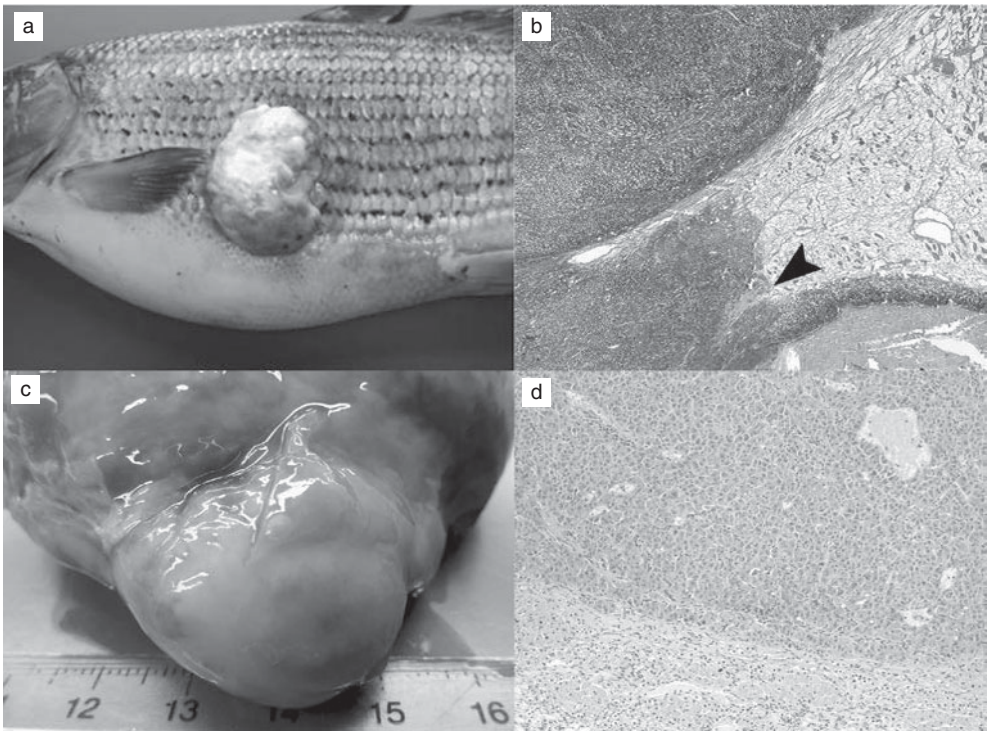


Fig. 4.8. (a), (b) Iridophoroma in a grayling (*Thymallus thymallus*). (a) Macroscopically, a firm whitish mass on the right flank is visible. (b) histologically the neoplastic cells are arranged in bundles, the cytoplasm is filled with an olive-green pigment, the arrow demonstrates the transition between hyperplastic pigment cell layer and neoplastic lesion, H&E stain. (c) Leukaemia with involvement of liver in a rainbow trout, *Onchorynchus mykiss*. (d) Hepatocellular adenoma. The neoplastic cells are well differentiated, the cytoplasm is basophilic and there is a high nuclei/cytoplasm relationship. The surrounding tissue is compressed, H&E stain.

Gills and pseudobranchs

Tumours of the gills are rare. In Atlantic and Pacific cod, tumorous lesions in the pseudobranchs are described mostly as bilateral masses at the dorsolateral part of the gill cavity, containing large pale cells similar to the X-cells seen in Pacific flatfish papilloma (Watermann and Dethlefsen, 1982; Watermann *et al*., 1982). Incidence is largely confined to fish less than 4 years old (Sindermann, 1990). Similar lesions are also found in dab from the North Sea (McVicar *et al*., 1987; Watermann *et al*., 1993). Comparable to the X-cell lesion in the skin, the cause is still not known, as viral particles are in the lesion, but X-cells are now supposed to be of parasitic origin (Watermann *et al*., 1993; Miwa *et al*., 2004). According to Grizzle and Goodwin (2010) these lesions are also classified as pseudoneoplasm. Branchioblastoma and gill epithelial cell tumours can be chemically induced (e.g. medaka) (Brittelli *et al*., 1985) or occur spontaneously in brown and rainbow trout and in Atlantic salmon (Roberts, 2012b; Schmidt-Posthaus, own observation).

Kidney

Nephroblastomas and adenocarcinomas are reported (Lumsden and Marshall, 2003). In nephroblastomas, pluripotent embryonic cells proliferate to form most of the excretory elements usually present in the trunk kidney, like epithelial cells forming tubuli and glomeruli. Fibrous tissue and cartilage may be present in variable amounts (Martineau and Ferguson, 2006). These tumours can be seen protruding from the posterior kidney in adult salmon, with none or minimal clinical

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consequences. One speciality are renal adenocarcinomas described in oscars (*Astronotus ocellatus*) which are now classified as originating from the proximal tubules (Hochwartner *et al*., 2010).

Haematopoietic tissue

The majority of tumours in haematopoietic tissues originate from lymphoid cells (Harada *et al*., 1990). Spontaneous lymphosarcomas have been diagnosed in Atlantic and Chinook salmon (Speare, 2002), infiltrating different inner organs, particularly the kidney interstitium. Lymphsarcomas of thymic origin (thymomas) are reported in salmonids (Herman, 1970; Warr *et al*., 1984; Martineau and Ferguson, 2006), also with a leukaemic component involving the kidney. Plasmacytoid leukaemia principally affects cage-cultured Chinook salmon (Kent *et al*., 1990; Kent and Dawe, 1993). These tumours are transmissible and a retroviral etiology was identified (Kent and Dawe, 1990, 1993; Eaton and Kent, 1992). Occasionally, leukaemia with involvement of other organs, e.g. liver, can be seen in rainbow trout (Schmidt-Posthaus, own observations) (Fig. 4.8c).

Liver

Hepatocellular adenomas and carcinomas, as well as bile duct adenomas and carcinomas, are relatively common in different fish species. Both tumours, originating from hepatocytes and bile ducts, can occur in the same individual. As mentioned in the section on 'Nutritional and Feed Related Problems' rainbow trout are especially sensitive to aflatoxin intoxication inducing hepatocellular and bile duct carcinoma. This is one of the few examples where neoplasms in fish metastasize, often to the spleen and kidney (Majeed *et al*., 1984; Martineau and Ferguson, 2006).

Liver tumours can be used to assess environmental pollution (Harshbarger and Clark, 1990) and rainbow trout can serve as a model for liver tumour induction by environmental toxicants (Bailey *et al*., 1996; Williams, 2012). The presence of hepatocellular neoplasms, from benign to highly malignant forms, could be correlated to levels of polychlorynated biphenyls (PCBs) and other chlorinated hydrocarbons or benzo[a]pyrene (Pierce *et al*., 1978; Smith *et al*., 1979; Hendricks *et al*., 1985; Cormier *et al*., 1989).

Cystadenomas of the gall bladder are reported as an occasional finding in Chinook salmon (Lumsden and Marshall, 2003).

Gastrointestinal tract and swimbladder

Compared to mammals, neoplasms arising from the gastrointestinal tract are rare in fish. Teeth tumours, like ameloblastomas (invasive tumours of the odontogenic epithelium), are reported in salmonids (Gorlin, 1972). An epizootic of fibrosarcomas arising from the swimbladder and associated with a retrovirus infection was reported in caged Atlantic salmon in Scotland (Duncan, 1978). A similar condition, also caused by a retrovirus, was described in Atlantic salmon on the western seaboard of the Atlantic Ocean (Paul *et al*., 2006).

Reproductive system

Tumours arising from the different cell components of the ovary and testis are known; however, these tumours are far more common in warmwater species than in coldwater finfish.

Endocrine system

To distinguish thyroid tumours, like adenomas or carcinomas, from thyroid follicular hyperplasia (goitre) can be challenging, especially as transition is often blurred and thyroid follicles in fish are non-encapsulated and ectopic follicles can exist in various organs (Baker *et al*., 1955; Harada *et al*., 1996).

Physical Deformities

The most common external physical deformities in fish are skeletal (vertebral, opercular, jaw and fins) (Fig. 4.9) and eye deformities. Consequences depend on the degree and type of deformity, ranging from asymptomatic to poor growth and performance, poor welfare, decreased market value and mortality.

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Fig. 4.9. Farmed rainbow trout *(Oncorhynchus mykiss)* showing skeletal deformity due to fused vertebrae. The origin in this case is unknown. Image courtesy of D.W. Bruno, Marine Scotland Science.

Shortened opercula, per example, compromises the pumping water capacity of the fish and increases gill exposure to environmental and infectious stressors. Factors causing physical deformities may be genetic, environmental, nutritional or a combination of these. Traumatic injuries and infections (e.g. rainbow trout fry syndrome caused by *Flavobacterium psychrophilum* or infection with *Myxobolus cerebralis*) can also lead to deformities. Congenital physical deformities have low incidence but are more common in captive than in wild fish due to the absence of natural selective pressures (Sadler *et al*., 2001). In salmon aquaculture, the induction of triploids is used to avoid problems associated with sexual maturation, such as lower growth rates, increased susceptibility to diseases and deterioration of organoleptic properties (Piferrer *et al*., 2009). However, Atlantic salmon triploids have been associated with higher prevalence of skeletal deformities and cataracts after sea transfer (Taylor *et al*., 2011; H. Migaud, Inverness, 2012, personal communication). O'Flynn *et al*. (1997) reported that Atlantic salmon triploids and diploids showed similar freshwater growth and survival; however, triploids grew better but had higher deformities and lower survival in sea water. The faster growth showed by triploids in sea water may explain some of the observed deformities (H. Migaud, Inverness, 2012, personal communication). High growth rate is known to induce inferior bone quality in other animal species (e.g. broilers, pigs) (Julian, 1998; Silverstone and Hammell, 2002). In commercial diets, minerals supply can be a limiting factor which is a challenge in fast growing animals. The high growth rate of Atlantic cod during juvenile stages has also been proposed as a potential factor associated with the high number of skeletal deformities under culture conditions (Fjelldal *et al*., 2009). However, different nutritional and environmental requirements, and different smolt window timings for triploids in comparison with diploids need to be considered and further studies are needed. Triploids have been proven to be more susceptible to higher temperatures and low oxygen conditions (Ojolick *et al*., 1995; H. Migaud, Inverness, 2012, personal communication), to show absence of primary gill filaments and reduced gill surface area (Sadler *et al*., 2001), and different heart morphology than diploids (H. Migaud, Inverness, 2012, personal communication). Triploids are also used to eliminate the risk of genetic interaction with wild populations when escapes occur, minimizing the ecological effect of farmed salmon (Cotter *et al*., 2000).

Inappropriate rearing conditions (regarding light, temperature, salinity, dissolved oxygen, flow rate and pH parameters), poor diet and exposure to toxins during egg incubation and larval stage have been associated with deformities (Silverstone and Hammell, 2002). Stages with no finished ossification are more susceptible to malformations. Temperature stress is recognized as a teratogenic factor that induces deformities during the embryonic development of teleosts (Takle *et al*., 2005). A range of malformations was induced in Atlantic salmon exposed to temperatures above 8°C during egg incubation (Takle *et al*., 2005).

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8°C is recognized as the upper limit for normal embryonic development for this species. Castro-Sánchez *et al*. (2011) showed that exposure to hypoxia (60% O_2 saturation) during the early post-hatching period induced a higher frequency of vertebral column deformities in Atlantic salmon. Hypoxia altered the type and distribution of collagen in the notochord sheath and retarded the mineralization process of the vertebral body. The structural properties of the notochord allow movement and provide anchoring points for the miotomal segments (Castro-Sánchez *et al*., 2011). Avoiding hypoxic conditions by controlling oxygenation, water temperature and stock density are recommended to avoid potential skeletal deformities. As seen previously (section 'Nutritional and Feed Related Problems'), deficiencies or toxicities with minerals (Ca, P, Zn, Se and Mn) and vitamins (A, D, C, E and K), as well as their interactions and lipid peroxidation, can also cause skeletal deformities (Lall and Lewis-McCrea, 2007; Fernández and Gisbert, 2011).

Poor development or non-inflation of the swim bladder is another problem that can lead to deformities. There are two different types of swim bladder, physostomous (e.g. salmonids) and physoclistous (e.g. flatfish). In physostomous fish, the swim bladder remains connected to the oesophagus and fish are able to regulate it by rising to the surface to swallow air. In physoclistous, however, the swim bladder loses its connection with the oesophagus after the first inflation, and the gas is then regulated by blood vessels. Swim bladder inflation is a key step during early larval stage of most fish, and occurs when air gulped from the surface is transferred to the swim bladder by the pneumatic duct. Water with excessive surface oil will impair their ability to fill the swim bladder (Ferguson, 2006). The use of surface skimmers to prevent build-up of surface oils can help to avoid this. Lordosis is commonly encountered in individuals with uninflated swim bladders (Kitajima *et al*., 1981).

Internal malformations of the soft tissues are also reported from culture fish: such as aplasia of the septum transversum (Poppe *et al*., 1998), abnormal shaped hearts (Poppe and Taksdal, 2000), anomalies of the swim bladder and situs inversus of the liver (Poppe *et al*., 1997). Heart deformities, e.g. ventricular hypoplasia or rounder hearts, can cause poor cardiac function, reduced tolerance to stress, and subsequent mortalities (Poppe *et al*., 2003).

Coloration Abnormalities in Flatfish

Pigmentation abnormalities occurring at larval stages are a common and important problem in flatfish hatcheries, which decrease the market value and consumer acceptability of the affected individuals. During metamorphosis, flatfish undergo a substantial transformation involving shift of the body axis, eye migration and pigmentation changes. Flatfish larvae have pigmented cells on both sides of the body, but during metamorphosis larval melanophores disappear and adult melanophores develop on the ocular (dorsal) but not on the blind (ventral) side (Bolker and Hill, 2000). Pigmentation abnormalities can occur on both sides. Non-pigmented areas on the ocular side are known as hypomelanosis or pseudo-albinism (Fig. 4.10), while hypermelanosis or ambicoloration refer to the presence of pigmented areas on the blind surface (Venizelos and Benetti, 1999). Both types of malpigmentation may be related to inappropriate hatchery conditions e.g. diet and lighting (Bolker and Hill, 2000).

Larvae are more sensitive to dietary deficiencies or supplementations during premetamorphosis than during metamorphosis (Lund *et al*., 2008, 2010). Dietary factors affecting normal pigmentation include concentration and ratio of marine fish essential fatty acids (especially docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA)), vitamin A and nutrients necessary for the synthesis of thyroid hormone. Metamorphosis and development of pigmentation are both under neural and neuroendocrinological control (Estévez *et al*., 1999). DHA is important for the correct neural development and function, including retinal development and vision (Bell and Tocher, 1989; Bell and Sargent, 1996), while ARA and EPA are involved in the production and modulation of eicosanoids (Bell *et al*., 1995a, b). Improvements in dorsal pigmentation

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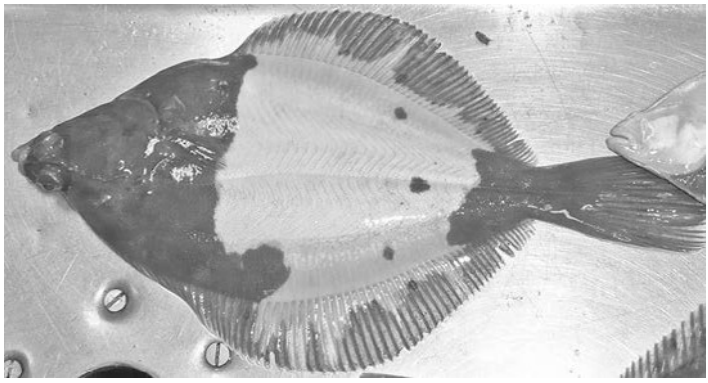


Fig. 4.10. Plaice (*Pleuronectes platessa*) from the North Sea showing pseudo-albinism. Note the large non-pigmented area on the ocular side.

in cultured turbot and halibut can be achieved by providing a ratio of DHA:EPA >2:1 and, potentially more important, an EPA:ARA ratio >5:1 (Bell *et al*., 2003). Atlantic halibut juveniles fed with *Artemia* during larval development show higher malpigmentation rates than larvae fed with copepods (McEvoy *et al*., 1998; Hamre *et al*., 2007). This is due to copepods containing much more EPA and DHA and lower levels of ARA than *Artemia*; even when *Artemia* are enriched with marine oil emulsions. Although the use of wild copepods has proved beneficial, seasonal fluctuations in their availability and potential biosecurity risks limit their use (Bell *et al*., 2003). A deficiency in vitamin A disrupts the pathway for melanin synthesis resulting in malpigmented fish (Daniels and Watanabe, 2010). However, excessive vitamin A supplementation can result in skeletal deformities (Takeuchi *et al*., 1995; Bolker and Hill, 2000). Thyroid hormones are key regulators of the metamorphosis in flatfish. Iodine, an element required for thyroid hormone synthesis, is present in higher levels in copepods than in *Artemia* (Hamre *et al*., 2005), which can also explain the higher incidence of malpigmented fish in captive conditions. Okada *et al*. (2003) showed that treating larvae with an inhibitor of thyroid hormone synthesis inhibited pigmentation.

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5 Infectious Diseases of Warmwater Fish in Marine and Brackish Waters

Angelo Colorni* and Ariel Diamant
National Center for Mariculture, Israel Oceanographic and Limnological Research Ltd., Eilat, Israel

Introduction

Warmwater mariculture in floating net pens or cages is practised in South-east Asia, a few islands in the Pacific Ocean, and in the subtropical regions of Western Eurasia and Eastern Asia. It includes brackish estuarine (salinities of 0.5–30 ppt) or truly marine environments in which temperatures rarely drop below 20°C. Nevertheless, fluctuations in physico-chemical parameters quite often occur suddenly or widely in coastal waters when cages are subject to inadequate water exchange, exposed to swift season changes, or in proximity to heavily urbanized shores. Stressful conditions will not only predispose the fish to disease but can be directly responsible for a wide range of pathogenic processes whose progression in warm waters is particularly rapid. As the fish grow (increase in biomass) water quality may deteriorate, particularly as the fish approach harvest size. Also, farmers often feed excessive amounts of energy-rich diets to their fish, which can result in fatty degeneration of the liver and extensive somatic fat deposition. Proper nutrition is critical to maintain the health of cultured fish, and the role of nutrition in health management through the modulation of immune response and disease resistance is a high-priority research topic (Oliva-Teles, 2012; Pohlenz and Gatlin, 2014). Phyto-planktonic flagellates, stinging jellyfish, oil spills, industrial toxic run-offs and other stress factors may also contribute to fish mortalities. Once the fish immunity is compromised the most significant health problem generally relates to infections. Pathogens may be greatly amplified in the cage environment and may also express an increased virulence, particularly in exotic fish species that do not have previous history of exposure to them. This may have important ecological implications, as the naturally low prevalence of pathogens and disease incidence in feral fish were observed to increase in areas peripheral to cage farms (Diamant *et al*., 2000, 2005; Colorni *et al*., 2002). In recent years there is heightened public environmental awareness worldwide and this has brought about implementation of stricter regulations to curb detrimental impacts of cage farming on coastal ecosystems. Also, management procedures are increasingly aimed at disease prevention, since treating diseases in these culture systems is often logistically unworkable, economically unsustainable and hardly ever environmentally compatible. This approach includes positioning the cage rafts in deep water sites, where flushing currents allow for a better water exchange and waste dispersal, avoiding fish

* E-mail: angelo@ocean.org.il

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over-crowding, formulating specific and balanced diets, performing frequent cage-net cleaning and ensuring that staff follow strict sanitary directives.

Cage and net pens are 'artificial islands' that act as a point of congregation for diverse vertebrates and invertebrates. The farmed fish, often kept at high stocking densities, come in close contact with wild fish that find shelter under the floating structures and enjoy a steady supply of feed. Small sized fish easily pass through the cage nets and mingle with the farmed stocks. Since cage farming is normally a continuous cycle production, stocks at different ages and growth stages are often cultured concurrently. Two-way transfer of infectious organisms may occur under such circumstances. Also, in the early days, when quarantine restrictions were rarely implemented, pathogens were transported freely over considerable geographical distances and probably found susceptible hosts and favourable conditions for proliferation. Thus, native hosts have become reservoirs for the introduced pathogens.

Viral Diseases

Over 125 different viruses have been identified in fish (Essbauer and Ahne, 2001), but as new species of marine fish acquire economic importance, additional diseases of viral aetiology are emerging. These viruses are temperature-dependent, are confined to poikilothermic species, often host-specific and usually affect young fish more severely while older fish become carriers. The taxonomy of these viruses is in many cases undergoing revision as a result of molecular technology.

Members of the *Iridoviridae* family are known to cause severe diseases in many fish species (Chinchar *et al*., 2005). The genus *Megalocytivirus* includes a number of closely related iridoviruses that produce a highly characteristic cytopathology.

Within the *Nodaviridae* family, the existence of a distinct fish-infecting genus has been suggested (Nishizawa *et al*., 1995; Nagai and Nishizawa, 1999), which lead to division of the Nodaviridae into two genera: the *Alphanodavirus* primarily infecting insects and *Betanodavirus* infecting fish.

Lymphocystis disease (LCD)

Lymphocystis is a highly contagious disease with worldwide distribution. It has been described in a large number of cultured and wild freshwater, brackish and marine fish (Lawler *et al*., 1977). The disease agent is a DNA virus belonging to the family *Iridoviridae*. Its host specificity is high. Transmission is waterborne, and port of entry supposedly through abrasions. Incubation time is temperature and host dependent, and around 10 days at 25°C (Bovo and Florio, 2008). In any given species, younger fish are most susceptible.

Lymphocystis viruses have a strong affinity to fibroblastic cells, in which they induce extreme hypertrophy. Affected fish develop macroscopic wart-like clusters of mainly fibroblasts that arise from the dermal layer of the skin and are usually covered by epithelium (Fig. 5.1). During the clinical stage, a presumptive diagnosis can be reached by observing the typical conspicuous skin and fin lesions. Histopathology on the basis of the characteristic basophilic cytoplasmic inclusions and the surrounding smooth, eosinophilic, hyaline capsule may provide a definitive diagnosis. Although LCD viruses are typically dermatotropic, invasion of visceral organs, in particular spleen and heart may also occur (Schäperclaus, 1992; Colorni and Diamant, 1995). Electron microscopy (EM) may be used to confirm presence of large (130–380 nm), icosahedral virions. The LCD virus is culturable in various fish cell lines (Bovo and Florio, 2008). The disease follows a chronic, usually benign, self-limiting course, with skin lesions eventually healing (4–6 weeks from first clinical signs) and leaving behind little or no scar tissue. Molecular methods like polymerase chain reaction (PCR) have been devised for definitive diagnosis and characterization of the virus also in asymptomatic fish. In experimentally infected fish, LCD virus was detected almost 2 weeks before appearance of external signs, and remained detectable in skin, caudal fin and eyeball for up to 4 weeks after external signs of infection had cleared (Cano *et al*., 2006a, b; Kvitt *et al*., 2008). Mortalities are generally limited to those individuals whose swimming, breathing or feeding behaviour are severely impaired by particularly large and cumbersome growths

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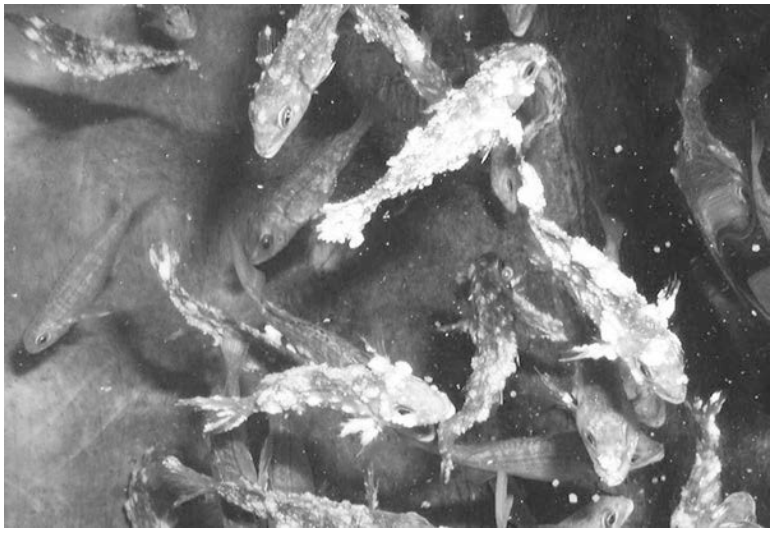


Fig. 5.1. Gilthead sea bream *Sparus aurata* fingerlings heavily infected with *Lymphocystis Disease Virus* (LCDV). Whitish clusters are hypertrophic LCDV-infected host dermal cells.

(Paperna *et al*., 1982). High stocking density and secondary skin bacterial infection caused by cannibalism and biting off cutaneous nodules may be aggravating factors. Prompt culling of the most heavily infected fish and reduction of stock density are the only palliative, as there is no effective therapy. Fish surviving infection do not re-acquire it, whether by becoming immune or more simply by outgrowing the critical age or size of this typically juvenile disease.

Red sea bream iridoviral disease (RSID)

The red sea bream iridovirus (RSID) is responsible for a serious disease. It was first detected in cultured red seam bream (*Pagrus major*) in Japan (Inouye *et al*., 1992; Nakajima and Sorimachi, 1994) and rapidly spread to other East and South-east Asian countries (OIE, 2003). The virus is a member of the genus *Megalocytivirus* which includes a number of closely related and highly cytopathogenic iridoviruses. The host range is wide and many other cultured sparid as well as non-sparid host species (e.g. yellowtail (*Seriola quinqueradiata*), Japanese seaperch (*Lateobrax* sp.), Japanese parrotfish (*Oplegnathus fasciatus*)) have been reported infected in Japan, China, Korea, Taiwan, Singapore and Thailand (Matsuoka *et al*., 1996; Kawakami and Nakajima, 2002; OIE, 2003; Do *et al*., 2005; Bovo and Florio, 2008). Fish vary in susceptibility to different RSID strains. Transmission is by horizontal contagion, either by ingestion of infected tissues or contaminated water. Disease typically occurs when water temperature is >20°C (Noga, 2010). Cage-cultured juveniles are particularly susceptible (Matsuoka *et al*., 1996), but high mortalities have also been reported in market-size fish (Nakajima *et al*., 1998). Infection in farmed orange-spotted grouper (*Epinephelus coioides*) was detected at juvenile stages of rearing (Kuo *et al*., 2012).

Diagnosis can be confirmed using monoclonal antibodies in indirect fluorescent antibody test (IFAT) (Nakajima and Sorimachi, 1995; Nakajima *et al*., 1995), polymerase chain reaction (PCR) or loop mediated isothermal amplification (LAMP) assays (Caipang *et al*., 2003, 2004), or by observing the icosahedral, 200–240 nm virions in EM. The virus is culturable in a number of fish cell lines (Bovo and Florio, 2008).

Affected fish are lethargic, anaemic, with haemorrhagic petechiae in the gills and splenomegaly. A key feature of *Megalocytivirus* infections is the formation of inclusion body-bearing, basophilic, hypertrophic cells within

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infected organs (spleen, kidney, digestive tract, occasionally also in heart and gills) and revealed by H&E or Giemsa staining (Inouye *et al*., 1992; Noga, 2010).

The immune responses to formalin-fixed (Nakajima *et al*., 1999) and DNA antigens (Caipang *et al*., 2006) have been tested, and a commercial vaccine is available in Japan (Nakajima *et al*., 1997, 1999).

Viral encephalopathy and retinopathy (VER)

This disease is still widely known as Viral Nervous Necrosis (VNN), based on its clinical signs (Yoshikoshi and Inoue, 1990), although it has been renamed Viral Encephalopathy and Retinopathy (VER) (OIE, 2003). The VER virus belongs to the genus *Betanodavirus*. It has been detected in a wide range of fish hosts, both cultured and from the wild, belonging to at least 16 families (Munday *et al*., 2002; Gomez *et al*., 2004). Like other members of *Betanodavirus*, the VER virus is a small (25–30 nm), non-enveloped, single stranded RNA virus with an icosahedral capsid. Two pieces make up its genome: RNA1 gene encodes the RNA-dependent RNA polymerase and RNA2 gene encodes the coat protein (Mori *et al*., 1992). On the basis of the partial nucleotide sequences of the RNA2 (T4 region), Nishizawa *et al*. (1997) suggested a classification into four clusters or genotypes, to which a fifth putative one was later added by Johansen *et al*. (2004).

Although transmitted both horizontally (Hick *et al*., 2011; Korsnes *et al*., 2012) and vertically (Kuo *et al*., 2011, 2012), VER is probably most commonly spread by sub-clinically infected broodstock to progeny during spawning. Neurotropic viruses can reach the central nervous system (CNS) via the bloodstream (viraemia) and the peripheral nerves, and the brain, spinal chord and retina appear to be the main sites of virus replication. High temperatures may shorten the incubation period, which varies between 4 and 30 days; the length of incubation period also depends on the fish species. VER viruses can persist in the marine environment for months (Frerichs *et al*., 2000). Clinical disease is most commonly observed in larval and juvenile stages, where mortality may reach 100% within 1 week from the onset of the neurological signs, but tends to decline as the fish grow. The infection, however, persists at chronic levels and surviving fish invariably become asymptomatic carriers. Mortalities have been documented in cage farmed European sea bass *Dicentrarchus labrax* in the Mediterranean as early as the mid 1990s (Le Breton *et al*., 1997). Since then, VER has assumed a worldwide distribution and is now known from over 40 marine fish species belonging to different families (Bovo and Florio, 2008). VER infections have already had significant economic and environmental impact in several areas: through direct losses, preclusion of trade for established and emerging mariculture species, and concerns of contagion to native fish species due to translocation of the virus (Munday *et al*., 2002). Diagnostic methods include histopathological examination of fixed nervous tissues, immunological methods using a specific antibody probe, and molecular methods using a specific gene probe. Viral propagation in various cell cultures (Bovo and Florio, 2008) is often an indispensable diagnostic step. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) is used to detect the virus in fresh material (Dalla Valle *et al*., 2000, 2005; Grotmol *et al*., 2000; Starkey *et al*., 2004; Ucko *et al*., 2004). Real-time reverse-transcription PCR (qRT-PCR) and in situ PCR have also been successfully used to detect infections in groupers, sea bass and other farmed species (Hick and Whittington, 2010; Hodneland *et al*., 2011; Mazelet *et al*., 2011, Kuo *et al*., 2012).

Pathogenesis of VER is related to the neuroinvasive nature of the virus and the degenerative effects it has on nervous tissues (including brain, spinal cord, ganglia in the peripheral nervous system) are most evident in the extensive spongiosis (vacuolization) of the granular layers of the retina. Clinically, VER is characterized by neurological signs such as impaired coordination, loss of equilibrium, whirling, erratic swimming, blindness with consequent changes in pigmentation (darkening), and swimbladder hyperinflation. Other signs are lethargy, anaemia and hyperexcitability in response to noise and light. In larvae and fingerlings, histopathological changes also include muscular degeneration

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(Gallet de Saint Aurin *et al*., 1990; see review by Munday *et al*., 2002).

Several research teams have been testing various kinds of experimental vaccines (Tanaka *et al*., 2001; Lin *et al*., 2007; Kai and Chi, 2008; Kai *et al*., 2010; Nishizawa *et al*., 2012). However, commercial vaccines are not yet available and no treatment has been devised for VER. As the disease has been reported primarily in juvenile fish, vaccination may be more effective if it is used on broodstocks to reduce the risk of vertical transmission (Bovo and Florio, 2008). Survivors from an outbreak may acquire a degree of immunity but typically become carriers. Non-lethal molecular diagnostic methods are currently being developed (Tarrab *et al*., 2012). Strict sanitary measures should be adopted. Iodophors (25–100 mg per l) and chlorine have proved to have a strong virucidal effect (Arimoto *et al*., 1996; Frerichs *et al*., 2000; Maltese and Bovo, 2001).

Bacterial Diseases

A bacterial species, with its binomial nomenclature of convenience and cohorts of atypical strains within the genus, has always been a strictly empirical entity. The advent of molecular-based methods of identification has provided a phylogenetic approach to determining bacterial taxonomic position by grouping closely related organisms that share a relatively recent ancestry into clusters. Unfortunately, the definition of species as a 'discrete cluster of strains with a relatively recent common ancestor' is no less vulnerable to arbitrary subjectivity, so that the question remains of how much internal diversity is permissible in a discrete cluster for it to be regarded as a single species or subspecies. Although specific primers for the identification of aquatic bacteria are continuously being reported, they need to undergo a frequently long period of validation before being accepted as diagnostically reliable. Nevertheless, PCR and 16S rDNA sequencing are increasingly being used instead of, or in addition to, biochemical profiles in the identification of bacteria of uncertain affiliation. From a more practical point of view, a distinction should be made between primary true pathogens, which are able to invade the tissues of healthy individuals and cause a characteristic disease, and opportunist pathogens, which are normally unable to invade the tissues of healthy fish, unable to cause a contagious disease, and unable to render the fish more resistant to a re-infection. Under stressful conditions, as in intensive culture, this distinction is not always maintained, as fish can occasionally lower their natural defences and become susceptible to normally avirulent commensal or saprophytic bacteria (Austin and Austin, 2007; Noga, 2010).

Vibriosis

Vibriosis is caused by several members of the genus *Vibrio* and related genera within the *Vibrionaceae* family. This bacterial family thrives in warm brackish and sea water and is widespread in coastal marine and estuarine environments. As a result of global warming, *Vibrio* bacteria have also spread to new temperate ecosystems (Baker-Austin *et al*., 2012). Vibrios are in many cases facultative pathogens that can readily colonize external lesions or, as part of the fish normal intestinal flora, set off an infection, presumably when a predisposing stress factor generates a failure in the mechanism regulating posterior gut permeability. Virtually all species of marine and estuarine fish of all ages are susceptible. Epizootic events in cage farms may also constitute a challenge pressure to the surrounding environment.

Vibrios are Gram-negative, aerobic or facultative anaerobic, short (up to 3 μm) rods, motile by at least a single polar flagellum, and positive to the cytochromoxidase enzyme test. Blood, spleen and kidney are the best isolation sources, and salt-supplemented media should be used for their culture. Most species can grow on thiosulfate citrate bile sucrose agar (TCBS), a medium relatively selective for *V. cholerae* and other *Vibrio* spp., and are sensitive to 2,4-diamino 6,7-di-isopropyl pteridine phosphate (vibrio-static agent 0/129), to which the closely related aeromonads are resistant. *Vibrio* (*Listonella*) *anguillarum*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, *V. harveyi*

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(*carchariae*) and *V. ordalii* have been associated with mortalities in farmed and feral fish in temperate and warm waters (Austin and Austin, 2007; Actis *et al*., 2011). Identification of the species may present some difficulties as the taxonomy of the *Vibrionaceae* has undergone numerous recent revisions and is still 'fluid'. Within the species, further categorizations into serotypes, serovars, biotypes, subgroups, etc. have been attempted, to distinguish the few pathogenic strains from the environmental strains which are the majority. A comprehensive molecular phylogenetic approach has been proposed, based on the study of a set of multilocus gene sequences (Thompson *et al*., 2005).

Fish vibriosis is characterized by a systemic haemorrhagic septicaemia. Anaemia, lethargy, skin darkening, corneal thickening, erythema of the vent and the base of the fins, congested visceral blood vessels and fluid accumulation in the intestines are the most common signs. Vibrios produce haemolysins which cause anaemia and the heavy haemosiderin deposition in melanomacrophage centres observed histologically in spleen and kidney, and a wide variety of proteases responsible for the extensive tissue damage (Hjeltnes and Roberts, 1993). In advanced cases, intestinal haemorrhage, destruction of the tunica mucosa, accumulation of serosanguinous fluid, mucoid abdominal exudate, congestion and haemorrhage of the liver, enlargement and liquefaction of the spleen, liver and kidney can be observed.

Treatment with medicated feed (e.g. oxytetracycline, nitrofurans, potentiated sulfonamides, oxolinic acid) can be effective if done at the initial stage of the disease, when the fish are not entirely anorexic (Noga, 2010). Commercial bacterins are available for certain *Vibrio* spp. (Noga, 2010). Vaccination by immersion or, when the fish are >25 g, intraperitoneal injection was reported to provide a satisfactory level of protection (Toranzo *et al*., 2009; P. Varvarigos, 2012, personal communication).

Photobacteriosis

The disease is caused by *Photobacterium damselae* subsp. *piscicida*, a halophilic member of the *Vibrionaceae* family. Based on phenotypic characteristics, it was originally placed in the genus *Pasteurella,* and the disease named 'pasteurellosis'. The disease was reported in a number of Mediterranean countries, in the United States and Japan. Transmission can be vertical, through the ovarian and seminal fluids from apparently healthy broodstock (Romalde *et al*., 1999), but experimental infections using bath challenges support the hypothesis that waterborne infection is the most likely pathway, with the bacterium able to infect its host through the gills, the gastrointestinal tract and possibly the skin (Magariños *et al*., 1995). *P. damselae* subsp. *piscicida* has a wide range of susceptible hosts, both wild and cultured (Toranzo *et al*., 1991) and the disease it causes is one of the most devastating in warm and temperate mariculture. Young fish tend to be more susceptible to the pathogen (Toranzo *et al*., 2005). Seasonal changes in water temperature are often critical in the epidemiology of the disease. Warm temperatures seem to be a predisposing factor, as above 25°C the probability and severity of the outbreaks increase (Hawke *et al*., 1987; Kitao, 1993a). At temperatures lower than 21°C mortality may decrease but fish become carriers (Magariños *et al*., 2001; Toranzo *et al*., 2005). The pathogen can enter a viable but not culturable (VBNC) state, and survive in the water column and the sediment (Magariños *et al*., 1994). The ability of the pathogen to survive for undetermined but presumably long periods in the environment in a VBNC-state or in carriers during the colder months suggests that once the pathogen contaminates an area it is likely to become a threat to the cage farm located in the vicinity.

Presumptive identification of the bacterium is carried out according to its morphological and biochemical features (rather unreactive, Gram-negative, non-motile, oval-shaped, 0.5 × 1.5 μm rod, with pronounced bipolar staining). At least three genetically distinct clone lineages (European, Japanese and Israeli) can be distinguished (Kvitt *et al*., 2002). An agglutination test kit based on polyclonal anti-*Photobacterium damselae* subsp. *piscida* antibodies is commercially available. PCR-based assays have been developed (Osorio *et al*., 1999, 2000; Kvitt *et al*., 2002; Osorio and Toranzo, 2002).

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Infected fish may appear darkened and in some cases petechiae are visible on the head, gills, operculum and fin bases, but clinical signs are generally few. The infection develops rapidly into an acute septicaemia characterized by splenomegaly, which becomes the organ of choice for isolation. Isolation is best done on tryptic soy agar (TSA), brain heart infusion agar (BHIA) or blood agar, all supplemented with 1–2% NaCl. In advanced cases, typical foci of bacterial microcolonies and severely compromised tissue appear as whitish spots and patches on the spleen surface (Colorni, 1998). Histologically, multifocal necrosis can be observed in splenic and hepatic tissues (Toranzo *et al*., 1991).

An antibiogram should be performed before any antibiotic treatment, as effectiveness of antibiotics is gradually diminished by the resilience of this bacterium that tends to develop resistance (Kitao, 1993a).

Commercial vaccines against *P. damselae* subsp. *piscicida* are available. The protection level of these vaccines, however, depends on the fish species, size and vaccine formulation (Toranzo *et al*., 2009; P. Varvarigos, 2012, personal communication). In general, their effectiveness has been modest and of relatively short duration. Positive effects on the protection of sea bream larvae were reported when the parental broodstock was vaccinated, and approximately 90 day-old larvae were immersed in the vaccine (Hanif *et al*., 2005).

Edwardsiellosis

Edwardsiellosis is a systemic disease in warm freshwater and marine fish. *Edwardsiella tarda* has been particularly devastating to flounder (*Paralichthys olivaceus*) culture in Japan, Taiwan and South-east Asia (see Plumb, 1993; Leong and Colorni, 2002; Evans *et al*., 2011, and refs. therein), as well as in turbot (*Scophthalmus maximus*) in Spain (Padrós *et al*., 2006). Whether *E. tarda* should be regarded as a primary or opportunistic pathogen may still be debatable (Austin and Austin, 2007), but it is noteworthy that this normally enteric bacterium is capable of surviving in phagocytes (Srinivasa Rao *et al*., 2001, 2003) whereas bacteria that are easily ingested and killed by phagocytes are generally unsuccessful as pathogens. *E. tarda* has been associated with a large variety of potential carriers (freshwater snails, sea urchins, frogs, turtles, snakes, alligators, gulls and humans, see Austin and Austin, 2007; Noga, 2010, and refs. therein). It has also been suggested that it may die quickly outside its host (Mamnur *et al*., 1994) or enter a viable but not culturable (VBNC) state (Sakai *et al*., 1994).

E. tarda is a Gram-negative, motile rod with peritrichous flagella and a member of the *Enterobacteriaceae*. It is best isolated from kidney. On Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar it forms small, round, transparent colonies. Diagnosis is based on standard biochemical features using commercial multi-testing kits. Presumptive identification may be confirmed by 16S rDNA sequencing. A loop-mediated isothermal amplification (LAMP) has been developed for rapid and sensitive detection of *E. tarda* (Savan *et al*., 2004). Histopathology analyses reveal severe suppurative nephritis, with abscesses of various sizes and bacteria conspicuously visible within masses of degenerate macrophages, neutrophils and fibrin layers (Padrós *et al*., 2006).

Clinical signs of edwardsiellosis vary with the species of infected fish. In groupers (*Epinephelus aeneus*) skin ecchymosis and ulceration, fin and tail erosion and, occasionally, exophthalmia and cataracts are observed. The gills appear pale and inflamed. Internal organs appear haemorrhagic and oedematous. The kidney in particular appears enlarged. Whitish granulomatous-like lesions, often visible on its surface, may gradually liquefy into large abscesses and spread into the surrounding musculature (Colorni, unpublished).

Traditional oral antibiotic treatment should be preceded by an antibiogram, as drug-resistant strains have been observed. Vaccines administered by immersion have proved to offer moderate to effective protection (Austin and Austin, 2007).

Infections by *Tenacibaculum maritimum* and other flexibacters

Tenacibaculum maritimum (*Flavobacteriaceae*) is an opportunistic Gram-negative bacterium

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commonly found in sea water (Salati *et al*., 2005) and formerly known as *Cytophaga marina* or *Flexibacter marinus* or *maritimus*. It is perhaps the major cause of flexibacteriosis, also commonly known as 'gliding bacterial disease', 'eroded mouth syndrome' and 'black patch necrosis' in marine fish. Isolates, often unspeciated, have been reported from Japan, USA and Europe (Austin and Austin, 2007). Fluctuations in water temperature, environmental stressors and skin abrasions may trigger the development of the disease (Toranzo *et al*., 2005). Host specificity is low, and a large variety of fish cultured in cages in East and South-east Asia and in the Mediterranean Sea has been reported to be infected with the bacterium (Leong and Colorni, 2002; Vatsos, 2007). The condition is particularly severe in younger fish (Wakabayashi, 1993). Mortalities are generally limited but can occasionally reach high (100%) levels (Vatsos, 2007). Its importance as a pathogen may be underestimated because the isolation of this halophilic bacterium requires selective low-nutrient culture media, not normally used in routine laboratory work (Toranzo *et al*., 2005; Austin and Austin, 2007).

Bundles of long, gliding rods in wet mounts and gram-stained preparations obtained from the lesions can serve as a presumptive identification. On selective culture media such as Anacker and Ordal, Marine Agar, or Flexibacter maritimus medium (FMM) (Pazos *et al*., 1996) pale yellow, flat, thin colonies are formed. In cultures, the long rods observed in wet mounts or Gram-stained preparations from gills or skin lesions tend to break up into shorter rods within the first 24–36 hours after the inoculum (Colorni, unpublished). PCR can be used to confirm a presumptive identification and to target *T. maritimum* in mixed bacterial populations (Toyama *et al*., 1996; Avendaño-Herrera *et al*., 2004). The mouth of an infected fish may appear eroded and haemorrhagic, fins and tail appear frayed and foci of gill rot may develop, while lesions may occur in the skin. The disease can also become systemic, involving internal organs (Toranzo *et al*., 2005). Skin and fin abrasions (e.g. following netting or rough handling during grading procedures in nurseries, lesions as a result of feeding activities by parasitic flukes on gill or isopods on the skin) may quickly become infected with *Tenacibaculum*.

Streptococcoses, lactococcoses and other infections by aquatic lactic acid bacteria (LAB)

Infections by Gram-positive chain-forming bacteria were recognized in the late 1990s as an emerging problem in worldwide aquaculture (Austin, 1999). *Streptococcus iniae* is perhaps the most virulent and cosmopolitan member of this group (Agnew and Barnes, 2007), but several other species are known to be highly contagious and cause lethal septicaemia in fish (Toranzo *et al*., 2005; Austin and Austin, 2007; Ucko and Colorni, 2014). While the origin, reservoirs and paths of infection of this group of bacteria often remain elusive, the fact that bacteria generally associated with udder inflammation in livestock (e.g. *Streptococcus parauberis*) or in the production of cheese (e.g. *Lactococcus lactis*) seem to be able to infect fish underscores the urgent need for thorough epidemiological and molecular phylogenetic studies. The latter in particular should be aimed at determining whether an extraordinary adaptability of this group of bacteria allows the same strains to occupy a wide spectrum of ecological niches or, conversely, intraspecific variants, often phenotypically indistinguishable, exist, differentiating between freshwater and marine strains, aquatic and terrestrial strains, or pathogenic and harmless environmental strains. The diseases by Gram-positive chain-forming bacteria may have both a sporadic and epizootic character, but farms in many parts of the world have suffered serious economic losses. In 1996, *S. iniae* was isolated for the first time in red drum (*Sciaenops ocellatus*) reared in sea cages off the shore of Eilat's northern coast (Israel, Red Sea). Transmission of *S. iniae* from sea cages to wild fish was suspected (Colorni *et al*., 2002; Kvitt and Colorni, 2004), whereas in the Mediterranean coast transmission was suspected from wild fish to inland farmed fish (Zlotkin *et al*., 1998a). Interaction of *Streptococcus* spp. in cage-cultured yellowtail (*Seriola quinqueradiata*) and wild fish was demonstrated

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in Japan, in particular when fresh trash fish was used in the fish diet (Minami, 1979; Kusuda and Kawai, 1982). The same strain of *Streptococcus agalactiae* was isolated from cultured gilthead sea bream (*Sparus aurata*) and wild mullet (*Liza kluzingeri*) in Kuwait, Arabian Gulf (Evans *et al*., 2002).

Bacteriological isolations are often from the spleen, kidney or blood of fish that present obvious signs of disease. Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar are better if supplemented with 5% blood. Cultures generally grow faster in CO_2 than in atmospheric ambient. Miniaturized commercial kits can be used to obtain a biochemical profile but a positive identification cannot always be reached with these systems alone. Confirmation should be sought using molecular methods. Specific primers for *S. iniae* and *Lactococcus garvieae* deduced from the 16S rRNA gene were developed in Israel (Zlotkin *et al*., 1998b; Bachrach *et al*., 2001; Ucko and Colorni, unpublished). A multiplex PCR has reportedly been developed, and successfully recognized the four species of Gram-positive chain-forming cocci most commonly associated with infections in marine fish (*S. iniae*, *S. parauberis*, *S. agalactiae*, *L. garvieae*) from cultures and fish tissues (Mata *et al*., 2004).

Clinical signs vary with the species of coccus, and the species and size of the host affected. In general, fish become lethargic and swim erratically or in spiral fashion as a result of an evident meningo-encephalitis. Exophthalmia and hyphema (bloody eyes), petechial haemorrhage, oedema with accumulation of serosanguinous fluid in the peritoneal cavity and intestine, a pale liver and a dark red spleen are the most common clinical signs. The ability of streptococci to survive in macrophages reduces the effectiveness of antibiotic treatment, as the macrophages will actually protect the bacteria from the antibiotic; infected macrophages later rupture to release the bacteria back into the blood stream (Zimmerman *et al*., 1975). Dead fish are a major source of re-infection for other fish and should be removed promptly from the cages. Surviving fish probably serve as carriers (Kitao, 1993b). *Streptococcus* spp. tend to remain viable for prolonged periods in the environment (Kusuda and Kawai, 1982). An experimental vaccine was developed in Israel, but resistant strains have rapidly turned up (Bachrach *et al*., 2001).

Mycobacteriosis

Fish mycobacteriosis has worldwide distribution in both freshwater and marine environments, from cold waters to tropical regions. It is a chronic disease and can remain subclinical for extended periods, stunting fish growth. The pathogenicity of mycobacteria is mainly related to their ability to survive even when ingested by macrophages and, at least in some aquatic species, to produce cytotoxic mycolactones (Ranger *et al*., 2006; Yip *et al*., 2007). Hundreds of fish species have been reported to be susceptible to various *Mycobacterium* spp., suggesting that virtually all species of fish can be infected. Contagion likely occurs by ingestion of contaminated particles. Transovarian transmission was also demonstrated in some fish (Conroy, 1966; Chinabut, 1999). Transmission of *Mycobacterium marinum* between sea cage stocks and wild fish was reported in the Red Sea, Israel (Diamant *et al*., 2000). Diseased fish are unmarketable, and the zoonotic potential of *M. marinum* is considerably high.

Mycobacteria are Gram-positive, acid-fast, aerobic, non-motile, often pleomorphic, 0.2–0.6 by 1.0–10.0 μm rods. Presumptive identification can be done by staining a thin, air-dried smear of material from a lesion using the Kinyoun or Ziehl-Neelsen method. These acid-fast bacteria appear bright red on a blue background. The bacteria can similarly be detected in histological sections. However, acid-fastness may be dependent on the metabolic state of the bacteria and can be lost, for example in early granulomatous lesions (Nyka, 1974; Colorni et al., 1998; Gauthier et al., 2003).

Two phylogenetically separate mycobacteria groups (Stahl and Urbance, 1990) can be distinguished: fast growers (forming visible colonies within 1 week, and often on general culture media), and slow growers (not forming visible colonies before 2–4 weeks, and only on media specific for mycobacteria, such as Löwenstein-Jensen medium or Middlebrook 7H10 agar medium). Ubiquitous

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saprophytic or opportunist mycobacteria generally belong to the former group, whereas *M. marinum* and other virulent species commonly identified in marine fish belong to the latter. Isolation is most effectively done from the spleen or kidney. At 24±1°C, *M. marinum* colonies become visible to the naked eye in about 4 weeks after inoculation; colonies turn bright yellow after exposure to light. Reliable identification is based on molecular methods. Specific primers for *Mycobacterium* spp. deduced from the 16S rRNA and *hsp65* genes and other genetic characterization techniques have been developed for the detection and identification of *M. marinum* (Knibb *et al*., 1993; Sechi *et al*., 2002; Ucko *et al*., 2002; Ucko and Colorni, 2005; Kaattari *et al*., 2006; Gauthier and Rhodes, 2009).

Infected fish may display only a few external signs such as skin ulcers likely caused by the rupture of shallow lesions, exophthalmia, and emaciation. Internal organs, however, such as spleen and kidney, are severely affected, appearing enlarged and granulomatous (Fig. 5.2). In advanced cases, the characteristic lesions (whitish nodules) also appear in the mesenteries, liver and heart.

No vaccines are available against fish mycobacteriosis and currently the disease is virtually impossible to control with antibiotics. Isoniazid, rifampicin, streptomycin, erythromycin, ethambutol, clarithromycin and other antibiotics used in human tuberculosis under prolonged administration regimes might be effective in reducing clinical signs (Kawakami and Kusuda, 1990; Austin and Austin, 2007). There is evidence that the disease can regress under certain circumstances, but recovered fish still probably carry a latent infection (Colorni *et al*., 1998). Once established in a facility, mycobacteriosis is difficult to eradicate, as the bacteria can survive for years in the environment (Reichenbache-Klinke, 1972). Intermediate or low level disinfectants (e.g. quaternary ammonium compounds) are ineffective (Mainous and Smith, 2005), while some *Mycobacterium* species have developed resistance to chlorine (Vaerewijck *et al*., 2005).



Fig. 5.2. *Mycobacterium*-induced splenomegaly in sea bream *Sparus aurata*.

Epitheliocystis

Epitheliocystis is an infection caused by a group of obligate intracellular prokaryotes related to the order Chlamydiales (Paperna, 1977; Desser *et al*., 1988; Nowak and LaPatra, 2006). They have a high degree of host specificity and a similar pathology in different hosts (Paperna *et al*., 1981; Nowak and Clark, 1999; Nowak and LaPatra, 2006).

The infection has been found in both wild and farmed fish, and is characterized by the formation in the host gills and skin of spherical or ellipsoid 'cysts' which are actually hypertrophic epithelial cells filled with minute Gram-negative coccoid organisms. They are more conspicuous in the gill filaments, where hyperplasia, fusion of adjacent lamellae and extensive destruction may occur. Clinical signs of epitheliocystis include lethargy, flared opercula and respiratory distress. Heavy infections and mortalities occur mainly in juvenile fish (Frances *et al*., 1997). Fish with opercular malformations appear to be more susceptible to infection (Colorni and Diamant, unpublished). The disease was diagnosed in numerous sparids (*S. aurata*, *P. major*, *Acanthopagrus schegeli*, *Dentex dentex*, *Diplodus puntazzo*, etc.) farmed in Mediterranean countries, Japan and Korea (Colorni and Padrós, 2011). Subclinical infections in juvenile gilthead sea bream are relatively common. Stress factors such as overcrowding, transportation or inadequate feeding may produce clinical signs, as well as favour co-infections with monogenean

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worms (Padrós and Crespo, 1995), *Trichodina* spp. and *Vibrio* (Cruz E Silva *et al*., 1997).

The natural transmission pathway of epitheliocystis is still unclear but, as some cohabitation experiments have shown, apparently occurs by horizontal transmission. Contaminated nets or other equipment may also be responsible for the rapid spreading of the infection in culture facilities (Paperna, 1977).

The epitheliocystis organism has yet to be cultured. Tentative diagnosis is usually made by observing the typical 'cysts' on the gills or skin in wet mounts, appearing as translucent capsules, 10–400 μm in diameter. All major types of epithelial cells can be infected, including chloride and goblet cells (Ferguson, 2006). Histologically, such hypertrophic cells appear to have a uniform, finely granular, basophilic structure, frequently surrounded by squamous or cuboidal epithelial cells (Turnbull, 1993). Treatment of fish with antibiotic-medicated feed has produced uncertain or very modest results. Oxytetracycline-medicated feeds may be worth experimenting (Goodwin *et al*., 2005) despite the decline in effectiveness of this antibiotic in sea water (Herwig, 1979; Lunestad and Goksøyr, 1990).

Zoonoses

Although it is reasonable to presume that within a given species only certain strains pose a health risk to humans, the zoonotic potential of some fish bacteria is not negligible (Novotny *et al*., 2004). Cases of human infections by aquatic mycobacteria have been reported with increasing frequency in ichthyopathological and medical literature (referred to in the latter as 'fish tank granuloma' or 'swimming pool granuloma').

Some *Vibrio* species are well known human pathogens: infections by *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus* occur through ingestion of contaminated or raw fish or seafood. These bacteria damage the inner wall of the human intestine, causing diahorrea. In addition, wound infections by *V. vulnificus* in individuals with a compromised immune system, may lead to a fatal septicaemia (Bisharat *et al*., 1999, 2007).

In humans, *E. tarda* can occasionally cause gastro-enteritis (Vandepitte *et al*., 1983; Verjan *et al*., 2005), as well as extraintestinal infections (Clarridge *et al*., 1980; Sechter *et al*., 1983; Hashavya *et al*., 2011; Ota *et al*., 2011). As for *S. iniae*, there have been several cases of human illness in North America all due to puncture wounds or injuries when handling *S. iniae*-contaminated fish (Weinstein *et al*., 1997). Infections by *L. garvieae* have been similarly described in recent years in medical case reports (Aubin *et al*., 2011; Watanabe *et al*., 2011; Fog-Møller and Andersen, 2012). The zoonotic potential of *M. marinum* is particularly high. *M. marinum* gains access through skin abrasions and generally produces superficial and self-limiting lesions involving the cooler parts of the body such as hands, forearms, elbows, buttocks and knees (Giavenni, 1979; Huminer *et al*., 1986; Ucko and Colorni, 2005). These infections are usually treatable with clarithromycin but require a lengthy systemic treatment. Persistent lesions occasionally require surgical debridement.

Parasitic Diseases

The protistan ectoparasites

A large number of unicellular eukaryotes live as facultative or obligate parasites of fish in warmwater mariculture. The two most devastating parasitic diseases, amyloodiniosis ('velvet disease') caused by the dinoflagellate *Amyloodinium ocellatum* (Fig. 5.3a) and cryptocaryonosis ('white spot disease') caused by the holotrichous ciliate *Cryptocaryon irritans*, however, are virtually not seen in cage cultures (Leong and Colorni, 2002). Presence of these organisms on cage-cultured fish suggests that the cages have been deployed in water too shallow. If logistically feasible, the cages should be moved to deeper waters, where more effective currents increase dispersal of the infective stages and reduce chances for re-exposure. Risks of disease outbreaks caused by organisms with a direct life cycle, enabling fish-to-fish transmission, remain high in these culture systems (Nowak, 2007).

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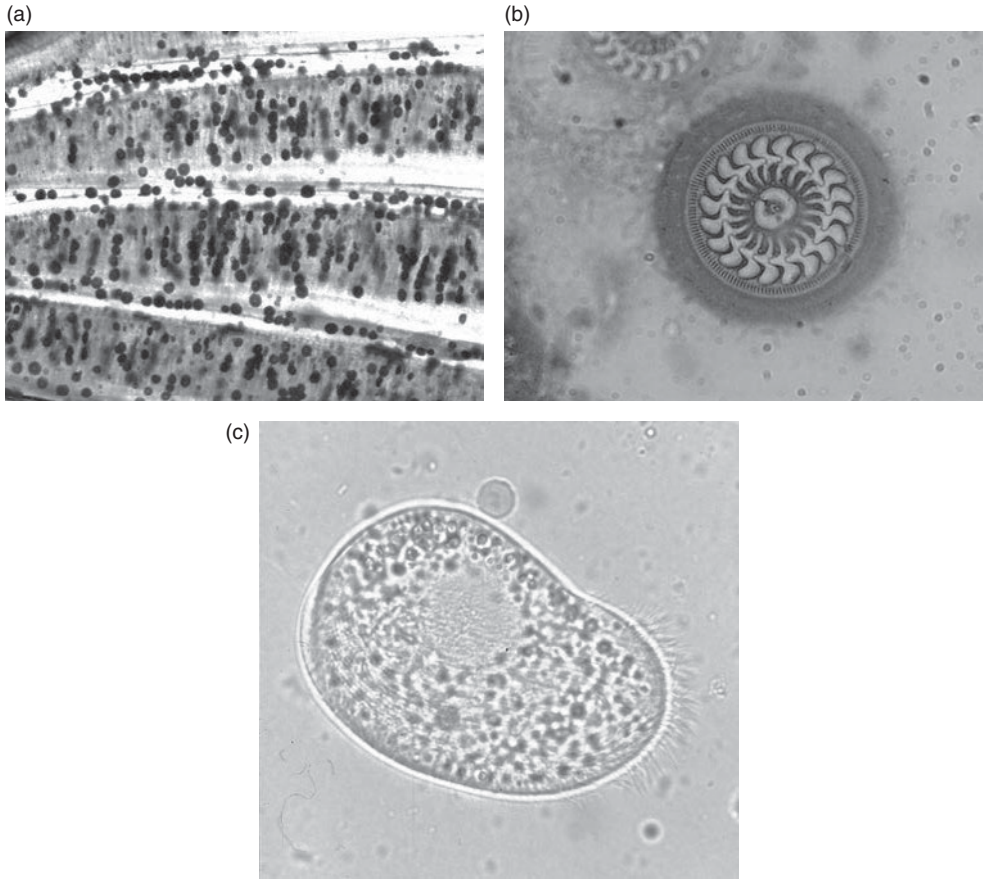


Fig. 5.3. Gill protistan parasites. (a) Heavy infection of *Amyloodinium ocellatum* on the gills of sea bream *Sparus aurata*. (b) *Trichodina* sp. from the gills of sea bass *Dicentrarchus labrax*. Klein's dry silver impregnation method. (c) *Brooklynella hostilis* from sea bream *Sparus aurata* gills.

Ciliates of the genera *Trichodina* (Fig. 5.3b), *Brooklynella* (Fig. 5.3c) and *Uronema* are cosmopolitan, opportunistic pathogens with a tendency to proliferate on stressed or debilitated hosts. They feed on cell debris and occasionally ingest blood cells (Colorni, 2008). Trichodinids in particular, with their repetitive adherence and suction activity, can erode and damage gill epithelium. *Brooklynella hostilis* can cause severe gill congestion and skin sloughing in captive fish (Gallet de Saint Aurin *et al*., 1990; Blasiola, 1992; Landsberg and Blackesley, 1995; Diamant, 1998a). Similarly, the free-living scuticociliate *Uronema marinum*, once regarded a harmless scavenger, is capable of invading the musculature and induce deep ulcers in fish predisposed by environmental stress. *U. marinum* has been observed in a wide range of temperatures and salinities and has no evident host specificity (Colorni, 2008). *U. nigricans* has caused lethal encephalitis in cage-cultured southern bluefin tuna *Thunnus maccoyii* in Australia (Munday *et al*., 1997).

The protistan endoparasites

Protistan endoparasites are generally considered to be more ancient than the ectoparasites, and thus better adapted to their fish hosts, with which they often co-exist causing them little harm (Lom, 1984). Some, however, may significantly alter appearance, texture, taste

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and odour of the affected fish, and may thus have considerable economic importance.

The microsporidians

The Microsporida are eukaryotic, obligate intracellular, spore-forming parasites related to the fungal phylum Zygomycota (Mathis, 2000; Dyková, 2006). They tend to be cell-specific, targeting a wide array of host tissue and cell types. Genera that infect fish are seldom encountered in other host groups, and most species are monoxenous. However, in some, small crustaceans may act as intermediate or paratenic hosts (see Moodie, 2005). Spores are small (2 to 10 μm, usually 7 μm or less) and may be observed in fresh infected tissue mounts, or in stained histological sections (Lom, 2008; Noga, 2010). Many of the genera parasitic in fish establish a complicated coexistence with their host cell. Some (e.g. *Glugea*) develop in great numbers, causing hypertrophy of the host cell and forming a xenoma. When in clusters, xenomas may generate a large pseudotumour.

Microsporidans are thought to be transmitted directly between fish through ingestion of infective spores (Lom and Nilsen, 2003; Lom, 2008). The highly resistant spores are dispersed either by excretion, shedding with necrotic tissue via skin/gill lesions or following host death (Johnson, 2000; Lom, 2008; Noga, 2010). While infections by microsporidians are clearly more commonly observed in wild-caught or pond-raised fish (Noga, 2010), economic losses in cage-farmed fish have been documented (Lom, 2008). In the western Mediterranean, a microsporidian infection described from young cultured gilthead sea bream was attributed to *Glugea* sp. (Mathieu-Daude *et al*., 1992). Abela *et al*. (1996) observed muscle lesions induced by *Pleistophora* in gilthead sea bream juveniles. Some additional case reports of microsporidian infections in the musculature of gilthead sea bream originated in Greece (Athanassopoulou, 1998), Italy (Fioravanti *et al*., 2006a) and Spain (Colorni and Padrós, 2011).

In Japan, *Microsporidium seriolae* causes 'beko disease', characterized by unsightly cysts, in yellowtail and red sea bream (Egusa, 1982; Egusa *et al*., 1988). Recently, a microsporidan which resembles *M. seriolae* but is a distinct species, was found at a high prevalence in the trunk musculature of Pacific bluefin tuna *Thunnus orientalis* in Japan (Zhang *et al*., 2010).

Molecular diagnostic methods for detecting microsporidians include *in situ* hybridization (Lee *et al*., 2003) and use of the ribosomal RNA and the genes encoding the RNA (Lom and Nilsen, 2003) for accurate identification and classification. Oral administration of Fumagillin has been reported to be partially effective in some spontaneous and experimental infections. The toxicity of this drug, however, is significant and its safety margin is narrow (Noga, 2010).

The apicomplexans

Apicomplexans are obligate protistan parasites common in both marine and freshwater fish. Members of this large group are characterized by a unique 'apical complex', an array of several ultrastructural elements at the anterior end of certain developmental stages. Piscine apicomplexans include the Coccidea (the majority) and Haematozoa, the latter known mainly from marine fish.

Fish coccidia seem to be less host specific than mammalian coccidia, although exceptions do exist (Molnár, 2006). A wide range of cell types and target organs are invaded, including the intestine, swimbladder, liver, spleen, testes, kidney, gills, blood cells, etc. (Davies and Ball, 1993; Perkins *et al*., 2000; Molnár, 2006; Steinhagen and Davies, 2008). While coccidiosis may cause substantial losses in freshwater culture (Molnár, 2006), the impact of this group on marine fish farms (apart from *Cryptosporidium molnari* in gilthead sea bream, see below) is largely unknown. As low intensity infections are generally subclinical and may go undetected, coccidiosis is likely far more widespread than previously thought. Cryptosporidiosis in particular is considered an emerging concern in farmed marine fish (Baragahare *et al*., 2011). Coccidian infections in wild sparids have been known for many years (see Duszynski *et al*., 2000). Reports include *Eimeria maggieae* in common pandora (*Pagellus erythrinus*) (Lom and Dyková,

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1981a, b), *Eimeria* sp. in axillary sea bream (*Pagellus acarne*), goldlined sea bream (*Rhabdosargus sarba*), yellowfin bream (*Acanthopagrus australis*), red pandora (*Pagellus bellotii*), bluespotted sea bream (*Pagrus caeruleostictus*) and striped sea bream (*Lithognathus mormyrus*). Haemogregarine-like organisms were reported in cage-cultured gilthead sea bream in the Red Sea (Paperna, 1979). In Spain, *Eimeria sparis* and *Goussia sparis* were recorded in the intestinal tract of gilthead sea bream (Álvarez-Pellitero *et al*., 1995, 1997; Sitjà-Bobadilla *et al*., 1996). Sporadic cases of *Eimeria* sp. infections have also been reported from sea bream in the central and eastern Mediterranean (Athanassopoulou *et al*., 1999; Fioravanti *et al*., 2006a). *C. molnari* typically infects the gastric epithelia of 30–100 g gilthead sea bream juveniles (Álvarez-Pellitero and Sitjà-Bobadilla, 2002; Sitjà-Bobadilla and Álvarez-Pellitero, 2003; Sitjà-Bobadilla *et al*., 2005). *C. molnari* has been reported in sea cages in Spain and Greece, apparently through stocked juvenile fish carrying the infection from the nursery system. Even high prevalence infections tend to disappear with fish growth, usually within a few months (A. Sitjà Bobadilla, personal communication, 2012).

Coccidiosis is diagnosed by identification of stages in fresh preparations of infected tissue, or in the case of intestinal coccidians, by examination of faeces. Autofluorescence of oocysts may be observed with epifluorescent microscopy (Davies and Stewart, 2000). Histological sections stained with Mallory's stain are particularly suitable, with mature oocytes staining yellow (Molnár, 2006). No effective commercial solutions are currently available and relatively few experimental treatments against coccidiosis have been attempted in marine fish.

The metazoan parasites

The metazoan microparasites (the myxozoans)

Myxozoans are obligate parasites of fish, which, while being microscopic, are multicellular with functional cellular specialization (Lom and Dyková, 1992). The Myxozoa are now classified as Cnidaria (Siddall *et al*., 1995; Holland *et al*., 2010; Ringuette *et al*., 2011). Histozoic myxosporeans invade cells, intercellular spaces or blood vessels, while coelozoic forms inhabit body cavities such as gall and urinary bladder. Most myxosporeans target specific organs and tissues, oftentimes infecting a cluster of closely related taxa. The life cycles of numerous freshwater species, involving alternating phases of asexual reproduction (in the fish) and sexual reproduction (in an annelid), are known (Yokoyama *et al*., 2012). Alternate development has also been demonstrated in a few marine myxozoans (Køie *et al*., 2004, 2007, 2008). At least one marine genus (*Enteromyxum*) is unique in that it is transmitted directly from fish to fish, through host ingestion of excrement containing vegetative developmental stages (Diamant, 1997; Redondo *et al*., 2002; Yanagida *et al*., 2004). While many myxosporean species are benign and elicit moderate host reactions, others can be highly pathogenic. Molecular methods for the genetic identification of myxozoans are being developed, which enable identification of species regardless of spore presence.

ENTEROMYXUM. The genus *Enteromyxum* includes three species, of which two, *E. leei* (formerly *Myxidium leei*) and *E. fugu*, are devastating pathogens of cage farmed fish, inducing severe, chronic desquamative enteritis. A third species, *E. scophthalmi*, is known only from pond-cultured flatfish (Sitjà-Bobadilla and Palenzuela, 2012). *E. leei* is non-fastidious in its host selection, with infections documented in dozens of fish species over a wide geographical distribution. It has been diagnosed in at least eight wild fish species (*Liza ramada*, *Siganus rivulatus*, *Siganus luridus*, *Trachurus indicus*, *Neopomacentrus miryae*, *Decapterus russelli*, *Diplodus noct* and *Teixeirichthys jordani*) associated with cage farms on the Israeli Red Sea and Mediterranean coasts (A. Diamant, unpublished).

Clinical signs include emaciation, poor growth, bloated abdomen, sunken eyes and death. Fish with severe enteromyxosis have a typical bony 'knife edge' dorsal ridge and emit a characteristic foul odour.

The direct transfer of *Enteromyxum* between fish renders it particularly risky to densely stocked farm systems. *E. leei* suspensions may

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remain viable and infective to naïve fish for 24 h (Yokoyama *et al*., 2009). Consequently, *E. leei* spread rapidly after its initial appearance (Diamant, 1992) to sea bream farms in most Mediterranean countries (Álvarez-Pellitero and Sitjà-Bobadilla, 1993a; Diamant *et al*., 1994; Le Breton and Marques, 1995; Sakiti *et al*., 1996; Tarer *et al*., 1996; Caffara *et al*., 1999). Different stocks of gilthead sea bream display different susceptibility and severity of clinical signs, presumably due to host genetic variability (Palenzuela, 2006). In a recent study, severity of the disease has been linked to the nutritional background of the host (Estensoro *et al*., 2011). *E. leei* has been reported from cage farmed sparids: gilthead sea bream, sharp snout sea bream (*D. puntazzo*), white sea bream (*Diplodus sargus*), two-banded sea bream (*Diplodus vulgaris*), red porgy (*Pagrus pagrus*) and red sea bream (*Pagrus major*) (Diamant *et al*., 1994; Kent *et al*., 2001; Padrós *et al*., 2001a; Athanassopoulou, 2007a), as well as European sea bass (*Moronidae*) and red drum (Diamant, 1998b). Sharp snout sea bream is particularly susceptible, with mortalities reaching 100% in cage farms (Montero *et al*., 2007; Álvarez-Pellitero *et al*., 2008). Water temperatures above ~ 20°C are required for onset of full-blown clinical enteromyxosis, while infections remain latent at lower temperatures (Fleurance *et al*., 2008). In Japan, mortalities occur in cage-cultured tiger puffer (*Takifugu rubripes*) and olive flounder (*Paralichthys olivaceus*) (Tun *et al*., 2000; Yasuda *et al*., 2005).

Diagnosis is based on presence of sporoblasts and spores in fresh gut mucosa squashes or histological sections (Fig. 5.4a). Molecular identification has been used in both diagnosis and epidemiological studies of enteromyxosis (Palenzula *et al*., 2004; Yanagida *et al*., 2004).

POLYSPOROPLASMA SPARIS. *Polysporoplasma sparis* (Fig. 5.4b) infects the renal glomeruli of gilthead sea bream, common pandora and sharp snout sea bream and is associated with poor growth and chronic low level mortalities, typically with a mild host immune and renal pathology (P. Varvarigos, 2012, personal communication). *P. sparis* has been reported from Spain, the Adriatic Sea and Greece (Sitjà-Bobadilla and Álvarez Pellitero, 1992, 1995; Palenzuela *et al*., 1999; Rigos *et al*., 1999; Mladineo, 2003a; Athanassopoulou *et al*., 2004b). Infections may reach high prevalences, but no clear seasonal pattern has been determined.

MYXOBOLUS. *Myxobolus* sp. sporadically occurs in the intestine of annular sea bream (*Diplodus annularis*) (Radujković and Raibaut, 1989) and sharp snout sea bream in the Adriatic Sea (Mladineo, 2003a, 2005). Kidney infections have been reported in white sea bream (*D. sargus*) and sharp snout sea bream in Greece (Golomazou *et al*., 2006, 2009). *Myxobolus* cysts containing trophozoites and mature spores may also develop in the interstitial renal parenchyma (Athanassopoulou *et al*., 2004c). In Israel, *Myxobolus* sp. was observed in striped bass (*Morone saxatilis*) spleen cultured in the Red Sea (Colorni and Diamant, unpublished). In Japan, *Myxobolus acanthogobii* causes skeletal deformities in yellowtail and Pacific mackerel (*Scomber japonicus*) (Yokoyama *et al*., 2005a). Myxozoan-induced encephalomyelitis in cultured yellowtail in western Japan is possibly due to *Myxobolus*. Although mature spores were not found in the spinal cord, 18S rDNA analysis identified the pathogen as *Myxobolus spirosulcatus,* a previously recognized, benign species from the bile duct of the same host species (Maeno *et al*., 1995). The condition involved reduced feeding, abnormal swimming, skin ulceration and redness of the brain. Histopathology revealed gliosis, multifocal glial nodules, nerve cell necrosis with neurophagia, brain haemorrhage, swollen eosinophilic nerve fibres and degenerative axons (Yokoyama *et al*., 2011).

CERATOMYXA. *Ceratomyxa* spp. are commonly found in the gall bladder, and to a lesser degree, the urinary system. *Ceratomyxa* infections are typically innocuous, but some exceptions exist. Diagnosis is based on the typical spore shape. Recent molecular studies have shown that the related genus *Leptotheca* is invalid, and its species have been reassigned to other genera (Gunter and Adlard, 2010). *C. sparidarum* is one such example. Previously known as *Leptotheca sparidarum,* it was observed in the kidney of gilthead sea bream and common dentex (*D. dentex*) in Spain (Álvarez-Pellitero *et al*., 1995; Sitjà-Bobadilla

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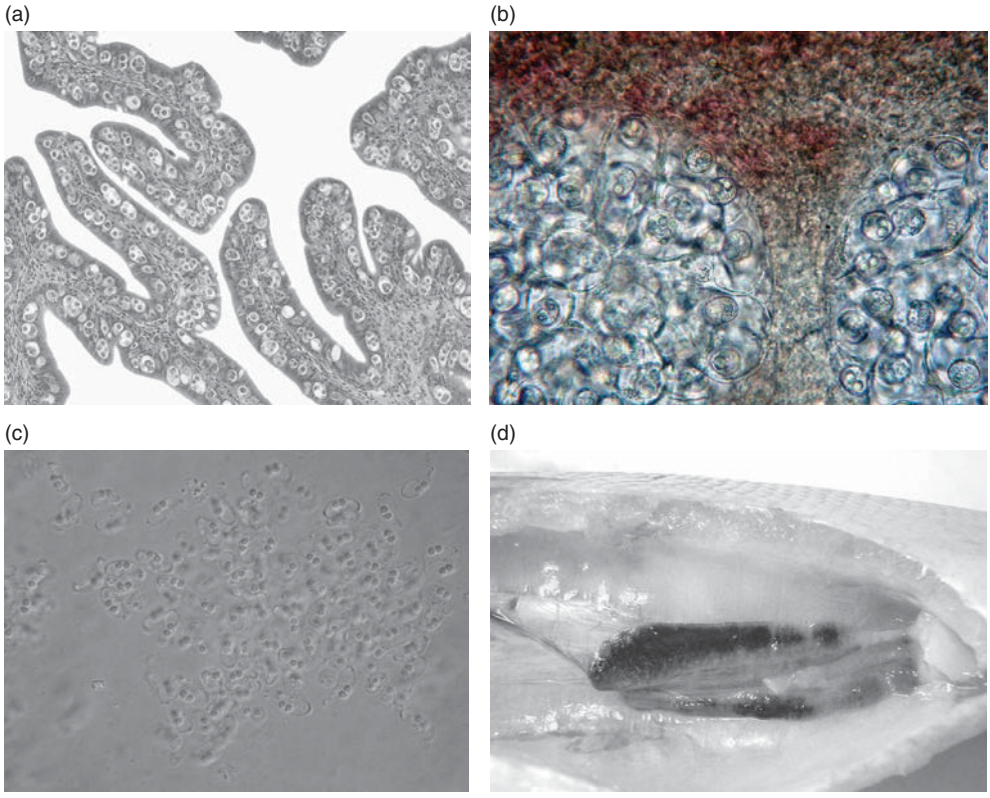


Fig. 5.4. Myxozoan parasites. (a) Histological section through the intestine of sea bream *Sparus aurata,* showing developing stages of *Enteromyxum leei* (H&E). (b) Live spores of *Polysporoplasma sparis* in the kidney of gilthead sea bream *Sparus aurata* (Image courtesy of Panos Varvarigos, Vet Care, Athens, Greece). (c) *Ceratomyxa* cf. *sparusaurati* spores in a fresh bile sample from sea bream *Sparus aurata* (Phase contrast). (d) *Sphaerospora testicularis* infection in the testes of sea bass *Dicentrarchus labrax*. Image courtesy of Marialetizia Fioravanti, University of Bologna, Italy.

and Álvarez-Pellitero, 2001), Italy (Fioravanti *et al*., 2006a) and Greece (Athanassopoulou, 2007a). Infected renal tubuli and Bowman's capsule display atrophy, flattening and vacuolation of the epithelium (Sitjà-Bobadilla and Álvarez-Pellitero, 2001). Infection with *C. sparusaurati* (Fig. 5.4c), *C. labracis*, *C. diplodae* and *Ceratomyxa* sp. have been reported in common dentex, sharp snout sea bream, gilthead sea bream, red porgy, European sea bass and various other non-sparid species (Álvarez-Pellitero and Sitjà-Bobadilla, 1993b; Álvarez-Pellitero *et al*., 1995; Palenzuela *et al*., 1997; Rigos *et al*., 1997; Athanassopoulou *et al*., 1999; Angelucci *et al*., 2008). *C. sparusaurati* in gilthead sea bream can cause gall bladder inflammation, ascites and abdominal distension and may induce low-level mortalities (Palenzuela *et al*., 1997). In Japan, *Ceratomyxa seriolae* and *C. buri* found in the gall-bladder of cage-cultured yellowtail cause no pathology, but a possible link between these species and 'green liver' syndrome (Egusa, 1983) was pointed out by Yokoyama and Fukuda (2001) as warranting further study.

SPHAEROSPORA SPP. *Sphaerospora* spp. are generally considered harmless to sea bass except in massive infections (Sitjà-Bobadilla and Álvarez-Pellitero, 1993). *S. dicentrarchii* was found in the intestines of cage farmed sea bass in Greece (Colorni, unpublished). *S. testicularis* (Fig. 5.4d) affects the gonad in male Mediterranean sea bass (Sitjà-Bobadilla and

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Álvarez-Pellitero, 1993). *S. epinepheli* infects the kidney of malabar grouper (*Epinephelus malabaricus*) in Thailand, causing disorientation and renal haemorrhage (Supamattaya *et al*., 1991). *Sphaerospora* (previously *Leptotheca*) *fugu* infects the gut of tiger puffer and is thought to contribute to host emaciation (Tun *et al*., 2000), although it is difficult to resolve its net effect as it tends to co-occur with *E. leei* and *E. fugu*.

KUDOA. *Kudoa* (order Multivalvulida) is a histozoic genus with four or more valves and polar capsules that affect various fish tissues, predominantly trunk muscle (Whipps *et al*., 2004). Diagnosis is based on the presence of macroscopic, whitish pseudocysts and the observation of the characteristic four polar capsules in fresh squashes, stained organ imprints (e.g. with Gram or Giemsa), or histopathological examination. In the Mediterranean, *Kudoa* sp. has been detected in white, gilthead and axillary sea bream (see Diamant *et al*., 2005). *Kudoa* pseudocycts measuring several millimeters scattered throughout the somatic muscle can significantly downgrade the market value of the fish (Langdon, 1991). Proteolytic enzymes released by the parasite may cause flesh spoilage due to post-mortem myoliquefaction ('post-harvest soft flesh' or 'jelly meat') (Kudo *et al*., 1987; Álvarez-Pellitero and Sitjà-Bobadilla, 1993a; Maeno *et al*., 1993; Rigos *et al*., 1999).

K. lateolabracis causes post-mortem liquefaction in Chinese sea bass *Lateolabrax* sp. (Yokoyama *et al*., 2004). Pseudoplasmodia may interfere with normal muscle function. Abnormal swimming behaviour was observed in at least five cage-farmed species infected with *Kudoa yasunagai*: Japanese sea bass (*Lateolabrax japonicus*), spotted knifejaw (*Oplegnathus punctatus*), yellowtail, tiger puffer and Pacific bluefin tuna (Zhang *et al*., 2010). *Kudoa neurophila* causes meningoencephalomyelitis in juvenile striped trumpeter (*Latris lineata*) in Australia (Grossel *et al*., 2003). *Kudoa shiomitsui* induces cysts in the heart of tiger puffer and Pacific bluefin tuna in Japan; however, the pathogenic potential of this species is still unclear (Zhang *et al*., 2010). Similarly, the pathological effects of the recently described *Kudoa prunusi* from the brain of cage-cultured bluefin tuna have yet to be determined (Meng *et al*., 2011). There are case reports in which *Kudoa* spp. has transferred from native wild populations to infect introduced cage-farmed stocks. In the Red Sea, *Kudoa* sp. first reported in gilthead sea bream (Paperna, 1982) was subsequently identified as *Kudoa iwatai,* a parasite described from cage-cultured red sea bream and spotted knifejaw in Japan (Egusa and Shiomitsu, 1983). The Red Sea isolate of *K. iwatai* was found in ten species of native host fish, and was presumed to have transmitted to locally cage-farmed sea bream and sea bass (Diamant *et al*., 2005). *Kudoa thyrsites* caused extensive post-mortem liquefaction in caged mahi mahi (*Coryphaena hippurus*) in Western Australia, to which the parasite was apparently transmitted from local clupeoid reservoir hosts (Langdon, 1991; Langdon *et al*., 1992). *Kudoa amamiensis* infections were reported in cage-farmed yellowtail and amberjack (*Seriola dumerilii*) (both *Carangidae*) in Amami and Okinawa Islands, Japan. Both farmed fish species were exotic to the area (Yokoyama *et al*., 2000) and were putatively exposed to local infection reservoirs within the local pomacentrid coral reef species populations (Egusa and Nakajima, 1980; Sugiyama *et al*., 1999; Whipps *et al*., 2003).

Kudoa in fish has been linked with cases of human allergic gastrointestinal signs (Martínez de Velasco *et al*., 2008).

HENNEGUYA. *Henneguya pagri* is considered highly pathogenic to red sea bream in Japan (Yokoyama *et al*., 2005b). Sporadic reports indicate the presence of this species also in the Mediterranean in both wild and farmed sharp snout and gilthead sea bream (Bahri *et al*., 1996; Caffara *et al*., 2003; Athanassopoulou, 2007a) with gill and cardiac bulb involvement but no significant pathology or associated mortalities. Cardiac lesions and enlargement of the bulbus arteriosus due to *H. lateolabracis* cause low level daily losses that tend to occur in a seasonal pattern in Chinese sea bass in Japan. Infected fish are anaemic, anorectic and lethargic and spores may cause congestion of gill capillaries and lamellar distortion (Yokoyama *et al*., 2003).

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Diagnosis and identification are based primarily on spore morphology. Polar capsules stain intensely with Wright's Giemsa or toluidine blue (Noga, 2010), or Gram stain (Schachner and Holzer, 2001). Experimental treatments have focused mainly on using coccidiostats. Feeds supplemented with drugs (such as fumagillin, toltrazuril, clamoxyquin, proguanil, the combination of sulphoquinoxaline and amprolium, narasin, nicarbazin), as well as natural bioactive extracts have all been tried, with partial success (Alderman, 1986; Hedrick *et al*., 1988; Yokoyama *et al*., 1990, 1999; Athanassopoulou *et al*., 2004b, c; Karagouni *et al*., 2005a, b; Golomazou *et al*., 2006; Palenzuela *et al*., 2009; Jones *et al*., 2011). Withdrawal periods of orally administered drugs for permissive fish tissue levels at marketing are largely undetermined for these chemicals. No approved anti-myxosporean drug is currently available. An interesting, but uniquely different strategy, based on supplementing fish feed with recombinant bacterial chitinase (ChiCD3) intended to damage the shell valves of myxosporean spores infecting the fish, has recently been proposed (Liu *et al*., 2011). The application of physical means for exclusion of myxozoans from culture systems has yielded some good results. Strong water flow has been shown to reduce severity of myxozoan infections (Hoffman, 1990; Hallett and Bartholomew, 2008; Bjork and Bartholomew, 2009). Various types of mechanical filtration of incoming water have proven partly effective in controlling myxozoan infections (Moran *et al*., 1999; Arndt *et al*., 2006; Cobcroft and Battaglene, 2012). Both UV and ozone are effective in eradicating *Kudoa neurophila* infective stages in seawater (Cobcroft and Battaglene, 2012). These measures can be useful in pond systems, but are not applicable to cages, where parasite access is largely uncontrollable. Here, sanitary management measures involving daily removal of dead fish can be effective. In Israel on the Red Sea, daily removal of carcasses with an air pump and a lift hose from a sack device located at the bottom of the floating cages helped keep *E. leei* infection levels significantly low in an offshore sea bream farm (Fig. 5.5a, b), while infection levels in an adjacent facility lacking such a routine were rampant (A. Diamant, unpublished).

The metazoan macroparasites (flatworms, flukes and 'sea lice')

The phylum Platyhelminthes (flatworms) includes dorsoventrally flattened, bilaterally symmetrical, unsegmented and acoelomate (no body cavity other than a digestive tract) worms. They usually lack an anus and regurgitate undigested material. They also lack specialized circulatory and respiratory systems.

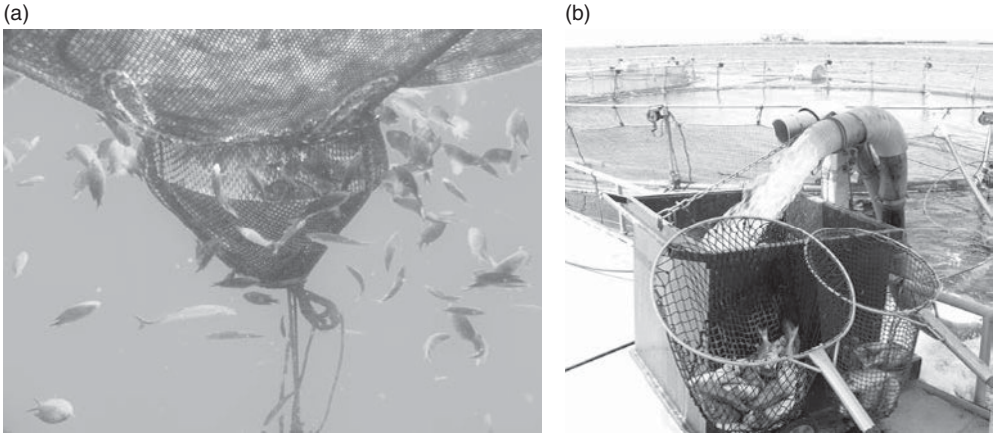


Fig. 5.5. (a) Sea bream cage bottom fitted with a cone shaped collector connected to a ~10 m long siphon. Wild fish attracted to the farm are seen congregating alongside. (b) Dead fish at the cage bottom are siphoned with an airlift to a collecting tank for sanitary disposal.

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Oxygen, carbon dioxide, nutrients and certain waste products pass through their compressed bodies by diffusion. The nervous system is concentrated in the cephalic portion of the body from which a nerve web innervates the periphery. The great majority of Platyhelminthes genera are monoecious. The mesenchyme (space between the skin and gut in which all internal organs are contained) contains a relatively loose connective tissue with fixed and pluripotent cells reinforced by collagen fibres, to which muscle fibres are attached. Traditionally, flatworms are classified into four groups: Turbellaria, Trematoda, Monogenea and Cestoda. The Digenea are classified as a subclass of the Trematoda (Olson *et al*., 2003; Cribb, 2005).

The 'sea lice' include a large number of copepod and isopod taxa that are adapted to a parasitic lifestyle, and responsible for acute tissue inflammation and necrosis of host tissues. Intensive fish farming in coastal waters provides an ideal environment to these crustaceans.

As all are invertebrates, metazoan marine parasites are osmoconformers, i.e. their body fluids are iso-osmotic with the ambient sea water (Bradley, 2009). Many species are sensitive to sudden fluctuations in salinity, so that whenever logistically feasible and dealing with euryhaline fish, a freshwater dip should be tried first as the preferred control treatment.

THE MONOGENEA. The monogeneans are mostly ectoparasitic, all are hermaphrodite and all have a single-host life cycle. They possess a posterior haptor armed with hooks and/or clamps or suckers by which they attach to the host (gills, scales or fins). They use proteolytic enzymes to break up the epithelial cells on which they feed, causing irritation, hyperplasia, haemorrhage and anaemia. At the anterior tip they usually have an additional attachment organ (adhesive glands). Monogeneans are mostly egg-layers, and eggs usually attach to the host by means of a coiled filament. The oncomiracidia (free-swimming ciliated larvae) emerging from the egg must locate a suitable host fish within a few hours, after which infectivity decreases significantly. Exceptions to this type of life cycle are the Gyrodactylids, which are viviparous and give birth to individuals identical to the parent and bearing well-developed embryos. The monogenea are geographically ubiquitous, affecting diverse species of fish and are thus the most frequently encountered worms in mariculture systems, either grazing on tissues or drawing blood from the host. Most have a restricted host specificity: for example, *Sparicotyle chrysophrii* and *Furnestinia echeneis* are known to have sea bream as preferential host, whereas *Lamellodiscus* spp. infest mainly sharp snout sea bream and *Diplectanum aequans* mainly sea bass (Fioravanti *et al*., 2006b; Sitjá-Bobadilla *et al*., 2010; P. Varvarigos, 2012, personal communication). Monogenea prefer rather specific sites on the fish but these may become less pronounced under intensive fish culture conditions. Massive fish mortalities during severe epizootics are usually the result of a breakdown in the normal host–parasite relationship created in part by the artificial farming conditions, i.e. when the oncomiracidia gain prompt access to stressed, immunocompromised hosts.

The class Monogenea is divided into two subclasses, the Monopisthocotylea (e.g. gyrodactylids, diplectanids, capsalids), which feed on epithelial cells and mucus and are characteristically motile, and the Polyopisthocotylea (e.g. microcotylids), which are haematophagous and principally stationary (Whittington and Chisholm, 2008). Haematophagous monogeneans may cause severe anaemia in captive fish (Paperna *et al*., 1984). However, because of the delicate manner of attachment to the secondary gill lamellae and the subtle manner in which blood is drawn, they usually cause comparatively less damage than the group of tissue grazers, which attach to the skin epithelia and feed in a more disruptive and destructive manner.

Monopisthocotylea. *Gyrodactylus* sp. has occasionally been found on fins and body surface of gilthead sea bream cultured in the Red Sea (Fig. 5.6a) and the Spanish Mediterranean coasts. *Gyrodactylus orecchiae* (*Gyrodactylidae*) caused heavy infections on juvenile fish in inshore cages in the Adriatic Sea. Clinical signs include hypermelanosis, lethargy, anorexia and weight loss (Paladini *et al*., 2009). Members of the family *Diplectanidae* are perhaps the most frequent monogeneans parasitizing

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sparids. They have four eyespots close to the anterior tip. The haptor is a single unit comprising several large, centrally located, sclerotized anchors (hooks or hamuli) and often small marginal hooklets. *Furnestinia echeneis* (*Diplectanidae*) (Fig. 5.6b) is frequently observed on gilthead sea bream in the Mediterranean and Red Sea fish farms. Many species of the genus *Lamellodiscus* have been described as parasites on sparids in the Mediterranean Sea, in Australia, and elsewhere. Some species present low host specificity (see Colorni and Padrós, 2011, and refs. therein). *Neobenedenia melleni* (Fig. 5.6c) and other members of the *Capsalidae* injure their host by feeding on mucus and epidermis, inducing ulcerative lesions that serve as ports of entry for secondary bacterial invaders.

Polyopisthocotylea. In this group, the opisthohaptor consists of a battery of small, muscular, adhesive suckers or clamps that are supported by cuticular sclerites. Contrary to the monopisthocotyleans whose anchors or hooks pierce tissue, the polyopisthocotyleans grasp host tissue between opposing clamps (Noga, 2010). Many Polyopisthocotyleans have been recorded worldwide.

Sparicotyle chrysophrii (Fig. 5.6d) is an important pathogen for cultured gilthead sea bream in the Mediterranean (Faisal and Imam, 1990; Sanz, 1992; Padrós and Crespo, 1995), and it causes significant problems in cage farms. In addition to anaemia, the haematophagous activity of this species (and in general, all microcotylids) may induce inflammation, epithelial hyperplasia and necrosis

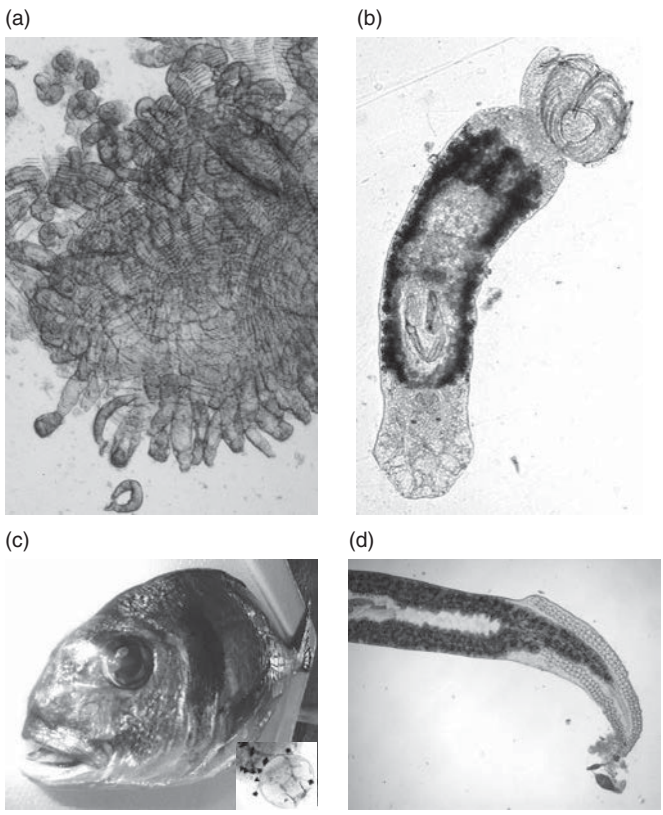


Fig. 5.6. Monogenean infections. (a) Fresh skin scraping of sea bream *Sparus aurata* infested with *Gyrodactylus* sp. (b) *Furnestinia echeneis* from sea bream *Sparus aurata* gills. (c) Sea bream *Sparus aurata* infested with *Neobenedenia* cf. *mellenii*. Inset: *Neobenedenia melleni* with eggs. (d) *Sparicotyle chrysophrii* from sea bream *Sparus aurata* gills.

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of tissues due to the long-term attachment of the multiple clamps to the lamellae. Secondary infections by *Tenacibaculum* and *Vibrionaceae* are commonly observed. A *Microcotyle* sp. has been described from gills of cultured red porgy from sea cages off Crete (Katharios *et al*., 2006).

Polylabroides multispinosus (*Microcotylidae*) was identified from the gills of yellowfin bream in Australia (Diggles *et al*., 1993; Roubal and Diggles, 1993; Roubal, 1995). External signs are often inconspicuous before a heavy infestation, but restlessness, respiratory distress, 'flashing', mucus hyperproduction and blindness may indicate infections. Capsalid worms are rather transparent and despite their size (up to 5–6 mm long) their presence may be overlooked. Although generally sensitive to formalin, hydrogen peroxide or, as in the case of *Neobenedenia melleni*, short freshwater exposure, eggs are more resistant and may survive even these therapies.

THE DIGENEA. The digeneans form a subclass of endoparasitic, heteroxenous worms that require one or two intermediate hosts to complete their life cycle (Bullard and Overstreet, 2008). All major groups of vertebrates serve as hosts for the adult stages. In fish, Digenea can be found as larvae or juveniles (usually encysted) and as adults (usually free). Most are hermaphrodite. Adult digeneans are recognizable by two suckers, one at the anterior end (oral) and one ventrally located, usually at the anterior half of the body. Miracidia and cercaria have a single pair of eye spots. Other stages may have vestigial photosensitive organs. Digeneans of fish are oviparous (egg laying) and in most cases the egg hatches outside the host to release a miracidium (ciliated free-swimming larva). This stage can survive for several hours, during which time it must locate and infect the first intermediate host: a mollusc, usually a gastropod or bivalve. Within this host, the parasite undergoes asexual reproduction which eventually (through sporocyst and redial stages) brings about the liberation of free-swimming cercariae. These may survive for up to 24 hours in the water column, during which time they must locate a suitable (second intermediate) host in which they develop into a metacercaria. In some digenean species the cercariae may simply encyst on vegetation or a substrate. When the metacercaria is ingested by the definitive host, it develops into a sexually mature adult.

In sanguinicolid infections (blood flukes) fish in proximity to an infected intermediate host (i.e. snail, bivalve, or as in the case of marine aporocotylid sanguinicolids, a polychaete) may accumulate a large number of the worm in their blood system. The eggs are released into the fish's vascular system, become sequestered in gills, heart, kidney, liver, spleen, pancreas, or other organs, where they hatch, cause inflammation and compromise the physiological and mechanical efficiency of these organs, which may occasionally kill the host (Bullard and Overstreet, 2002).

Adult digenean trematodes of the intestinal tract or in other host body cavities (peritoneum, swimbladder) are not considered particularly harmful to the fish.

Cercariae and metacercariae as well as haematoparasites (fish blood flukes) can have a serious detrimental effect. Acute infections by cercariae have been occasionally described due to severe damage in the host tissues during cercarial penetration and migration. Small fish would be most susceptible (Noga, 2010). Once encysted, metacercariae do not produce further tissue damage. However, intense melanization reaction around the cysts may reduce the market value of affected fish. Sanguinicolid adults reside in blood vessels and in heart or peritoneal cavity. The embryonated eggs they release become visible when lodged in the small capillary networks of the gills. Affected lamellae appear swollen and ischemic. Severe gill lesions caused by the fully developed miracidia emerging from the eggs and making their way into the water through the gill tissue may be observed (Noga, 2010). Sanguinicoliasis has caused mass mortalities of fish reared in ponds and cages in North America, Europe and Asia (Bullard and Overstreet, 2002). In caged gilthead sea bream cultured in different sites along the Spanish Mediterranean coast, massive presence of aporocotylid eggs and miracidia clogged the gill capillaries causing severe local tissue damage, while the adults could be found in the vessels of the renal

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parenchyma or in the afferent vessel of individual gill arches (Padrós *et al*., 2001b; Holzer *et al*., 2008; Alama-Bermejo *et al*., 2011). Unidentified aporocotylid sanguinicolids were reported in gilthead sea bream also in Spain, Italy, Croatia and Greece (Álvarez-Pellitero, 2004; Fioravanti *et al*., 2006a; Alama-Bermejo *et al*., 2011). Sanguinicolids (*Paradeotacylix* spp.) have caused mass mortalities in cultured amberjack in Japan (Ogawa *et al*., 1993).

It has also been strongly suggested that fattening Atlantic bluefin tuna (*Thunnus thynnus*, in sea cages in the Mediterranean) by feeding with fresh pilchard, anchovy, sprat, mackerel, Spanish mackerel, etc. may lead to digenean infections (e.g. *Didymozoidae*) (Mladineo and Tudor, 2004).

Chemical therapies and biological controls are often impractical or ineffective in large-scale aquaculture operations (Paperna and Dzikowski, 2006), and intermediate hosts are often unknown.

THE TURBELLARIA. The turbellarians are lower flatworms and comprise a polyphyletic group of mostly free-living organisms with simple life cycles. *Ichthyophaga* and *Paravortex* are generally regarded as parasitic on fish. They form shallow pouches in the host's gills or body wall, feeding on blood or body fluids. During the parasitic stage (approximately 6 days) the worms reach about 450 μm in length. Upon leaving their host the worms grow and mature on the sea bed, each eventually releasing up to 160 free-swimming infective larvae. The life cycle takes about 10 days at 24.5°C to complete (Kent and Olson, 1986). Transmission can also be directly, from fish to fish, as adults are able to crawl from one host to another. They were previously described in wild or captive tropical aquarium fish, in which they caused negligible pathology (Cannon and Lester, 1988); however, infections by (prob.) *Ichthyophaga* reached epizootic proportions in cultured carangids (lookdown, *Selene vomer*, and Florida pompano, *Trachinotus carolinus*) in North Carolina (Noga *et al*., 1999; Noga, 2010) and more recently *Paravortex* was a problem in a sea cage farm in Mexico (Yechiam Shapira, Campeche, Mexico, 2012, personal communication). The latter group seems to be an emerging disease risk in warmwater mariculture. Infested fish may display rapid breathing, erratic swimming and attempts to scratch themselves against the cage net. The larvae encyst in the skin, where they induce hypermelanized tissue reactions that appear as tiny black spots, and in the gills where they feed on the blood of their host. Lesions closely resemble those caused by digenean metacercariae. Two or three consecutive formalin or organophosphate treatments or freshwater baths are effective to control the infection (Noga, 2010).

THE PARASITIC CRUSTACEA. A large number of copepod and isopod species parasitize the integuments of fish. Some species are sedentary, others move freely over the host surfaces, grasping, anchoring and feeding on skin and gills. They all cause irritation, infiltration of macrophages and lymphocytes and induce epithelial proliferation. The gill filaments can be severely damaged and skin erythema and haemorrhage typically occur in heavy infestations. Opportunistic bacteria frequently infect the lesions. Like all arthropods, these parasitic crustaceans have to undergo several moults during growth and maturation, resulting in discrete developmental stages. Transmission is direct.

Copepoda. About 1700 species of copepods are known to be parasitic, mostly on marine fish (Noga, 2010). Copepod morphology, structure, biology and ecology are highly diversified. Eggs hatch to release free-swimming nauplii without a gut. These moult at intervals to give rise to a succession of naupliar stages, the last of which moults to form the first of a series of copepodids, each with a mouth cone. In parasitic species, the copepodids are the infective stages. The adults can be highly modified in their morphology as an adaptation to their parasitic life, while also exhibiting distinct sexual dimorphism, the ovigerous female being larger than the male. A common feature among copepods is a partial or total loss of segmentation (Boxshall, 2005). Caligiforms, Ergasiliforms and Lernaeids/Pennellids are the main groups found in the marine environment. The majority of copepods parasitize a single host; the Pennellids are the exceptions (Piasecki and Avenant-Oldewage, 2008).

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Copepods often attach to the host by pressing their shield-like cephalothorax onto the skin like a sucker, using the second antennae and maxillipeds as clamps. They feed mainly on mucus and epidermis. In Asia, various ergasilids and caligid species have been described in cultured red sea bream (Yamashita, 1980; Ogawa and Yokoyama, 1998a, b), while in black sea bream, (*A. schegeli*), several species of *Caligus* (*C. epidemicus*, *C. multispinosus*, *C. acanthopagri*, *C. latigenitalis* and *C. rotundigenitalis*) were recorded (Lin and Ho, 1993; Lin et al., 1994; Izawa and Choi, 2000). Copepods belonging to other families were also observed. In Japan, *Alella macrotrachelus* was described in black sea bream (Muroga *et al*., 1981).

In Australian captive sparids (e.g. yellowfin bream), *Ergasilus australiensis*, *Bomolochus stocki*, *Alella macrotrachelus*, *Lernanthropus atrox* and *Lerneanthropus chrysophhrys* were reported (Roubal, 1995). The impact of these parasites on maricultured fish was reviewed by Johnson et al. (2004). Copepod on fish has rarely been associated with mortalities in warm waters, but caligids in particular can proliferate and spread to epizootic proportions.

Chemotherapeutants for control of 'sea lice' include formaldehyde, organophosphate insecticides and antiparasitic compounds such as ivermectin, pyrethrum, carbaryl and diflubenzuron. However, different species of copepods or different developmental stages within the same species have different degrees of susceptibility to these chemicals. The therapeutic dosage and safety margin for the hosts are often dangerously close (Noga, 2010). Some species die only after laying eggs resistant to these treatments. As exposures to fresh water have proved effective with some species, when logistically feasible, this treatment should be tried first. Interestingly, as juveniles, some sparid species such as white and sharp snout sea bream show a cleaning behaviour feeding on other fish parasitic *Caligidae* (Mariani, 2001).

Isopoda. About 500 parasitic isopods are known from nearly all fish host groups (Noga, 2010). Two families (suborder Flabellifera) with prominent differences in morphology and ecology (Kabata, 1984; Brandt and Poore, 2003) are of importance to the fish farmer. The great majority of isopod fish parasites belong to the family *Cymothoidae* whose members can reach 6 cm in length and tend to be associated with their hosts through their entire life. In the *Gnathiidae*, only the larval stages (praniza) are parasitic. Among the first family, *Ceratothoa* spp. are constantly fertile, and remain in reproductive condition throughout the year. A mature gravid female isopod releases about 400–550 larvae at a time. Fecundity and hatching rate increase as water temperature rises (Varvarigos, 1998). *Ceratothoa oestroides* is not host-specific. In the Mediterranean, wild species, such as grey mullet (*Mugil* sp., *Liza* sp.), bogue (*Boops boops*), goldline (*Sarpa salpa*), striped sea bream (*L. mormyrus*) and white sea bream (*D. sargus*) aggregate around floating cage farms, feeding on uneaten pellets and acting as vectors for the transmission of *C. oestroides* to the farmed sea bream and sea bass. High fish density in the cages favours transmission (Horton and Okamura, 2001a, b). Strong water currents may affect the dispersal of the manca larvae, hindering their ability to intercept a fish.

Cymothoids are protandrous hermaphrodites, i.e. they first develop as males and later turn into females that carry eggs and then larvae in a ventral brood pouch. Because of their large size, parasitic isopods can cause considerable damage with their sharp biting and sucking mouthparts and hooked legs (Noga, 2010). Cymothoids are usually found paired, attached to the buccal and branchial cavities, presumably capable of impairing the intake of the food pellets. However, as the female faces the mouth opening and the dorsal curvature fits the contours of the fish's palate, the chitinous segments of her armour, imbricated like roof tiles with their free margin directed backward, seem perfectly adapted to performing the same gripping function as the fish's tongue (Colorni *et al*., 1997). Heavy infestations of *C. oestroides* larvae may kill smaller fish (about 5–20 g of weight) (Athanassopoulou, 2007b). Chemotherapeutants for control of isopods include the use of organophosphates, but in fact no radical effective treatment has been devised (Athanassopoulou *et al*., 2001, 2004a).

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The most common isopods in Mediterranean fish are *Ceratothoa parallela* (Papapanagiotou and Trilles, 2001) that parasitizes gilthead sea bream, and *Ceratothoa oestroides* and *Nerocila orbignyi* that parasitize European sea bass (Šarušic, 1999). *C. oestroides* has occasionally been observed also on sea bream (Mladineo, 2003b).

Gnathia spp. feed on fish blood. The thoracic somites of the pranizae are supple and the larvae can thus engorge large quantities of blood. Particularly vulnerable to attack are cage fish injured or stressed by handling in the first days post stocking, as skin wound exudates appear to attract the pranizae (Colorni and Padrós, 2011). Adult gnathiid isopods are non-parasitic, free-living and reside in the meiobenthos, hiding in mud tubes or inside sponges. *Gnathia piscivora* and *Elaphognathia* sp. were described in the Red Sea, attaching to cage-cultured gilthead sea bream at night time (Paperna and Por, 1977). Once engorged with blood, the pranizae sequentially abandon and return to the fish, feeding and moulting three times before maturation into non-parasitic adults.

The body of flabelliferid isopods is dorsoventrally flattened, consisting of seven peraeonal somites (anterior segments, with seven pairs of pareopods) and six pleonal somites (abdominal segments) with five pairs of pleopods and the pleotelson. There is no carapax, and paired compound eyes consist of numerous ommatidia (Piasecki and Avenant-Oldewage, 2008).

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6 Infectious Diseases of Warmwater Fish in Fresh Water

Gilda D. Lio-Po[1]* and L.H. Susan Lim[2]
[1]*Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, the Philippines;* [2]*Institute of Biological Sciences, Universiti Malaya, Kuala Lumpur, Malaysia*

Introduction

Cage culture of freshwater fish in Southeast Asia (Cambodia, Indonesia, Philippines, Malaysia and Vietnam) is either at the subsistence, semi-intensive or intensive level. Fish cultured in cages include different species of tilapia (*Oreochromis* spp.), carp (*Cyprinus carpio*, *Aristichthys nobilis*, *Ctenopharyngodon idella*, *Labeo* spp., *Catla spp.*, *Cirrhina* spp., *Hypophthalmichthys* spp., *Leptobarbus* spp.), snakeheads (*Channa* spp.), eleotridids (*Oxyeleotris marmoratus*), milkfish (*Chanos chanos*) and catfish (*Clarias* spp., *Pangasius* spp., *Hemibagrus* spp., *Pangasianodon* spp.) including the channel catfish (*Ictalurus punctatus*) introduced from USA (Guerrero, 1979; Natarajan *et al*., 1983; Lio-Po and Lim, 2002; Molnár *et al*., 2006a, b; Székely *et al*., 2009a, b; Najiah *et al*., 2012).

Reports on diseases of feral and cultured fish in warm freshwater, do not distinguish between diseases found in cage culture and pond culture (Davy and Chouinard, 1982; Lio-Po *et al*., 1982; Lio-Po, 1984; Kabata, 1985; Arthur, 1987, 1992; ADB/NACA, 1991; Lim, 1991d, 1992; Paperna, 1991, 1996; Dharma *et al*., 1992; Nasution *et al*., 1992; Alawi and Rusliadi, 1993; Aqua Farm News, 1993; Thune *et al*., 1993; Arthur and Lumalan-Mayo, 1997; Lio-Po *et al*., 2001; Lio-Po and Lim, 2002; Lio-Po and Inui, 2010). Epizootics in cage-cultured fish have greater impact because of high stocking densities, close proximity of cultured fish with each other as well as with feral fish and intermediate hosts of parasites. For example, fish kills of cage-cultured carp attributed to koi herpesvirus occurred in Indonesia in 2002 (Sunarto *et al*., 2005a). In addition, there were 64% reported disease outbreaks in cage-cultured channel catfish in the USA during the 1990s with mortality in 91% of these cases (Masser *et al*., 1991). In addition, diseases occur more frequently in cages than in ponds (Collins, 1988). Fish reared in cages may also present a potential health threat to man, especially when they are reared in unsanitary waters in areas where fish-borne zoonotic diseases are prevalent (Ko, 1995) or when located in polluted areas. For example, *Piscinoodinium pillulare*, the causative agent of velvet disease of cyprinids was first reported on pond-reared fish but is now found on cage-cultured stock (Székely *et al*., 2009). Hence, diseases that are important in pond aquaculture are potential problems in cage culture.

* E-mail: liopo@seafdec.org.ph

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Viral Infections

Viral infections often cause mass mortality among fry or fingerlings, although older fish may either develop pathological signs and die or may appear unaffected but are carriers. Most viral infections in fish occur at lower water temperatures of 15–25°C (Lio-Po *et al*., 2001; Goodwin, 2005; Dishon *et al*., 2007). In tropical South-east Asia, such conditions often occur annually, during the months of October to February. Stress from handling, poor water quality, water temperature, age of fish, high stocking density and poor nutrition are factors that facilitate the development of viral diseases (Plumb, 1999b).

Viral infections of freshwater fish in tropical Asia include Koi Herpesvirus (KHV) Disease, Epizootic Ulcerative Syndrome (EUS), Grass Carp Reovirus Disease (GCRVD), Channel Catfish Virus Disease (CCVD) and Spinning Tilapia (ST) Syndrome (Table 6.1). The koi herpesvirus disease has the most impact on cage-cultured carp (Sano *et al*., 2004a; Sunarto *et al*., 2005a) while the Spinning Tilapia (ST) Syndrome has been reported once since its outbreak in Australia (Ariel and Owens, 1997) and reviews on this disease have been published (Lio-Po, 2001; Lio-Po and Lim, 2002; Lio-Po, 2010). On the other hand, EUS is associated with rhabdovirus, *Aeromonas hydrophila* and the pseudofungus *Aphanomyces invadans*, and discussed in the subtopic on Disease of Complex Infectious Aetiology in ths chapter.

Koi herpesvirus (KHV) disease

Outbreaks of Koi Herpesvirus Disease (KHVD) also known as Carp Nephritis and Gill Necrosis (CNGV) (Pikarsky *et al*., 2004) had been reported in England, Germany, Israel, USA, Belgium, South Africa, Switzerland, the Netherlands, France, Denmark, Austria, Italy, Japan, Luxemburg and Poland (Perelberg *et al*., 2003; Haenen *et al*., 2004; Bergmann *et al*., 2006, Way and Dixon, 2007).

In Asia, the first outbreaks of KHV caused mass mortalities of cultured common carp and koi carp in Israel in the spring of 1998 when temperatures were 18–28°C (Perelberg *et al*., 2003) Its occurrence was reported in koi in Hong Kong (Sunarto and Rukyani, 2005); common carp in Indonesia (Sunarto *et al*., 2005a); koi in Taiwan (Tu *et al*., 2004a); common carp in Japan (Sano *et al*., 2004a), koi in Thailand (Tandavanitj *et al*., 2005)

Table 6.1. Taxonomic classification and physical characteristics of viral pathogens of economically important tropical finfishes commonly cultured in fresh waters (modified from Lio-Po, 2010).

Disease	Virus Agent	Genus	Family	Nucleic Acid	Size (nm)	Shape	Enveloped
Koi Herpesvirus Disease (KHVD)	*Cyprinid herpesvirus 3* (CyHV-3) or KHV	*Herpesvirus*	*Alloherpes-viridae*	dsDNA	~100–110	Icosahedron	Yes
Grass Carp Haemorrhagic Disease (GCHV)	GCRV or CGRV or GSV	*Aquareovirus C*	*Reoviridae*	dsRNA	~60–70	Icosahedron	No
Epizootic Ulcerative Syndrome (EUS)	EUS Rhabdovirus	*Rhabdovirus*	*Rhabdo-viridae*	ssRNA	~65 × 175	Bullet-shaped	Yes
Channel Catfish Virus Disease (CCVD)	CCV	*Herpesvirus ictaluri*	*Herpes-viridae*	dsDNA	~90–100	Icosahedron	Yes
Spinning Tilapia Syndrome	Spinning Tilapia Virus	*Iridovirus*	*Adeno-viridae*	dsDNA	~110–140	Icosahedron	No

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and koi broodstock in Korea (Gomez *et al*., 2011; Lee *et al*., 2012). The KHV was also detected in Malaysia but not at epizootic levels (Musa *et al*., 2005) and isolated from Malaysian koi imported into the UK (Gilad *et al*., 2003).

In Indonesia, heavy mortalities attributed to KHV first occurred among cage-cultured common carp in Blitar, East Java, in March 2002 (Sunarto *et al*., 2005a). Subsequent outbreaks were observed in Subang Regency, West Java; Cirata Reservoir, West Java. By 2006, the disease had spread to more sites in the country. The KHV outbreaks were associated with 95% mortality and losses amounting to approximately US$0.5 million within a 3-month period (Sunarto *et al*., 2005b).

In Japan, KHV was first reported among cage-cultured common carp in Lake Kasumigaura, Ibaraki Prefecture in October 2003 (Sano *et al*., 2004a). The disease gradually spread to other prefectures and by 2006, 45 prefectures had confirmed outbreaks and involved koi carp for the first time (Sano *et al*., 2004b; T. Iida, personal communication). Approximately 1200 t of common carp equivalent to 20% of the annual production were lost through KHV (Yuasa and Sano, 2009).

The high mortalities and dramatic spread of the disease in Indonesia and Japan created an acute awareness of its emergence as a new disease in the region (Lio-Po, 2007, 2009; Yuasa and Sano, 2009). The virus was isolated by cell culture from infected stocks of koi or common carp in the USA, UK, Israel, Japan, Malaysia, Indonesia, Thailand and Taiwan (Hedrick *et al*., 2000; Gilad *et al*., 2003; Perelberg *et al*., 2003, Sano *et al*., 2004a; Sunarto *et al*., 2011). However, the virus was not detected during annual active surveillance from 2004 to 2006 in Cambodia, Lao PDR, Myanmar, Philippines and Vietnam (Lio-Po *et al*., 2009).

Causative agent

The koi herpesvirus (KHV) or *Cyprinid herpesvirus* 3 (CyHV-3) belongs to the family, *Herpesviridae* (Waltzek *et al*., 2005). This dsDNA virus has an icosahedral nucleocapsid measuring 101.9 ± 10.3 nm (Sunarto *et al*., 2011). The KHV has a total genome length of approximately 295 kbp (Aoki *et al*., 2007). Sequence analyses showed the KHV isolated in the UK, USA and Israel had 100% homology. The genotypes of the USA isolate are classified as E1, E6 in Israel while E1, E2, E3, E4 and E5 are in the Netherlands (Kurita *et al*., 2009). The Japanese and the PR China KHV belong to the A1 strain while the Indonesian and Taiwan strains are A1 and A2, which indicates that strains in Europe, America and Israel are closely related to those in Asia. Sunarto *et al*. (2011) reported that sequence analysis of all Indonesian KHV isolates displayed a marker I allele (I++) previously identified only in isolates of the Asian lineage; and the marker II allele, which has been reported previously only amongst isolates of European lineage. Recently, Kim and Kwon (2013) provided evidence of two genotypes from diseased carp in South Korea.

Pathology

Infected fish typically have white, necrotic patches on the gill filaments (Fig. 6.1). Affected fish often swim on the surface and exhibit respiratory distress. Sunken eyes, haemorrhage on the body surface and excessive mucus production with rough pale patches of the skin may be associated with the infected fish.

Infected fish gills develop lamellar epithelial degeneration, focal areas of necrosis and exfoliation. Histopathology consists of hyperplasia and fusion of the secondary gill lamellae (Tu *et al*., 2004b). Intranuclear

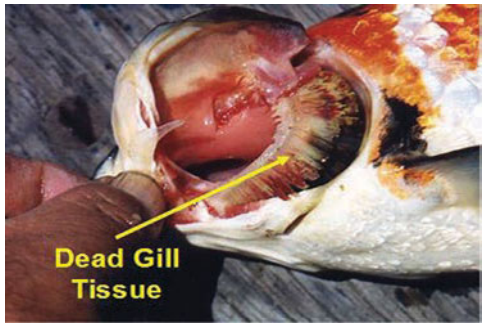


Fig. 6.1. Koi carp with KHV infection showing whitish grey, necrotic gill tissue. Image courtesy of A. Goodwin, University of Arkansas, USA.

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inclusions in the branchial epithelium may be observed. Other than the gills, necrotic tissues are also in the liver, spleen and kidney parenchymal cells (Hedrick *et al*., 2000). In addition, non-specific secondary infections of bacterial, parasitic and fungal origin may be associated with KHV infections such as secondary gill infections attributed to *Flavobacterium columnare* and *Aeromonas* spp. (Sunarto *et al*., 2005b).

Koi herpesvirus infects common carp, koi carp and ghost carp (hybrid of koi and common carp) (*Cyprinus carpio*) although there are conflicting reports on the susceptibility of other fish species to KHV. Experimental transmission of the infection from common carp to goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) or Nile tilapia (*Oreochromis niloticus*) was unsuccessful (Perelberg *et al*., 2003). In Indonesia, naturally infected cage-cultured common carp did not infect tilapia in adjacent cages. Likewise, Nile tilapia (*O. niloticus*) and sutchi or striped catfish (*Pangasius hypophthalmus*) (now known as *Pangasianodon hypoththalmus* cohabitated with KHV-infected common carp were not infected (Yuasa, 2004). In Japan, ayu (*Plecoglossus altivelis*), goldfish, gibel (*C. auratus langsdorfii*), common dace (*Tribolodon hakonensis*), common minnow (*Zacco platypus*), dark chub (*Zacco temminckii*) experimentally-exposed to KHV by immersion did not develop the disease (Ito *et al*., cited by Yuasa and Sano, 2009). However, Hedrick *et al*. (2006) reported that goldfish x common carp hybrids were moderately sensitive to KHV.

Natural infections of susceptible fish result in 80–90% mortality within a week after the onset of clinical signs (Hedrick *et al*., 2000; Neukirch and Kunz, 2001; Perelberg *et al*., 2003; Sano *et al*., 2004a; Tu *et al*., 2004b; Sunarto *et al*., 2005b). The virus can be transmitted from infected fish to naïve koi or common carp via intraperitoneal injection, by bath or by cohabitation with infected koi or common carp at 18–25°C. However, the virus is not infectious to humans.

Experimental exposure of healthy common carp to KHV at 22°C can cause up to 82% mortality within 15 days (Ronen *et al*., 2003). The virus appears to be more virulent to 14 g common carp dying 6 to 9 days post inoculation (dpi) compared to 6 g test fish which succumb in 10 to 14 dpi (Lio-Po *et al*., 2006). Other experimental studies, showed that 3-day old common carp fry (mean TL: 7.5 and 8.7 mm) were not susceptible, while 69–100% mortalities were observed in juveniles (mean TL: 13.8 and 29.2 mm) reared at 24°C (Ito *et al*., 2007). The incubation period of the disease is 5–7 days, characterized by onset of clinical signs, and rapid spread to koi and common carp when water temperatures were 15–25°C (Gilad *et al*., 2003; Ronen *et al*., 2003).

The KHV were detected by immunofluorescence and by semi-quantitative PCR in the kidney but in lesser amounts in the liver and brain of infected fish (Ronen *et al*., 2005). Furthermore, interstitial nephritis was detected 2 dpi that increased in severity through 10 dpi. Gilad *et al*. (2004) found the highest KHV DNA concentrations with virus genome equivalents of 10^8 to 10^9 per 10^6 host cells in the gill, kidney and spleen by real-time TaqMan PCR. High levels of KHV DNA were also found in the mucus, liver, gut and brain. At the same time, Yuasa *et al*. (2005) detected the virus in several organs including the gills at 3–40 dpi by PCR in common carp fingerlings after experimental immersion in 10^3 $TCID_{50}$ per ml at 23°C, but did not detect the virus in any organ at 60 dpi. The virus titres increased and peaked at 7–9 dpi in the gills, fin, scales, kidney and intestine. The virus was frequently isolated by cell culture from the gills and kidney at 6–17 dpi but not after 20 dpi. Subsequent studies showed that viable KHV was detectable in the fish brain even at 145 dpi but were infectious only for up to 28 dpi (Yuasa *et al*., 2007). In Korea, KHV was detected in the gills, intestine, kidney, spleen, heart and liver of infected common carp (Lee *et al*., 2012).

Temperature is a critical factor in the pathogenesis of KHV infection. Cell cultures inoculated with KHV and incubated at optimum temperature develop typical vacuolation. However, the vacuolated cells may revert to normal and plaques may disappear when the cells are shifted to non-permissive temperatures, and can reappear again after transfer to permissive temperature (Dishon *et al*., 2007). *In vivo*, the virus

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induces infection/mortalities at 18–25°C. However, when fish are maintained at 13°C and 30°C, no infection or mortality develops (Goodwin, 2005). Furthermore, fish exposed to KHV at 20–24°C for 3 days then transferred to non-permissive temperatures survived the disease. However, fish held at 13°C for 30 days and shifted to 22–24°C develop the disease with rapid onset of mortality. Likewise, Yuasa *et al*. (2008) showed that common carp experimentally exposed to KHV at 16°C, 23°C and 28°C died from infection in 21–54, 5–20 and 7–14 dpi, respectively, but cohabitation of infected carp with naïve koi carp continuously shed the virus for 34 days (7–40 dpi) at 16°C, for 14 days (1–14 dpi) at 23°C, and for 12 days (3–14 dpi) at 28°C. Experimental immersion of common carp (body weight = 30–50 g) into a 1:1000 dilution of a KHV stock at 23°C for 1 h resulted in cumulative mortality of 80%. Fish developed haemorrhage of the skin, fin congestion, exfoliation of the epidermis and sluggishness 3–4 dpi, but survivors recovered subsequently. KHV was reisolated from the gills ($10^{2.05}$–$10^{3.55}$ $TCID_{50}$ per g) at 3–14 dpi and from the kidney ($10^{5.05}$–$10^{5.55}$ $TCID_{50}$ per g) and brain ($10^{3.05}$–$10^{3.55}$ $TCID_{50}$ per g) 7–14 dpi (Yuasa *et al*., 2012).

Strain differences in virulence were also experimentally demonstrated by Yuasa *et al*. (2007). Strain 1 caused 50% mortality while strain 2 caused 60–75% mortality in common carp and detected by PCR in the gills, scales, kidney, spleen, liver, heart, intestine and brain 14 dpi and in the gills, scales and kidney 28 dpi. Strain 2 was initially detected in the 8 organs much later at 28 dpi and in the gills, scales, kidney, intestine and brain by 60 dpi. Moreover, strain 2 was detected in the brain for up to 360 dpi. At 70 dpi, > 10,000 genome copies per mg wt of the brain and mean of < 1000 genome copies per mg wt of the kidneys of surviving fish were detected. In Japan, Ito *et al*. (2007) reported that the indigenous strain of common carp showed higher sensitivity to the virus than the Eurasian strain of common carp or koi carp. In Europe, the ghost carp shows higher sensitivity to the virus than koi carp.

The virus can remain latent in the host for long periods without manifesting clinical signs of KHVD, becoming active only at permissive temperatures. In such cases, the fish host then becomes a carrier. Studies confirmed that the KHV persists in the brain of its fish host for at least a year (Yuasa *et al*., 2007). The KHV infection recurs in KHVD fish survivors when stressed. Thus, even after 7 months post infection wherein the virus can be found in the gills, kidney, spleen and leukocytes, the virus can be reactivated at permissive water temperature. Such reactivation was confirmed by detection and transmission of the virus by cohabitation with naïve carp introduced 150 days after initial virus challenge. Recent studies by Sunarto *et al*. (2012) confirmed that the KHV ORF134 transcribes a spliced transcript encoding a 179-amino-acid interleukin-10 (IL-10) homologue (khvIL-10) which was observed to be highly expressed during the acute and reactivation phases of the infection but expressed at low levels during the low temperature-induced persistence. At the same time, KHV early helicase and DNA polymerase and late intercapsomeric triplex protein (ITP) and major capsid protein genes were also expressed at high levels during the acute and reactivation phases, but only low-level expression of the ITP gene was detected during the persistent phase.

Diagnosis

The virus may be isolated in susceptible fish cell lines such as koi fin (KF-101), koi fin (KCF-1), koi tail (KT-2) or common carp brain (CCB) (Hedrick *et al*., 2000; Neukirch and Kunz, 2001; Lio-Po and Orozco, 2005; Ilouze *et al*., 2006; Sunarto *et al*., 2011). Tissue filtrates are prepared from the gills, kidney, spleen or leukocytes after homogenization and filtration through 0.45 µm membrane filters. The KF-1 cells inoculated with KHV exhibit typical vacuolations at 20–25°C in 7–14 days (Fig. 6.2). In contrast, survival of KHV in CCB cells was maintained for 30 days at 30°C (Dishon *et al*., 2007).

In fresh samples, infectivity titre of KHV from gills and kidney stored at 23°C for 6 h ranged from $10^{4.05}$–$10^{4.55}$ $TCID_{50}$ per g which dropped if extended to 12–18 h. Storage of fresh gill and kidney samples at 4°C up to 7 days can yield KHV titre ranges

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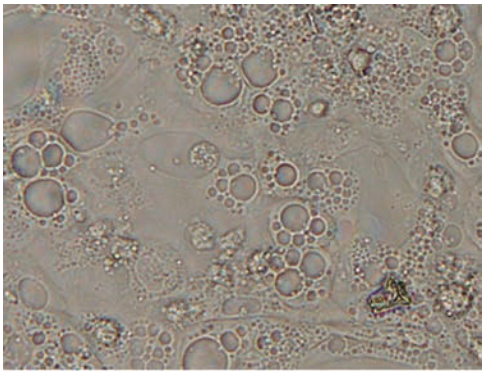


Fig. 6.2. KF-1 cells infected with KHV showing typical vacuolations, 100×.

of $10^{3.8}$–$10^{4.55}$ $TCID_{50}$ per g while at −30°C KHV titres comparable to 6 h at 23°C temperature storage were obtained until 14 days (Yuasa *et al*., 2012).

Histopathology of the gills of infected fish shows that the presence of severe gill hyperplasia with lamellar fusion and epithelial necrosis with prominent nuclear swelling and eosinophilic intranuclear inclusions is diagnostic for KHV infection (Hedrick *et al*., 2000; Tu *et al*., 2004b). Necrosis may be observed in the liver, spleen and kidney parenchymal cells.

The virus can also be visualized by transmission electron microscopy (TEM). In addition, the enzyme-linked immunosorbent assay (ELISA) allows detection of antibodies to KHV in the serum of koi and common carp previously exposed to the virus (Adkison *et al*., 2005; St-Hilaire *et al*., 2009). This method confirms the presence of anti-KHV antibodies found in the sera of survivors from KHV outbreaks that become carriers of the virus.

To date, the Polymerase Chain Reaction (PCR) is one of the most sensitive, specific and rapid tests for KHV detection (Gilad *et al*., 2002; Gray *et al*., 2002; Bercovier *et al*., 2005; Yuasa *et al*., 2005; El-Matbouli *et al*., 2007). To standardize the KHV PCR detection methods used in 21 laboratories from 19 countries, Way *et al*. (2008) organized a ring trial in 2006 and in 32 laboratories from 27 countries in 2007. The ring trial confirmed that the Bercovier-TK (1) and modified Gray SpH (2) primer sets were the most robust for detection of KHV DNA. In addition, real-time PCR for the quantitative estimation of the KHV virus has been developed using the TaqMan real-time PCR (Gilad *et al*., 2004).

The Loop-Mediated Isothermal Amplification (LAMP) test was developed as equally sensitive but also a rapid method for the detection of KHV (Gunimaladevi *et al*., 2004; Yoshino *et al*., 2006). A non-destructive sampling of suspected fish, particularly the expensive koi fish, just use the fish mucus. Other DNA-based methods are the Reverse Transcriptase (RT)-PCR targeting the mRNA terminase (Yuasa *et al*., 2005) and the *in situ* hybridization (ISH) (Haenen *et al*., 2004) which can also detect KHV in paraffin-embedded tissues of the gills, intestine, kidney, spleen, heart, liver of infected common carp. Strong ISH-positive responses were detected within the secondary gill lamellae epithelial cells and mucus cells of the basal area (Lee *et al*., 2012).

A less expensive detection test for KHV is by bioassay using tissue filtrates prepared from KHV-infected fish tissues then injected intraperitoneally to healthy common carp. Development of typical disease signs of KHV infection in the naïve fish reared at 23–28°C for at least 2 weeks is a presumptive diagnosis. However, for expensive koi carp, KHV infection can be established by a modified bioassay test called 'Sentinel Method' wherein an expensive koi carp is cohabited with KHV-free common carp at 23–28°C for 2–4 weeks. The development of KHV in the naïve common carp is a positive indication of KHV infection in the suspected koi carp (Lio-Po, 2010).

Prevention and control

A live, attenuated KHV commercial vaccine was developed in Israel for the prevention of KHV infection (Ronen *et al*., 2003; Perelberg *et al*., 2005). Vaccinated carp develop high antibody titre resulting in a Relative Percent Survival (RPS) of 80–95%. Protective immunity after vaccination lasts for at least 8 months. Similarly, Yasumoto *et al*. (2006) used a formalin-inactivated KHV vaccine entrapped within the liposomal membrane experimentally sprayed on dry pellets that

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were fed to common carp yielding an RPS of 74.4% and 65% when challenged with its homologous virus 22 days after vaccination.

KHV infection occurring at permissive temperatures can be controlled by increasing temperature to 30°C (Ronen *et al*., 2003). In a related study, shifting of rearing water temperature at 3 days interval to 30°C for 5 days was effective in reducing mortality in KHV-infected fish. However, results also suggested that the treated fish became carriers of the virus (Fukuda *et al*., 2007). Also, KHV at non-permissive environmental conditions can be significantly reduced within 3 days after inoculation (Shimizu *et al*., 2006). Experiments on viability of KHV indicated that the virus at 20–30°C sustains virus viability for up to 3 days while at –5°C, 4°C and 15°C for up to 5 days (Lio-Po *et al*., 2006). The virus can be killed by 200 mg per l iodophor, 60 mg per l benzalkonium chloride or 30% ethyl alcohol for 20 min (Kasai *et al*., 2005).

The transboundary introduction of KHV into non-endemic countries should be closely monitored. Somga *et al*. (2010) intercepted an illegal importation of koi carp found positive for KHV in Manila. Although Lio-Po *et al*. (2009) did not detect the virus in Cambodia, Lao PDR, Myanmar, the Philippines, Vietnam during active surveillance from 2004 to 2007, adequate restriction on importation of koi and common carp should be in place.

Grass carp reovirus (GCRV) disease

Grass Carp Reovirus (GCRV) disease was previously known as Haemorrhage of Grass Carp (Nie and Pan, 1985), Haemorrhagic Disease of Grass Carp (Jiang, 2009) and Chinese Grass Carp Reovirus (CGRV) Disease in China. This is the most serious viral infection reported in grass carp in PR China causing approximately 80% mortalities and was first reported in Hubei Province in 1972 (Jiang, 2009). Outbreaks in central, southern and eastern PR China were subsequently observed at 24–30°C with annual losses. The virus also infected grass carp in northern Vietnam, in Mar to Apr and Oct to Nov 1996 (Jiang, 2009). However, annual, active surveillance of the virus in that country in 2004 to 2007 did not detect the presence of GCRV in cultured or wild grass carp (Lio-Po *et al*., 2009). In the USA, a similar disease called the Golden Shiner Virus (GSV) Disease (Plumb *et al*., 1979) was first detected in cultured golden shiner (*Notemigonus crysoleucas*) in the summer of 1977 (Plumb *et al*., 1979). Outbreaks of a haemorrhagic disease similar to GCRV has occurred in channel catfish (*I. punctatus*), introduced in China in 1984, but specifically named channel catfish reovirus (CCRV) disease (Xu *et al*., 2013a). The infection caused mortalities of up to 60% in cultured fingerlings <12 cm in length at 28°C.

Causative agent

Aquareovirus C is specifically known as Grass Carp Reovirus (GCRV) or Chinese Grass Carp Reovirus (CGRV) or Golden Shiner Virus (GSV) or Golden Shiner Reovirus (GSRV). The virus has a dimension of 60–80 nm belonging to the family *Reoviridae*. Two serotypes with distinct antigenicities were reported in China, i.e. GV-87/3 from Hunan Province and GV-90/14 from Hubei Province (Jiang, 2009). However, the CCRV-730 reported in channel catfish measures 60–70 nm in diameter with 99–100% nucleotide sequence similarity to the GCRV-873 strain.

Pathology

In PR China, epizootics occur in grass carp (*C. idellus*), black carp (*Mylopharyngodon piceus*), topmouth gudgeon, chebacheck or stone moroko (*Pseudorasbora parva*) and rare minnow (*Gobiocypris rarus*) (Wang *et al*., 1994). However, the virus can replicate subclinically in silver carp (*H. molitrix*) and Chinese minnow (*Hemiculter bleekeri*) (Ding *et al*., 1991). Other cyprinids such as bighead carp, common carp and golden carp can be asymptomatic carriers (Jiang, 2009).

In the USA, the GSV infects golden shiner and grass carp (McEntire *et al*., 2003) and is also isolated from fat head minnow (*Pimephales promelas*) and creek chubs (*Semotilus atromaculatus*) (Goodwin *et al*., 2006). The virus induces low-grade mortality (Plumb *et al*., 1979). However, under crowded conditions and at high water temperatures, acute epizootics

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caused 50–75% mortality (Schwedler and Plumb, 1982).

Exophthalmia, darkened body colour, haemorrhage of the mouth cavity, haemorrhagic or pale gills, fin bases and gill covers are typical signs of infected grass carp (Fig. 6.3). Affected golden shiners exhibit listless swimming near the surface, petechial haemorrhage in the cornea, dorsal musculature (reddish back), petechiae on the ventral body surfaces, in visceral fat and in the intestinal mucosa.

Internally, affected fish develop haemorrhage in the musculature, intestine, liver, spleen and kidney. Degeneration and necrosis of the liver cells, hyperaemia and haemorrhagic vessels of the liver and spleen occur (Guo and Jiang, 1993). The virus replicates rapidly in the head kidney of infected fish causing an acute infection and significant mortalities of up to 80% among fingerlings and sometimes among yearlings. Experimental transmission of the virus by immersion in water containing GCRV resulted in disease and mortality within 1 to 2 weeks of virus exposure of naïve fish at 25–28°C. The serotype GV-90/14 showed increased virulence, inducing 100% mortality in 3–5 days in experimentally-exposed grass carp fingerlings while only 38% mortalities in 5–10 days was attributed to the serotype GV-87/3 (Jiang, 2009).

On the other hand, the CCRV infected channel catfish exhibit pale gills, liver with localized haemorrhage as well as dark and enlarged spleen, empty gut and haemorrhage on the intestinal wall with clear yellow to blood-tinged fluid in the abdomen (Xu *et al*., 2013a). Experimental infection yielded initial deaths at 2 dpi reaching up to 50% mortalities in 7 dpi. Clinical signs were similar to that observed in natural infection. Diseased channel catfish fingerlings (6–12 cm) become lethargic, float at the surface, with spiral swimming and die within 24 h manifesting severe haemorrhage at operculum, lower jaw and fin bases, body colour depigmentation, bilateral exophthalmia and abdominal distension.

Diagnosis

Detection of GCRV is based on typical clinical signs as well as isolation of the virus in cell cultures of CK, CG and CF at 25°C. Although the GV-87/3 strains of GCRV can replicate and induce CPE in CK cells, this does not take place with the GV-90/14 (Jiang, 2009). *In vitro* replication is considered optimum between 28°C and 30°C inducing CPE in 3 to 7 dpi (Deng *et al*., 1985). The GSV can also be isolated in fat head minnow (FHM) cells (Plumb *et al*., 1979). Cytopathic effects in CCRV exposed CCK cells occurs 48 h post-inoculation (Xu *et al*., 2013a).



Fig. 6.3. Grass carp infected with GCRV. Image courtesy of Y.L. Jiang, Laboratory of Aquatic Animal Diseases, Shenzhen, China.

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Transmission electron microscopy (TEM) of negatively-stained purified virus can also detect the virus.

Neutralization test, ELISA or RT-PCR can also detect the virus (Jun *et al*., 1997; Li *et al*., 1997). Seng *et al*. (2004) developed an RT-PCR detection of not only GCRV but also other Aquareoviruses such as threadfin reovirus (TFV) and guppy reovirus (GPV) in low titre virus-infected cell cultures by using a pair of degenerate primers (450 bp in length).

Prevention and control

In China, vaccination of naïve fingerlings with formalin-inactivated GCRV with lipopolysaccharide (LPS) induced immunity up to 90%, a week after vaccination at 20°C or above (Chen *et al*., 1985; Yang and Zuo, 1990, 1994; Jiang *et al*., 1991). However, inactivated vaccine prepared from organs of infected fish in one region was effective in the same region but may not be as effective when applied to fish in another region (Yang *et al*., 1989). Vaccine administration by immersion yielded lower protection rate; however, by hyperosmotic infiltration the protection rate can be increased (Ye *et al*., 1992). The development of an attenuated vaccine was reported by Jiang (1995) but protection was poor. Since the application of the GCRV vaccines in China, grass carp fingerlings mortality has been reduced from over 80% to less than 30% (Jiang, 2009).

Channel catfish virus disease (CCVD)

Channel catfish virus (CCV) is a viral pathogen of fry and fingerling channel catfish and can cause significant losses in commercially reared fish. The first outbreak of CCVD occurred in the USA, in 1968. Since then, the virus has occurred in most southern states and in several other areas of the USA as well as in other countries where channel catfish had been introduced (Plumb, 1999b). The channel catfish (*I. punctatus*), is the primary fish host species of CCV. Other catfish species such as the African catfish (*Clarias gariepinus*), walking catfish (*Clarias batrachus*) and European catfish (*Silurus glanis*) were resistant to experimental challenge with CCV (Plumb *et al*., 1985; Boon *et al*., 1988).

Causative agent

Channel catfish virus (CCV) or *Herpesvirus ictaluri* measuring 90–100 nm.

Pathology

Clinical signs are abdominal distension, exophthalmia, pale or haemorrhagic gills, petechial haemorrhage at the base of the fins and throughout the skin on the ventral surface. In 20–50% of the epizootics, affected fish swim in an upright or hanging position. Weak fish sink to the bottom and respire weakly which increases prior to death (Plumb, 1999b).

Acute infection occurs primarily in cultured channel catfish fry and fingerlings < 10 g in size following waterborne exposure to CCV (Plumb, 1999b). The disease develops into a haemorrhagic viraemia after initially replicating in the kidney and then in the spleen (Plumb, 1971). Thereafter, the virus is transported to the intestine, liver, heart and brain. After experimental immersion of channel catfish fingerlings in water inoculated with CCV, positive isolations of the virus from day 2 through 8 dpi in the skin and blood and through 10 dpi in posterior kidney and gills were obtained (Kancharia and Hanson, 1996). Necrosis of the renal hematopoietic tissue and tubules; oedema, necrosis and congestion of the liver; intestinal oedema; congestion and haemorrhage in the spleen are characteristic histopathological findings (Wolf, 1988). In addition, skeletal muscle haemorrhage among experimentally infected fish had been observed. Severe mortality of sometimes nearly 100% occur among young-of-the-year channel catfish at water temperatures of 25°C or higher within 7–10 days. It causes moderate mortalities at 21–24°C and almost no mortalities below 18°C. Hence, in the USA, outbreaks of CCVD commonly occur between June to October.

The virus is transmitted horizontally from fish to fish or through the water. Experimental infection is possible by injection (IM or IP), incorporation of the virus into

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the feed, swabbing gills with virus suspension and by immersion in virus inoculated water. Fish up to 10 g size can be infected by CCV by water-borne transmission while for larger fish, injection of the virus is required. Infection of 1-year old fish results in low mortality and usually associated with transport or handling stress (Plumb, 1999b).

Channel catfish fingerlings that survive a primary CCV epizootic become carriers of the virus. Such virus latency was hypothesized when *in vitro* replication of the virus in cloned B cells, T cells and non-cloned macrophages was reported (Chinchar *et al*., 1993). Subsequent studies confirmed that experimentally-challenged channel catfish fingerlings yielded 22% survivors, which at 140 dpi showed the intestine, kidney, liver, brain and peripheral blood leukocytes were PCR positive for CCV (Gray *et al*., 1999b). Survivors of experimental CCVD averaged only two-thirds the length and one-seventh the weight of control fish 6 months after a standardized feeding regime (McGlamery and Gratzek, 1974).

The portal of entry for CCV from water is via the gills and the gut. The virus can be isolated from the kidney of fish with active infection using channel catfish ovary (CCO) or brown bullhead (BB) cells, which develop CPE 24–48 h post inoculation (hpi). Optimum replication was observed at 25–30°C. The virus is readily transmitted from fish to fish during an epizootic. In natural and experimental infections, exposed fry die within 3 dpi and within 7–10 days after the first mortality. The virus also persists in apparently healthy adult broodfish in a latent state. The CCV can occur in conjunction with a secondary bacterial infection with *Flavobacterium columnare* that prolongs mortality (Plumb, 1999b).

DIAGNOSIS. The CCV replicates in CCO and in BB cells at 25–33°C (Wolf and Darlington, 1971; Bowser and Plumb, 1980). The virus can also be detected by TEM, serum neutralization test and indirect fluorescent antibody technique (IFAT). In addition, ELISA can detect antibodies against CCV in the serum of previously CCV-exposed fish (Crawford *et al*., 1999).

A rapid, sensitive and specific test for CCV detection is by PCR, which can detect the virus in brain, blood, intestine, kidney and liver of acutely infected fish (Gray *et al*., 1999a). Likewise, a nested PCR assay can detect fewer than 10 copies of CCV DNA of approximately 1 femtogram (fg) in the blood of asymptomatic broodstock carriers (Baek and Boyle, 1996).

Prevention and control

Experimental studies have shown that attenuated CCV can induce protective immunity to channel catfish after injection or bath exposures (Walczak *et al*., 1981). Passive transfer of adult channel catfish sera with anti-CCV neutralizing antibody activity also protected juvenile channel catfish from challenge with CCV (Hedrick and McDowell, 1987). Subsequent reports, likewise, indicated that vaccination of channel catfish with a combination vaccine pair (ORF 59 and ORF 6) provided strong protection from CCV challenge (Nusbaum *et al*., 2002). These results showed that virus neutralizing antibodies were elicited with an anamnestic response upon viral challenge. Liu *et al*. (2012) reported that in a viral neutralization assay the antiserum was able to inhibit CCV infection of channel catfish ovary (CCO) cells. This suggests that viral protein is likely to play an important role in the CCV infection of channel catfish ovary cells.

Bacterial Diseases

High stocking density of fish leads to increased feed ration and waste with concomitant increase in ammonia and nitrite toxicity (Mitchell, 1997). This increases the organic load of the rearing water and permits rapid bacterial multiplication. Stress and trauma from handling are also predisposing factors. In addition, most bacterial pathogens elaborate enzymes that facilitate their entry/invasion into the fish host tissues. Although they may cause primary infection, they may also act as secondary disease agents to a primary virus or parasite. The major bacterial infections among warm freshwater fish are Motile

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Aeromonas septicaemia, *Pseudomonas* septicaemia, Edwardsiellosis, Enteric septicaemia, Columnaris disease and Streptococcal septicaemia/meningoencephalitis.

Motile *Aeromonas* septicaemia (MAS)

This is the most common bacterial infection in fish (Cipriano, 2001), but known previously as haemorrhagic septicaemia, infectious dropsy, infectious abdominal dropsy, red pest, red disease or red sore. Motile *Aeromonas* septicaemia affects freshwater and occasionally brackish-water and marine warmwater fish worldwide. It was the most severe disease problem among cage-cultured channel catfish in USA between 1972 and 1980 (Plumb, 1994). Subsequently, it became the third most common bacterial infection (1987 to 1991) among cage-cultured channel catfish in USA, accounting for 13–22% of disease outbreaks (Duarte *et al*., 1993). In the tropics, MAS infections are often reported in pond- and pen-cultured milkfish; common carp, grass carp, Nile tilapia and giant gourami (*Osphronemus goramy*) (Kou, 1972; Ruangpan *et al*., 1985; Karunasagar *et al*., 1986; Lio-Po *et al*., 1986; Saitanu *et al*., 1986; Supriyadi, 1986; Areerat, 1987; Angka *et al*., 1988; Okaeme *et al*., 1989; Yambot, 1997). Moreover, *A. hydrophila* has been associated with EUS-affected striped snakeheads (*Channa striata,* also known as *Ophicephalus striatus*) and walking catfish (*C. batrachus*) in the wild as well as in ponds and cages (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Subasinghe *et al*., 1990; Torres, 1990; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Rahman *et al*., 1999).

Causative agent

A. hydrophila, previously known as *A. punctata* or *A. liquefaciens* is the etiologic agent of MAS. Other species such as *A. sobria* and *A. caviae* are considered non-pathogenic and are rarely associated with fish epizootics. *A. hydrophila* is a free-living, mesophilic bacterium found in the soil, freshwater lakes, ponds, streams, bottom mud, domestic tap water and sewage. It is often associated with the normal flora of fish. Thus, the bacterium has been isolated from both healthy and diseased fish (Lio-Po and Duremdez-Fernandez, 1986; Lio-Po *et al*., 1986; Torres, 1990; Lio-Po *et al*., 1992). It causes infections not only in aquatic animals but also in avian hosts, cows and humans.

Pathology

Infected fish lose appetite, become lethargic and swim near the surface. External signs may vary according to fish species but are generally similar to clinical signs of other bacterial septicaemia infections in fish, i.e. exophthalmia, distended abdomen. However, septicaemia in acute MAS can be fatal with no clinical signs. Among milkfish reared in pens in a freshwater lake in the Philippines, acute signs of petechial haemorrhage of the skin and fin bases including dermal and caudal fin rot were observed (Lio-Po *et al*., 1986). Fingerlings to adult Nile tilapia can be infected by *A. hydrophila*. Yambot (1997) isolated *A. hydrophila* from cage-cultured tilapia with haemorrhagic skin, ulceration, loss of scales, eye abnormalities, fungal growth and/or tail and fin rot in the Philippines. A case of natural infection in tilapia caused by *A. hydrophila* was associated with severe epidermal lesions so severe that the vertebrae were exposed (Lightner *et al*., 1988). This condition is common among EUS-affected fish (Roberts *et al*., 1994b). Hence, it is not surprising that the bacterium has been consistently isolated from EUS-affected fish (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Subasinghe *et al*., 1990; Torres, 1990; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Rahman *et al*., 1999). *A. hydrophila* was also isolated from diseased catfish *P. hypophthalmus* in association with *Edwardsiella ictaluri* and *Clostridium* sp. (Ly *et al*., 2009).

In channel catfish, *A. hydrophila* infection has three categories: (i) motile aeromonad septicaemia with external signs; (ii) cutaneous, manifesting lesions that are limited to the skin and underlying muscle; and (iii) latent septicaemia with no external signs (Grizzle

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and Kiryu, 1993). Internal clinical signs include oedema, haemorrhage and necrosis. The disease is acute in very young fish while adults generally develop chronic infections (Plumb, 1994). In USA, *Aeromonas* was diagnosed in 9.9% of catfish pond operations with mortalities in 2009 (USDA, 2010).

Motile aeromonads are most commonly isolated from mucosal surfaces and internal organs of healthy fish (MacMillan, 1985; Harikrishnan and Balasundaram, 2005). Infections induced are predisposed by stress from temperature shock, low dissolved oxygen, high ammonia, handling or hauling, scale loss and an ongoing primary infection (Plumb *et al*., 1978; Lio-Po *et al*., 1986). These predisposing conditions are possibly immunodepressive plus the virulence of the *Aeromonas* strain are important factors in the development of MAS epizootics (Thune *et al*., 1993). Moreover, *A. hydrophila* is often reported in mixed infection either with *Edwardsiella tarda*, *E. ictaluri*, *F. columnare*, *Streptococcus* spp., or with parasites (Kanai *et al*., 1977; Liu *et al*., 1990; Duarte *et al*., 1993, Ly *et al*., 2009). In Carp Dropsy, *A. hydrophila* was a component in the pathology of the disease that is attributed to a virus as the prime aetiological agent (Roberts, 1993).

Experimental *A. hydrophila* infections were induced in milkfish (*C. chanos*) fingerlings with up to 100% mortality in 2 days after immersion exposure of scarified fingerlings but not in fish with intact skin (Lio-Po and Duremdez-Fernandez, 1986). In addition, intraperitoneal injection of milkfish with the bacterium caused mortalities within 12 h of injection. In walking catfish and snakeheads, *A. hydrophila* induced dermal lesions after intramuscular injection of at least 10^5 cells per fish that eventually ulcerated (Lio-Po *et al*., 1992). Compared with other bacteria associated with EUS lesions such as *Pseudomonas* sp., *Aquaspirillum* sp. and *Streptococcus* sp., *A. hydrophila* induced the most severe lesions upon intramuscular injection of snakeheads (Lio-Po *et al*., 1998). Likewise, experimental infection of Nile tilapia fingerlings by immersion yielded an LD_{50} of 1.5×10^6 cfu per ml with 100% mortality at 10^8 cfu per ml and no mortality at 10^3 cfu per ml (Yambot, 1997). In channel catfish with mechanically abraded skin, *A. hydrophila* experimentally induced systemic infection in 80% of exposed fish while cutaneous lesions developed in the remaining fish (Matsche and Grizzle, 1999). Moreover, challenge of tra catfish (*P. hypophthalmus*) with (*A. hydrophila*) induced external signs of haemorrhagic infection (Ly *et al*., 2009).

The infection elicits an intense inflammatory response, with massive infiltration of monocytic and granulocytic cells into infected tissues (Huizinga *et al*., 1979; Ventura and Grizzle, 1988). Infected goldfish are anaemic, e.g. low red blood cell counts, haematocrit and haemoglobin (Brenden and Huizinga, 1986). In addition, there is a shift in the differential counts of lymphocytes to a predominance of neutrophils. Walking catfish injected intraperitoneally with *A. hydrophila* developed focal necrosis in the liver, kidney, intestine and dorsal musculature (Angka, 1990). Histopathologically, marked necrosis of the muscle fibers occurred in snakeheads intramuscularly injected with *A. hydrophila* (Lio-Po, 1998).

Motile aeromonads secrete extracellular products (ECP) and these include toxins, protease, cytotoxin, haemolysin, leucocidin, gelatinase, elastase, staphylolysin, caseinase, enterotoxin and a dermonecrotic factor (Hsu *et al*., 1981; Olivier *et al*., 1981; Kanai and Wakabayashi, 1984; Lallier *et al*., 1984; Krovacek, 1989; Yadav *et al*., 1992). Dermonecrotic strains of this bacterium secrete haemolysin at 10°C and 30°C (Olivier *et al*., 1981). Also, such cytotoxin-producing strains were associated with EUS-affected fish (Yadav *et al*., 1992). However, correlation between virulence and ECP production was not consistent (Leaño *et al*., 1996). In 2000, Cascon *et al*. described the molecular characteristic of an elastase secreted by *A. hydrophila* that is important in its pathogenicity. This protease has a high amino acid sequence similarity to proteases secreted by *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Vibrio* spp. An investigation of twelve strains of *A. hydrophila* isolated from fish showed that protease production varied among strains with peak protease production at 27.6 ± 4.9°C (Uddin *et al*., 1997). The protease levels increased during the late log phase to early stationary phase. Siderophore production

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was also reported but was not related to virulence of the bacterium (Santos *et al*., 1988; Leaño *et al*., 1995).

Dooley *et al*. (1986) described a crystalline surface layer, or S-layer of 52 kDa protein and further correlated this to strain virulence (Murray *et al*., 1988; Ford and Thune, 1991). Virulence of *A. hydrophila* varies even among isolates from the same epizootic (Lio-Po *et al*., 1992). Also, Rahman *et al*. (1997) reported that *A. hydrophila* stored in 0.60% and 0.85% NaCl solutions had higher virulence than the cultured bacterium when injected intraperitoneally to carp and goldfish. Attachment of *A. hydrophila* to carp epithelial cells is attributable to a 43-kDa outer membrane protein adhesin because of an abundance of this particular receptor on the cell surface (Lee *et al*., 1997). Subsequent studies showed that a significantly higher number of starved *A. hydrophila* adhered to the skin of crucian carp (*Carassius carassius*) (Rahman and Kawai, 1999).

Diagnosis

Motile *Aeromonas* spp. are flagellated, Gram-negative, short rods. These do not produce pigments and are resistant to 10 or 150 µg vibriostat 0/129 (2, 4-diamino-6,7-diisopropylpteridine phosphate). The bacteria grow between 18–39°C (Uddin *et al*., 1997). In tryptic soy agar (TSA) or in brain heart infusion agar (BHIA) at 25–30°C incubation for 24–48 h, *Aeromonas* spp. produce white to creamy, convex, moist colonies. In Rimler-Shotts medium, the bacterium forms orange-yellow colonies at 35°C (Shotts and Rimler, 1973). The three important *Aeromonas* spp. in fish can be differentiated using biochemical tests (Lio-Po *et al*., 1992; Plumb, 1994). Joseph and Carnahan (1994) further classified aeromonads into six species: *A. hydrophila*, *A. veronii*, *A. caviae*, *A. schubertii*, *A. jandaei* and *A. trota*. Molecular identification can be applied by ribotyping of restriction genomic DNAs of aeromonads using different fragments of 16S rDNA gene of *Escherichia coli* as a probe (Lucchini and Altwegg, 1992). Also, the amplified fragment length polymorphism (AFLP) is a high-resolution genotype tool for classification of *Aeromonas* spp., this and the pulse-field gel electrophoresis (PFGE) are rapid techniques for typing of *A. hydrophila* (Huys *et al*., 1996; Talon *et al*., 1996). Igbal *et al*. (1998) recommends the application of genetic identification using DNA hybridization.

Both virulent and non-virulent strains have been isolated from diseased fish (Torres *et al*., 1990; Lio-Po *et al*., 1992; Leaño *et al*., 1996). Definitive identification of either strain is a major difficulty. Cartwright *et al*. (1998) developed monoclonal antibodies for detection of virulent strains using either ELISA or FITC-immunofluorescence. Virulent strains of *A. hydrophila* require about 30 min to induce cytopathic effects in EPC cells while avirulent strains do not induce this pathologic effect (Leung *et al*., 1996). A PCR method that is reported to be rapid, sensitive and specific for the detection of virulence factors of *Aeromonas* spp. has been developed (Bin Kingombe *et al*., 1999). Likewise, identification of the genetic differences and virulence genes among different strains of *A. hydrophila* using suppression subtractive hybridisation (SSH) technique was reported (Zhang *et al*., 2000).

Prevention and control

Outbreaks of *A. hydrophila* infections in fish-pen reared milkfish are usually related to transport and handling stress, adverse environmental conditions of low oxygen concentration, low pH, and increased levels of ammonia and carbon dioxide (Walters and Plumb, 1980; Lio-Po, 1984; Lio-Po *et al*., 1986). Moreover, tilapia fingerlings during seining can get caught between nets and subsequently develop MAS causing more than 25% mortality after stocking in cages (J.A. Plumb, personal communication). Thus, prevention of stressful conditions in cultured fish will minimize MAS outbreaks.

Limited success has been achieved with vaccination via hyperosmotic infiltration of milkfish against MAS (Lio-Po, unpublished data) but vaccination proved protective in tilapia (Ruangpan *et al*., 1985). Indian major carp vaccinated with *A. hydrophila* yielded increased agglutinating antibody titre (Karunasagar *et al*., 1991). Vaccination by intraperitoneal injection of formalin-killed *A. hydrophila* to catfish (*C. macrocephalus*)

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is more effective than immersion or oral administration (Areechon *et al*., 1992). Biofilm vaccine at 10^{13} cfu per g of *A. hydrophila* in catla (*Catla catla*), rohu (*Labeo rohita*) and common carp for 15–20 days elicited high serum antibody titre and protective response for 60 days (Azad *et al*., 1999). Similarly, studies by Swain *et al*. (2007) showed that vaccination using formalin-killed whole cell antigens of *A. hydrophila* singly or as a polyvalent vaccine consisting of *A. hydrophila*, *E. tarda* and *Ps. fluorescens* provided protection to rohu, *L. rohita*. The RPS reported was 80% in both single and polyvalent vaccines.

Blue gourami (*Trichogaster trichopterus*) when intraperitoneally immunized with major adhesin (43 kDa) in Freund's complete adjuvant, developed protective immunity to challenge by homologous and heterologous strains of *A. hydrophila* and one virulent strain of *Vibrio anguillarum* (Fang *et al*., 2000). Vaccination studies in carp immunized with crude lipopolysaccharide showed that the mechanism of immunity is attributed to a sensitized thymocyte-macrophage system (Baba *et al*., 1988a, b). Also, ECP as a vaccine conferred protection in common carp, *C. carpio* (Chandran *et al*., 2002).

Recently, immersion vaccination of carp with *A. hydrophila* bacterins showed a distinct increase of lysozyme level in fish mucus with stronger bacteriolytic properties 7 and 28 days after immunisation (Kozinska, 2000). Moreover, a single intraperitoneal injection of 20 mg per kg β(1,3)-D-glucan to blue gourami enhanced immune response against *A. hydrophila* for up to 29 days (Samuel *et al*., 1996). Subsequent studies showed that the use of four glycans, namely: Bar (glycan extracted from Barley), krestin, scleroglucan and zymosan, significantly increased survival rates of tilapia and grass carp after infection with *A. hydrophila* (Wang and Wang, 1997).

Prophylactic bath treatments with 1–3% NaCl will help reduce post-handling infections. Likewise, bath treatments with 2–4 mg per l potassium permanganate are also effective for external lesions. Medicated feed with 2–4 g oxytetracycline per kg feed (50–100 mg per kg fish) for 14 days is recommended (Plumb, 1994). However, drug resistant strains of *A. hydrophila* may evolve (Aoki, 1999).

***Pseudomonas* septicaemia**

In freshwater culture systems, *Pseudomonas* septicaemia has been implicated in epizootic outbreaks in Nile tilapia, grass carp, silver carp and bighead carp (*Aristichthys nobilis*) (Miyashita, 1984; Lio-Po and Sanvictores, 1987; Thune *et al*., 1993).

Causative agent

Pseudomonas fluorescens is the aetiological agent of the disease. It is a normal flora of tilapia gut (Sugita and Kadota, 1980).

Pathology

The clinical signs in fish affected with *Pseudomonas* septicaemia are very similar to those with Motile *Aeromonas* septicaemia. Gross signs include ascites, exophthalmia, septicaemia and ulcers. The infection may be acute or chronic with the latter commonly associated with skin lesions. The bacterial pathogen has likewise been associated with fin rot. Histopathological findings in Nile tilapia include focal necrosis, abscess and granulomas in the eyes, gills, liver, swim bladder, kidney and spleen (Miyashita *et al*., 1984). The bacterium also causes mortalities in 2-week old Nile tilapia fry (Lio-Po and Sanvictores, 1987). It remains viable in fresh water for up to 150 days (Duremdez and Lio-Po, 1984) and secretes an extracellular proteinase (Li and Fleming, 1967).

Diagnosis

Clinical signs of *Pseudomonas* septicaemia resemble those of MAS. As such, isolation and identification of the bacterial pathogen is required. *Ps. fluorescens* is a Gram-negative rod with one to three polar flagella. It can be cultured on nutrient agar, *Pseudomonas* F agar and blood agar (Austin and Austin, 1987). For strains pathogenic to fish, the optimum growth temperature is 20–25°C. These secrete oxidase, catalase and gelatinase but not amylase, galactosidase, urease or hydrogen sulphide. It is citrate positive, oxidative for glucose and produces a fluorescent pigment (Plumb, 1994).

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Prevention and control

Stress from low dissolved oxygen, high stocking density, physical trauma and poor nutrition are predisposing factors in the development of *Pseudomonas* septicaemia (Post, 1983). Therefore, avoidance of these conditions is necessary to prevent epizootic outbreaks. Suggested bath treatments during the early stage of the disease include 1–2 mg per l benzalkonium chloride for 1 h (Austin and Austin, 1987).

In addition, Swain *et al*. (2007) showed that vaccination using formalin-killed whole cell antigens of *Ps. fluorescens* singly or as a polyvalent vaccine with *A. hydrophila*, *E. tarda* and *Ps. fluorescens* provided protection to rohu (*L. rohita*), yielding RPS of 80% and 70% in single and polyvalent vaccines, respectively.

Edwardsiellosis

The disease has been reported in at least 25 countries worldwide (Austin and Austin, 1987). It affects eels, mullet, channel catfish, tilapia, carp, striped bass and olive flounder (Meyer and Bullock, 1973; Wakabayashi and Egusa, 1973; Kubota *et al*., 1981; Lio-Po *et al*., 1982; Sae-Oui *et al*., 1984; Herman and Bullock, 1986; Han *et al*., 2006).

Causative agent

Edwardsiella tarda is the causative agent of Edwardsiellosis. This bacterium is synonymous to *Paracolobactrum anguillimortiferum* and to the *E. anguillimortiferum* described by Wakabayashi and Egusa (1973) and Kuo and Kuo (1977), respectively.

Pathology

Infected fish may exhibit haemorrhagic areas on the body and malodorous lesions on the flank or caudal peduncle and swollen abdomen. Larger fish (>40 cm) are commonly infected. Internally, white nodules may develop in the liver (Fig. 6.4) (Lio-Po *et al*., 1982). Histopathology of Edwardsiellosis in tilapia consists of liquefactive necrosis of infected tissues with bacterial infiltration of macrophages engorging bacterial cells. This eventually leads to granuloma formation in the liver, spleen and kidney (Miyazaki and Kaige, 1985). Virulence factors of *E. tarda* include: haemolysin (Kusuda and Kitadai, 1993); dermatotoxin (Ullah and Arai, 1983); siderophore (Kokubo *et al*., 1990); and superoxide dismutase and catalase (Han *et al*., 2006). Infection is usually prevalent in channel catfish at 30°C. *E. tarda* can also cause infections in humans.



Fig. 6.4. Nile tilapia (*O. niloticus*) with white nodule in liver attributed to *Edwarsiella tarda*.

Diagnosis

E. tarda infection can be diagnosed based on typical clinical signs and isolation/identification of the pathogen *in vitro*. This Gram-negative, motile bacillus can be isolated on TSA at 25–30°C yielding small, grey, circular, transparent colonies. Identification is based on either conventional biochemical tests or determined using the API 20E kit.

Prevention and control

Vaccination by hyperosmotic infiltration of *Sarotherodon niloticus* (now known as *O. niloticus*) did not provide protection against *E. tarda* infection (Lio-Po and Wakabayashi, 1986). In contrast, vaccination by intraperitoneal injection using formalin-killed whole cell antigens of *E. tarda* singly or as a polyvalent vaccine consisting of *A. hydrophila*, *E. tarda* and *Ps. fluorescens* provided protection to rohu, *L. rohita*. A RPS of 80% in either single or polyvalent vaccine was observed (Swain *et al*., 2007).

Enteric septicaemia of catfish (ESC)

ESC is also known as *Edwardsiella ictaluri* infection. It is a major disease of cage-reared channel catfish (*I. punctatus*) in USA and accounts for about 30% losses in south-eastern USA. The estimated annual loss attributed to this pathogen is US$20–30 million (Plumb and Vinitnantharat, 1993). The majority of cases occur in May and June and again in September and October between 22°C and 28°C (Plumb and Schwedler, 1982). As high as 68% morbidity rate was observed in May 1987 and an estimated 10–32% yearly morbidity rate among the primary diagnostic cases of cage-cultured and pond-cultured channel catfish in 1987–1991 (Duarte *et al*., 1993). In 2009, 36.6% of catfish losses in all pond operations were attributed to enteric septicaemia (USDA, 2010). Likewise, outbreaks were reported in Indonesia, Thailand, Vietnam, Japan and Australia.

Causative Agent

Enteric septicaemia in freshwater fish is attributed to *E. ictaluri*.

Pathology

E. ictaluri is pathogenic to channel catfish but very slightly pathogenic to blue catfish (*Ictalurus furcatus*). White catfish (*Ameiurus catus*) and brown bullhead (*Ameiurus nebulosus*) are occasionally infected while natural infections of walking catfish (*C. batrachus*), striped catfish (*P. hypophthalmus*), yellow catfish (*Pelteobagrus fulvidraco*), Nile tilapia (*O. niloticus*), wild ayu (*Plecoglossus altivelis*) have been reported (Plumb, 1999a; Crumlish *et al*., 2002; Yuasa *et al*., 2003; Sakai *et al*., 2008; Ly *et al*., 2009; Soto *et al*., 2013).

Infections induced by *E. ictaluri* may be either acute or chronic. In the acute form, bacteria are ingested and enter the blood stream through the intestine, and colonize various organs causing necrosis and ulceration (Noga, 2010). The peritoneal cavity secretes bloody or clear fluid, haemorrhage and necrosis of the liver and splenic and renal hypertrophy. Fish exhibit abdominal distension, exophthalmia, and pale gills. In the chronic form, bacteria invade the olfactory organ through the nasal opening and spread from the meninges to the skull and skin forming a hole in the head lesion (Shotts *et al*., 1986). Recent studies by Shoemaker *et al*. (2012) demonstrated the presence of *E. ictaluri* in the brain, gill, kidney and liver of fish infected with this pathogen.

In channel catfish, *E. ictaluri* infection results in suppurative inflammation (Miyazaki and Kaige, 1985). Experimental infection of channel catfish showed that fish previously exposed to *E. ictaluri* and then infected by *Ichthyophthirius multifilis* (Ich) yielded significantly higher mortality of 71.1% versus 26.7%, 28.9% and 0% for fish infected by *E. ictaluri* only, by Ich only and non-infected control, respectively (Shoemaker *et al*., 2012). At day 8 following experimental infection, *E. ictaluri* parasitized fish had significantly more bacteria present in the brain, gill and liver, with no bacteria detected in these organs in the *E. ictaluri*-only treatment, suggesting that the bacteria persisted longer in parasitized fish. Furthermore, channel catfish naturally coinfected with Ich and *E. ictaluri* could concomitantly transmit both pathogens to naïve channel catfish (Xu *et al*., 2013b).

In Thailand, in 1985, *E. ictaluri* was reported in diseased walking catfish (*C. batrachus*) (Boonyaratpalin and Kasornchandra, 1985; Kasornchandra *et al*., 1987). In Vietnam, in 1999, Ferguson *et al*. (2001) reported a bacillary necrosis of *Pangasius* with multifocal, irregular white lesions of varying sizes on several organs (liver, spleen, kidneys) that were associated with high mortality. By March 2002, Crumlish *et al*. (2002) isolated and identified *E. ictaluri* from *P. hypoththalmus*. Also in Vietnam, Ly *et al*. (2009) isolated *E. ictaluri* from cultured tra catfish (*P. hypophthalmus*) in association with *A. hydrophila* and *Clostridium* sp.

In Central Sumatra, Indonesia, mass mortalities of 50–100% in sutchi catfish (*P. hypoththalmus*) occurred in two farms of freshwater ponds during a 2–3 week period in 2002. Affected fish showed pale gills, abdominal swelling and haemorrhage of the skin, swelling of the internal organs with white patches. *E. ictaluri* isolated from the internal organs induced 100% mortalities to *P. hypoththalmus* after intraperitoneal injection with

1.2–2.0 × 10^5 cfu per fish at 28°C within a week showing the same clinical signs as natural infection (Yuasa *et al*., 2003). In Vietnam, challenge experiments on tra catfish with *E. ictaluri* exhibited gas bubbles in the stomach and gut with a foul smell (Ly *et al*., 2009). Moreover, Nusbaum and Morrison (2001) reported that superinfection of *E. ictaluri* infection with latent *A. hydrophila* may enhance clinical signs of ESC.

Infected tilapia manifest swollen abdomen due to dropsy, protruded bloodshot anus and cloudy eye. Internally, ascites and white nodules in the liver, spleen, kidney, gills and rarely in the intestine are associated with this infection. Moreover, it was associated with granulomatous inflammation preceded by macrophage infiltration (Kubota *et al*., 1981). Outbreaks occur at 24–28°C (Francis-Floyd *et al*., 1987). Studies by Soto *et al*. (2013) suggested the cutaneous and oral routes as the main portals of entry of the bacteria with the spleen and head kidney as major targets of infection.

Diagnosis

E. ictaluri produce pinpoint to small-sized colonies (average 0.14 ± 0.13 mm) that appeared off-white and translucent with irregular surface and edge. After 48 h growth in TSA at 28°C, biochemical profile can be determined using the API 20E kit and by 16S rRNA Genbank analysis (Crumlish *et al*., 2002). In addition, quantitative PCR can detect *E. ictaluri* in the brain, gill, kidney and liver of channel catfish (Shoemaker *et al*., 2012). Macrorestriction analysis is proposed as a typing method to determine intraspecific diversity among isolates (Bartie *et al*., 2012).

Prevention and control

In USA, immersion vaccination of catfish against ESC is recommended when the fish are in the fry stage (Bebak and Wagner, 2012; USDA, 2010). Macrophage chemotaxis in channel catfish in response to an *E. ictaluri* exoantigen was significantly different among unfed fish, feeding once daily to satiation, feeding every other day to satiation, and no feeding for 3 weeks followed by feeding once daily to satiation during the fourth week. The lowest response was exhibited by fish that were not fed while the highest in the fish group that were fed daily (Lim and Klesius, 2003). Moreover, mortality from ESC was significantly lower for fish fed daily or every other day than for fish that were not fed before and after challenge.

Columnaris disease

Columnaris disease is an acute to chronic infection of freshwater fish and a common bacterial infection in south-eastern USA during warmer months of March to September with peaks in June (Duarte *et al*., 1993; Mitchell, 1997). The infection caused fish losses in approximately 39% of all channel catfish pond operations in 2009 (USDA, 2010). The disease also occurs in Asia and Europe affecting warmwater fish like channel catfish and other ictalurids, walking catfish, eels, common carp and tilapia (Plumb, 1994; Verma *et al*., 2007).

Causative agent

The pathogen is *Flavobacterium columnare*, formerly called *Flexibacter columnaris*, *Cytophaga columnaris*, *Chondrococcus columnaris* and *Bacillus columnaris*.

Pathology

Clinical signs include frayed fins with greyish to white margins, depigmented, necrotic skin lesions with yellowish or pale margins, which can develop into shallow ulcers, yellowish mucoid material at the mouth and light to dark brown gill discoloration. Infection primarily begins at the mouth, fins and gills. Gills or dermal/muscular capillaries of infected fish become congested and degenerated (Plumb, 1994). Lesions of gills initiate at the distal end of the filaments that extend to the base. Epithelial vacuolation, necrosis, congestion, oedema, fusion and degeneration of the secondary lamellae subsequently follow. Acute mortality is usually associated with gill lesions. Internal pathology or host

inflammatory response may occur, and the pathogen may be isolated from internal tissues (Thune *et al.*, 1993; Plumb, 1994; Shotts and Starliper, 1999; Welker *et al*., 2005).

Farkas and Olah (1986) described three stages of gill necrosis. The first stage is initiated and maintained by environmental stress (probably ammonia, pH, temperature or any toxins in the rearing water) but *F. columnare* is seldom detectable on the gills that are pale or dark purple. The second stage consists of bacterial invasion of the damaged gill above 20°C causing gill necrosis, resulting in a grey-white coating of the gills. In the third stage, the white coating of the gills has disappeared and with the infected gills becoming distorted. Different stages of gill necrosis may be observed in the same fish population.

Transmission of the bacterium is via water. The disease is most commonly associated with stress from high temperatures, elevated organic loads, high stocking density, low dissolved oxygen and trauma from excessive handling. In channel catfish, it occurs more often at 25–32°C with significant mortality. Young fish are more susceptible than older fish. It may occur as a primary infection or as a mixed infection with another bacterium, *E. ictaluri* or *A. hydrophila* or in association with a parasite, e.g. *Henneguya* sp. or *Ichthyobodo* sp. (Hawke and Thune, 1992; Duarte *et al*., 1993; Plumb, 1994). Columnaris disease appears to follow outbreaks of other diseases (Duarte *et al*., 1993).

Survivors of Columnaris disease release the pathogen into the water at rates of up to 5×10^3 cells per ml per h (Fujihara and Nakatani, 1971), and surviving fish may release the bacterium for up to 140 days post infection. The severity of the lesion depends on the virulence of the strain and the ability of the pathogen to elaborate proteolytic enzymes. *F. columnare* produces an extracellular chondroitin AC lyase that degrades chondroitin and hyaluronic acid in fish connective tissue (Griffin, 1991). Bertolini and Rohovec (1992) also reported four extracellular proteases with molecular weights of 32, 34, 40 and 47 kDa. Newton *et al*. (1997) further observed that more protease is secreted into a medium with low nutrients and salt (Ordal's medium) than in media with high concentrations of nutrients or salt such as TYES, Hsu-Shotts, modified Shieh's media.

Kuo *et al*. (1981) showed that survival of fish given 0.35–1.4 mg iron per 100 g fish prior to challenge with the pathogen was reduced from 3 days to 1 day. Furthermore, highly virulent strains of *F. columnare* adhere more readily to the gills than low virulent strains, and are enhanced in ion-rich water, in the presence of nitrite or organic matter and at 28°C (Decostere *et al*., 1999).

The bacterium can survive up to 16 days at 25°C in hard, alkaline water with high organic load and survival decreases at pH 7 or less and in waters with less than 50 mg per l $CaCO_3$ and with low organic matter. In sterile mud at 25°C, the organism survives for 16 days (Becker and Fujihara, 1978).

Diagnosis

F. columnare is a slender, Gram-negative, non-flagellated rod (about 0.5 by 4-12 µm) with gliding motility and forms 'hay stacks' or columns. Primary isolation of the pathogen can be achieved on selective Cytophaga agar supplemented with 5 µg per ml neomycin and 200 IU per ml polymyxin B (Hawke and Thune, 1992). Colonies are yellow to orange and rhizoid. This aerobic organism cannot tolerate more than 0.5% NaCl and grows between 4°C and 36°C, producing gelatinase, caseinase, catalase, oxidase and chondroitin sulfatase (Song *et al*., 1988).

Diagnosis of the disease is dependent on the appearance of typical lesions on the skin, fins and gills including the detection of the filamentous bacterial cells in wet mounts made from lesions. Based on the gene sequence of the 16S ribosomal RNA of the bacteria, Bader and Shotts (1998) designed primers for its detection using PCR.

Prevention and control

Maintenance of fish under optimal environmental conditions, proper handling of fish, prophylactic treatment and good health management practices are recommended for disease prevention (Plumb, 1994). Daily oral vaccination with heat killed *F. columnare* for

4 weeks reportedly reduced mortality of rainbow trout from 48% to 8% with protection correlated with antibody levels (Fujihara and Nakatani, 1971). Similarly, tilapia also mounts a significant humoral response (mean titre 11,200 which increased to 30,600 following secondary immunization) in plasma and cutaneous mucus to *F. columnare* after IP immunization with formalin-killed sonicated cells of *F. columnare* in Freund's complete adjuvant within 2 weeks. The mean titre remained significantly elevated above controls even at 10 weeks post-immunization (Grabowski *et al*., 2004). The Columnaris vaccine became available to the catfish industry in USA in March 2005 and by 2009, 3.9% of operations stocked fish were vaccinated with Columnaris in grow-out ponds (USDA, 2010).

In addition, experimental application of FCP1 phage therapy via intramuscular route in walking catfish (*C. batrachus*), provided protection against *F. columnare* infection. After treatment, gross signs disappeared and all experimental fish survived (Prasad *et al*., 2011).

Therapeutic agents such as potassium permanganate at 5 mg per l (depending on the organic load of the rearing water) in combination with oxytetracycline added to feed at 50 mg per kg fish per day for 10 days is effective in controlling outbreaks in cages (Duarte *et al*., 1993).

Streptococcal septicaemia/ meningoencephalitis

In freshwater cage-cultured Mozambique tilapia (*Oreochromis mossambicus*) epizootics attributed to Streptococcal septicaemia was reported in Taiwan (Tung *et al*., 1985). Other outbreaks include the disease in Nile tilapia, hybrid tilapia (*O. niloticus* × *O. aureus*), red hybrid tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*), channel catfish and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) in Israel, Japan, Malaysia and USA (Kitao *et al*., 1981; Eldar *et al*., 1994; Perera *et al*., 1994; Baya *et al*., 1996; Stoffregen *et al*., 1996; Camus *et al*., 2008; Najiah *et al*. 2012). Streptococcal infections were also reported among wild and cultured marine fish and are discussed in detail in Chapter 5.

Causative agent

Streptococcal species pathogenic to freshwater fishes are *Streptococcus agalactiae*, *S. iniae*, *S. ictaluri*, *S. difficile* and *S. shiloi* (Tung *et al*., 1985; Eldar *et al*., 1995a; Shoemaker *et al*., 2000; Camus *et al*., 2008; Evans *et al*., 2008; Najiah *et al*. 2012).

Pathology

Among cage-cultured tilapia (15–20 cm in length), this bacterium caused cumulative mortality of 50–60% within one month (Tung *et al*., 1985). Clinical signs include unilateral and bilateral exophthalmia with or without conjunctiva haemorrhage and corneal opacity. Petechiae occur on the underside of its operculum, around the anus, caudal and pectoral fins and mouth, with darkening of the body and discoloration of the dorsal and lateral trunk and peduncle with nodular or abscess formation. Abdominal swelling with ascites is common. Affected fish are anorexic, swim in a circle sluggishly, turning laterally and eventually die.

Internal signs include petechiae and haemorrhage of the intestinal tract, liver and pyloric caeca. Systemic infection has been observed with evidence of bacterial dissemination in the heart, liver, kidney, stomach, small intestine, brain, eyes and musculature. Multiple necrosis with granuloma occur in the hepatic parenchyma. The spleen develops hyperplasia of the reticuloendothelial cells with necrotic foci. Degenerative changes in the renal tubules, catarrhal enteritis in the small intestine and stomach, bacterial meningitis and abscess formation in the muscle have been noted.

Mass mortality of cage-cultured red hybrid tilapia (*O. niloticus*) in Como River, Kenyir Lake, Malaysia was associated with *S. agalactiae*, *Burkholderia cepacia* in the fish liver and *Staphylococcus aureus* in the fish eyes (Najiah *et al*., 2012). Moribund fish exhibit erratic swimming, sluggishness and haemorrhagic eyes.

The disease was experimentally reproduced in trout and tilapia using 10^7 and 10^8 cfu

of *S. shiloi* and *S. difficile*, respectively, with virulence increased to 10^2 and 10^5 cfu after *in vivo* passage (Eldar *et al*., 1995a). *Streptococcus* is also more pathogenic to Nile tilapia than to channel catfish (Chang and Plumb, 1996). In a mixed infection experiment with *Streptococcus* sp. and *A. hydrophila* as inocula, mortality was higher among experimental fish inoculated with both bacterial pathogens compared with those inoculated with either *Streptococcus* or *A. hydrophila* (Liu *et al*., 1990). Infection via the nares is a potential route of infection in Nile tilapia and hybrid striped bass (Evans *et al*., 2000). Experimental transmission occurs by immersion, injection, oral or cohabitation and is enhanced by injury to the skin or stressful environment. Sources of infection are water, mud, contaminated feed or carrier fish (Plumb, 1994).

Environmental factors influenced the development of Streptococcal disease in Nile tilapia. Shoemaker *et al*. (2000) showed that significantly higher mortality (about 28.4%) develops in medium (11.2 g per l) as against 4.8% in low (5.6 g per l) fish density treatments exposed to 2.5×10^7 cfu per ml *S. iniae* by immersion. Moreover, the infection can be transmitted by cohabitation with *S. iniae* infected Nile tilapia for 48 h. In another study, Bunch and Bejerano (1997) demonstrated that low oxygen and high nitrite levels increased mortality in hybrid tilapia exposed to *Streptococcus* sp. However, these factors had no additive effect. Later studies though, in Nile tilapia (*O. niloticus*), showed that exposure to sublethal dissolved oxygen stress on blood glucose caused significantly higher mortality rates (27% and 80%) after infection with *S. agalactiae* at 7.5×10^2 cfu than fish exposed to acceptable DO (Evans *et al*., 2003). Furthermore, streptococcal infection in young channel catfish (*I. punctatus*), occurred in association with *Trichodina* infestation (Evans *et al*., 2007). Channel catfish were only susceptible to high concentrations of *S. ictaluri*, i.e. 7% CPM (cumulative percent mortality) in fry at 10^8 cfu by immersion dose; 20% and 10% CPM in fingerlings and juveniles, respectively at 10^7 cfu per fish by IP injection (Pasnik *et al*., 2009).

Diagnosis

Streptococcal organisms can be isolated in culture from the brain, kidney, heart, spleen and exophthalmia in Todd-Hewitt (TH) broth (DIFCO), nutrient agar (NA) supplemented with sheep or goat's blood, brain heart infusion agar (BHIA) or TSA for 24–48 h at 20–30°C (Kitao *et al*., 1981). Modified Hucker's Gram staining showing small, Gram-positive cocci, approximately 0.3–0.5 µm in diameter, most often occurring in chains is a presumptive diagnosis. These organisms are non-motile and encapsulated. Plumb (1994) divided *Streptococci* associated with fish epizootics into four major groups: 1) group β is non-haemolytic, 2) group D alpha and group D are β-haemolytic, 3) alpha haemolytic strains that do not react with Lancefield antisera, and 4) other *Streptococci* from freshwater and marine fish. The pathogen does not grow in 40% bile, 6.5% saline, 0.1% methylene blue milk or at 10°C and 45°C (Kusuda and Salati, 1999). Details on the classification of *Streptococci* spp. based on biochemical and serological tests are in Kitao *et al*. (1981) and Plumb (1994). All isolates from freshwater fish are beta haemolytic (Kitao *et al*., 1981; Tung *et al*., 1985). The BIOLOG profile (MicroLog3 system) was used in confirming identification of *S. ictaluri* (Pasnik *et al*., 2009).

Prevention and control

Avoidance of stress due to adverse or poor water quality, rough handling, high stocking density, removal of infected or dead fish and overfeeding should be followed. Medicated feed with enteroflaxin at 5 mg per kg body wt for 10 days (Stoffregen *et al*., 1996) or with erythromycin-doxycycline mixture at 100 mg and 70 mg per kg body wt for 6 days are also effective (Tung *et al*., 1985).

Formalin-killed *S. difficile* vaccine injected intraperitoneally protects tilapia (Eldar *et al*., 1995b). In addition, Klesius *et al*. (2000) showed that intramuscular injection of a combined vaccine prepared from two strains of *S. iniae* obtained from Nile tilapia provided relative per cent survivals of 63.1% and 87.3% when challenged with its homologous pathogens. Furthermore, anti-*Streptococcus iniae*

whole sera provided immunity to Nile tilapia (*O. niloticus*) against *S. iniae* (Shelby *et al*., 2002). Subsequent studies showed that Nile tilapia (*O. niloticus*) when injected IP with *S. iniae* bacterin vaccine yielded RPS of 79–100% upon challenge with heterologous *S. iniae* derived from diverse geographical locations (Shoemaker *et al*., 2010).

Pseudofungal Diseases

Stramenopiles are pseudo-fungal organisms previously classified as mycotic microbes (Alexopoulos *et al*., 1996). Infections induced by the stramenopiles (Family *Saprolegniaceae*, Class *Oomycetes*) are commonly called 'water mould infections', cotton tuft disease or Saprolegniasis. Branchiomycosis and mycotic granulomatosis also occur in cultured fish in fresh waters. The Epizootic Ulcerative Syndrome (EUS) is associated with rhabdovirus, the bacterium, *A. hydrophila* and/or the stramenopile, *Aphanomyces invadans* (see subtopic on Disease of Complex Infectious Aetiology in this chapter).

Saprolegniasis

The Oomycetes are distributed worldwide and affect warmwater fish in ponds, lakes, dams and rivers. In India, *Achlya* spp., *Aphanomyces*, *Dictyuchus*, *Saprolegnia* and *Pythium* were isolated from rohu, grass carp, common carp, catla, banded gourami (*Colisa fasciatus*), *Labeo bata*, climbing perch (*Anabas testudineus*) and giant snakehead (*Channa marulius* also known as *Channa micropeltes*); *Aphanomyces* spp. from rohu and *Puntius ticto*; *Saprolegnia* spp. from dwarf gourami (*Colisa lalia*), banded gourami, *Nandus nandus*, *Heteropneustis fossilis* and *Notopterus notupterus* (Srivastava, 1980; Bisht *et al*., 1996). Saprolegniasis was also reported in Nile tilapia, mango tilapia and common carp in Taiwan, Egypt, Nigeria and Hungary (Chien, 1981; Okaeme *et al*., 1989; ElSharouny and Badran, 1995; Jeney and Jeney, 1995). In USA, Saprolegniasis caused 20.6% catfish losses in ponds in 2009 (USDA, 2010).

Pathology

Aphanomyces piscicida causes Mycotic Granulomatosis in ayu (*P. altivelis*) and dwarf gourami. External clinical signs include red spots on the body surface due to fungal growth, swelling, erosion and ulcers. Histologically, fungal-like hyphae and granulomas are seen in the internal organs and musculature. It is also highly pathogenic to goldfish (*C. auratus*), *Rhodeus ocellatus*, bluegill (*Lepomis macrochirus*) and crucian carp (Hatai and Egusa, 1977; Hatai *et al*., 1994). In experimentally infected ayu, typical mycotic granulomatosis occurred while in common carp no inflammatory response was observed (Wada *et al*., 1996).

Fungal-like *Aphanomyces* spp., *Achlya*, *Allomyces* and *Saprolegnia* are also associated with EUS in snakeheads (Roberts *et al*., 1993; Paclibare *et al*., 1994; Willoughby *et al*., 1995). However, only *Aphanomyces* has been experimentally shown to induce lesions in naïve snakeheads (Chinabut *et al*., 1995; Lilley and Roberts, 1997). Bruno *et al*. (2011) provide a recent review on Saprolegniasis. Oomycete infections are also discussed in detail in Chapter 3.

Branchiomycosis

Another fungal-like pathogen, *Branchiomyces* was also implicated as a cause of loss of 85% of juvenile red tilapia hybrid (*Oreochromis niloticus* × *O. mossambicus*) and green tilapia hybrid (*O. niloticus* × *O. aureus*) in Israel (Paperna and Smirnova, 1997). An outbreak among farm-reared channel catfish was likewise reported (Khoo *et. al*., 1998). Carp are also susceptible (Post, 1983).

Pathology

Affected fish are lethargic with ragged or corroded gills that are either bright red or white to brown depending on the degree of necrosis. Histological examination of the gill filaments of infected fish demonstrates the proliferation of hyphae of up to 11 µm in diameter. At the onset of sporulation, the hyphae contain multinucleated plasmodia

that develop into daughter plasmodia. The final stage of cell division yields a sporont filled with spores. Spores are released from the necrotic gills and remain suspended in the water or fall to the bottom.

In severe infection, some filaments undergo complete degeneration with necrotic residues of the pseudofungus. As a result, the pseudofungi reduce the blood supply to the gills causing necrosis and sloughing away of the gill tissue. Hence, the disease is commonly named 'gill rot' (Post, 1983). Secondary bacterial invasion of the filament edges follows.

The presence of organic matter, algal blooms, dissolved fertilizer, low dissolved oxygen, pH between 5.8 and 6.5, high stocking density and 25–32°C are predisposing factors. Under favourable conditions, the disease may develop in 2–4 days although *in vitro* culture of the pathogen produced spores in the 14th day of culture (Post, 1983).

Diagnosis

Two species have been described: *Branchiomyces sanguinis* and *B. demigrans*. Squash preparations of the gills examined using light microscopy will differentiate the two species. *B. sanguinis* has a thin hyphal wall (0.2 µm), spores of 5–9 µm diameter and affects the gill lamellar capillaries. *B. demigrans* has a thicker hyphal wall (0.5-0.7 µm), spores of 12–17 µm diameter and infects the parenchyma of the gills (Post, 1983; Noga, 2010).

Prevention and control

Affected fish should be burned and/or buried taking into account local regulations. Survivors of the epizootic are carriers of the pathogen and should not be cultured with naïve fish or transported into *Branchiomyces*-free geographical areas.

Disease of complex infectious aetiology: Epizootic Ulcerative Syndrome (EUS)

The epizootic ulcerative syndrome (EUS) is a seasonal epizootic condition of freshwater and estuarine warmwater fish of complex infectious aetiology characterized by the presence of invasive *Aphanomyces* and necrotizing ulcerative lesions typically leading to a granulomatous response (Roberts *et al*., 1994a). It affects wild and cultured snakeheads, catfish (*Clarias* spp.), *Mastacembelus armatus*, *Puntius* spp., giant snakehead, marble goby (*Oxyeleotris marmoratus*), *Glossogobius giurus*, blue gourami, snakeskin gourami (*Trichogaster pectoralis*), *Trichopsis vittata*, Siamese fighting fish (*Betta splendens*), swamp eels (*Monopterus albus*) and several wild fish species (Lilley *et al*., 1998). Major outbreaks initially occurred in Malaysia in 1979, in Indonesia in late 1980, in Thailand in 1981, in Kampuchea, Myanmar and Lao PDR in 1984, in the Philippines in 1985, in Sri Lanka in 1987, in Bangladesh and India in 1988, in Bhutan and Nepal in 1989 (Tonguthai, 1985; Lilley *et al*., 1998; Lio-Po, 1998). In addition, EUS has been observed in Vietnam, Singapore and Pakistan. EUS-like lesions on fish were also reported in 1972 in Australia (Rodgers and Burke, 1977) where infected fish included mullet (*Liza* spp.; *Mugil* sp.), sand whiting (*Sillago ciliata*), *Acanthopagrus australis* and *Arrhamphus sclerolepis*. The disease was then called Red Spot Disease (RSD). Similarly, in Papua New Guinea *Toxotes chatareus*, *Kurtus gulliveri*, *Bunaka* spp., goby, freshwater anchovy and spotted scat (*Scatophagus argus*) were severely affected in 1975 (Haines, 1983). Fish with EUS were found in all types of freshwater systems, including lakes, rivers, streams, culture ponds, rice paddies, irrigation canals and reservoirs. Cage-cultured snakeheads in the Philippines are very susceptible to the disease (Lio-Po *et al*., 1992). In addition, EUS was reported among cage-cultured silver barb (*Puntius gonionotus*) and mad barb (*L. hoevenii*) (Christensen, 1989).

Causative agents

This disease is associated with a rhabdovirus (65 × 175 nm), *A. hydrophila* and *Aphanomyces invadans* (= *A. piscicida*). The expert consultation on EUS conducted in 2002 cited assertions on the polymicrobial etiology of the disease (Baldock *et al*., 2005).

EUS-ASSOCIATED VIRUS. The presence of a virus in EUS fish was initially reported by Wattanavijarn *et al*. (1983, 1985), Roberts *et al*. (1986) and

Saitanu *et al*. (1986). However, the identification of a rhabdovirus in EUS fish in Thailand and in the Philippines was first reported by Frerichs *et al*. (1986) and by Lio-Po *et al*. (2000), respectively. The virus is bullet-shaped, typical of the genus rhabdovirus (Family: *Rhabdoviridae*) and induces CPE in monolayer cells of snakehead (SSN-1), catfish spleen (CFS), snakehead spleen (SHS), snakehead liver (SHL) and CCO yielding virus titre of 10^6 $TCID_{50}$ per ml at 25°C in 2–3 days (Lilley and Frerichs, 1994; Lio-Po *et al*., 2000). Optimum replication in SHS cells is at 15–25°C. Characterization and serological comparison of the virus to other fish rhabdoviruses associated with EUS-affected fish in Thailand showed that the Philippine virus isolate is morphologically similar and slightly antigenically related to the ulcerative dermal rhabdovirus (UDRV) isolated in Thailand (Kasornchandra *et al*., 1992; Lio-Po *et al*., 2000; Kreethachat and Kanchanakhan, 2005).

In Thailand, Kasornchandra *et al*. (1992) characterized the snakehead rhabdovirus (SHRV) serotypes Sh as a lyssavirus rhabdovirus while Kanchanakhan *et al*. (1999) isolated nine rhabdoviruses from EUS snakehead (*C. striata*) and three-spot guorami (*T. trichopterus*) during the 1993–94 EUS epizootic in Thailand; nine rhabdoviruses from snakeheads during the 1995–96 EUS epizootic; two rhabdovirus from giant gourami (*Ophronemus goramy*) and climbing perch (*Anabas testudineus*) in 1996–97 and named as ulcerative dermal rhabdovirus (UDRV) (vesiculovirus rhabdovirus). Lilley and Frerichs (1994) reported the homogeneity and antigenic relatedness of six EUS rhabdovirus isolates from Thailand, Sri Lanka, Myanmar and Australia to UDRV, serotype Ud.

In the Philippines, EUS rhabdovirus (ERV) was, likewise, isolated from EUS-affected *O. striatus*, *Anabas* sp. and *T. pectoralis* using catfish and snakehead cells (Lio-Po *et al*., 2000). The ERV replicates at 25°C with a titre of 3.02×10^6 $TCID_{50}$ per ml with optimum replication at 15°C (Lio-Po *et al*., 2000). Positive isolations were predominantly from fish with slight lesions (Lio-Po, 1998).

EUS-ASSOCIATED BACTERIA. *A. hydrophila* has been consistently isolated from lesions of EUS-affected fish (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Pal and Pradhan, 1990; Subasinghe *et al*., 1990; Torres, 1990; Lio-Po *et al*., 1992; Cruz-Lacierda and Torres, 1994; Karunasagar and Karunasagar, 1994; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Rahman *et al*., 1999).

EUS-ASSOCIATED PSEUDOFUNGI. *Aphanomyces* spp., *Achlya*, *Allomyces* and *Saprolegnia*, were also reported in EUS-affected snakeheads (Roberts *et al*., 1993; Paclibare *et al*., 1994; Willoughby *et al*., 1995).

Pathology

Lesions associated with EUS are characterized by severe, ulcerative, dermal necrosis with extensive erosion/sloughing of the underlying musculature. The necrotic muscular tissue emits a foul odour. Fish have frank ulcers that consist of eroded dermal layer, exposing the underlying musculature that may be haemorrhagic. In less severe infections, there is scale loss with erosion of the skin surface with or without haemorrhagic signs (Lio-Po *et al*., 1992).

In general, EUS outbreaks show a seasonal pattern (Phillips and Keddie, 1990). In Laguna de Bay, the Philippines, the EUS morbidity rate among snakeheads was estimated to be 59% in January, 1986 (Mines and Baluyot, 1986). Outbreaks are more common from September to March which correlate with the period when water temperatures in the region are at their lowest range of below 25°C. Such a low temperature reduces the immune response of fish (Catap and Munday, 1998).

The spreading pattern of outbreaks of EUS in South-east and East Asia strongly indicates the infectious nature of the aetiologic agent. The actual pathogen of this disease has been in dispute for years. Rhabdovirus, *A. hydrophila* and *A. invadans* have been associated with EUS-affected fish (Frerichs *et al*., 1986; Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Chinabut *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Kanchanakhan, 1996; Lilley and Roberts, 1997; Lilley *et al*., 1998; Lio-Po *et al*., 2000).

EUS-ASSOCIATED VIRUS. Earlier experiments on the pathogenicity of rhabdovirus from EUS fish

were not demonstrated (Frerichs *et al*., 1993). However, in subsequent studies ERV experimentally induced dermal lesion development and mortality of snakeheads reared at 20–22.5°C but not at 28–32°C (Lio-Po *et al*., 2001). Slight dermal lesions developed 3 to 10 days following IM inoculation which progressed to moderate lesions at 10–12 dpi but not deep ulcers. Similarly, Kanchanakhan (1996) reported that rhabdovirus can experimentally cause skin damage in juvenile snakehead at ~ 20°C. This lower temperature corresponds to the cooler months of December through February when outbreaks of EUS among freshwater fish occur in the Philippines and in other EUS-affected countries.

Moreover, naïve snakehead fry and fingerlings exposed to ERV by bath manifested significant mortalities ($p < 0.01$) of 100% at 5 dpi and 9 dpi, respectively, with no apparent lesions (Lio-Po *et al*., 2001). Kanchanakhan *et al*. (2002) also exposed snakehead fry to the EUS rhabdovirus isolated from Thailand and obtained similar results.

Stocking of naïve snakeheads with EUS fish in lakes and cohabitation with apparently healthy snakeheads in lakes led to EUS-like lesion development attaining 100% morbidity by days 12 and 14, respectively at 23–26°C (Lio-Po *et al*., 2003) while naïve fish stocked with EUS fish in aquifer water did not manifest EUS-like lesions. The study demonstrated the transmission of the virus from infected and apparently healthy snakeheads held in endemic areas to naïve fish.

EUS-ASSOCIATED BACTERIA. Pure cultures of the bacterium inoculated intramuscularly induce dermonecrotic lesions in healthy catfish and snakeheads (Lio-Po *et al*, 1992, 1996, 1998; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995). This bacterium grows between 18–39°C and secretes a dermonecrotic factor at 10°C and 30°C (Olivier *et al*., 1981; Uddin *et al*., 1997). Moreover, cytotoxin-producing strains were associated with EUS-affected fish and hypothesized to play an important role in the pathogenesis of the disease (Yadav *et al*., 1992).

EUS-ASSOCIATED PSEUDOFUNGI. Isolates of *A. invadans* were experimentally shown to induce lesions in most test snakeheads or sand whiting (Roberts *et al*., 1993; Chinabut *et al*., 1995; Catap and Munday, 1998). Other studies reported that the pseudofungi grow invasively through the fish muscle causing severe myonecrosis (Callinan *et al*., 1995; Chinabut *et al*., 1995; Lilley and Roberts, 1997). Granuloma development was observed at 26°C or above while fish at lower temperatures showed acute inflammation (Chinabut *et al*., 1995). In addition, Catap and Munday (1998) observed that sand whiting injected with zoospores of *Aphanomyces* sp. at 26°C developed highly inflamed, haemorrhagic external lesions while similarly-treated fish held at 17°C had slightly inflamed injection sites. The temperature-related growth rate of this pathogen appears to correlate with the findings that *Aphanomyces* isolates from EUS-affected fish generally thrive better at 26-30°C than at lower temperatures (Lilley and Roberts, 1997).

In eastern USA, Atlantic menhaden (*Brevoortia tyrannus*), develop ulcerative lesions identical to the EUS in Asia. Subsequent infection experiments inoculated *A. invadans* (80 zoospores per fish) to Atlantic menhaden, hogchoker (*Trinectus maculates*), striped killifish (*Fundulus majalis*) and mummichog (*F. heteroclitus*). Killifish developed frank lesions in approximately 5–10 days and after that was similarly observed in menhaden (Johnson *et al*., 2004). Hogchoker and mummichog did not develop gross skin lesions other than reddened areas under the epidermis.

Diagnosis

The EUS-associated virus can be isolated from organ tissues of catfish and snakeheads in cell monolayers of CFS, SHS, SHL, CCO, BF2, SSN-1 (Frerichs *et al*., 1989; Kasornchandra *et al*., 1992; Kanchanakhan *et al*., 1999; Lio-Po *et al*., 1999; 2000). Filtrates derived from the visceral organs of EUS-affected fish can induce CPE when inoculated into susceptible cells.

The bacteria, *A. hydrophila* and the pseudofungus, *Aphanomyces* sp., can be isolated from ulcerated muscles of EUS-affected fish in TSA medium and in Czapek Dox medium, respectively (Lio-Po *et al*., 1992; Callinan *et al*., 1995; Willoughby *et al*., 1995). Histological

sections of muscular lesions of EUS-affected fish show the development of a necrotic granulomatous mycosis that may eventually invade the abdominal viscera (Lilley *et al*., 1998). Bacterial colonies are also histologically demonstrated in EUS-affected snakeheads (Cruz-Lacierda and Torres, 1994; Cruz-Lacierda, 1995).

Prevention and control

Quarantine and restricted movement of EUS susceptible fish from endemic areas to nonendemic sites should be practised. Prophylactic treatment with 5 mg per l Coptrol (a chelated copper compound) was reported to prevent induction of EUS lesions while a proprietary mixture, CIFAX, may be curative (Lilley *et al*., 1998). Moreover, recent studies showed that fish fed with the immunostimulant, Salar-bec, survived better when challenged with *A. invadans* (Miles *et al*., 2001).

Parasitic Diseases

Although there is information on parasitic diseases of fish in tropical aquaculture (Kabata, 1985; Lim, 1991d, 1992; Paperna, 1991, 1996; Arthur, 1992; Arthur and Lumalan-Mayo, 1997), there is little or no information on parasitic diseases in cage culture systems. This paucity of information on pathogenic parasites, their control, lack of regulations concerning the movement of diseased fish, and mandatory reporting of diseases and mortalities in developing countries have made management of parasitic diseases a difficult task. The diseases and specific identity of the parasites infecting warm freshwater cultured fish (in particular cage cultured fish) are seldom known and at best only the genera are recorded (Paperna, 1991). Overall, there is also a lack of knowledge about the actual disease patterns, the pathology and prevailing factors predisposing fish to the disease (Christensen, 1989; Dharma *et al*., 1992; Nasution *et al*., 1992; Alawi and Rusliadi, 1993). The lack of comprehensive investigations into the diseases encountered in cage culture has resulted in the abandonment of some promising projects such as the culture of the marble goby (*O. marmorata*), in Thailand (ADB/NACA, 1991). Thus, our discussions on parasitic diseases are at the generic and other taxonomic groupings rather than at the species level.

Generally, wild/feral fish have greater parasite species diversity but lower population abundance and the converse is true for cultured fish (Lim, personal observation; Lersutthichawal, 1999). Personal observations, discussions with tropical fishery scientists and the current literature indicate that not all the parasites known from other culture systems have similar impact on cage-cultured fish.

Diseases caused by protistans

The protistan parasites that cause disease in fish include the Amoebozoa, Dinoflagellata, Ciliophora, Parabasalia, Euglenozoa, Myxozoa, Microspora, Sarcomastigophora and Apicomplexa (Basson and Van As, 2006; Dickerson, 2006; Dyková, 2006; Feist and Longshaw, 2006; Molnár, 2006; Noga and Levy, 2006; Woo, 2006; Zilberg and Munday, 2006). The commonly reported pathogenic protistans in/on fish reared in cages in warm waters include the myxosporeans, trichodinids and the dinoflagellates (Christensen, 1989; T.T. Dung, personal communication; F. Shaharom, personal communication). The mad barb (*L. hoevenii*) cultured in cages in Indonesia are infected with myxosporeans (Christensen, 1989). In Vietnam, fish are plagued by *Trichodina*, *Balantidium* (in the intestine of catfish) and *Glossatella* (T.T. Dung, personal communication). The oodinid dinoflagellate *Piscinoodinum* sp. infects grass carp and bighead carp and silver barb (*P. gonionotus*) in pond culture as well as catfish and tilapia in cage culture (Shaharom-Harrison *et al*., 1991; F. Shaharom, personal communication). Various other protistan parasites have also been recorded but their prevalence is unknown. For example, *Ichthyobodo* (*Costia*) and *Oodinium* are known to affect hybrids of *Clarias* in tropical warm freshwaters (Paperna, 1991) resulting in pale gills and excessive mucous secretions, causing the fish to gasp for air. The lack of reports of protistan diseases in warmwater cage culture systems could be a lack of expertise to

diagnose the disease and/or the absence of reporting procedures rather than the absence of the disease agents.

Movement of fish for culture has contributed to the worldwide distribution of many of their parasites especially parasitic protistans. For example, *Eimeria cheni* and *E. sinensis*, originally found in farmed carp in China are now found in Europe (Molnár, 1976). Nile tilapia imported into Thailand from Egypt were also infected with *Eimeria vanasi* (Paperna, 1991), while cichlid fish farmed in Israel (Landsberg and Paperna, 1985) were infected by *Eimeria vanasi* and *Gousia cichlidarum*. A few protistan diseases found in coldwater fish could be regarded as emerging disease problems in cage culture in warm waters.

Myxosporean diseases

Myxosporeans are observed as cysts, infecting the skin and subcutaneous layer, muscle, gills, central nervous system as well as visceral organs. These cause extensive lesions when cysts rupture and mortality occurs in cultured as well as feral fish (Lom and Dykova, 1995). In most cases in South-east Asia, the specific myxosporidean pathogens are unknown and at best the identification is at generic level. *Thelohanellus* (Myxobolidae), *Myxobolus* (Myxobolidae) and *Myxosoma* (Myxidiidae) have been reported from exotic carp and indigenous cyprinids in India, South-east Asia and China (ADB/NACA, 1991; Paperna, 1991). *Thelohanellus* has been reported on silver barb (*P. gonionotus*), common carp and *Clarias* spp. in the Peninsular Malaysia (ADB/NACA, 1991; Paperna, 1991). Myxosporean is a major problem in Central Java (Indonesia), infecting mad barb (*L. hoevenii*) reared in cages and ponds and silver barb (*P. gonionotus*) reared in ponds (Christensen, 1989). *Myxobolus koi* has been found on the gills of common carp and goldfish in Japan (Egusa, 1992) and from fish farms in Israel, Indonesia and the Indian continent causing high mortality among the younger fish (ADB/NACA, 1991; Paperna, 1991) while *Myxobolus artus* is on common carp in East and South-east Asian countries (Lom and Dykova, 1995). In addition, Okoyama (2003) reviewed the Myxosporeans.

Sutchi catfish (*P. hypophthalmus*), cage-cultured in Terengganu River, Malaysia was infected by six myxozoan species: *Hennegoides berlandi*, *H. malayensis*, *H. pangasii*, *H. shariffi*, *Myxobolus baskai* and *M. pangasii*; with five myxozoans infecting the gills and one on the spleen (Molnár *et al*., 2006a). Myxozoans infecting the gills were characterized by a specific site selection. *H. shariffi* and *H. berlandi* formed plasmodia in the multi-layered epithelium of the gill filaments. Of the two vascular species, *H. pangasii* develops in the gill arteries, while *M. baskai* infected the capillary network of the gill lamellae. Plasmodia of *H. malayensis* were found inside the cartilaginous gill rays of the filaments. Large plasmodia of *M. pangasii* were located in the spleen but they only affected the serosa layer (Molnár *et al*., 2006a). In another study, Asian redtail catfish (*Hemibagrus nemurus*) also cage-cultured in Terengganu River, Malaysia, were infected by *H. mystusia, H. hemibagri* and *H. basifilamentalis. H. mystusia* formed plasmodia in the multi-layered epithelium between the gill lamellae and in the non-lamellar edge of the gill filaments, while *H. hemibagri* developed in the capillary network of the lamellae. *Henneguya basifilamentalis* had large oval plasmodia located deep among the filaments just above the gill arch (Molnár *et al*., 2006b).

In 2009, new myxosporean species were found in fish cultured in ponds in Kelantan State and in net-cages in Terengganu State, Malaysia. *Myxobolus omari* and *M. leptobarbi* were found in the muscles of striped catfish (*P. hypophthalmus*) and mad barb (*L. hoevenii*), respectively, while plasmodia and spores of *Thelohanellus zahrahae* and *Henneguya daoudi* were detected in the gills of Java barb (*Barbonymus gonionotus*) and blue guorami (*Trichogaster trichopterus*), respectively. Plasmodia and spores found in these fish differed from the known myxosporean species in their morphology, tissue tropism and 18S rDNA structure (Székely *et al*., 2009a). In another report, infections with four new myxosporeans (two *Myxobolus* spp., *Henneguya* sp. and *Thelohanellus* sp.) were found in fish cultured in Tasik Kenyir Water Reservoir, Malaysia (Székely *et al*., 2009b)

Pathology

Myxobolus koi infections on the gills of common carp and goldfish result in many small white cysts to large pinkish to reddish cysts in the gill tissue (Paperna, 1991; Egusa, 1992). Large cysts are enclosed in the host connective tissues that turn dark red due to haemorrhaging and this leads to congestion and degeneration of the gill capillaries. The movement of the opercula and respiratory processes are further affected by increased mucus production and epithelial proliferation. Spores of *M. koi* were also observed in the heart, liver, kidney and intestine (Hoshina, 1952). According to Lom and Dykova (1995), *Thellohanellus pyriformis* form large plasmodia in the subcutaneous tissue and muscle of cyprinids causing fatal epizootic in Indonesia. Little is known about the pathology caused by the other myxosporeans. In Malaysia, the intensity of infection varied and no disease signs were seen in infected fish (Molnár *et al*., 2006a, b; Székely *et al*., 2009a, b).

Diagnosis

One characteristic sign of myxosporean infections are small white and/or large cysts on the gills. For example, *M. koi* occurs as small white cysts and large pinkish to reddish cysts in the gill tissues of the gills of common carp and goldfish (Paperna, 1991; Egusa, 1992). Opercular movements of infected fish are hampered and respiration is affected by the increased mucus secretion and epithelial proliferation (Hoshina, 1952). Some myxosporeans are confined to the body and these occur as white cysts under the scales often near the tail or fins resulting in sores or ulcers on the skin (Christensen, 1989). Identification of the myxosporeans is based on the morphological characteristics of the spores. Briefly, cysts on the skin or gills are removed and gently broken to release the spores (preferably) on glass slides. The multicellular spores are unique in possessing nematocyst-like polar capsules (Lom and Dykova, 1995). They are usually oval-pear to round shape, anterior end pointed, posterior end rounded, 1–2 polar capsules with polar filaments, sporoplasm with or without iodophilic vacuole and with or without posterior processes (Shulman and Shtein, 1962; Lom and Dykova, 1995).

The spores of *Myxobolus* are oval- to pear-shape with two polar capsules at the pointed anterior end; the posterior end is rounded and lacks processes. The spores of *Henneguya* are round, oval or fusiform with two anterior polar capsules and valves with two caudal processes from the posterior end. The oval, round spores of *Myxosoma* are different in having two polar capsules at one end and lack processes and iodophilic vacuoles, while *Thelohanellus* has oval, round spores with smooth valves without processes and one medially displaced polar capsule.

Prevention and control

There is no effective treatment and the best method is to remove and destroy heavily infected fish from cages (Christensen, 1989). In light (early) infections, the cysts can be carefully removed and destroyed. Treatment with saline (0.23–5.0%), copper sulfate (0.025–0.05%), potassium permanganate, formalin, methylene blue, glacial acetic acid or phenol, is not effective (Hoshina, 1952) as myxosporean spores are highly resistant to chemicals. The inclusion of certain drugs (such as Proguanil and furazolidone) into the fish feed reduced spore production and alleviated lesions (Lom and Dykova, 1995). Although the life cycles of some species of myxosporeans are known to involve intermediate hosts such as oligochaetes (Lom and Dykova, 1995), for the majority of cases, the life cycles have not been elucidated and the actual intermediate hosts not identified. Hence, control of myxosporeans via eradication of intermediate hosts (oligochaetes) is not a viable option at the present time. Eradication of heavily infected hosts appears to be the most viable option for the moment.

Diseases caused by ciliates

The ciliates (Phylum Ciliophora) are common ectoparasites of fish, especially in hatcheries and on young fish in grow-out ponds. *Ichthyophthirius multifilis* is the most well known pathogenic ciliate and it is related to

the marine pathogen, *Cyrptocaryon irritans*. Others include the trichodinids and *Chilodonella*. However, the specific identities of these ciliates are unknown in the majority of reported cases in tropical aquaculture. Besides the known obligatory parasitic (pathogenic) ciliates, there are also facultative parasites (*Tetrahymena* for example), which are opportunistic organisms.

Trichodinid diseases

Pathogenic trichodinids include *Chilodonella*, *Trichodina*, *Tripartiella* and *Trichodenella*. A large number of trichodinids are associated with the goldfish, common carp, grass carp, silver carp and bighead carp and these were introduced into Israel and South-east Asia from China (Chen, 1955; Paperna, 1991). The trichodinids (*Trichodina acuta*, *T. centrostrigeata* and *T. heterodentata*) from African cichlids have also been introduced into South-east Asia (Albaladejo and Arthur, 1989; Bondad-Reatanso and Arthur, 1989).

Trichodinids are found on snakeheads and *Pangasius conchophilus* cultured in cages in Vietnam (T.T. Dung, personal communication). Although chilodonellosis occurs mainly in cold waters, *Chilodonella hexasticha* has also been found on the bighead carp in Malaysia (Shariff, 1984).

Trichodinids commonly cause fish mortality in hatcheries and they may continue to be a problem after fish are transferred to cage culture systems. Trichodinids are prevalent on young clariid hybrids of African catfish (*C. gariepinus*) and *Clarias* sp. in cages. These are also found on silver carp, bighead carp and grass carp in hatcheries in China and Vietnam, and are also on pangasiids and *Catla* sp. in cage culture. In Nepal, trichodinids cause mortality among the fry during spring and autumn. Although there are many species of trichodinids, only a few are known to be pathogenic (Lom, 1995).

Pathology

Pathological effects are dependent on the host's response, the intensity of infections and environmental conditions since stressful conditions can compromise the host's ability to counteract infections. Some trichodinids live specifically on the body surface or on the gills, while others are found both on the skin and the gills (Paperna, 1996). In skin infections, the preferred sites are the bases of fins where they damage epithelial tissue through adhesion and crawling actions. They feed on the epithelial cells causing abrasion and some trichodinids may withdraw cellular contents damaging cells that degenerate and disintegrate resulting in erosion and desquamation of the epidermis. The host responds to the infection by increased mucus secretion and epithelial hyperplasia, cellular destruction and inflammation. The damaged gills and epidermal tissues are targets for bacterial invasion. The infected epidermis thickens, becomes turbid with mucus and sloughed epithelial cells, and the fish becomes emaciated. When the gills are infected, excessive mucus is produced, massive destruction of the gills, and proliferation of epithelial cells occur causing difficulty in respiration. Trichodinids are usually found in association with monogenean and other protistan infections. Massive infections causing damages in the epidermis as described above result in mortality due to disruption in respiratory functions of the gills. Young fish in overcrowded and confined stressful habitats are usually heavily infected with trichodinids, while older fish have fewer but more host-specific species (Paperna, 1996).

Diagnosis

Trichodinids are easily observed microscopically from skin and gill scrapings (Paperna, 1996). Taxonomy of the trichodinids is based on the structure of the buccal ciliature, the morphology of the adhesive disc and the number and size of its components (Lom, 1995). Trichodinids are essentially a flat disc, with somatic ciliature consisting of 3–4 ciliary wreaths around the aboral surface of the body that is transformed into an adhesive disc. The disc is a proteinaceous skeleton, composed of a ring of hollow conical denticles. The denticle consists of blades (centrifugal flat projections) and horns (rod-like centripetal projections), connected to each other by radial pins (Fig. 6.5).



Fig. 6.5. *Trichodina acuta* from the skin of *Ctenopharyngodon idellus* (Klein's silver impregnation). Image courtesy of R. Arthur, Canada.

There are five genera of fish trichodinids (Lom, 1995). In warm freshwater cage-culture systems, trichodinids have not been identified to species. *Trichodina* is characterized by denticles with massive central conical parts, flat semicircular blades, straight thorns and a diameter of 50–100 µm. For general identification, skin and gill smears containing trichodinids should be air-dried, fixed in Bouins for 20 min, washed in 70% ethanol, rehydrated and stained with haematoxylin, dehydrated and mounted. For specific identification, the adhesive disc is studied using a silver impregnation method (Welborn, 1967; Paperna, 1996). Air-dried smears should be fixed in 2% silver nitrate for 7–9 min in the dark, washed in distilled water and exposed to sunlight or ultraviolet light for 5–10 min.

Prevention and control

Outbreaks of trichodinid infections are usually the result of adverse environmental conditions that are common in intensive culture systems. The best preventive measure is to ensure that good quality environmental conditions are maintained. To eliminate trichodinids from aquaculture systems, several chemicals have been recommended (Lom, 1995): saline solution (0.1–0.2% as a dip for 1–2 days), formalin (150–250 mg per l as a dip for 30–60 min), acriflavine (indefinitely in water at 10–20 mg per l) and potassium permanganate (0.1% as a dip for 30–45 min). Formalin has been used effectively to control trichodinids in warm waters. The efficacy of formalin in controlling trichodinids depends on water quality (pH, salinity and ambient temperature) and species of fish treated. Van As *et al*. (1984) showed that 25 mg per l for 24 h was effective in cleaning infected carp, while 45 mg per l for 24 h was needed to clean tilapia.

Ich or white spot disease

Ichthyophthirius multifiliis is a pathogenic ciliate infecting freshwater fish causing Ichthyophthiriosis (also known as Ich or white spot). This pathogen was first reported from China (Dickerson and Dawe, 1995), but is now a cosmopolitan pathogen in temperate and tropical warmwater fish (ADB/NACA, 1991). It is predicted to spread with the increase in aquaculture activities and also via the aquarium trade (Paperna, 1996). The outbreaks of Ich is dependent on water temperature and as temperature increases the life cycle of this parasite is completed in a shorter time (Dickerson and Dawe, 1995; Dickerson, 2006), making them a potential danger to cage culture systems in tropical warm waters. In USA, 4.9% catfish losses in ponds were attributed to Ich infestation in 2009 (USDA, 2010). This parasite is maintained within the fish at a low subclinical (enzootic) infection and as encysted tomont. It persists in the environment, becoming epizootic clinical infections when fish are stressed as a result of poor management practices (e.g. poor feed, overcrowding and poor sanitation). The pathogen is not host-specific and recovery from the disease confers resistance to reinfection (e.g. Paperna, 1996; Dickerson, 2006).

Pathology

The feeding or trophont stage is located within the epidermis (gills or skin) of the fish (feeding on the basal layer of the epidermis). The matured tomonts leave the fish and damaging the epidermis causing detachment

from its basal membrane, they secrete a gelatinous cyst wall and divide asexually to form tomites that differentiate into infective theronts that are released into the water. The tomites develop into infective theronts that penetrate the epidermis of the fish, becoming established in the basal layer of the epithelium just above the basal membrane, and feed on epithelial cells. The rate of development of these stages is dependent on water temperature (see above). Intense and prolonged infections cause epithelial proliferation, haemorrhagic inflammation and subsequent disintegration of the integument.

Diagnosis

Clinical signs include anorexia, lethargy and the disease is characterised by white spots on the skin and gills (Dickerson and Dawe, 1995). The ciliate (1 mm in diameter) has a small cytostome, a large crescent-shaped macronucleus and small micronuclei in fixed and stained specimen (with either Giemsa or haematoxylin).

Prevention and control

This pathogen is particularly difficult to control. An integrated approach incorporating appropriate culture practices (locating cages in areas where water movement is continuous and stocking of clean and healthy fish), immunization and chemotherapy in cases of heavy infestations are probably the most effective means to disease control (Dickerson and Dawe, 1995; Paperna, 1996; Dickerson, 2006).

The chemicals recommended for treatment include sodium chloride, malachite green, formalin and potassium permanganate (Dickerson and Dawe, 1995; Paperna, 1996). The efficacy of these chemicals is dependent on a number of factors such as environmental conditions, fish species and developmental stages of the parasites (see below). For example, the encysted tomonts in the environment are resistant to antiparasitic chemicals (Paperna, 1996). The stages of the parasite that can be destroyed are the dividing tomonts and the newly released tomites. Several chemicals have been listed for use against this pathogen and the cost-effective chemicals suitable to large-scale farming system are malachite green (0.05–0.15 mg per l continuously for 3–4 days) and a mixture of formalin and malachite green (50 mg per l and 0.05 mg per l) (Paperna, 1996). The fish species has to be taken into consideration when chemicals are used since some species, especially catfish, do not respond well to malachite green (Paperna, 1996). Potassium permanganate has been used successfully in ponds to control Ich but its effectiveness is affected by the amount of organic matter in the waters (Dickerson and Dawe, 1995). Malachite green in a non-water soluble formulation in feed had been reported to be effective against the trophonts (Schmahl *et al*., 1992). However, malachite green has been reported to be carcinogenic and its use is limited to aquarium fish and should not be used in fish cultured for human consumption (Dickerson and Dawe, 1995). Fish infected with Ich and immersed in Toltrazuril or triazinone (10 µg per ml) for 4 h (repeated daily for 3 days) have been shown to be effective against trophonts (Dickerson and Dawe, 1995). Subsequently, Straus (2008) reported the effective control of trophonts in channel catfish fingerlings using 1.1 mg per l copper sulphate ($CuSO_4$, $5H_2O$) every other day for four treatments.

Recent reports cited that a combination of sulfonamide-diaminopyrimidine (Sulfamonomethoxin (SMMX)-Ormethoprim (OMP) (112.5 and 37.5 mg per kg body weight) in feed given to goldfish (*C. auratus*) with *I. multifilis* infection resulted in significant and highly significant reduction of parasites on the body surface, 3 days and 7 days, respectively, after treatment (Kawano and Hirazawa, 2012). In addition, there was a significant difference in mortalities.

Studies have shown that fish recovered from Ich infections developed immunity against the parasites (Dickerson and Dawe, 1995). Immunization and vaccination offer another way to protect fish against Ich. Experimental immunization using killed vaccines, intraperitoneal inoculation with live theronts and controlled exposure to infective tomites have been used (Paperna, 1996; Sin *et al*., 1996). An experimental recombinant

vaccine (from a 316 bp gene fragment of the immobilizing antigens or i-antigens of *I. multifiliis* and expressed in *E. coli*) has been developed for ichthyophthiriosis (Woo, 1998). Goldfish inoculated with the recombinant protein vaccine in Freund's adjuvant survived a parasite challenge (He *et al*., 1997).

Disease caused by dinoflagellates

There are five genera of parasitic oodinid dinoflagellates: *Amyloodinium*, *Piscinodinium*, *Crepidoodinium*, *Ochthyodinium* and *Oodinioides* on fish (Noga and Levy, 1995, 2006). The ichthyotoxins produced by dinoflagellates cause massive mortality in cultured and feral fish (Steindinger and Baden, 1984). The important freshwater pathogenic dinoflagellate in fish is *Piscinodinium*, which is closely related to the marine dinoflagellate pathogen, *Amyloodinium*. *Piscinodinium* is not host-specific and has been reported on feral, aquarium and cultured food fish species from diverse families in warm waters (Lom and Schubert, 1983; Paperna, 1991, 1996; Shaharom-Harrison *et al*., 1991).

Velvet or rust disease

Fish with excessive mucus covering the body, together with a rust-coloured appearance on the skin, is infested with *Piscinodinium pillulare*, the causative agent for velvet rust diseases, gold dust disease, pillularis disease and freshwater *Oodinium* disease (Shaharom-Harrison *et al*., 1991). *Piscinodinium*, like its marine relative *Amyloodinium* is found on a wide range of host species and is known to cause mortality in warmwater fish (Paperna, 1996).

Piscinodinium pillulare has been reported from 14 tropical ornamental fish species as well as cultured carp and cyprinids (Shaharom-Harrison *et al*., 1991; Noga and Levy, 1995). In Peninsular Malaysia, *P. pillulare* occurs on aquarium fish, cultured grass carp, bighead carp, silver barb (*P. gonionotus*) and mad barb (*L. hoevenii*) and causing mortality in the latter fish (Shaharom-Harrison *et al*., 1991). This pathogen also causes disease in cage cultured *Hemibagrus nemurus* in the Trengganu River and in tilapia cultured in Kenyir Dam, Malaysia (F. Shaharom, personal communication) although not to the same extent found on pond-cultured fish.

Pathology

Histopathological changes of gill structure occur with a massive proliferation of the gill epithelium, fusion of adjacent lamellae and separation of the gill respiratory epithelium resulting in a severe hyperplasia of the entire gill filament (Shaharom-Harrison *et al*., 1991). The trophonts of *P. pillulare* penetrate host cells by nail-like extensions resulting in degeneration and collapse of the cells, leading to focal erosion of the epithelium, proliferation of the epithelium and obliteration of the gill lamellae. The inner strata of the epithelium become spongious and may undergo complete lysis (Lom and Schubert, 1983; Paperna, 1991).

Diagnosis

Initial diagnosis can be based on clinical signs and confirmed by microscopic examination of the trophont stage. *Piscinoodinium* infect skin and gills with clinical signs similar to amyloodiniosis. Infected fish have a yellow to rust (velvety) coloured skin, dense covering of mucus resulting in darkening of the skin, dyspnoea, anorexia and skin ulcers (Shaharom-Harrison *et al*., 1991).

All oodinids have a parasitic trophont stage and a sessile, stalked, sac-like trophozoite stage that feeds on the skin and gill epithelia. The trophont has a prominent stalk, which anchors the parasite to the host. It likely uses the stalk to absorb nutrients. After feeding, the trophont detaches, withdraws the stalk and forms an encysted tomont (reproductive cysts). The tomont divides asexually forming dinospores, the mobile infective stages. The trophonts and tomonts are important for definitive diagnosis and microscopic identification of these stages is necessary. Trophonts are oval with smooth walls and usually visible to the naked eye as white spots (80–100 µm) and in Lugol's iodine turn dark blue.

Piscinoodinium is distinguished from other oodinid dinoflagellates on the morphology of

the trophont, especially the type of host attachment and mode of nutrition (Lom, 1981). Fish should be examined live or immediately after death and snips of the gills be removed and examined. Trophonts are removed by brushing the fish gently in a dish of water and the sediment examined under the microscope. The trophont of *Piscinoodinium* is a yellow-green, pyriform or sac-like cell, almost round, 12 × 29 µm, with a rudimentary sulcus and a short stalk with an attachment disc extending from its base and thin holdfasts (rhizocysts) radiating from the stalk (Lom and Schubert, 1983). Head parts of the rhizocysts are inverted in separate compartments (rhizothecas) in the sole of the disc while their shafts are firmly embedded in the host cell cytoplasm. The theca covers the entire cell except for the area of the attachment disc.

Prevention and control

Outbreaks of oodinid infections result from stress due to poor environmental conditions. Hence, environmental manipulation is probably a viable approach to prevent outbreaks of *Piscinoodinium*. Formalin detaches trophonts, but does not inhibit division (Paperna, 1996). A copper ion concentration of about 0.15 mg per l (mixture of 5-hydrate copper sulfate with citric acid monohydrate) in water is effective in controlling *Piscinodinium* (Paperna, 1996). A salt dip for 1–3 min dislodges the trophonts, while immersion for 3–5 days in a combination of 7 g per l salt and 40 mg per l of potassium permanganate is also effective. However, freshwater fish cannot tolerate high salt concentration and potassium permanganate higher than 2 mg per l (van Dujin, 1973; Plumb, 1979).

Diseases caused by monogeneans

Monogeneans are among the most commonly reported parasitic agents of fish (ADB/NACA, 1991). They are mainly ectoparasitic on gills, buccal cavity, body surface and fins of freshwater fish although some are endoparasitic (Gussev and Fernando, 1973; Euzet and Combes, 1998). Monogeneans are oviparous with the exception of the viviparous gyrodactylids. Although they rarely cause disease in wild fish, except the benedenids (Paperna, 1975), they are important pathogens in intensive fish culture (Paperna *et al*., 1984). Their direct life-cycle results in rapid and continuous recruitment especially in warm waters; this makes monogeneans especially dangerous in intensive culture. Disease caused by monogeneans is normally debilitating and subsequent fish mortality is usually attributed to a viral or a bacterial infection. Monogeneans stress the hosts by destroying the epidermal integrity of the fish, thus predisposing their hosts to other pathogens. Cone (1995) suggested that monogeneans could be the mechanical vectors of bacterial and viral diseases, but further confirmation is needed. In intensive culture systems, where intensity of infection can be high on the gills, monogeneans can cause death directly by inhibiting respiration through physical damage of the gills. Fish mortality from monogenean infections may result from damage to gill tissues and skin caused by attachment organs, and by feeding on the integument which stimulates cell proliferation and secretion of copious amounts of mucus (Paperna, 1991). Cage culture with high stocking density in tropical areas is usually conducive for the perpetuation of parasitic diseases. The nets trap eggs, infective larvae and food debris around the cages, which attract carrier/reservoir feral fish.

Most monogenean genera are specific to a group of related host species. *Dactylogyrus* are found on cyprinids and catfish harbour *Thaparocleidus*. Although most species are specific to a particular host, some parasites, like *Thaparocleidus caecus*, are found on a number of pangasiids (Lersutthichawal, 1999).

Many monogeneans on warm freshwater cultured fish have not been identified or are incorrectly classified. For example, *Dactylogyrus* spp. had also been incorrectly implicated to be pathogenic to snakeheads, tilapia and clariids cultured in South-east Asia (Kabata, 1985). These fish possess their own unique and specific monogeneans (Lim and Furtado, 1983, 1986; Lim, 1986, 1991a). *Trianchoratus* is found on snakeheads other than the giant snakehead, which is infected by *Sundanochus* spp., while *Cichlidogyrus* spp.

infect tilapias and *Quadriacanthus* spp. and *Bychowskyella* spp. infect South-east Asian clariids. The gyrodactylids, are ubiquitous and may be host-specific.

The most commonly reported monogeneans on warm freshwater cultured fish are the *Dactylogyrus* on carp, *Cichlidogyrus* on cichlids, *Bychowslyella* and *Quadriacanthus* on clariids, *Trianchoratus* on snakehead, *Pseudodactylogyroides* on marble goby, *O. marmorata*, *Thaparocleidus* on catfish other than clariids and *Gyrodactylus* (see later). *Pseudodactylogyrus* have been recorded from eels (*Anguilla* spp.) in warmwaters of Indonesia (K. Buchmann, personal communication) and *Pseudodactylogyrus* have caused massive mortalities of the cultured eels especially *Anguilla japonicus* in Europe in the 1980s (Buchmann *et al*., 1987; Buchmann, 1997).

In the majority of cases, the specific identity of the pathogenic monogeneans, signs and pathology of the infection, disease mechanism, and control and preventive measures have not been elucidated nor documented. For instance, it is known that *Thaparocleidus siamensis* occur in greater intensity than *T. caecus* on cultured striped catfish (*P. hypophthalmus*) in Peninsular Malaysia and Thailand (Lim, 1990, 1996; Lersutthichawal, 1999), but it is unknown which species is pathogenic. The translocation of monogeneans along with their hosts has been well documented for *Dactylogyrus* spp. on imported Chinese carp, *Cichlidogyrus* spp. on tilapia and *Quadriacanthus clariadis* on *C. gariepinus* imported into Thailand (Paperna, 1991; Lersutthichawal, 1999).

Disease caused by *Dactylogyrus* species

Dactylogyrus species are specific to the *Cyprinidae* although they are also found on *Hemiramphidae* (Lim, unpublished data) and one species on a catfish (Gussev, 1976). This genus has been listed frequently as a disease-causing agent since cyprinids are the most cultured fish group.

Dactylogyrus has also been shown to cause mass mortality of fry, small fish and broodfish (Paperna, 1991). The four important species of *Dactylogyrus* that cause disease in cultured common carp in Israel are *Dactylogyrus anchoratus*, *D. extensus*, *D. minutus* and *D. vastator* (Paperna, 1991). They have different temperature preferences: for example, *D. extensus* flourishes at low water temperature (optimum temperatures of 16–17°C), while *D. vastator* prefers warmer waters (20–24°C). *D. minutus* has been found on common carp in Taiwan (Paperna, 1991). The grass carp are infected with *D. lamellatus* and *D. ctenopharyngodonis*, silver carp with *D. hypophthalmichthys*, *D. suchengtaii* and *D. scriabini* and bighead carp with *D. aristichthys* and *D. nobilis* (Paperna, 1991). In Peninsular Malaysia, *D. nobilis* and *D. aristichthys* are found on cultured bighead carp and *D. lamellatus* on grass carp (Shaharom, 1988). Silver barb (*P. gonionotus*) and mad barb (*L. hoevenii*) are infected with *D. leptobarbus* and *D. lampam* (Mizelle and Price, 1964; Lim and Furtado, 1986; Lim, 1991b), respectively. In Thailand, however, there are seven species of *Dactylogyrus* on feral silver barb (*P. gonionotus*) (Chinabut and Lim, 1993).

Pathology

Dactylogyrus spp. cause disease in exotic carp in Europe, but not in indigenous cyprinids in South-east Asia. Feeding on epithelial cells and attachment by the monogeneans causes severe destruction of the gills resulting in haemorrhage and metaplasia of the gill tissue. Secondary bacterial infections usually occur and result in death of the fish. The pathology caused by *D. vastator* and *D. lamellatus* are similar (Molnár, 1972; Paperna, 1991). *D. vastator* causes severe hyperplasia of the epithelium of gill filaments. Extensive proliferation of the respiratory epithelium of the gills interferes with respiratory functions and may be a direct cause of death. The sites of proliferation are dependent on the monogenean species. *D. vastator* prefers the tips of gill filaments and it causes massive mortality in young fish but seldom on fish greater than 32–35 mm since the functions of the remaining gill filaments are not affected. Massive infestations of *D. extensus* can cause mortality in 4–7 kg broodfish (Paperna, 1991).

Diagnosis

Fish infected with *Dactylogyrus* species are lethargic and usually found swimming on the surface of the water. Heavily infected fish have pale to greyish gills, swollen at the edges, the opercula appear to open wider than normal and secrete excessive amounts of mucus (Christensen, 1989). They are also anorexic and are usually found gasping for air and have abnormal behaviour such as jumping out of the water.

Dactylogyrus are usually found on the gills although in massive infections they are also in the buccal cavity. The parasite can kill directly by damaging gill structures and affecting respiration especially in warm eutrophic waters with low dissolved oxygen. *Dactylogyrus* infections usually result in secondary bacterial infections with subsequent mortality.

The signs and pathology of monogenean infections are not generic nor species specific. Hence, diagnosis of monogenean infection is based on the identification of the pathogen. Correct diagnosis requires proper preparation of specimens. Briefly, gills can either be completely removed or gill clippings taken from the infected fish. Each parasite is removed carefully under a dissecting microscope, placed on a slide, and covered with a coverslip. Excess water is removed and the corners of the cover slip sealed with nail polish to prevent the cover slip from moving (Lim, 1991c). Ammonium picrate is added underneath the cover slip to clear and fix the specimen, which is examined using a phase contrast microscope. Monogenean species are usually identified on the basis of the sclerotized reproductive and haptoral armaments on the cleared and flattened specimens. *Dactylogyrus* are oviparous parasites with or without 4 eye-spots, 14 marginal hooks on its haptor, 2 anchors, 1-2 connective bars and 2 needle-like structures and spindle-shaped dactylogyrid-type of seminal vesicle. The descriptions for the various *Dactylogyrus* for imported carp are in Gussev (1985); Lim and Furtado (1986) and Chinabut and Lim (1993) for silver barb (*P. gonionotus*) and Mizelle and Price (1964) for mad barb (*L. hoevenii*). Other diagnostic techniques (such as immunological) are unknown.

Prevention and control

The main method for control of monogeneans is the application of chemicals. Chemotherapeutic treatments include dips or baths in salt, formalin or organophosphates (Dylox, Dipterex, Neguvon, Chlorophos), Bromex-50 and potassium permanganate (Paperna, 1996; T.T. Dung, personal communication; T.S. Thana, personal communication). The recommended doses and concentrations vary according to host and parasite species as well as physico-chemical properties of the waters. One-hour bath with formalin at 1:4000 (< 10°C), 1:5000 (10–15°C) or 1:6000 (15°C) and bath with 3–5 mg per l of potassium permanganate for 1–2 h (Hoffman and Meyer, 1974) has been recommended. Trichlorfon (Dylox) may be added in the food (50 mg per kg fish) four times at 3 days intervals each month during the critical periods. Lime and other chemicals have been recommended for pond applications. 0.4–0.5 mg per l of trichlorphon (0.0-dimethyl-2,2,2 trichloro-1-hydroxyethyl phosphanate) in Japan (dispersing 0.2 mg per l dimethyl-1,2 dibromo-2,2 dichloroethyl phosphate (Bromex)). These chemicals will be effective if the cages are in ponds. However, they will not be effective for large bodies of water and rivers where cages are usually located. When chemicals prove ineffective most farmers will just destroy their heavily infected fish (personal communication with farmers). Eradication of feral reservoir fish from ponds is possible but not when the cages are in rivers or large lakes. The best alternative management strategy includes good husbandry based on the knowledge of the reproductive biology and ecological requirements of the parasites such as temperature dependency. Using healthy fish fry from reliable hatcheries, limiting stocking density, providing good quality feed and sanitation of nets will help to keep infestation at a low level. Some fish are able to acquire immunity against monogenean infections (Paperna, 1964, 1991) and more studies should be done to see if this could be used in the control of monogenean infections.

Disease caused by *Cichlidogyrus* species

Cichlids are cultured in warm freshwater cages as well as in warm estuarine waters.

Tilapias cultured in freshwater are affected by *Cichlidogyrus* spp., while in marine waters they are infected by marine monogenean, *Neobenedenia melleni*. Tilapia (cichlids) is cultured in cages in freshwater in Indonesia, Vietnam and the Philippines as well as Malaysia. Cichlids are hosts to species of *Cichlidogyrus*, *Onchobdella* and *Enterogyrus* (an endoparasitic monogenean present on Sri Lankan cichlids). Several species of *Cichlidogyrus* and *E. chichlidarium* have been introduced with their fish hosts into the Philippines, Indonesia and Peninsular Malaysia (Lim, unpublished data; Shaharom, 1985). The *Cichlidogyrus* species on tilapia in Indonesia have been incorrectly identified as *Dactylogyrus* species (ADB/NACA, 1991). *Cichlidogyrus* species is also found on tilapia in cages in Vietnam (T.T. Dung, personal communication).

Pathology

As noted by Paperna (1980) and Paperna *et al*. (1984), no report of mortality due to *Cichlidogyrus* species has been described, but *C. sclerosus* were found to cause severe gill damage in tilapias cultured in the Philippines (Kabata, 1985). *Neobenedenia* species found on tilapia in cages in estuarine waters are more pathogenic than *Cichlidogyrus* species.

Diagnosis

The behaviour of the fish can indicate the presence of parasites and this is similar to *Dactylogyrus* infection. However, accurate diagnosis requires removing the gills or gill clippings and the monogeneans collected and prepared as stated above for *Dactylogyrus*. *Cichlidogyrus* could be distinguished from other monogeneans in having a haptor with four anchors, two bars of which one is v-shaped and the other is made up of three parts. To identify the different *Cichlidogyrus* species consult Paperna (1980).

Prevention and Control

As for *Dactylogyrus* infections.

***Trianchoratus* and *Sundanonchus* infections**

Monogenean species belonging to two genera are found to infect the channids. *Sundanonchus* species are restricted to giant snakehead (*C. micropeltes*) while *Trianchoratus* species are found on other channids. Although these monogenean species are found on and known to plague cultured snakeheads, there is no report on mortality due to these monogeneans.

Diagnosis

Methods for collecting and preparation of monogenean species for diagnosis are the same as for the *Dactylogyrus* species above. *Trianchoratus* species has four anchors of which one pair is vestigial, with connective bars, 14 marginal hooks and dactylogyrid-type of seminal vesicles (Lim, 1986), while *Sundanonchus* species, infecting giant snakeheads could be differentiated from the other monogeneans in having four anchors, with two connective bars (dorsal bar may be split into two), 16 marginal hooks, dactylogyrid-type of seminal vesicle and x-shaped vitelline duct (Lim and Furtado, 1985; Kritsky and Lim, 1995).

Prevention and control

As for *Dactylogyrus* infections.

Diseases caused by *Pseudodactylogyroides marmoratae*

Pseudodactylogyroides marmoratae has been found on cage-cultured marble goby (*O. marmorata*), a highly priced fish cultured in cages in Malaysia and Vietnam (Leong and Wong, 1998; T.T. Dung, personal communication). Marble goby (*O. marmorata*) is cultured in cages in Peninsular Malaysia, Indochina and Thailand. However, this fish is no longer cultured in Thailand because of disease problems (ADB/NACA, 1991). Other than the fact that this parasite causes disease, practically nothing is known about the signs, its pathology or how to control this pathogen.

Diagnosis

Monogeneans are usually collected, prepared and studied as described above for *Dactylogyrus*. *Pseudodactylogyroides* (Fig. 6.6) has four anchors, of which one pair is usually underdeveloped and small, the larger pair has a patch-like inner root, two connective bars, 14 marginal hooks and a dactylogyrid-type seminal vesicle (Lim, 1995).

Prevention and control

As discussed above for *Dactylogyrus*.

Diseases caused by Thaparocleidus species

This monogenean is on cultured pangasiids and bagrids in South-east Asia (Lim, 1990; Lersutthichawal, 1999; A. Pariselle, personal communication). As with *Pseudodactylogyroides*, little is known about the pathology caused by this group of monogeneans.

Diagnosis

Thaparocleidus (Fig. 6.7) has four anchors, two connective bars of which one could be whole or separated into two units, 14 marginal hooks and a sac-like seminal vesicle (Lim, 1996).

Prevention and control

As discussed above for *Dactylogyrus*.

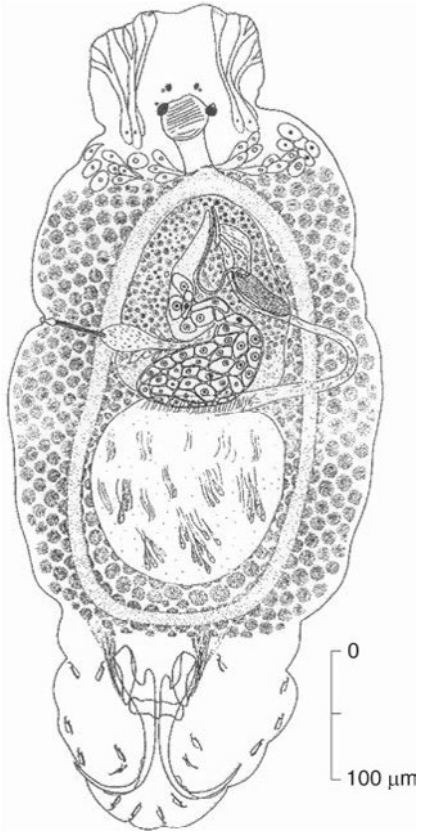


Fig. 6.6. *Pseudodactylogyroides marmarotae* from the gills of *Oxyeleotris marmorata*. Reproduced with permission from Systematic Parasitology.

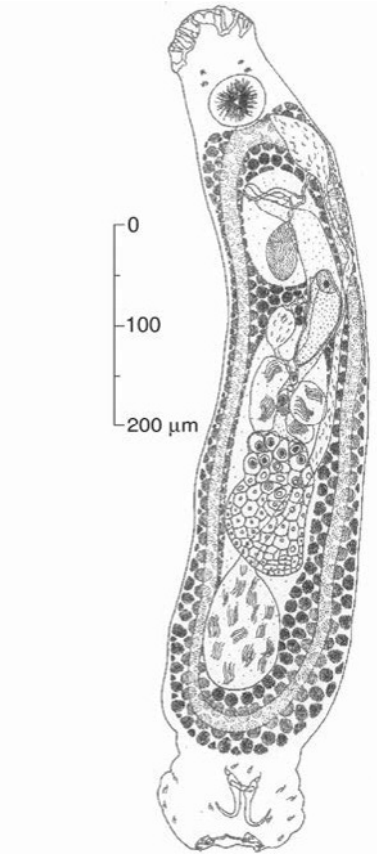


Fig. 6.7. *Thaparocleidus caecus* from the gills of *Pangasius hypophthalmus*. Reproduced with permission from Raffles Bulletin.

Diseases caused by *Gyrodactylus* species

The gyrodactylids are easily differentiated from most mongeneans since they have developing embryos in their uterus (viviparous). The young gyrodactylids do not need to search for a host. Gyrodactylids are able to disengage and re-attach on new hosts especially under intensive culture where fish are in close proximity to each other.

Some *Gyrodactylus* species have wide host specificity and cause fish mortality. Gyrodactylids are easily translocated via the live fish trade, for example *G. turnbulli* is spread from Singapore via the aquarium trade to England, New England States, Nova Scotia and Peru (Cone, 1995). Although the gyrodactylids are important pathogens in warm water culture systems, there is paucity of information on this group. Studies on the pathogenicity of the *Gyrodactylus* were conducted mainly on temperate species (Paperna, 1991; Cone, 1995). In South-East Asia, *Gyrodactylus* is common on *Clarias* spp. such as the *C. batrachus*, *C. macrocephalus*, *C. gariepinus* and the hybrid of *C. macrocephalus* and *C. gariepinus* reared in cages in Thailand (Aqua Farm News, 1993). Paperna (1991) reported *G. rysavyi* and *Macrogyrodactylus* on *C. gariepinus* in Africa. These parasites may have an impact on the future of cage culture of *C. gariepinus* in the Ivory Coast as well as the *Clarias* culture in Thailand, the Philippines and Indonesia. *Gyrodactylus fuscus* has been found on *Clarias fuscus* in North Vietnam. Unidentified *Gyrodactylus* spp. infects cage-cultured mad barb (*L. hoevenii*) and silver barb (*P. gonionotus*) in Indonesia (Christensen, 1989). *Gyrodactylus* infests tilapia cultured in fresh water and brackish waters (Natividad *et al*., 1986).

Pathology and clinical signs

Gyrodactylus are usually found on the skin and fins, although there are species on the gills. These parasites are also found in conjunction with protistan and bacterial infections.

Heavily infected fish secrete copious amounts of mucus secretion, their fins become frayed, skin ulcerated, and gills damaged by the feeding and attachment processes of the worm. They also exhibit abnormal behaviour which includes rubbing against the net, anorexia, hyperproduction of skin mucus, haemorrhagic ulcers on the body sides, fin rot (mainly anal and caudal fins), thickening and opacity of the eye cornea. At this stage, it is easy to detect parasites on the eyes, skin and fins. The skin usually appears whitish. At the later stage of infections, reddish inflamed areas develop on the skin and the eyes may become opaque and blind (Christensen, 1989).

Diagnosis

Initial diagnosis can be based on clinical signs and confirmation by detection and examination of the parasite. The anterior region of the gyrodactylid is divided into two lobes with two sets of head glands. Its haptor is armed with 16 hinged, marginal hooks, two anchors and two connective bars. *Gyrodactylus* species are difficult to identify (Paperna, 1991). The body size, excretory systems, dimensions and morphology of the sclerotized parts (reproductive spines, anchors, marginal hooks, connective bars) are important criteria for species differentiation (Malmberg, 1970). *Gyrodactylus* species in the tropical regions are poorly studied and more investigations are required.

Prevention and control

A formalin bath using 20–25 ml of 40% formalin in 100 l water for 30 min in well aerated, clean water is effective in getting rid of an infection. Other formulations include formalin at 1:2000 for 10 min and ammonia solution at 1.5 ml ammonia per 1. The latter two reduce infections but do not eradicate them. Trichlorphon (0.25 mg per l) is also effective (Meyer, 1968).

Diseases Caused By Other Helminths

Although there are pathogenic trematodes, nematodes, cestodes and acanthocephalans in tropical aquaculture (Paperna, 1996), the pathogenic species causing disease in tropical cage culture systems are unknown. For instance, trematodes and cestodes have been

found in cage cultured *Pangasius bocourti* and in snakeheads in Vietnam but their identities are unknown (T.T. Dung, personal communication). With the introductions of more exotic fish (from where?) into tropical waters there will likely be reports of helminthic parasites in the future. The impact of helminthic infections is unknown. The acanthocephalan *Gorgorhynchus ophicephali* has been detected in intestines of feral giant snakeheads and in some fish there were intestinal perforations (personal observation); however, the impact of the parasite on cultured giant snakeheads is unknown. Since there are no reports of massive fish mortalities caused by cestodes, trematodes or nematodes, the following sections will briefly deal with known helminth infections in fish that are cultured in cages in warm waters.

Trematode infection

Sanguinicola (blood fluke) has been recorded on exotic cultured grass carp and bighead carp (Anderson and Shaharom-Harrison, 1986). Thus far, no *Sanguinicola* has been reported on clariids of South-east Asia although *Sanguinicola dentata* is found on *C. lazera* (now known as *C. gariepinus*) from Africa (Paperna, 1996) and this species has been imported into Thailand for culture purposes. Metacercariae causing 'black spots' in cichlids and clariids in Africa (Paperna, 1996) could spread to other tropical waters. Kabata (1985) noted the presence of clinostomatids and heterophyids in farmed fish in the warm waters, but thus far none has been reported among cage cultured fish.

Nematode infection

Nematodes are common on feral as well as food fish (Kabata, 1985). The nematode, *Anguillicola crassa,* could become important since its host, *A. japonicus,* is cultured in Taiwan and on a smaller scale in Indonesia. The other nematode of importance is *Philometroides cyprini* in common carp (Paperna, 1996). Camallanids are common on feral catfish (Lim, unpublished data; Kabata, 1985) but little is known about their effect in cage culture.

Cestode infection

The adult Asian tapeworm, *Bothriocephalus acheilognathii* causes mortality in heavily infected grass carp in Europe (Paperna, 1996). This tapeworm is not confined to cultured imported carp but has spread to native fish in warm waters of Asia (Peninsular Malaysia) and Israel with the imported grass carp, silver carp and bighead carp (Shaharom, 1985 ADB/NACA, 1991; Paperna, 1991, 1996). Paperna (1996) has provided a detailed account of the disease caused by this cestode species. Cestodes are also present in cultured and wild fish in warm waters. *Lytocestus* spp. are found in cultured and wild walking catfish (*C. batrachus*), while *Senga* spp. are found in cultured and wild snakeheads, *Channa* spp. (Furtado, 1963; Furtado and Lau, 1971; Furtado and Tan, 1973).

Cestode infections in fish and resulting mortality are sporadic. Fish infested with intestinal (adult) cestodes have retarded growth, erratic swimming behaviour, distended abdomen, become emaciated, cease to feed, develop a haemorrhagic enteritis caused by the destruction of the intestinal epithelium and heavily infected fish have varying degrees of aseptic dropsy (Paperna, 1996). The cyclopoid copepod is the intermediate host, and the cestodes could be an important pathogen in cage culture systems since fish are in intimate contact with the environment.

Diseases Caused By Parasitic Arthropods

Lernaea and *Ergasilus* spp. (Copepoda), *Argulus* (Branchiura) and *Alitropus* (Isopoda) have been recorded on a wide range of cultured fish species (Kabata, 1985; ADB/NACA, 1991). *Lernaea* and *Argulus* cause the most problems in warm-water aquaculture in South-east Asia and India. The parasites were introduced into these countries via fish importation (ADB/NACA, 1991) and will be discussed in detail below.

The isopod, *Alitropus*, is another common arthropod in aquaculture systems (Fig. 6.8) and is associated with poor fish growth and increased fish mortality (Lim, unpublished data; Lester and Roubal, 1995). It could be a potential pathogen in warm freshwater cage cultures. However, nothing much is known about its impact in aquaculture. Infestation of the copepods, *Ergasilus*, besides causing cage-cultured fish to lose weight and appear unsightly, cause gill damage and in heavy infestations result in gill dysfunction (Kabata, 1985). *Ergasilus* was recorded to cause fish mortality in Indonesia, especially in young fish (ADB/NACA, 1991). *Ergasilus* is a common crustacean parasite of fish and a potential pathogen in cage-culture systems, however little is known about its ecology or pathology.

Lernaea infections

Lernaea or anchor worm causes the most damage in warm freshwater fish and is usually associated with high fish mortality. *Lernaea* species seem to prefer warm waters of 26–30°C (Shields and Tidd, 1974). Although it is known that this parasite causes disease in cage cultured fish in South-east Asia, the extent of its impact and damages to aquaculture has not been estimated (Kabata, 1985).



Fig. 6.8. *Alitropus* species (Isopoda) found on the skin of *Channa micropeltes*. Image courtesy of K.S. Liew.

Pathology

Lernaea cyprinacea is distributed widely with the global translocation of carp and is now recorded in 45 species of cyprinids as well as in other orders of fish especially the siluriforms (Lester and Roubal, 1995). *Lernaea* is in India, Nepal, Bangladesh, Thailand, Indonesia, Peninsular Malaysia, Vietnam, China and Japan (ADB/NACA, 1991). Lernaeosis occurs in China on silver carp, bighead carp, grass carp and black carp; in India and in Bangladesh on all the major carp; in Vietnam on bighead carp, grass carp, silver carp, common carp, crucian carp, snakehead and in Indonesia on common carp, silver barb, spotted gourami, mudfish and catfish. In 1976, these parasites reached epizootic levels destroying about 30% of fish in over 7500 ha of ponds, ricefields and open waters in West Java and North Sumatra (ADB/NACA, 1991). In South-east Asia, *Lernaea polymorpha* is found on bighead carp and silver carp (Shariff and Sommerville, 1986).

Haemorrhaging and gross lesions occur at the site of *Lernaea* infections and are associated with bacterial and other secondary infections. There are relatively few studies on the effects of anchor worm infection on the fish hosts in warm waters. Some authors suggest that the attached females feed on host blood, while others suggest that they probably ingest host cells and absorb tissue fluids (Egusa, 1992). Lester and Roubal (1995) provided detailed information on the other signs associated with *Lernaea* infections and these include blindness, epidermal and dermal necrosis and haemorrhage, and encapsulation of the embedded horns of *Lernaea*. Copepodids of *Lernaea* may cause disruption and necrosis of the gill epithelia and large numbers of larvae on the gills may cause fish mortality. Lesions caused by penetration of metamorphosing females are generally associated with punctuate haemorrhage and

muscle necrosis is evident at the point of penetration of parasites (Khalifa and Post, 1976). Penetrating female *L. polymorpha* causes punctuate haemorrhage in bighead carp causing mortality in heavily infected fish (Shariff and Sommerville, 1986). *Lernaea cyprinacea* in the eyes cause blindness.

Diagnosis

There are over 40 species of pathogenic *Lernaea* (Kabata, 1983), but in most outbreaks, the specific identity of the parasite is unknown. *Lernaea* spp. is macroscopic and easily seen with the naked eye on the surface of fish. Only females of *Lernaea* are parasitic and are highly modified so these do not resemble free-living copepods. Adult *Lernaea* females have their anterior end embedded into the body musculature of its host, while their long rod-shaped body with two egg sacs protrudes outside the host tissue. The anterior head region is modified as a small hemispherical cephalothorax, which contains the mouth, with a well-developed holdfast, bifurcate dorsal process and simple ventral process (anchor). The anterior region might even penetrate into the body cavity and embed into visceral organs. *Lernaea* spp. are distinguished by the shape of the anterior anchors that may be modified by bone or other structures encountered during development in their host tissue. *Ergasilus*, is recognisably a copepod with a second antenna modified for attachment and a pair of multiseriate egg sacs arising from the genital segment (Lester and Hayward, 2006).

Prevention and control

Several chemicals are recommended but their efficacy requires further careful testing (Kabata, 1985; Egusa, 1992; Paperna, 1996). Kasahara (1962) effectively used Dipterex (organophosphate trichlorphon) to control and eradicate the larval stages of *L. cyprinacea* in the water column. At temperatures of 20–27°C, concentrations of 0.5 mg per l and 0.2 mg per l kill the nauplii in 1 and 2 days, respectively. Copepodid stages are killed in 24–36 h at 0.2 mg per l and in 12–18 h at 0.5 mg per l at 20°C, but Dipterex is not effective on adult females. However in cages located in rivers or large lake systems, the use of chemicals is ineffective and dipping fish in chemicals seems to be insufficient to get rid of all the copepodid stages (Lester and Roubal, 1995; Lester and Hayward, 2006).

***Argulus* infections**

The majority of the branchiurans are freshwater parasites (about 75% of the 120 species of *Argulus*), with few estuarine or marine species (Kabata, 1985). *Argulus* or fish louse (Fig. 6.9) is macroscopic and easily observable on the skin and fins and also in the oral cavity. Infected skin becomes opaque with frayed fins. This ectoparasitic crustacean feeds on the mucous layer, flesh and blood of the fish. The prolonged feeding and strong attachment of *Argulus* by its suckers onto the host results in direct mechanical damage to the skin, disruption in epithelial structure, resulting in lesions and subsequent invasions by opportunistic pathogens such as pseudofungi (Singhal *et al*., 1986; van der Salm *et al*., 2000). There are at least four species of argulids (*Branchiura, Argulidae*) which are economically important as parasites of fish in warm freshwater aquaculture, viz. *Argulus japonica*, *A. foliaceus*,



Fig. 6.9. *Argulus* species (Branchiuran) found on the skin of *Channa micropeltes*. Image courtesy of K.S. Liew.

A. indicus and *A. siamensis* and these have been introduced along with their cyprinid hosts and are now reported from both local indigenous cyprinids and non-cyprinid hosts in the introduced areas (Paperna, 1991). *Argulus japonica* is in Israel, while *A. foliceaus* is in Thailand, Peninsular Malaysia and Sri Lanka on carp and native cyprinid species (Kabata, 1985). *A. indicus*, an Asiatic species is on anabantids, chanids, tilapias and native cyprinids in Indonesia, Thailand and India. *A. siamensis* is reported in Thailand from anabantids and *Cirrhina* spp. (Gopalakrishnan, 1968; Kabata, 1985) and in India on a snakehead species (*Channa gachua*) (Ramakrishna, 1951). *A. japonica* is an important parasite of warm freshwater fish, while *A. coregoni* parasitized cold freshwater fish (Lester and Hayward, 2006).

Pathology

This parasite is not host specific and is found on a wide range of fish species from cyprinids to siluriforms and perciforms (see above). The life-cycle of the parasite is direct and the egg hatches into free-swimming larvae that must find a host within 2–3 days. It is reported to cause massive mortality of fish in Bangladesh and in the majority of cases the outbreaks were seasonal, usually in the colder months (Kabata, 1985). *Argulus* usually infect the young fish from spring till early summer. The parasite is also common in India affecting the major Indian carp especially *Rohu* spp. In Peninsular Malaysia, argulids have been found on wild fish such as *C. micropeltes* (Lim, unpublished data), the imported fry of bighead carp and grass carp (Shaharom, 1988). *Argulus* spp. are found on the sand goby and snakeheads in cages in Vietnam (T.T. Dung, personal communication). Heavily infected fish are lethargic, listless, cease to feed and rub themselves on the substrate in attempts to dislodge the parasite.

The lesion or wound made by the feeding *Argulus* may be restricted to the epidermis or may penetrate through to the stratum spongiosum of the dermis and even stratum compactum turning the dermis oedematous (Lester and Roubal, 1995). The area may become necrotic with secondary bacterial and fungal infection. Mortality may be associated with changes in the ionic and osmotic homeostasis, anorexia and secondary infections. Kabata (1970), Paperna and Zwerner (1976) and Paperna (1980) noted that lytic and toxic substances secreted into the dermal area while feeding cause acute haemorrhagic, inflamed wounds. *Argulus* feeding on blood causes fish to become anaemic and its piercing proboscis stylet causes haemorrhagic spots on the epidermis. The spots are formed by epidermal hyperplasia. Bacterial infections occur around the site of infection. *Argulus* may also be vectors of viral infections. Ahne (1985) showed that spring viraemia of carp (SVC) was transmitted by *A. foliaceus* and in Israel, carp pox (carp papilloma) occurred in conjunction with *A. japonica* infestation (Sarig, 1971).

Diagnosis

The parasite is oval-round, dorso-ventrally flattened (about 4–8 mm in diameter), with a pair of modified sucker-like first maxillae. Its proboscis or feeding organ is for inserting into the epidermis and the underlying tissue of the fish hosts to feed on blood.

Prevention and control

Several chemicals, especially organophosphate insecticides formalin, chlorine, sodium chloride, even antimalarial drugs are recommended (Kabata, 1985; Egusa, 1992; Lester and Roubal, 1995; Paperna, 1996), but their efficacy in different types of waterbodies are unknown. Studies carried out in warm waters of Israel and Africa show that some (see below) of the insecticides are effective in killing argulids with safety margin for fish (Paperna, 1996). Lindane has been used to clean fish of argulids prior to marketing (Paperna, 1996). The chemicals in use are gemmexane (this is toxic to fish and man), Pyrethrum (Paperna, 1996), Dipterex, tricholorphon, Neguvon, malathion, formalin and antimalarial drugs such as quinine hydrocholoride (Puffer and Beal, 1981; Kabata, 1985; Singhal *et al*., 1986). However, not all the chemicals are equally effective for

the different developmental stages of argulids. For example, Dipterex is effective against the adults and larvae causing them to fall off the fish and die but has no effects on the eggs (Egusa, 1992). The water chemistry and temperature are important factors in the use of these chemicals. The occurrence of these parasites, despite the amount of chemicals used, indicates that the eggs are still in the system and that the chemicals used are not effective in destroying the eggs. The strategy of not stocking the ponds until the larval stages have died could be effective. Other methods of control include the use of substrates such as wooden slats to trap eggs, filtering incoming water to remove larval stages, stocking clean fish, quarantine incoming fish with treatment if necessary before stocking, and stocking with argulid-predatory fish (Kabata, 1985).

Conclusions and Recommendations for Future Research

Microorganisms and parasites are normal flora and fauna inhabiting the skin, fins, gills and the gastrointestinal tract of fish. Under normal conditions, many of these organisms do not induce disease in their fish host. However, man-made pollutants and/or intensification of fish culture results in increased environmental changes, which may be stressful to fish. Bacterial multiplication, for instance, is enhanced with increasing organic matter from uneaten feeds. The stress predisposes fish to invasion by opportunistic pathogens and subsequent morbidity and mortality. Stress is also associated with handling, stocking, grading and shipping of fish (Plumb, 1999b).

Fish mortality is often attributed to several factors (e.g. fish condition, pathogens and environment) and it is difficult to determine the significance of any one of these factors (Mitchell, 1997). Recently, viral pathogens caused fish epizootics which are associated with significant mortalities in cage-cultured fish. Similarly, some bacterial pathogens are equally virulent. However, parasites may not have a direct effect on fish mortality but they debilitate the fish and make them more susceptible to other pathogens (Ahne, 1985; Shoemaker *et al*., 2012). In addition, their organs of attachment usually create portals of entry for viral, bacterial and pseudofungal pathogens of fish. In addition, some parasites are reservoirs of viral pathogens. Related developments are reports of multiple infections such as the case of EUS and multiple bacterial pathogens' interaction in enhancing disease (Lio-Po, 1998; Ly *et al*., 2009). The impact of climate change on the epidemiology of emerging fish diseases should also be considered.

Despite the long history of aquaculture in the tropics and the importance of disease in aquaculture there have been little concerted efforts to document and investigate the diseases of fish cultured in cages and ponds. This may be attributed to lack of trained manpower and lack of institutional support. The diversity of fish cultured in warm waters does not help to alleviate this problem. The usual approach to disease and health management is to use chemicals (usually indiscriminately) or if this does not work to discard the fish species and start afresh with another species. It was only in recent years that reporting of fish death and fish disease outbreaks in many of the developing and underdeveloped countries became mandatory through the auspices of the Network of Aquaculture Centers in Asia (NACA) and the World Organisation for Animal Health (OIE). In some instances, fish are imported and stocked without quarantine. It must be considered that the movement of fish, especially across international boundaries, may transfer fish pathogens as well (Hedrick, 1996; Lavilla-Pitogo and Nagasawa, 2004). In this regard, the provisions on the Aquatic Animal Health Code (OIE, 2013) should be adhered to. As such the essential role of biosecurity in disease prevention cannot be ignored.

The lack of institutional support results in reduced research on pathogens and consequently an inability to control and prevent diseases. There is also lack of trained personnel in disease management and little reliable information on the specific identity of pathogens. Trained competent fish disease managers, who are able to diagnose diseases, detect

pathogens, and are capable of dispensing proper prevention and control measures are important to sustain aquaculture. A related issue is the lack of legislation and guidelines pertaining to the use of drugs and chemicals in aquaculture. Currently, drugs and chemicals are used indiscriminately and usually the aetiologic agents are not identified without any specific withdrawal period prior to the sale of the fish, notwithstanding the public health implications of drug resistance. Bear in mind that early detection and prompt diagnosis of fish infections are key steps toward application of effective control measures. As such, the development of more sensitive and rapid DNA-based techniques such as PCR/RT-PCR, multiplex PCR, LAMP, microarray analysis, 16S rRNA analysis and macrorestriction analysis for pathogen screening, characterization and identification can greatly enhance prompt diagnosis of infections (Gray *et al*., 1999a; Crumlish *et al*., 2002; Seng *et al.,* 2004; Bercovier *et al*., 2005; Yuasa *et al*., 2005; Yoshino *et al*., 2006; Bartie et al., 2012; Shi *et al*., 2012; Shoemaker *et al*. 2012).

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7 Non-infectious Disorders of Warmwater Fish

Florbela Soares,[1]* Ignacio Fernández,[2] Benjamín Costas[3] and Paulo Gavaia[2]
[1]*IPMA – National Institute for the Ocean and Atmosphere, Olhão, Portugal;* [2]*CCMAR – Centre of Marine Sciences (CCMAR/CIMAR-LA), University of Algarve, Campus of Gambelas, Faro, Portugal;* [3]*CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal*

Introduction

The aquaculture sector has expanded, intensified and diversified in the past decade. This expansion has mainly been due to breakthroughs in research and development, compliance with consumer demands, and improvements in aquaculture policies and governance. Advances in aquaculture research and development in Europe have been remarkable, in particular the improvements in the efficiency of production systems and the quality of the fish produced, as well as mitigating environmental impacts. Examples of new technologies include: the increase in types of recirculation systems; development of cages and nets that can be used in higher energy locations; and the development of integrated multitrophic production systems. These technological advances have strengthened the field and allowed diversification. Several new species have emerged in Mediterranean aquaculture and they include meagre (*Argyrosomus regius*), tuna (*Thunnus thynnus*), white sea bream (*Diplodus sargus*) and sharp snout sea bream (*Diplodus puntazzo*). Some species such as European seabass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) have reached production volumes sufficient for the development of stable markets.

In warmwater fish species, mainly European seabass and gilthead sea bream, the dominant intensive ongrowing systems are floating sea cages. These may be used in lagoons, sheltered bays or semi-exposed and offshore conditions (EFSA, 2008). Juveniles (about 2.5–5.0 g) are usually transferred from the pre-growing tanks into the sea cages. However, for the gilthead seabream it is possible to stock juveniles at smaller (0.3–2 g) or, more recently, at larger sizes (8.0–10.0 g). Farmers prefer to stock fish of 2.5 to 150 g at a density of 5 to 10 kg per m^3 and older fish (>150 g) at a density of 10 to 20 kg per m^3. Initially cages used to be placed at well-protected, largely enclosed sites. Issues related to oxygen deficits and cage fouling during the summer months, coupled with the scarcity of suitable sites and questions related with the management of the coastal zone, resulted in the development and implementation of offshore cage technology. Still, the ability to control offshore environmental conditions is very limited. Interventions related to routine management

* E-mail: fsoares@ipma.pt

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during the intensive ongrowing phase in sea cages (e.g. antifouling treatments and noise), development of morphological-anatomical abnormalities and other anthropogenic factors such as pollutants and contaminants, will have a significant impact on the farm revenue. European seabass and gilthead sea bream are exposed to disturbances that may cause stress during ordinary farming procedures. Potential stressors include handling and manipulation, cleaning routines, grading, crowding and confinement, transportation between units, prophylactic measures and use of chemicals. The presence of predators, boats and divers are also factors of disturbance for ongrowing fish kept in sea cages. All these conditions are a matter of concern among fish farmers since tertiary stress responses may take place. These responses to chronic stress extend to the level of the organism and population and refer to aspects of whole-animal performance such as changes in growth (Ellis *et al*., 2012).

Commercial feeds have improved and are now produced by major manufacturers in highly sophisticated facilities. Although nutrient deficiencies and toxic contamination may occur, they are less frequent. Therefore, nutritional imbalances or excess of particular nutrients can lead to undetected subclinical disorders such as slower fish growth, losses due to disease, and other more baffling problems when a new fish species is cultured. The dependence on fishmeal has been reduced for several species, i.e. its replacement with plant-protein ingredients varies from 50–95% without jeopardizing growth performance and feed utilization in European seabass, gilthead sea bream, sharp snout sea bream, black sea bream and meagre (Kaushik *et al*., 2004; Dias *et al*., 2009; Mérida *et al*., 2010; Estévez *et al*., 2011; Ngandzali *et al*., 2011). However, alternatives such as soybean, corn meal and many other protein sources have not been sufficiently adjusted to meet fish requirements, reducing the content in anti-nutrients (natural or synthetic compounds that interfere with the absorption of nutrients) such as phytosterols and protease inhibitors (Krogdahl *et al*., 2010).

Other issues such as inbreeding or climate change are also matters of concern. Aquaculture in temperate zones will be more affected by waters warming to levels that will exceed the physiological limit for many farmed species. The increase in extreme weather events (e.g. storms) may affect aquaculture through physical destruction of aquaculture facilities, with increased risks in farms located in more open and exposed sites; while the appearance, spreading, incidence or mortality due to pathogenic diseases could be also increased due to climate change (Harvell *et al*., 2002; Pounds *et al*., 2006; Lafferty, 2009). Both situations might imply loss of stocks. Moreover, little is known about the influence of fish domestication and/or selection on behaviour and adaptation within the context of fish culture. European seabass and gilthead sea bream industry has been based on empirical criteria for genetic selection and systematic genetic improvement programmes have only recently been implemented (Navarro *et al*., 2009; Borrell *et al*., 2011).

The purpose of the present chapter is to give an overview on aspects of current finfish cage culture, such as environmental and anthropogenic related problems, stress and nutritional imbalance and the development of abnormalities which may affect growth and increase disease susceptibility.

Disorders

Environmental and anthropogenic related problems

Since the outgrowing phase in warmwater fish species is mainly done in offshore locations, fish farmers are not able to control environmental conditions and their success relies on environmental factors. This is in contrast to hatchery and nursery phases where farmers have tight control over most of the environmental factors. There are bidirectional relationships between cage aquaculture and environment, which may limit the growth of this economic activity. Environmental factors may impact fish physiology while some aquaculture activities may lead to environmental impacts that can negatively affect the fish. For example, the feeding activity

(rates and regimens) may increase water eutrophication through increase of phosphorus. In addition, several anthropogenic activities have been found to affect aquaculture suitability such as vessel traffic or waste water flow from urban communities, intensive agriculture industry or antifouling treatments.

We will briefly discuss environmental factors (water temperature, dissolved oxygen, ammonia concentration and algal blooms), anthropogenic activities that modify environmental conditions (presence and concentration of pollutants, underwater noise, fouling processes and antifouling treatments) and fish physiology (vaccination procedures) that affect finfish in cage culture.

Water temperature

Inappropriate water quality can cause serious problems (e.g. high mortalities) to fish, particularly under intensive farming conditions. These problems depend on fish species, time and level of exposure and potential synergy with other coexisting factors (Vatsos and Angelidis, 2010). Water temperature is an important factor since fish are poikilotherms, the exception is tunafish species that can thermoregulate in a limited manner (Kubo *et al*., 2008). Growth and other key factors including feed intake, feed conversion rate, nutrient and energy retention and plasma enzymology are affected by temperature (Glencross and Rutherford, 2010). For example, gilthead sea bream cage culture is mainly located in southern Europe and Mediterranean where water temperatures are higher and are better thermal regimes for this species. However, European seabass do better in north-eastern Mediterranean coasts where average temperatures are lower throughout the year (Jover *et al*., 2009). Using a fish growth model, Hernández *et al*. (2007) showed a positive influence of the average water temperature over the optimal harvesting time and that the optimal food pellet size is related to water temperature. Recent research has demonstrated that 'thermal unit growth coefficient' (TGC) varies throughout the growth cycle of gilthead sea bream. Briefly, the growth rate is determined by temperature if the fish is below or above 117 g (Mayer *et al*., 2012). This proposed TGC model could be an important tool for fish farmers since it would help to decide on when, how and which fish sizes should be stocked to get better timing and management of cage units.

Suboptimal rearing temperature is easily detected by direct observation of fish since they decrease swimming activity at lower water temperatures (Hasler *et al*., 2009). This reduction of swimming activity is more pronounced during early winter as several fish species have a compensatory response in terms of contractile properties, myosin ATPase activity, proportion of red fibres in their axial musculature, and levels of aerobic enzymes in their musculature that will help temperature acclimation (Guderley and Blier, 1988). An alternative diagnosis involves feed intake analysis – lower feed intake with decreasing temperatures. In contrast, fish at temperatures higher than its optimum exhibit hyperventilation and the consequent higher metabolic rate. Recent work also showed that higher rearing temperatures increased the oxidative stress in European seabass. It was proposed that malondialdehyde and catalase activities could be good biomarkers of excessive rearing temperature (Vinagre *et al*., 2012).

A range of histopathological lesions could be induced due to inappropriate rearing temperatures. They include an abnormal proportion of red fibres in the axial musculature (Guderley and Blier, 1988). In order to prevent water temperature problems during the production cycle, a good selection of the location for cage aquaculture is critical and should be based on sound knowledge of water temperature regimes throughout the year. However, the site for offshore aquaculture is also affected by other coastal activities (e.g. marine industries such as harbours or tourism). The control of the impacts of suboptimal water temperature on fish production should be a well-programmed management activity which takes into account fish size and stocking time, feeding regimes and harvesting time.

Dissolved oxygen

Normal levels of oxygen (normoxia) for marine fish are 4–8 mg per l with lower and

higher values defined as hypoxia and hyperoxia, respectively (Shultz *et al*., 2011). Aquatic hypoxia takes place when water temperatures rise (mid-day during the summer), cloudy weather, early mornings due to oxygen depletion by algae during the night (Noga, 2000), excessive feeding, low seawater circulation, increased net fouling and/or with organic pollution, eutrophication and algal blooms. Also, hypoxia is a major stress factor (Karim *et al*., 2003) and has been implicated with the development of vertebral deformities (Castro-Sanchés *et al*., 2011), resulting in considerable economic losses to the industry. This problem is likely to be exacerbated in the coming years, partly because of increasing human activity such as coastal construction that changes the current and mixing patterns of water. Hypoxia is proposed as one of the greatest risks in tuna farming (Nowak, 2004). Low oxygen conditions result in hyperventilation in an effort to pass more water over the gills to obtain oxygen (Shultz *et al*., 2011). As a consequence fish under hypoxia conditions have ionic disturbances and an accumulation of lactate (Vanlandeghem *et al*., 2010), that reduces the ability to burst swim (Kieffer, 2000). Moreover, adult European seabass exposed to hypoxia conditions showed significantly lower ascorbate and α-tocopherol levels (Di Marco *et al*., 2008). Hypoxia can also affect growth, feed consumption and feed conversion efficiency (Pichavant *et al*., 2001), as well as affecting the immune system (Henrique *et al*., 2002) and making fish more susceptible to opportunistic pathogens. In contrast, hyperoxia reduces ventilation rates (Shultz *et al*., 2011) and the most serious consequence is an inability of fish to excrete wastes such as carbon dioxide (CO_2). High levels of circulating CO_2 cause respiratory acidosis and nephrocalcinosis (Gómez, 2000). Hyperoxia and high CO_2 levels could happen under intensive fish rearing conditions (e.g. in tanks), but none of those conditions have been reported in cage culture.

Aquatic hypoxia conditions in cage culture could be easily prevented by regular monitoring of dissolved oxygen, selecting cage sites with good water currents (Burt *et al*., 2012) and lower anthropogenic impacts through agriculture and urban activities (eutrophication and organic pollution). Reducing stocking densities (Pichavant *et al*., 2001), feeding ration and timing, as well as cleaning net cages to reduce fouling are recommended procedures to reduce the impacts of hypoxia on fish.

Ammonia concentration

Another important environmental factor is ammonia in the water. Ammonia and urea are the two main end products of nitrogen metabolism in fish. Increased levels of ammonia under intensive culture conditions can have a big impact on fish survival and growth (Person-Le Ruyet *et al*., 2003). Vatsos and Angelidis (2010) give a detailed description of its toxic effects on fish and how its levels in seawater depend on different environmental factors. In brief, ammonia could be more toxic in recirculating systems, particularly when the pH is high, which is not mostly the case in cage culture, where the pH is almost constant (between 7.8 and 8.2) with a sustained water circulation. Unionized ammonia concentration of 0.5 mg per l can also be harmful to finfish and crustaceans (Santacruz-Reyes and Chien, 2009). Average values of NH_4-N range between 0.60 ± 0.56 μM and 0.73 ± 0.60 μM in Mediterranean regions with low anthropogenic impact (Faragallah *et al*., 2009) are considered maximum values for finfish cage culture. Therefore, seawater ammonia content should not be considered a major environmental factor impacting cage culture, however since ammonia excretion mainly depends on the amount of protein content in fish feeds and the metabolic efficiency of the species (Vatsos and Angelidis, 2010), as well as local intensive agriculture outputs and water current regimes, fish farmers should pay attention to these factors in order to minimize the risk of an increase in ammonia levels.

Pollutants

Diverse human land-based and coastal activities have influenced the existence and distribution of pollutants, and undoubtedly increased and spread these to the aquatic environment. When pollutants enter this environment, they may lead to large-scale and sudden mortality of animal life, especially when the

compounds are in high quantities (e.g. large-scale releases of hydrocarbons from Exxon Valdez or Prestige oil spills). However, lower quantities can have an impact on marine organisms and may result in immunosuppression, physical damage to gills and epithelia, and adverse effects on metabolism, as well as increasing susceptibility to various infectious diseases. Several diseases including epidermal papilloma, fin/tail erosion, gill disease/hyperplasia, liver disease, neoplasia, parasitic diseases, skin disease/ulceration and viral infections have been linked to pollution (Austin, 2007).

The potential adverse effects of pollutants in farmed fish depend on fish size and species, exposure time and source of the pollutants, as well as on the environmental conditions (e.g. dissolved oxygen or pH). In addition, some species have the innate ability to accumulate heavy metals regardless of whether or not the habitat is contaminated, as it seems to be the case of copper or mercury accumulation in mullet (*Mugil cephalus*) and tuna, respectively. In addition, those adverse effects also depend on the type of pollutant (Austin, 2007). Metals, pesticides and hydrocarbons are taken up by fish through the gills, digestive tract and body surface and are important inducers of oxidative stress in aquatic organisms. Therefore antioxidant enzymes (such as metallothioneins or superoxide dismutase, catalase, glutathione peroxidase, and glutathione-s-transferase enzymes) could be used as biomarkers for oxidative stress produced by pollutant exposure. More than 90% of the heavy metal load in coastal aquatic systems are bound to suspended particulate matter and sediments; consequently, they are not available to pelagic fish and are effectively recycled back into the overlying water phase through a variety of biological (e.g. activities of bottom-dwelling fish) and physicochemical processes (e.g. pH changes, sediment oxidation, and heavy metal complexion by anions like chloride; in Sylaios *et al*., 2012).

Pollutants found in farmed fish could be the result of the presence of contaminants in the rearing water, coming from local sources such as aquaculture activities, as could be the case of copper used in some antifouling treatments (Varvarigos, 2007) or from neighbouring pollution sources such as industrial, agricultural or urban waste water discharges. In some cases, the contaminant concentration is increased with the trophic level (biomagnification), which occurs at different rates depending on the physicochemical properties of the different contaminants (Harmelin-Vivien *et al*., 2012). Worryingly persistent organic pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides and polybrominated diphenyl ethers (PBDEs), have been found in high and moderate concentrations in the feed and in salmon (Jacobs *et al*., 2002). Similarly, organochlorine pollutants were found in farmed, feed and wild gilthead sea bream from the Western Mediterranean (Spain) (Serrano *et al*., 2008). White sea bream and European seabass also have low levels of organochlorine (Ferreira *et al*., 2008; Schnitzler *et al.,* 2008). Interestingly, concentrations of organochlorine compounds in gilthead sea bream tissues were strongly correlated with seasonal changes and with the biological cycle of the species (Blanes *et al*., 2009). Nacher-Mestre *et al*. (2010) clearly showed that changes in diets led to different pollutant content in fish. In that study, gilthead sea bream was fed throughout a full production cycle (14 months) with feeds containing different proportions of fish oil replaced by vegetable oil, followed by a finishing phase with fish oil based diets. At the beginning of the study, fish had different levels of PAHs, which decreased after 330 days when fed with fish oil partially replaced with vegetable oils. This 'wash-out' of pollutants was confirmed in European seabass exposed to light cycle oil, a refined product of heavy fuel oil (Bado-Nilles *et al*., 2011). Fish showed severe external lesions (tissue necrosis, suppurative exudate and haemorrhagic areas) 3 days after the beginning of the recovery period. In addition, there were reduced phagocytic activity and lysozyme concentration, suggesting some degree of immunosuppression (Bado-Nilles *et al*., 2011).

In addition to diets, the rearing system also affects the level of pollutants in fish. Trocino et al. (2012) recently reported in European seabass that the levels of some contaminants such as dioxin-like polychlorinated

biphenyls and heavy metals (such as copper and mercury) were different if the fish were reared in extensive coastal lagoons, intensive inland basins and intensive sea cages.

A careful design of the facilities, the selection of the cage sites, use of specialized equipment, selection of feed and feed composition and the application of genetic selection programmes can minimize or even eliminate the effects of contaminants in fish (Vatsos and Angelidis, 2010). As an example, selecting the proper antifouling treatment in sea cages (see below), will reduce or eliminate the already reported exceed risk for algicides concerning seawater organisms (Muñoz *et al*., 2010). However, it is important to note that until now all residues and contaminants detected in farmed fish species are low and always below the guidelines recommended for human consumption (Ferreira *et al*., 2008; Blanes *et al*., 2009; Muñoz *et al*., 2010; Padula *et al*., 2012; Trocino *et al*., 2012).

Fouling and antifouling treatments

Offshore aquaculture requires an infrastructure that invariably consists of a complex assortment of submerged components: cages, nets, floats and ropes. The presence of such large and diverse surfaces provides for a broad diversity of epibiotic organisms to settle and grow (Fitridge *et al*., 2012). These marine algae and animals, collectively termed biofouling (Fig. 7.1), are severely problematic to culture operations and success and have significant economic impacts. The direct economic costs of biofouling control to the aquaculture industry are between US$1.5 to 3 billion per year (Fitridge *et al*., 2012). However, indirect effects of biofouling on the production remain largely unknown.

All the potential negative impacts of biofouling on offshore aquaculture and the potential solutions to minimize it, were intensively reviewed in the literature (Braithwaite *et al*., 2007; Raveendran and Mol, 2009; Magin *et al*., 2010; Stowe *et al*., 2011; Bixler and Bhushan, 2012), and a recent review is recommended for further reading (Fitridge *et al*., 2012). Cage nets used in marine finfish industry focus on the mitigation of net fouling, which leads to three main negative effects. Water exchange restriction is a key impact due to the net occlusion through the growth of fouling organisms (Madin *et al*., 2010), leading to poor water quality with low dissolved oxygen levels, inhibited removal of feed and waste excess. Therefore, fish could be subjected to stressful conditions regarding some environmental factors (hypoxia and high ammonia levels). Excess of biofouling can also compromise cage structure, causing deformation and structural fatigue (Swift *et al*., 2006), leading to potential fish escapes due to net damage. In addition, the third negative effect is related to increased risk of diseases, with the fouling communities acting as reservoirs for pathogenic microorganisms (macro- or

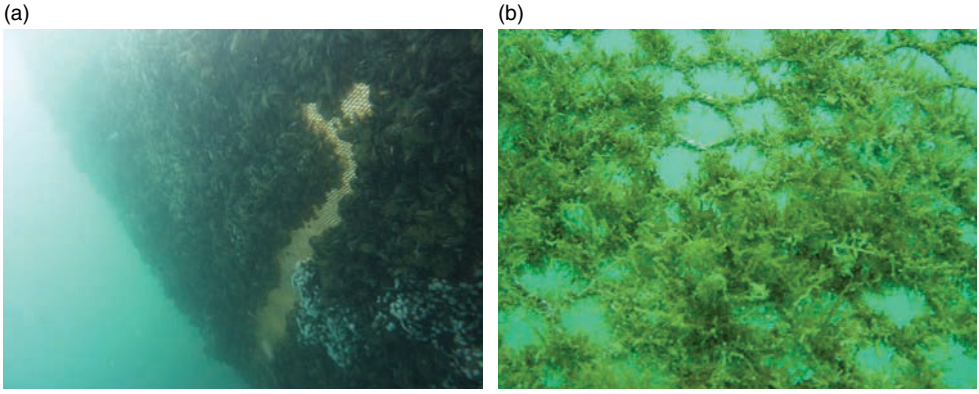


Fig. 7.1. Cage nets with heavy biofouling by mussels (a) and macro algae (b) reducing the water exchange and hampering the water quality for rearing fish. Photos by Pedro Pousão-Ferreira.

microbial fouling species), and an increased fish exposure and vulnerability to infectious diseases, through exposure to lower dissolved oxygen levels that lead to increased stress.

The operations performed in a commercial fish farm to minimize biofouling are a complex process and usually employ a multifaceted approach: net changing and cleaning; chemical treatment of structures to prevent the recruitment of fouling organisms; and biological control to graze biofouling (Fitridge *et al.*, 2012). Frequent net changing damages the infrastructure, increasing the risks of fish escape and disturbs the feeding regimes, which may induce lower growth rates. As an alternative to net replacement, nets can be cleaned *in situ*, primarily with cleaning discs on remote operating vehicles, or manually by divers. *In situ* cleaning through brushing increases fouling problems because it increases surface rugosity and loose filaments that are ideal settlement substrata for some fouling species (Carl *et al.*, 2011). The use of chemical antifoulants consists mainly of copper coatings that are toxic for most of marine invertebrates. Its application should be done each year in temperate regions (Braithwaite *et al.*, 2007). Recently, it has been shown that copper released from antifouling treated nets could be a risk factor for European seabass health (Cotou *et al.*, 2012) with low concentrations in the muscle (1.5 μg g^{-1}) and liver (117 μg g^{-1}). Moreover, although copper levels did not induce oxidative stress, it affected the immediate immune defence mechanism of European seabass, making them more vulnerable to diseases. Similarly to other antifoulants (zinc and different biocides), copper also causes negative impacts on non-target species such as the cultured fish (Mochida *et al.*, 2006). The European Commission is proposing to classify copper as a dangerous substance, based on the 67/548/EEC directive. As a consequence, new candidates to supplement or replace the use of copper as an antifoulant are being proposed (Fitridge *et al.*, 2012). Organic booster biocides were recently introduced (Guardiola *et al.*, 2012) as alternatives to the tributyltin (TBT) related compounds, formerly used in antifouling products, due to the restrictions of its use by their known negative impact on the marine environment. The third alternative against fouling is the use of herbivorous fish or invertebrates to control fouling; however, this biological control is constrained by the fact that the fish or invertebrates used should have a broad dietary range due to the high diversity of organisms of biofouling. Moreover, attempts to do so have been experimental and on a small scale.

New antifouling strategies in the aquaculture industry should be environmentally friendly, able to withstand onshore handling and cleaning, and economically viable. Fish farmers should consider the potential range of species that could foul their nets, mechanisms to control and remove fouling without affecting fish.

Algal blooms

An algal bloom is a rapid increase of algae in an aquatic system caused by phytoplankton species. This is usually accompanied by undesirable or harmful effects, which have important ecological, social and economic consequences. Among the thousands of marine phytoplankton species, some 300 species are known to produce algal blooms, but only 40 or so species have the capacity to produce potent toxins (Hallegraeff, 1993). Blooms are recognized by the coloration of the water resulting from the high density of pigmented cells (the so-called 'red tides'). Toxin producers can contaminate seafood or kill fish, and the high biomass producers can also cause anoxia and kill marine fauna (Smayda, 1990). Harmful algal blooms (HABs) are particular events involving toxic or otherwise harmful phytoplankton. HAB occurrence is becoming more frequent and problematic in some coastal regions in temperate seas like the Mediterranean basin. It is an emerging problem, where the local aquaculture industries and population may face a health risk that is difficult to measure (Hallegraeff, 1993; Masó and Garcés, 2006). Moreover, the monitoring of toxic algae species has mainly been associated with shellfish as they are filter-feeders.

In general, most algae-related fish kills are the result of oxygen depletion rather than the toxicity; however, some algal species can seriously damage fish gills, either mechanically or through production of haemolytic

substances. Fish death may be caused by capillary haemorrhage, dysfunction of gas exchange at the gills, suffocation from an overproduction of mucus, or even from secondary infection of the damaged tissue (Horner *et al*., 1991). The effects on aquaculture cage production were reported sporadically but revealed a tremendous economic impact when they occurred. Global annual impact of red tides has been estimated between US$13–25 million (Anderson *et al*., 2000) and the impacts of algal blooms on aquaculture are in Shumway (1990).

In addition to algal blooms, jellyfish blooms, mainly caused by *Cnidaria* and *Ctenophora* (Mills, 2001) have also been reported in the Mediterranean and may also interfere with aquaculture production (Purcell *et al*., 2007). Descriptions of algal and jellyfish bloom-forming species in the Mediterranean basin are provided in Nastasi (2010). No caged fish mortality has been reported in the Mediterranean Sea due to algal or jellyfish blooms, which does not mean that it could not happen in the future. There are reports of high abundances of at least five scyphozoan species and a cubozoan species in the Mediterranean and large-scale occurrence of some ctenophores (e.g. *Mnemiopsis leidyi*) that bloomed from Spain to Israel in 2009 (Purcell, 2012).

This kind of bloom could affect offshore aquaculture by limiting or prohibiting product commercialization due to human health risks associated with the consumption of fish containing algae toxins, rather than causing fish mortality. Thus, the presence of marine biotoxins in seafood products is regulated to ensure the consumer's safety. This regulation states that fishery products containing biotoxins must not be placed on the market (Regulations EC No 853/2004 and EC No 1021/2008). Biotoxins have been isolated in wild caught fish, however, we should not neglect their potential accumulation in caged fish that could eat mussels, ascidians and other planktonic filter-feeders from the net fouling.

Strategies to prevent future problems and reduce the potential risk of algal (toxic or non-toxic) and jellyfish blooms in offshore aquaculture can vary considerably (Anderson *et al*., 2005). The selection of cage site should be based on previous occurrence of such blooms, water current flows (particularly critical in semi-enclosed areas), local and massive nutrient inputs from intensive agriculture or urban centres, and seasonal temperature variations. Additionally, dissolved oxygen, cell counts and identification of phytoplankton species should be monitored routinely to mitigate HAB effects. Also, non-regular activities such as towing the fish net pens away from the sites of intense HABs should also be considered (Anderson *et al*., 2005). Advanced technological tools have also been developed, such as the solid-phase adsorption toxin tracking devices to detect dissolved biotoxins in sea water (MacKenzie, 2010), or molecular biology based detection methods (Dittami and Edvardsen, 2012). Highly sophisticated monitoring systems using buoys with fibre optical sensors and data transfer by satellite to detect blooms might be implemented as it has been done on the Norwegian coast (Hallegraeff, 1993). Alternatively simple procedures such as stop fish feeding can also reduce the potential fish losses in cages when a non-toxic algal bloom occurs, in order to minimize the fish oxygen demand when it is already compromised (Hallegraeff, 1993). There are four general strategies that can be used to combat or suppress an invasive or harmful species: (i) mechanical, by dispersing clay over the water surface; (ii) chemicals, attempted in 1957 against a Florida red tide but not being actively pursued by the HAB community due to its widespread impact in the marine fauna; (iii) biological; and (iv) genetic control (commonly used on terrestrial harmful species), with possible negative impacts through the introduction of indigenous or non-indigenous organisms into the ocean to control other organisms (Anderson *et al*., 2005).

Vaccination procedures

A relatively small number of pathogens are responsible for important economic losses in cultured fish (Toranzo *et al*., 2005; and Chapters 2, 3, 5, 6 and 8 in this book). These diseases have led to high fish mortalities and decreased incomes of the fish farming industry. Methods to prevent fish disease/mortality include the use of antibiotics, chemical products,

vaccines, as well as pre- and pro-biotics (Kiron, 2012; Oliva-Teles, 2012). Fish can be immunized by injection, immersion and oral administration. Although those therapies have been proven to be efficient to prevent fish pathologies, farmers often report poor return to feeding, occasional mortalities and also trauma in fish post vaccination. Side effects of vaccination procedures include residues in the fish muscle, development of resistance to antibiotics and environmental pollution. Although most of these reports are on coldwater fish species there are also reports from marine warmwater species.

Intraperitoneal injection is a usual way to immunize reared fish, but can induce granulomatous peritonitis. Bjorge *et al*. (2011) and Sørum and Damsgård (2004) described a significant reduced feed intake by Atlantic salmon post intraperitoneal injection and this would explain the lower weight in vaccinated compared to unvaccinated fish at harvesting (Aunsmo *et al*., 2008). Pathological changes induced by intraperitoneal vaccination include organ adhesions and sometimes deposition of melanin, spinal deformities and granulomatous uveitis (Bjorge *et al*., 2011). In addition, Koppang *et al*. (2008) found that farmed salmon injected with oil adjuvanted vaccines developed autoimmunity disorders similar to the mouse model of Lupus-like systemic autoimmune syndrome. Side effects depend on fish size, being lower when the vaccine is administered in larger fish (Berg *et al*., 2007). Moreover, vaccine-induced side effects can be reduced through selective breeding, as well as by other factors such as the formulation of the vaccine (Drangsholt *et al*., 2011).

A study on European seabass injected intraperitoneally with monovalent (*Photobacterium damselae* subsp. *piscicida* or *Listonella anguillarum*) and divalent (*Ph. damselae* subsp. *piscicida* and *L. anguillarum*) vaccines with or without adjuvants such as mineral oil, liposome or alginate (Afonso *et al*., 2005) showed high numbers of macrophages for up to 2 months in fish injected with the two oil adjuvanted vaccines, which indicated chronic inflammation. This chronic inflammation has been suggested to induce vertebral deformities (Gil-Martens, 2010). High neutrophil counts were maintained only in fish injected with antigens, which coincided with the most severe side effects. Although vaccination with formulations containing liposome did not cause side effects, the same authors suggested that the usefulness of liposome as a fish vaccine adjuvant deserves further investigation. Moreover, monovalent mineral oil formulated vaccines produced less pronounced lesions than the divalent ones, and European seabass injected with the water-based vaccine was the least affected. Overall, oil adjuvanted formulations tend to yield more severe lesions than those with other adjuvants and non-adjuvanted vaccines (Afonso *et al*., 2005).

Another therapeutic/prophylactic treatment is the administration of antibacterial agents through immersion. Potentiated sulfa antibiotics such as combination of trimethoprim and sulfamethoxazole (TMP-SMX) are currently used against bacterial pathogens in Mediterranean aquaculture (Rigos and Troisi, 2005). However, TMP-SMX induces mild short-term acute stress, characterized by an increase in plasma cortisol, glucose and disrupted hydromineral balance in gilthead sea bream and European seabass (Rigos and Troisi, 2005).

Since fish vaccination or antibiotic bath exposure are stressful for fish, careful handling before, during and after these procedures is important. Keeping fish submerged in clean water during vaccination (the 'wet' method), is considered to offer significant benefits over that of holding fish out of water while awaiting injection (the 'dry' method). Attention should be paid to fish behaviour, especially feeding and swimming activities, and presence of body lesions after vaccination.

Noise

Sound perception by fish is based on three interconnected systems: the auditory, the equilibrium and the lateral line. During the last decade, many studies have evaluated the effects of anthropogenic acoustic disturbance on marine organisms, thus increasing the awareness of the damage done to animals exposed to human related underwater sounds (National Research Council, 2000, 2003). These sounds are associated with shipping, seismic surveys, sonar, recreational boating and many

other anthropogenic sources that are known to induce several types of responses in fish (Slabbekoorn *et al*., 2010). Santulli *et al*. (1999) demonstrated typical primary and secondary stress responses to air gun detonations (variation in cortisol, glucose, lactate, AMP, ADP, ATP and cAMP levels) in different tissues of European seabass. Nevertheless, acoustic pollution is expected to have a major impact on finfish, so cage rearing sites should be restricted or closed to marine traffic. Low-frequency (100–500 Hz) ambient noise is detected by many fish species (Popper *et al.*, 2003), and they come from transport and recreational shipping (Ross, 2005). However, physiological responses to underwater noise are poorly understood. It has been shown that European seabass and gilthead sea bream exposed to a 0.1–1 kHz presented significant increases in swimming activity and in lactate and haematocrit levels (Buscaino *et al*., 2010) which indicated intense metabolic activity in white muscle anaerobic fibres. Furthermore, the increase of metabolic muscle activity implies a higher demand for oxygen, and thus an increase in respiratory rate. Another example is the altered fish behaviour of tuna (*Thunnus thynnus*) to sound generated by hydrofoil passenger ferries, small boats and large car ferries (Sara *et al.*, 2007). Tuna normally assumed a concentrated coordinated school structure with unidirectional swimming and without a precise shape. However, with noise from hydrofoils, tuna changed swimming direction and increased their vertical movement. This disturbed behaviour was most evident when the sound came from outboard motors of small boats. Another impact on fish behaviour was reported by Picciulin *et al*. (2012) who showed that noise from boats caused variations in brown meagre (*Sciaena umbra*) vocalizations with mean pulse rate increasing over multiple boat passages.

The effect of anthropogenic noise on fish deserves greater attention than what it has been paid by fish farmers. To reduce potential stress on fish by anthropogenic activities, limiting vessel traffic around fish cages as well as using newly developed engines (producing lower noise) in fish farming vessels should be considered.

Nutritional imbalance

General nutritional problems

INTRODUCTION. Diet formulation and manufacturing (e.g. grinding, heating, adding moisture, pelleting and drying) add an element of uncertainty to the stability and bioavailability of certain essential nutrients, and sometimes results in clinical deficiencies (Hardy, 2001). Deficiencies and toxic conditions are more likely to be associated with oxidation of dietary lipids, mould related to incomplete pellet drying, and deficiencies resulting from antagonistic interactions among feed ingredients in formulated feeds (Roberts, 2012). For instance, certain amino acids still chemically measurable may be rendered biologically unavailable or inactive, during processing. For instance, lysine may form a compound with carbohydrate in the feed, rendering it unavailable to the fish (Hardy, 2001).

Specific nutrient requirements are usually determined under standard and favourable environmental conditions. However, such ideal conditions are not always present in commercial farms, where animals are often stressed, confronted with pathogens or other unfavourable environmental conditions. In such conditions the requirements for certain nutrients are likely to increase, due to greater activity of the immune system and physiological activity (Maita, 2007), or decrease such as it is the case during the winter season (e.g. in 'Winter disease').

Deficiencies or imbalance can be categorized in two types: deficiency of macronutrients (protein, carbohydrate, lipid and fibre), and deficiency of micronutrients (vitamins and minerals). Lipid deficiency is the most serious problem, whether in relation to deficiency, oxidation or imbalance. Among the micronutrients, any of a wide range of components can exert an effect, especially on fast-growing younger fish (Roberts, 2002). Nutritional imbalance also includes the excess of particular nutrients; an example is excessive carbohydrate or lipid levels may result in hepatocyte degeneration and increased mortality (associated with fatty livers), respectively (Roberts, 2012).

IMPACTS ON FISH PRODUCTION. Since nutritional imbalances still occasionally occur in commercial production, undetected subclinical deficiencies may be more common, possibly contributing to inefficient fish growth, increased disease susceptibility, and unsolved problems encountered with attempts to culture new fish species. The most frequent indicator of an amino acid deficiency problem is impairment of growth and feed utilization. These include dorsal fin erosion, which has been associated with lysine deficiency, and cataract associated with methionine and tryptophan deficiency (Roberts, 2012). However, dietary problems associated with the lipid fraction of the diets appear to be more serious. Three long chain polyunsaturated fatty acids, namely docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) have a variety of relevant functions in fish. Inadequate essential fatty acids (EFA) give rise to several alterations such as reduction in erythrocyte volume with increased erythrocyte fragility, and lower haemoglobin content and number of red blood cells. Additionally, renal morphology is severely affected with extreme dilation of capillaries and occlusion of Bowman's capsules in glomeruli (Montero *et al*., 2004). Although the requirements for vitamins are small, deficiencies of these micronutrients can cause clinical signs ranging from poor appetite to severe tissue deformities (Lovell, 1998).

DIAGNOSIS. A wide range of specific clinical features has been described in experimental studies due to deficiency of dietary nutrients (Table 7.1). Additionally, starvation may occur in farmed fish due to complete deprivation of food, to inadequate feeding or to behavioural, physiological, or mechanical prevention of food intake.

CLINICAL SIGNS, GROSS AND HISTOPATHOLOGICAL LESIONS. The most frequent clinical description associated with nutritional deficiency diseases is inappetence characterized by loss of weight and decrease in condition factor. In European seabass this is also associated with darkening of the skin, lethargy and poor growth (Echevarría *et al*., 1997).

Table 7.1. Disorders associated to nutrient deficiencies in warmwater fish species.

Nutrient	Disorder	Species	Reference
Amino acid deficiencies			
Arginine, lysine and threonine	Decreased growth and feed efficiency	European seabass Gilthead sea bream	Tibaldi and Lanari (1991); Tibaldi *et al*. (1994); Tibaldi and Tulli (1999); Fournier *et al*. (2002)
Fatty acid deficiencies			
ARA	Lower hepatosomatic index	European seabass	Geay *et al*. (2011)
EPA	Growth and immune deficiencies Decreased antioxidant capacity	European seabass Gilthead sea bream	Montero *et al*. (1998, 2008); Saera-Vila *et al*. (2009); Geay *et al*. (2011)
DHA	Growth and immune deficiencies Decreased swimming activity and antioxidant capacity	European seabass Gilthead sea bream	Montero *et al*. (1998, 2008); Saera-Vila *et al*. (2009); Geay *et al*. (2011)
Vitamin deficiencies			
Vit B	Reduced haematocrit Atrophic pancreas	Gilthead sea bream	Morris *et al*. (1995)
Vit E	Decreased complement activity	Gilthead sea bream	Montero *et al*. (1998)

EFA deficiencies alters the oxygen-carrying capacity of blood in gilthead sea bream, immunosuppression of both cellular and humoral systems, and renal injury, that include renal tube degeneration and systemic glomerulonephritis (Montero *et al*., 2004). Vitamin B deficiency has been associated with significant reductions in haematocrit levels in gilthead sea bream. These vitamin-deficient fish show atrophic pancreas with an accumulation of pigmented granules around the organ. In addition, the normal homogeneity of the liver parenchyma is lost (Morris *et al*., 1995).

Lipid peroxidation, specifically polyunsaturated fatty acid (PUFA) oxidation, is acknowledged as being highly deleterious and resulting in damage to cellular biomembranes, which contain large amounts of PUFA. This is possibly the most extensively studied aspect of oxidative damage to biological systems. In fact, high PUFA content diets resulted in signs of increased peroxidative stress in juvenile gilthead sea bream, as evidenced by increased levels of tissue lipid peroxidation products (Tocher *et al*., 2002).

PREVENTION AND CONTROL. Careful management of fish nutrition and health are two critical factors in intensive aquaculture systems. The consequences of amino acid deficiencies are not catastrophic, usually causing a slower growth and higher diet conversion ratios, rather than fish mortality. Similarly, EFA imbalances are rare in commercial fish production, excluding spawning marine broodstock (Hardy, 2001). Vitamin and mineral deficiencies, although the easiest problems to avoid are the most common deficiencies observed in commercial aquaculture. A constant supply of essential water-soluble vitamins is required to prevent deficiencies, since these vitamins are not stored for long in body tissues (Lovell, 1998).

Winter disease

INTRODUCTION. The so-called 'winter syndrome' (WS) or 'winter disease' mainly affects farmed gilthead sea bream reared at low temperatures in many Mediterranean countries such as France, Italy, Portugal, Greece and Spain, and especially in the northern areas.

Winter syndrome was first detected in cultured gilthead sea bream in the Mediterranean area in 1991; sea bream are apparently most affected but during the cold season WS has also been described occasionally in amberjack reared in the Mediterranean area. However, other fish species, such as European seabass, that are frequently reared in the same facilities do not seem to be affected (Gallardo *et al*., 2003).

WS is associated with long-term exposure to low ambient temperatures during the winter months, when water temperature is below 13°C. The causes for WS are not clear and it likely involves several factors such as chemical stressors like water pollutants or biological stressors such as parasites (Lemly, 1996), a depressed immune status, nutritional imbalance and osmoregulatory problems (Tort *et al*., 1998b), and an increased susceptibility to opportunistic pathogens and decreased ability to fight infections (Pickering and Pottinger, 1985). *P. anguilliseptica*, *Aeromonas* and an unidentified enterobacteria have been detected in different outbreaks of winter disease in gilthead sea bream during winter months (Berthe *et al*., 1995; Bovo *et al*., 1995; Doimi, 1996). Most experimental studies show that the disease can be reproduced only in conjunction with a severe external stressor. Thus, the disease probably arises from the combined action of low temperature, nutritional imbalance, immunosuppression and the presence of *Pseudomonas anguilliseptica*.

IMPACTS ON FISH PRODUCTION. Although mortalities resulting from winter syndrome are usually 7–10%, in severe outbreaks mortality can be up to 30–50% (Coutteau *et al*., 2001) or even 80% (Padrós *et al*., 1998).

Mortalities can occur as acute peaks or constant mortalities. Winter syndrome affects individual fish weighing 100–300 g by reducing their feed uptake. Nevertheless, winter syndrome mainly affects 1-year-old gilthead sea bream.

DIAGNOSIS. When water temperature decreases below 13°C, fish reduce food ingestion, become lethargic, present disturbed swimming

movements, anaemic, darker colour, distended and haemorrhagic abdomen, corneal cloudiness and are marked by typical darkening of skin pigmentation forming the so-called 'stress bands'.

- *External clinical signs* include swimming bell-up or on the side, distended abdomen (slightly protruded red anus and small haemorrhages around the base of fins).
- *Internally fish have distended intestines filled with clear fluid*, ascitic fluid in abdominal cavity, distended gall bladder, pale liver with occasional blood-shot areas and enlarged spleen.

GROSS AND HISTOPATHOLOGICAL LESIONS. A variety of tissue lesions have been described in affected fish and they include: (i) a granular degeneration and necrosis in white muscle fibres, caused by starvation that can induce changes in muscle related to catabolic reactions (Gallardo *et al*., 2003); (ii) severe liver alteration characterized by a fatty degeneration in hepatocytes (Fig. 7.2) (Padrós *et al*., 1998; Tort *et al*., 1998b; Contessi *et al*., 2000); (iii) severe distension of the intestines, that appear distended and filled with a clear liquid and mucous casts indicating a potentially reduced nutrient absorption; and (iv) presence of lesions in the pancreas. Fish with WS also have increased plasma cortisol, decreased complement and lysozyme activities, and reduced circulating lymphocytes (Tort *et al*., 1998a) and red blood cells (Padrós *et al*., 1999). These suggest severe immunosuppression in fish and in some cases may allow outbreaks of diseases caused by opportunistic pathogens like *P. anguilliseptica* that has been isolated from the brain (Doménech *et al*., 1997).

PREVENTION AND CONTROL. Winter disease is considered a multifactorial disease; hence there are several ways to diminish, at least partially, the effects and the occurrence of this syndrome. They include restrict feeding throughout the winter season and restart feeding the fish only when water temperature exceeds 13°C; use lower dietary lipid content in fish feeds before the winter months, especially at the end of summer and the approaching winter season; improve the fish immunological status providing optimal feeding and avoid handling and stressful episodes during winter. High stocking density in cages is an additional stressor and may impair the physiological condition of

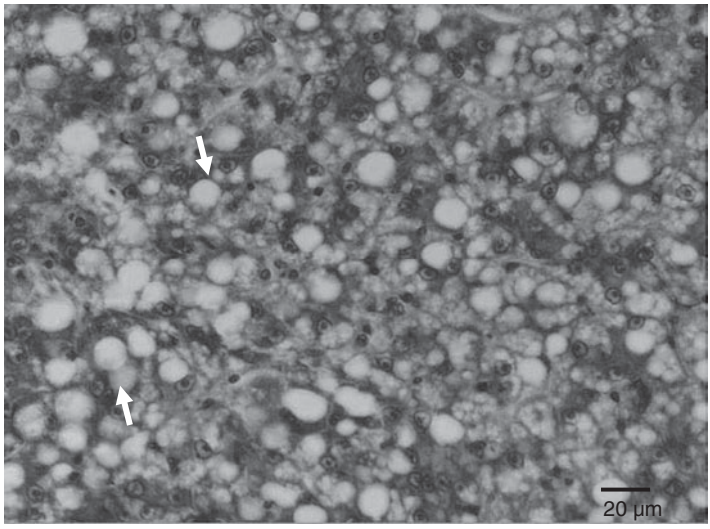


Fig. 7.2. Gilthead seabream (*Sparus aurata*) liver of a fish affected by winter disease (40×, HE stained). Notice the highly vacuolated hepatocytes (arrows).

the fish. Further work is required to characterize the cause of this condition.

Stress in warmwater fish species

Introduction

In fish, stress can be defined as a condition in which the dynamic equilibrium (homeostasis) is disturbed as a result of the actions of intrinsic or extrinsic stimuli (Wendelaar Bonga, 1997). The context, severity and duration of the stress and of the resultant response(s), must all be taken into account when assessing whether an adverse outcome is likely to occur (Pottinger, 2008). Acute stress is usually of short duration allowing the fish to respond to the stressor and recover, whereas chronic stress is a long-term condition with more severe effects (Barton, 2002).

Confinement, high stocking densities, poor water quality and transport are stress inducers that are highly relevant to aquaculture management and have received considerable attention (Wendelaar Bonga, 1997; Barton, 2002; Portz *et al*., 2006; Pottinger, 2008; Tort, 2011). Physiological responses to these stressors have been broadly grouped as primary, secondary and tertiary. First, the perception of an altered state initiates a neuroendocrine reaction that forms part of the generalized stress response in fish (Barton, 2002). This response includes the activation of the brain-sympathic-chromaffin cells axis with enhancing of plasma catecholamines (e.g. norepinephrine, epinephrine) as well as stimulation of the hypothalamic-pituitary-interrenal axis, resulting in the release of corticosteroids into circulation. The secondary stress response comprises the various biochemical and physiological effects associated with stress and mediated to a large extent by stress hormones. A number of metabolic pathways are activated resulting in alterations in blood chemistry and haematology (Mommsen *et al*., 1999). Secondary responses to stress also include measurable changes in blood haemoglobin and haematocrit as well as in plasma glucose, lactate and major ions (e.g. chloride, sodium and potassium). Tertiary responses are on the level of whole organisms and populations, including inhibition of growth and immune response as well as changes in metabolic rate, behaviour and survival (Wendelaar Bonga, 1997; Mommsen *et al*., 1999; Barton, 2002). The extent of tertiary responses may be directly related to the severity and duration of the stressor. Outcomes associated with exposure to unalleviated stressors, or frequent encounters with acutely stressful events include growth suppression, reproductive dysfunction, and loss of immunocompetence that leads to enhanced susceptibility to pathogens and parasites (Schreck, 1996; Wendelaar Bonga, 1997; Tort, 2011).

Impacts on fish production

Water quality is one of the most important contributors to fish health and stress level. For instance, temperature, dissolved oxygen, ammonia, nitrite, nitrate, salinity, pH and carbon dioxide are the most common water quality parameters affecting physiological stress (Portz *et al*., 2006). Therefore a chronic exposure to high ammonia and low dissolved oxygen will lead to decreased growth, higher feed conversion ratio and may increase mortality (MacIntyre *et al*., 2008). Some studies have suggested that fin damage intensifies with increasing stocking density and high temperature (25°C or 29°C) in European seabass (Person-Le Ruyet and Le Bayon, 2009). In gilthead sea bream cage culture, a possible impairment of the corticosteroid stress response from rearing at high densities could result in a reduction in the physiological ability to cope with social stressors from conspecifics or abiotic changes in their confined environment (Barton *et al*., 2005).

Diagnosis

Stress response in fish is usually evaluated by measuring levels of hormonal, metabolic, haematological and hydromineral parameters in the blood; while growth and feeding parameters, and organosomatic indexes are generally used as tertiary stress indicators (Barton, 2002). However, recent technologies such as proteomics, genomics and metabolomics could give better insights into the mechanisms involved in stress-related processes, thus facilitating the identification of stress and/or welfare indicators and proposing

different biomarkers for stress in fish (Prunet *et al.*, 2012; Rodrigues *et al.*, 2012). Studies on liver transcriptome showed significant changes in various functions including inflammation (acute phase proteins), immune response, gluconeogenesis and glycogenolysis, energy metabolism and protein degradation (Prunet *et al.*, 2012). Analysis of RNA from the liver of gilthead sea bream under confinement led to four major temporally defined gene expression profiles that comprise rapid metabolic readjustment followed by tissue repair and remodelling processes, and re-establishment of cellular homeostasis and regulation of the immune system (Calduch-Giner *et al.*, 2010).

ADVERSE WEATHER CONDITIONS. The environmental changes that take place in cage culture systems can affect very rapidly the physiology of fish. Therefore, both seabass and sea bream need to cope with rapid changes in water temperature and salinity. Estuaries or enclosed basins during raining seasons (autumn/winter) can have a decrease in water salinity, inducing osmotic and, consequently, metabolic problems (Vargas-Chacoff *et al.*, 2009). During winter, temperature reduction induces a reduction or cessation of feed intake, which alters both osmoregulatory and metabolic responses to salinity acclimation, compromising the osmoregulatory capacity (Polakof *et al.*, 2006). This lower feed intake due to a drop in ambient temperature usually initiates the mobilization of fat deposits and/or amino acids utilization in order to support the glucose demand during this period, since the fish cannot evade the environmental changes (Vargas-Chacoff *et al.*, 2009).

SUBOPTIMAL CULTURE CONDITIONS. There is a general trend to maximize stocking density; however this may exert adverse effects on fish health, and on the capacity of the producer to maintain good water quality. Inappropriate stocking densities lead to poor welfare and compromise the health conditions of fish, and thus profitability of the industry (Pottinger, 2008). Several studies have addressed the effects of handling and crowding conditions on oxidative stress in fish. A microarray analysis of the genes expressed during the time-course of stress response in gilthead sea bream after acute confinement (100 kg per m^3) highlighted a vast array of metabolic adjustments including the re-establishment of cellular homeostasis with increased intracellular trafficking and scavenging of reactive oxygen species (Calduch-Giner *et al.*, 2010). Additionally, heat shock cognate protein 70 k Da (chaperoning) was down-regulated in gilthead sea bream under chronic stress (Alves *et al.*, 2010) indicating immunosuppressive effects of stressors. Heat shock proteins play important roles in the host response to environmental pollutants, food toxins, inflammation and on the specific and non-specific immune responses to bacterial and viral infections in fish (Roberts *et al.*, 2010). Fish can also increase their swimming speeds in response to increased stocking density. In fact, individual and group swimming behaviour, respiratory activity, aggression, stereotypic and abnormal behaviour have been linked with acute and chronic stressors in aquaculture and can therefore be regarded as likely indicators of poor welfare (Martins *et al.*, 2012). Additionally, during transport fish have the risk of suffering physical damage through handling and increased stocking density that cause fin erosion, snout abrasion and eye damage. Any such damage will increase the risk of osmoregulatory disturbance, render the fish more susceptible to disease organisms and compromise subsequent performance (MacIntyre *et al.*, 2008).

Clinical signs, gross and histopathological lesions

There are a number of gross signs indicative of a tertiary stage stress response, such as reduction or cessation of growth and decrease in condition factor. An important effect of high stocking density can be haemoconcentration, affecting haematocrit, haemoglobin, red blood cell count and total plasma proteins, an effect that has been described as a strategy to increase oxygen carrying capacity of the blood during periods of high energy demand (Montero *et al.*, 1999a). Several histopathological lesions and immune parameters observed in chronically stressed fish are shown in Table 7.2.

Table 7.2. Gross signs, histopathological lesions and immune parameters indicative of a tertiary stage stress response in warmwater fish.

Stressor	Disorders	Species	Reference
High stocking density	Fin damage	European seabass	Tort *et al*. (1996);
	Chronic erosive dermatopathy	Gilthead sea bream	Montero *et al*. (1999a, b);
	Decreased complement activity and circulating lymphocytes	Sharp snout sea bream	Person-Le Ruyet and Le Bayon (2009);
	Increase of melano-macrophage centres in spleen	Meagre	Rigos and Katharios (2010)
Low temperature	Mucous hyperplasia in the intestinal mucosa	Gilthead sea bream	Tort *et al*. (1998a, 2004)
	Decrease circulating lymphocytes and plasma lysozyme		
High temperature	Fin damage	European seabass	Person-Le Ruyet and Le Bayon (2009)
High CO_2 levels	Increased mortality	European seabass	Grøttum and Sigholt (1996);
	Gas bubble disease		Athanassopoulou *et al*. (2004);
	Nephrocalcinosis		Vandeputte *et al*. (2009)
Osmotic stress	Cataract	European seabass Gilthead sea bream	Bjerkås *et al*. (2000)

Prevention and control

Improvement of aquaculture practices to minimize chronic stress is one of the main challenges in aquaculture research. The European Food Safety Authority has identified hazards and risk factors potentially affecting the welfare of European seabass and gilthead sea bream (EFSA, 2008). The most obvious approach to limiting the stress associated with a particular regime is to reduce the frequency, duration and severity of stressors. An interesting approach to reduce stress under intensive rearing conditions is to accelerate domestication through selective breeding programmes. Despite considerable research, the implications for the reduced endocrine stress response in aquaculture are still not clear (Barton *et al*., 2005). While it may be advantageous to select fish exhibiting low stress responses for intensive commercial aquaculture, those being reared for stock enhancement could conceivably be at a disadvantage when released into a natural environment (Barton *et al*., 2005).

Skeletal abnormalities

Introduction

The development of morpho-anatomical abnormalities constitutes an important problem for the fish farming industry and is considered one of the major constraints for intensive production (Boglione *et al*., 2013). However, despite being widely reported in cultivated fish and several studies that focused on finding causative factors, a definitive solution has not yet been found. Successful fish culture requires the use of healthy nondeformed fry, optimized feeding, good husbandry techniques, disease monitoring, surveillance and reporting (Sobhana, 2009). Since most of the problems related to deformities occur during early life stages, it is essential to have an effective selection process to cull out all the fry that show an altered phenotype (Boglione and Costa, 2011).

Axial deformities

The types of vertebral deformities in teleosts range from vertebral malformations,

as platyspondyly, caused by fusion or compression of vertebral bodies, or deformed arches and spines, to vertebral column curvatures (Favaloro and Mazzola, 2000, 2003, 2006; Fraser *et al*., 2004; Lewis *et al*., 2004) and they are classified according to the affected vertebral region (Matsuoka, 1987, 1997; Boglione *et al*., 2001). Macroscopically, the majority of vertebral deformations can be grouped into: (i) scoliosis, an abnormal lateral curvature; (ii) lordosis, an abnormal V-shaped dorsal curvature; and (iii) kyphosis, an abnormal inverted V-shaped ventral curvature (Koumoundouros, 2010; Cardeira *et al*., 2012). These deformities frequently co-occur in the same individuals, causing lordosis-scoliosis-kyphosis (LSK) and saddleback syndromes with various severity levels (Afonso *et al*., 2000; Sfakianakis *et al*., 2003). Lordosis and kyphosis are frequent in farmed pelagic fish such as gilthead sea bream (Chatain, 1994; Fernández *et al*., 2008), European seabass (Chatain, 1994; Koumoundouros *et al*., 2002), white sea bream (Fig. 7.3) or meagre (Fig. 7.4), and are also present in flatfish such as the Atlantic halibut (*Hippoglossus hippoglossus* – Lewis *et al*., 2004) or Senegalese sole (*Solea senegalensis* - Cardeira *et al*., 2012).

The occurrence of skeletal anomalies in cage cultured fish has been known since the reports from Backiel *et al*. (1984) where a high incidence of vertebral column anomalies (up to 94% incidence) have developed in a cultivation of common carp (*Cyprinus carpio*) in floating cages. There was a strong correlation of vertebral abnormalities with high water current. European seabass exposed to high water current developed a significant number of deformities with 77% of the population being affected and 20% were severely deformed fish with body curvatures like lordosis and kyphosis (Divanach *et al*., 1997). Saddleback syndrome have been described in cage cultured gilthead sea bream (Korkut *et al*., 2009), white sea bream (Sfakianakis *et al*., 2003), and common dentex (Koumoundouros *et al*., 2001) and is characterized by a severe alteration of the anatomy with partial or total loss of the dorsal fin and sometimes associated to vertebral deformities like fusions (Fig. 7.3b, c) or haemal lordosis and caudal fin anomalies (Sfakianakis *et al*., 2003). Fish displaying these types of anatomical alterations are unfit for commercial purposes and will affect consumers' perception of aquaculture products.

In European seabass, Loy *et al*. (2000) assessed the effects on body morphology under different rearing conditions during larval and post-larval rearing, and then reared in cages for 15 months. Differences in shape were evident in juvenile fish; meanwhile in adults the differences were smaller but still significant. More recent studies performed by

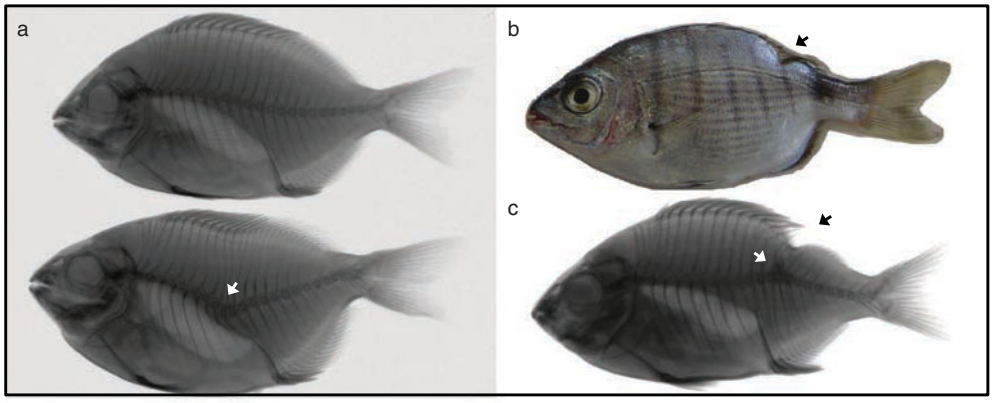


Fig. 7.3. Juvenile specimens of white sea bream (*Diplodus sargus*). (a) Radiography of a normal (top) and a lordotic (bottom) fish. (b) External appearance of a fish displaying saddleback syndrome (arrow). (c) Radiography of the same syndrome revealing also internal vertebral compressions (white arrow). Radiography on a Carestream Kodac DSX 4000 apparatus (35KVP, 45 seconds).

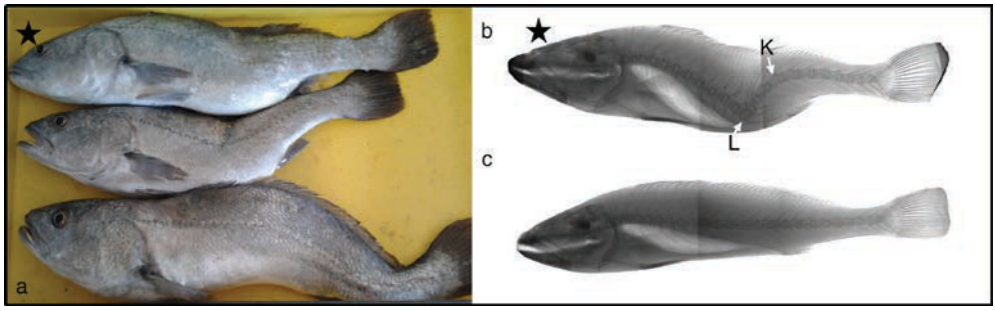


Fig. 7.4. Incidence of deformities in meagre (*Argyrosomus regius*). (a) Some lordo-kyphotic specimens of meagre captured at the end of the production cycle. (b) Composite radiography of a fish displaying a severe deformation (star on a) with a hemal lordosis (L) angle of 90° and a compensatory kyphosis (K). (c) Composite radiography of a normal fish. Radiography on a Carestream Kodac DSX 4000 apparatus (35KVP, 30 seconds).

Başaran *et al*. (2007, 2009) have shown that juveniles with vertebral deformities, at the age of transfer to rearing cages, have a significantly reduced swimming speed and affected their capacity to grow and convert food.

The incidence of vertebral column deformities in European seabass gradually decreases with growth since there is a high mortality of the affected fish during metamorphosis (Koumoundouros *et al*., 2002). However, surviving fish with lordosis or kyphosis will have a significantly altered body shape (Sfakianakis *et al*., 2006). The transfer of fry carrying a high incidence of this type of deformity into cages for on-growing leads to production losses by feeding of deformed fish with increased mortality and poor growth performance. European seabass fry are sturdier and can be moved from weaning tanks to on-growing facilities. They are usually graded 2–3 times for size separation and evaluation of deformities until they reach a size of 1–2.5 g. Juveniles produced for sea farming are kept in flow-through tanks until they reach approximately 10 g prior to transfer to sea cages (Moretti *et al*., 1999) and these fish must be carefully selected in order to minimize the passage of individuals carrying skeletal malformations.

Gill cover anomalies

Gill-cover anomalies are common in intensively reared species such as gilthead sea bream, European seabass with incidences up to 80% of the fish from hatchery. Fish with deformed gill covers have lower resistance to low oxygen levels (Paperna, 1978; Barahona-Fernandes, 1982; Francescon *et al*., 1988; Chatain, 1994; Verhaegen *et al*., 2007; Koumoundouros, 2010). Opercula deformities are usually unilateral (81.4%) with similar right/left frequency (Koumoundouros *et al*., 1997).

These anomalies were seen to develop in larvae during pre-flexion and flexion stages (Koumoundouros *et al*., 1997; Koumoundouros, 2010). In gilthead sea bream, the inwards folding of the opercular plate into the gill chamber is detectable in larvae after 25 days post hatch (Beraldo *et al*., 2003). However, during the on-growing stage in sea cages, some fish with monolateral inside folding of the gill-cover may have a partial recovery, but only if the anomaly is low (Beraldo and Canavese, 2011). Juvenile gilthead sea bream develop opercula plate anomaly when they are under intensive culture conditions; whereas siblings reared in semi-intensive conditions have relatively fewer deformed opercula plates (Boglione *et al*., 2013), an effect that can be due to a higher selective mortality of deformed fish under semi-intensive conditions. Opercula deformities may be reversed and this proceeds in different ways in relation to the anatomical structure, but regeneration may not be completed in 9 months (Beraldo *et al*., 2003).

Prevention and control

Grading of intensively farmed fish is performed 2–3 times for European seabass and gilthead sea bream during the weaning and pre-ongrowing phase. This activity is combined with an inspection for functional swim bladder and for skeletal deformities.

European seabass affected by axial malformations will be lethargic with poor swimming performance (Başaran *et al*., 2007). This can be used as a diagnosis of malformations. An obvious bending of the body is indicative of a vertebral curvature deformity and can be due to lordosis-kyphosis or scoliosis, which is sometimes accompanied with dorsal fin alterations such as saddleback syndrome. These fish are not fit for the market and should be removed. Small internal deformities that do not change the external morphology and can only be detected using radiographic examination or histological preparation might not impair growth and will also not affect the marketability of the fish.

A proper management of water current velocities and implementing feeding plans that ensure a balanced diet will help to prevent the occurrence of vertebral and opercular anomalies. Also, selection of juveniles from hatcheries with deformity free certification for stocking cages, will ensure a low incidence of deformed fish at the end of the production cycle.

Neoplasia

Introduction

Tumours or neoplasms are growth of abnormal cells that proliferate uncontrollably in tissues but generally they are not common in farmed fish.

Impact(s) on fish production

Neoplasias are sometimes known to cause problems in commercial stocks. Plasmacytoid leukaemia caused by a retrovirus is responsible for high mortality of Chinook salmon (*Oncorhynchus tshawytscha*) (Kent, 1997; Grizzle and Goodwin, 1998). Neoplasias normally do not cause high mortalities in fish, however, cutaneous abnormalities affect the marketability of fish.

Diagnosis

Neoplasias are rarely observed in cultured fish, possibly due to the limited lifetime in commercial operations. Factors identified as oncogenic agents include viruses, chemical compounds (e.g. pesticides and heavy metals), age, parasites, genetic background, and physical trauma-related husbandry practices (Hinton, 1989; Grizzle and Goodwin, 1998) but overall the causes are poorly understood.

There are four principal categories of neoplasms, and they affect epithelia, mesenchyme, pigment and neural cells.

Fibrosarcoma (mesenchymal) was described by Lucke and Schlumberger in 1941. Heavy metal pollution is the primary causative agent in black sea bream (*Spondyliosoma cantharus*) (Marino *et al*., 2010). In *Sparidae* were reported a case of ameloblastic fibroodontoma of the lips (Paperna *et al*., 1977), a case of branchial osteochondroma (Nash and Porter 1985), a shwannoma affecting the dorsolateral part of the fish body (Marino *et al*., 2008), and a neoplasia in the mouth (Gutierrez *et al*., 1977), all in farmed gilthead sea bream. The first two cases were from the Red Sea (Israel), the other from Spain and the last one from the South Tyrrhenian Sea (Italy). Neoplasia of haematopoietic origin has most frequently been observed in lymphoid tissues (Harshbarger, 1977). In addition, meagre juveniles maintained in intensive production developed a thymus sarcoma (Soares *et al*., 2012a) (Fig. 7.5), but the cause of that remains unknown as it is in general for the origin of all these neoplastic lesions.

Clinical signs, gross and histopathological lesions

Neoplasms are typically detected during gross examination of an external or internal abnormal tissue growth. The diagnosis is confirmed histologically. Benign neoplasia are normally well-differentiated and circumscribed, without invading surrounding normal tissue and do not metastasize. Malignant neoplasias are often not well-differentiated, may grow rapidly, infiltrating normal tissues and tending to metastasize (sarcoma or carcinoma).

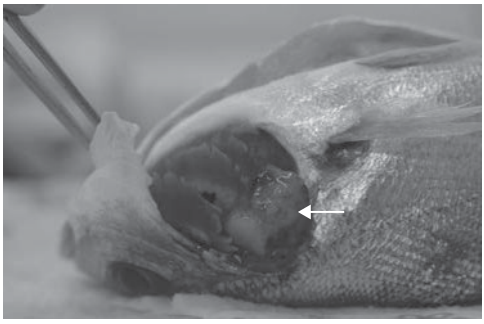


Fig. 7.5. Thymus sarcoma in meagre (*Argyrosomus regius*) juveniles.

In particular, juvenile meagre maintained under normal growing conditions developed lateral and/or bilateral circular shaped sarcoma within the opercular cavity. The sarcoma was dense, reddish and its growth from the branchial arch exerted pressure on the operculum, forcing it to open. Histologically, the neoplasm exhibited marked proliferation of mesenchymal connective tissue composed by fusiform cells, which developed in a solid pattern accompanied by abundant mononuclear cell types. Multifocal areas of discrete necrosis were also observed, compatible with a sarcomatous proliferation (Soares *et al*., 2012b).

Prevention and control

As far as we know, horizontal transmission of neoplasia does not occur, except in those caused by infectious viruses or parasites. Avoiding exposure to unfavourable biotic and abiotic factors that are potential causes of neoplasia in fish ('Diagnosis' section above) constitutes the main prevention measure. When neoplasias appear, a general screening of potential causative factors is recommended, and when identified the elimination of those factors should be mandatory.

Anti-nutrients

Introduction

The expansion of aquaculture has been accompanied by a rapid growth of fish feed production, which for carnivorous species has been largely based on fishmeal and fish oil. As the production of fishmeal and fish oil is stagnating there is a shortage of supply concomitant with the increase in price of these raw materials (Tacon and Metain, 2008). Thus replacement of fishmeal by alternative protein sources is necessary if growth in aquaculture production is to be pursued (Gatlin *et al*., 2007). The use of vegetable products has several disadvantages, particularly related to their protein contents, amino acid profiles and unsaturated fatty acid imbalances, which include endogenous anti-nutritional factors. These anti-nutrients (natural or synthetic substances found in feeds) may have harmful effects such as reduced palatability, decreasing efficient nutrient utilization for growth, altering nutrient balances, inhibiting growth, increasing intestinal dysfunction, altering gut microflora, inducing pancreatic hypertrophy and immune modulation causing hypoglycaemia and/or liver damage (Krogdahl *et al*., 2010).

Impacts on fish production

Fishmeal replacement with plant-protein ingredients varies from 50–95% without jeopardizing growth performance and feed utilization in European seabass, gilthead sea bream, sharp snout sea bream, black sea bream and meagre (Kaushik *et al*., 2004; Dias *et al*., 2009; Mérida *et al*., 2010; Estévez *et al*., 2011; Ngandzali *et al*., 2011). However, a high or total substitution of fish oil with rapeseed, linseed and/or soybean oils for several months caused a decrease of growth rate in gilthead sea bream and European seabass (Izquierdo *et al*., 2005; Montero *et al*., 2008, 2010; Geay *et al*., 2010). Other metabolic consequences were observed in fish fed high levels of vegetable oil mixtures (Benedito-Palos *et al*., 2008). European seabass fed a vegetable-based diet had lower expressions of genes related to immune response compared to those fed a fish-based diet (Geay *et al*., 2011). Sitjà-Bobadilla *et al*. (2005) also observed that gilthead sea bream fed a 75% fishmeal replacement (mixture of plant-protein sources) had decreased complement values. Long-chain polyunsaturated fatty acids are important

regulators of the inflammatory response in fish; hence their reduction in diets containing vegetable oils as a single lipid source may increase susceptibility to pathogens. Most anti-nutrients at current levels do not lead to mortality, but could produce adverse effects such as immune impairment, and decreased productivity.

Anti-nutrients due to fishmeal and fish oil replacement

Anti-nutrients can be broadly divided into four groups: (i) factors affecting protein utilization and digestion, such as protease inhibitors: tannins and lectins among others; (ii) factors affecting mineral utilization, which include phytates, gossypol pigments, oxalates, glucosinolates; (iii) anti-vitamins; and (iv) miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phyto-oestrogens and saponins (Francis *et al*., 2001). Effects of anti-nutrients seem to be important; however, the picture is complicated as the gut microbiota may modify the anti-nutrients and therefore their interactions and biological effects may be altered (Krogdahl *et al*., 2010). Protease inhibitors, phytates and antigenic compounds at levels likely to be present in fish diets containing commercially available plant-derived protein sources, are unlikely to affect fish growth performance. A number of studies in gilthead sea bream and European seabass have shown that partial replacement of fishmeal with plant proteins is possible (Robaina *et al*., 1995; Pereira and Oliva-Teles, 2003; Gómez-Requeni *et al*., 2004; Kaushik *et al*., 2004) but should be made taking into account the possible anti-nutritional effects.

Clinical signs, gross and histopathological lesions

Histological studies showed an increased deposition of lipids and decreased glycogen deposits in the liver with increased levels of fishmeal replacement (Robaina *et al*., 1995; Sitjà-Bobadilla *et al.,* 2005). The lipid accumulation in enterocytes also led to desquamation and degeneration of the epithelium in gilthead sea bream fed different lipid sources (Caballero *et al*., 2003). Moreover, a marked dilation of the gut submucosa (frequently with eosinophilic granular cells infiltration) and liver steatosis was also observed in fish fed diets with total fish meal replacement (Sitjà-Bobadilla *et al.,* 2005).

Prevention and control

Some anti-nutrients are easy to eliminate during processing, while others are not. Since organic acids are particularly heat-stable, a beneficial effect can be obtained from heat-treating proteic compounds (Guillaume and Métailler, 2001). In addition, oligosaccharides become more digestible after heat treatment, whereas the effect of thermal treatment on substances such as tannins remains uncertain (Francis *et al*., 2001). For all anti-nutrients, fermentation or enzyme treatments directly focusing on inactivation of a specific anti-nutrient may reduce content or activity of the ingredient (Krogdahl *et al*., 2010). Selective breeding and genetic modification of plants may also alter their anti-nutrient contents. It is therefore theoretically possible to create genetically engineered ingredients with low levels of anti-nutrients. Obtaining varieties which are free or practically free of anti-nutrients will provide a radical solution to the problems encountered by nutritionists (Guillaume and Métailler, 2001; Krogdahl *et al*., 2010).

Inbreeding

Selective breeding to help improve fish stocks in Mediterranean aquaculture is still not well established. Future research will focus on: (i) increasing the quality of fish in terms of organoleptic and morphological characteristics; (ii) improving the efficiency of fish growth, by increasing feed conversion rates and decreasing skeletal deformities; and (iii) decreasing fish mortality by increasing fish disease resistance. This genetic selection should be done without losing genetic variability, as it can make future selective breeding programmes ineffective. Consequently, DNA microsatellite analysis could ensure successful retrospective parental assignments in more than 99% of cases. Moreover it allows comparisons of

offspring viability with different parental origins after the common rearing and mass spawning of sea bream species (Borrell *et al*., 2007). This tool has improved gilthead sea bream production with controlled genetic selection resulting in a better growth and carcass traits (Navarro *et al*., 2009) or faster growth of offspring (Borrell *et al*., 2011). Nevertheless, uncontrolled or not well-designed genetic drift could also lead to undesired side effects which could be the case of higher incidence of deformity (Afonso *et al*., 2000; Castro *et al*., 2008). It has been shown that European seabass could have differential growth depending on its genetic background when fed diets with partial vegetable oil replacements (Le Boucher *et al*., 2010). Hence, holistic approaches are recommended in breeding programmes that consider different fish traits. In addition, most of the genetically improved strains reaching the industry were developed through traditional selective breeding (selection, crossbreeding and hybridization; Hulata, 2001). However, emerging and more modern technologies for genetic manipulation (chromosome-set, sex manipulations and transgenics) are more strictly regulated and are not as well understood by the public, and this could hamper the consumer's perception.

Aquaculture waste

The bi-directional interaction between offshore aquaculture and the environment has been described at the beginning of this chapter, and the ways by which this interaction may limit the aquaculture success and growth. In addition, most environmental conditions cannot be controlled by fish farmers. However, it is not the case with eutrophication and destruction of natural ecosystems, which, at least in part, are affected by the farm's husbandry and management practices. As an example, excessive feeding regimens and rates can lead to the release of organic compounds into the environment, which promotes eutrophication. Another example is the increased release of pollutants such as copper or irgarol associated with anti-fouling treatments. Therefore a controlled waste production strategy is necessary to maintain sustainable aquaculture growth in the future (reviewed by Amirkolaie, 2012). As food is the major source of waste in aquaculture, the management of aquaculture waste can be controlled by food producers through optimized diet formulations and through improved feeding strategies by fish farmers. Highly digestible diets have been introduced to reduce solid waste excretion. Further reductions in solid waste can be achieved through careful selection of feed ingredients and food processing to improve nutrient availability. A reduction in released dissolved nitrogen wastes can be achieved by ensuring a balance between protein and energy promoting fish to use non-protein sources as energy source. Phosphorous wastes can be decreased through careful ingredient selection and proper processing to improve diet digestibility. A proper food ration and feeding method for each species should be adopted, because feed leftovers constitute a large part of waste production. However, organic waste output from aquaculture feeding operations cannot be completely eliminated since fish do not retain all of the food they consume and some part of the food always remains uneaten.

Climate change

Climate change presents one of the most serious environmental threats for global ecosystems and its potential effects on aquaculture have been recently reviewed (Rosa *et al*., 2012).These authors address most of the relevant impacts of climate change (on temperature, eutrophication, harmful algae blooms, sea level rise, acidification and disease) and propose a wide range of adaptation and mitigation strategies that might be implemented to minimize the impacts.

Global climate change may impose severe risks for aquatic animal health, since increasing water temperature leads to an increased incidence of parasitic and bacterial diseases (Harvell *et al*., 2002; Pounds *et al*., 2006; Lafferty, 2009). This could occur through a temperature-driven effect on the epidemiology of the disease. Higher temperatures may

boost the rate of disease spread through positive effects on parasite fitness in a weakened host due to the thermal stress (Harvell *et al*., 2002). Increased temperature may also extend the transmission period leading to higher total prevalence of infection and more widespread epidemics. However, the direction and magnitude of disease outbreaks related to higher temperatures is known to vary between different parts of the world (Easterling *et al*., 2000). It has been shown that temperature variation has decreased in some areas due to an increase in minimum temperatures rather than in the maxima (Easterling *et al*., 1997), emphasizing the asymmetric nature of climate warming. The effects of global warming will also depend on the age of fish, disease ecology, farming practices and immunity (Karvonen *et al*., 2010). Another example is the spread of *Ciona intestinalis* and other invasive biofouling organisms, which increase the complexity of the strategies against fouling in sea cages (Fitridge *et al*., 2012). Global warming has many unpredictable effects on the marine environment and consequently on offshore aquaculture. Side effects of the global climate change also include the acidification of seawater and the rise of sea levels. Recent evidence suggests that both increased water temperatures and elevated levels of dissolved CO_2 can change the behaviour of fish in ways that reduce individual fitness and could have significant effects on the growth and survival of fish, as it was reported in juvenile reef fish (*Amphiprion melanopus*; Nowicki *et al*., 2012).

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8 Sporadic Emerging Diseases and Disorders

Simon R.M. Jones[1]* and Pedro A. Smith[2]
[1]*Pacific Biological Station, Nanaimo, British Columbia, Canada;*
[2]*Department of Animal Pathology, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile*

Introduction

An emerging disease is either a novel disease or an existing disease with novel characteristics including increased severity, and appearance in a new host species or in a new geographical area. Changes in disease characteristics, including clinical signs, among seawater netpen-reared fish can also be influenced by transitions in coastal ecosystems as a result of climate change (Harvell *et al*., 1999). Sporadic diseases are a subset of emerging diseases that are limited spatially, temporally or both. Awareness of emerging diseases in netpen-reared finfish over the last decade has been influenced most importantly by two trends: the application of highly sensitive gene-based diagnostic and screening technologies and the rapid expansion of netpen aquaculture, both in geographic range and species cultivated. Coincident with these trends has been the introduction of regulations that limit the regional and international movement of fish and their products based on a list of known pathogens. These regulations seek to limit the rapid emergence of diseases following introduction of exotic pathogens into a naïve ecosystem. An example of this is the first appearance of viral haemorrhagic septicaemia virus (VHSV) in the Great Lakes of North America in 2003. This outbreak affected and caused mortality in a large number of wild species including perch (*Perca flavescens*) and walleye (*Sander vitreus*) (Al-Hussinee *et al*., 2011) and was caused by the novel VHSV genotype IVb, with affinities to isolates from mummichog (*Fundulus heteroclitus*), striped bass (*Morone saxatilis*), 3-spine stickleback (*Gasterosteus aculeatus*) and brown trout (*Salmo trutta*) on the Atlantic coast of Canada (Gagné *et al*., 2007). Meanwhile, VHSV genotype IVa is enzootic on the Pacific coast of North America in salmonid and clupeid species (Meyers and Winton, 1995; Marty *et al*., 1998). The occurrence of VHSV in the Great Lakes elicited massive research efforts to understand the origin and impacts of the outbreak as well as efforts to minimize impacts to finfish populations in adjacent bodies of fresh water both in Canada and the USA (Elsayed *et al*., 2006; Al-Hussinee *et al*., 2011). The detection of infectious salmon anaemia virus (ISAV) in netpen-reared Atlantic salmon (*Salmo salar*) in Chile in 2007 is another example of the emergence of a disease following dissemination of the agent into a new region (Mardones *et al*., 2009). In Atlantic salmon, Chilean ISAV isolates caused high mortality,

* E-mail: simon.jones@dfo-mpo.gc.ca

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were shown to be most similar to strains belonging to a Norwegian clade of European isolates (genotype I) and were possibly introduced via salmon eggs around 1996 (Godoy *et al*., 2008; Kibenge *et al*., 2009; Vike *et al*., 2009). These examples underscore the observation that management and containment activities are typically unprepared for the rapidity and severity of emerging diseases in finfish.

Rather than being exotic, most diseases occurring in cultured finfish, whether sporadic or not, can be traced back to pathogens that are enzootic and their emergence reflects changes in aquaculture practices (Murray and Peeler, 2005). Among the examples cited in this chapter, most (e.g. *Francisella noatunensis*, piscine reovirus, piscine myocarditis virus, *Parvicapsula* spp., *Enteromyxum* spp.) are known to or suspected to occur in wild reservoir hosts. In these cases, their emergence reflects the introduction of susceptible hosts into an environment in which a natural cycle of infection already exists. In addition, haemorrhagic smolt syndrome, Pacific salmon jaundice and rainbow trout (*Oncorhynchus mykiss*) gastroenteritis are examples of sporadic diseases for which the aetiologies are not established but may be linked to one or more conditions in the aquaculture environment that may exacerbate the virulence of an unknown infectious agent. Although contemporary aquaculture husbandry practices in most regions seek to minimize the impacts of disease among farmed finfish, some practices such as increased total biomass at a site, selection for rapid fish growth and non-fish-sourced nutrients in commercial diets, may exacerbate the risk of diseases and disorders. Chemotherapeutic intervention and vaccines are not yet options for many diseases caused by parasites and viruses. In these cases, early detection and slaughter of infected stock can be the most effective control measures. Thus, advances in our knowledge concerning reservoirs of infection, the mechanism(s) of pathogen transmission and of host resistance will increase the likelihood of effective management or containment strategies (Rimstad, 2011). This chapter introduces and describes the impacts of a selection of diseases that have recently been of sporadic or emergent importance to the culture of finfish. For each, diagnostic approaches are reviewed along with a summary of clinical signs and characteristic lesions and possibilities for prevention, treatment or mitigation are discussed.

Piscine Myocarditis Virus (Cardiomyopathy Syndrome)

Introduction

Cardiomyopathy syndrome (CMS) is an endomyocarditis first observed in the mid-1980s among Atlantic salmon farmed in Norway (Amin and Trasti, 1988; Ferguson *et al*., 1990). The disease has since been reported from Atlantic salmon in Scotland (Rodger and Turnbull, 2000) and the Faroe Islands (Poppe and Seierstad, 2003). In Norway, CMS occurs throughout the year, although fewer cases were reported between July and September (Brun *et al*., 2003), and the disease is typically observed during the second production year in sea water (Ferguson *et al*., 1990). The cause of CMS was initially not known although nodavirus-like particles were observed in affected cardiac muscle (Grotmol *et al*., 1997) and Hodneland *et al*. (2005) obtained two isolates of a Norwegian salmon alphavirus (NSA virus) from salmon affected with CMS. The occurrence of typical CMS lesions in wild Norwegian salmon was also suggested as evidence of an infectious aetiology (Poppe and Seierstad, 2003). An infectious aetiology for CMS was later confirmed by the reproduction of characteristic lesions in naïve Atlantic salmon following inoculation with tissue homogenates prepared from CMS cases in Norway and Scotland (Bruno and Noguera, 2009; Fritsvold *et al*., 2009). Since then, genetic evidence of a previously undescribed virus belonging to the *Totiviridae* was found in association with CMS lesions (Løvoll *et al*., 2010). Subsequently, a totivirus, named piscine myocarditis virus (PMCV), was isolated from affected tissues and CMS lesions were reproduced in naïve salmon following inoculation with the cultured virus (Haugland *et al*., 2011). PMCV has since been detected in marine smelt

(*Argentina silus*) in Norwegian waters (Böckerman *et al*., 2011; Tengs and Böckerman, 2012).

Impact(s) on fish production

The significant economic impact of CMS reflects a high frequency of occurrence among near to market size salmon. Brun *et al*. (2003) examined 246 cases between 1998 and 2001, and estimated that CMS was associated with a direct cost to the Norwegian salmon aquaculture industry of €4.5M to €8.8M. Wiik-Nielsen, J. *et al*. (2012) have suggested that the present impact of CMS is even higher given the growth of the salmon aquaculture industry in Norway.

Diagnosis

Cardiomyopathy syndrome, heart and skeletal muscle inflammation (HSMI, see next section) and salmon pancreas disease are all associated with myopathies in farmed Atlantic salmon and their differentiation can present a diagnostic challenge (Table 8.1). Other conditions associated with cardiomyopathies in salmon include congenital defects, acute or chronic infections, connective tissue disorders, deformities, nutritional deficiencies and autoimmunity (Bruno and Noguera, 2009). Mixed infections of PMCV and the piscine reovirus (PRV, see next section) have been reported (Løvoll *et al*., 2010; Wiik-Nielsen, C.R. *et al*., 2012a). Typically CMS occurs in preharvest salmon although the lesions have been reproduced in experimentally infected postsmolts (Fritsvold *et al*., 2009), and PMVC has been detected in salmon fry (Wiik-Nielsen, C.R. *et al*., 2012a). The virus has a broad tissue tropism and tissue-specific viral titres, inferred from levels of genomic RNA correlate with cardiac histopathology (Timmerhaus *et al*., 2011). Thus, histopathological diagnosis of CMS may be confirmed with the detection of PMCV.

Table 8.1. A comparison of heart and skeletal muscle lesions in Atlantic salmon associated with heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and pancreas disease (PD) (Kongtorp *et al*., 2004b).

Lesions	HSMI	CMS	PD
Epicarditis	+	+	+
Myocarditis and degeneration of compact myocardium	+	–	+
Myocarditis and degeneration of spongy myocardium	+	+	+
Skeletal muscle inflammation and degeneration	+	–	+
Multifocal necrosis of hepatocytes	+	+/–	–
Necrosis of exocrine pancreas	–	–	+

Clinical signs, gross and histopathological lesions

Sudden mortality in the absence of prior clinical signs is a common feature of CMS, although anorexia, lethargy, haemorrhagic skin and oedema can be observed in affected fish (Rodger and Turnbull, 2000; Poppe and Seierstad, 2003). Findings at necropsy may also include a fibrinous peritonitis with ascites, atrial dilation and thrombosis (Fig. 8.1) and haemopericardium leading to cardiac tamponade (Ferguson *et al*., 1990; Rodger and Turnbull, 2000; Poppe and Seierstad, 2003). Microscopic lesions include a mononuclear cell infiltration (Fig. 8.2) affecting the myocardium and endocardium (Bruno and Noguera, 2009). In later stages of CMS, cardiac muscle fibres are replaced by inflammatory cells, a process rarely observed in HSMI (Bruno and Noguera, 2009).

Prevention and control

The occurrence of a marine reservoir for PMCV indicates the possibility of ongoing risk to farmed salmon along the entire coast of Norway. Preliminary surveillance, however, suggests PMCV occurs sporadically and only in association with outbreaks of CMS (Wiik-Nielsen, J. *et al*., 2012). The occurrence

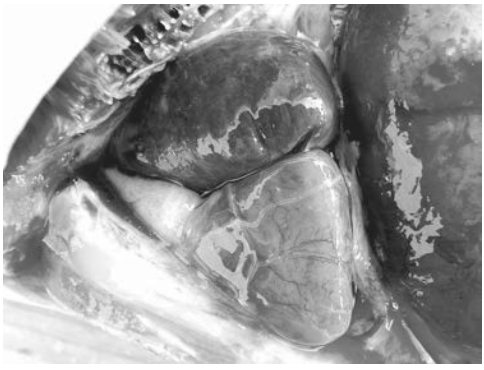


Fig. 8.1. Atlantic salmon (*Salmo salar*) affected with cardiomyopathy syndrome showing severely dilated atrium. Image courtesy of T. Poppe, University of Oslo.

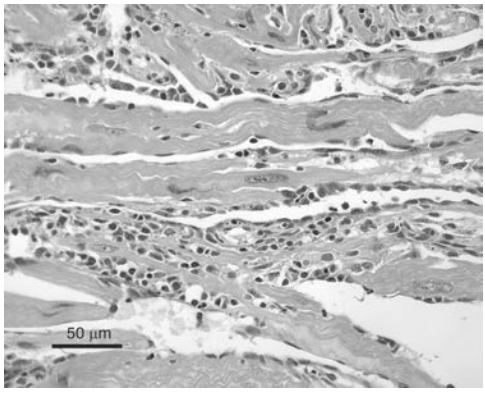


Fig. 8.2. Spongy myocardium of Atlantic salmon (*Salmo salar*) affected with cardiomyopathy syndrome showing cardiomyocyte degeneration and inflammatory infiltrate. H & E stain. Image courtesy of J. Wiik-Nielsen, University of Oslo.

of CMS in wild salmon indicates aquaculture conditions are not a strict requirement for disease development. However, in the absence of a vaccine or other treatments, optimizing husbandry and health management practices on farms would seem a prudent strategy to minimize the impact of CMS. As with HSMI, the presence of adverse environmental factors and other infectious agents (see Nylund *et al*., 2011), may increase the severity of CMS following infection with PMCV. Careful monitoring of the patterns of CMS-associated mortality among farm-reared Atlantic salmon may provide opportunities for improved disease management (Soares *et al*., 2011). An earlier suggestion that selective breeding may prove useful in reducing the incidence of CMS (Kent and Poppe, 1998) appears to be supported by recent research indicating divergent disease outcomes in high- and low-responder Atlantic salmon (Timmerhaus *et al*., 2012).

Piscine Reovirus (Heart and Skeletal Muscle Inflammation)

Introduction

Since its recognition as a distinct pathological condition in 1999 (Kongtorp *et al*., 2004a, b), heart and skeletal muscle inflammation (HSMI) has emerged as one of the most common diseases in farmed Atlantic salmon in Norway (Løvoll *et al*., 2012). A disease similar to HSMI was also reported in Scotland (Ferguson *et al*., 2005). The disease is principally recognized by a distinctive pattern of histopathological lesions in cardiac and skeletal muscles and in the liver. An infectious aetiology was suggested from controlled transmission experiments (Kongtorp *et al*., 2004a; Kongtorp and Taksdal, 2009) and from epidemiological data indicating increased spatial distribution of HSMI over time in Norway (Kongtorp *et al*., 2006). Morphologically distinct viral-like particles were subsequently observed in tissues from HSMI-affected Atlantic salmon (Watanabe *et al*., 2006) and a novel piscine reovirus (PRV), also referred to as the Atlantic salmon reovirus, has been correlated with the occurrence of HSMI (Palacios *et al*., 2010; Mikalsen *et al*., 2012). There is preliminary evidence for the presence of PRV in smelt (*A. silus*), Atlantic horse mackerel (*Trachurus trachurus*), Atlantic herring (*Clupea harengus*) and capelin (*Mallotus mallotus*) in Norwegian waters (Wiik-Nielsen, C.R. *et al*., 2012b). In addition, PRV and the poorly described Tasmanian salmon reovirus have been recovered from Atlantic salmon in the absence of HSMI in Norway, Chile and Australia (Palacios *et al*., 2010; Anonymous, 2012; Zainathan *et al*., 2012). Thus while the rapid emergence of HSMI in Norway may be related to the widespread distribution of PRV,

more research is required to understand the aetiology and pathogenesis of HSMI, including host and environmental factors that may trigger viral replication.

Impact(s) on fish production

HSMI mainly affects salmon several months after transfer to sea water, although the disease has been observed within 2 weeks of transfer (Kongtorp *et al*., 2004a). Most fish in affected net pens show signs of morbidity, and cumulative mortality may reach 20%. Some salmon farms in Norway experience annual outbreaks with associated financial losses (Kongtorp *et al*., 2006). An outbreak of an HSMI-like disease in Scotland was associated with 9% mortality over 3 months and resulted in slaughter of the entire stock (Ferguson *et al*., 2005).

Diagnosis

Primary diagnosis is based on a combination of clinical signs and histopathological lesions in cardiac and skeletal muscles and in the liver. Mikalsen *et al*. (2012) showed that PRV replicated and caused cytopathology in the GF-1 cell line from the grouper (*Epinephelus coioides*). Furthermore, inoculation with supernatant and cell lysates from infected cultures resulted in lesions consistent with HSMI in naïve salmon. In addition, HSMI lesions and genetic evidence of the virus occurred in naïve salmon cohabited with PRV-injected salmon (Mikalsen *et al*., 2012), providing evidence of a causal relationship between PRV and HSMI. Despite this, the apparently ubiquitous distribution of PRV in healthy farmed salmon and in wild salmon in Norway indicates that detection of the agent is not a particularly valuable primary diagnostic tool. Rather, the repeated demonstration that HSMI lesions are strongly correlated with viral genomic RNA copy number (Palacios *et al*., 2010; Finstad *et al*., 2012; Løvoll *et al*., 2012) suggests that additional host, environmental or infectious factors influence viral replication and the development of HSMI. Diagnostic rule-outs include CMS and pancreas disease (PD, McLaughlin and Graham, 2002), the pathologies of which are summarized in Table 8.1.

Clinical signs, gross and histopathological lesions

While morbidity is typically close to 100%, affected fish tend not to present externally visible signs other than being lethargic. Grossly visible lesions include pale flaccid heart, pericardial haemorrhage, ascites and pale or stained liver. Microscopic lesions include necrosis of cardiac myocytes with a concomitant massive mononuclear inflammatory infiltrate involving both compact and spongy layers of the ventricle (Kongtorp *et al*., 2004b). Epicarditis is frequently extensive and associated with the myocarditis. The pattern of inflammation in red skeletal muscle is similar to that observed in heart although not always observed. Focal necrosis in the liver in addition to oedema and congestion in multiple organs are consistent with circulatory failure. Research has begun to characterize the immunological characteristics of the inflammatory infiltrate and suggests a diversity of T-cell subsets with evidence of wound healing and apoptosis within the cardiac lesions (Mikalsen *et al*., 2012; Yousaf *et al*., 2012).

Prevention and control

Given the widespread occurrence of HSMI along the Norwegian coastline, prevention and control will require a much clearer understanding of the aetiology, including the relative roles of PRV, the environment, the host and perhaps other infectious factors on the development of the disease. It will also be important to understand how these factors influence PRV transmission and replication in the host. Observations from Scotland (Ferguson *et al*., 2005) suggested handling stress precipitated an earlier onset of mortality. This appears to emphasize a need to minimize stressors as part of an overall fish health management strategy. A stochastic model for

assessing transmission of HSMI (PRV) among salmon farms in Norway (Aldrin *et al*., 2010) may prove useful in developing disease management strategies. A vaccine is not presently available. However, salmon fed a diet supplemented with the saturated fatty acid tetradecylthioacetic acid (TTA) experienced a modest reduction in mortality (2.5%) and increased growth compared with controls (4.7% mortality), following an outbreak of HSMI in experimental seawater net pens (Alne *et al*., 2009). Increased expression of immune-related genes in TTA-fed salmon during HSMI was suggested to reflect increased immunocompetence in the heart (Grammes *et al*., 2012). Whether a TTA-supplemented diet reduces the prevalence of PRV or the prevalence and severity of morbidity is unknown.

Francisella noatunensis (Francisellosis)

Introduction

Bacteria of the genus *Francisella* have only recently been described as pathogens in aquatic animals, whereas *F. tularensis* in mammals has been recognized as the causative agent of tularaemia, a rare zoonotic disease with high mortality rate, for almost a century (Oyston, 2008). In this respect it is noteworthy that *F. tularensis* is one of the most virulent bacterial pathogens known and is a major concern for public health because of its potential as a biological weapon (Darling *et al*., 2002; Johannson *et al*., 2012). Bacteria in this genus are Gram-negative, non-motile, facultative intracellular, non-spore forming cocco-bacilli.

In wild and cultured finfish, francisellosis is caused by *Francisella noatunensis*. The infection ranges from an acute to chronic granulomatous disease. Although the nomenclature of fish-pathogenic *Francisella* isolates underwent an initial period of change (Mikalsen *et al*., 2007; Ottem *et al*., 2007; Ottem *et al*., 2008, Mikalsen and Colquhoun, 2009; Ottem *et al*., 2009), isolates from cases in cultured Atlantic cod (*Gadus morhua*) in Norway and cultured Atlantic salmon in Chile are now considered *F. noatunensis* subsp. *noatunensis* whereas those from cultured tilapia (family *Cichlidae*) in Taiwan, the USA, the UK and from the three-lined grunt (*Parapristipoma trilineatum*) in Japan are referred to as *F. noatunensis* subsp. *orientalis* (syn *F. asiatica*). *Francisella* spp. share phylogenetic affinity with *Piscirickettsia salmonis* (Gammaproteobacteria), another intracellular bacterial pathogen of fish. Neither *Francisella* spp. nor *P. salmonis* are related to the true *Rickettsia* spp., which belong to the Alphaproteobacteria. The direct transmission of *F. noatunensis* through sea water (Ellingsen *et al*., 2011) combined with the widespread geographic distribution of the disease indicates both the ubiquity of the causal agent and the risk to susceptible hosts maintained in high density under aquaculture environments.

Impact

Francisellosis is a threat to the development of the cod aquaculture industry. According to FAO (2010), approximately 21,000 t of cod were produced in 2009, mainly in Norway (18,000 t), Iceland (2,000 t) and in Canada and the USA combined (1,000 t). In contrast, annual landings from commercial fisheries are approximately 800,000 t. The bacterium was confirmed by analysis of archival tissues to have been present in wild cod from the southern North Sea as early as 1988 (Zerihun *et al*., 2011). All reports of the infection in farmed cod have come from Norway, as far north as Nordland (Ottem *et al*., 2008). In the two reported outbreaks, clinical signs were first observed among larger (2 to 3 kg) fish (Nylund *et al*., 2006; Olsen *et al*., 2006) but it is unknown when the infections were acquired. The prevalence of the disease in farmed cod was as high as 48% in one area; however, it is not clear whether moribund fish were selected or if sampling was random. An apparent association between high water temperatures and elevated francisellosis-associated mortalities, combined with a recent trend of increasing water temperatures along the coast of south-western Norway, indicates a likelihood that the disease incidence will continue to rise.

Following a 25-year span of intensive cultivation, the current annual global production of Nile tilapia is estimated to be 2.2 million t (FAO, 2010). Infections with unidentified rickettsia-like organisms (RLOs), often associated with elevated mortality and systemic granulomatous lesions have been reported in cultured tilapia over a wide geographic range (Chen *et al*., 1994; Chern and Chao, 1994; Fryer and Mauel, 1997; Mauel and Miller, 2002; Mauel *et al*., 2003; Mauel *et al*., 2005). Elevated mortality and similar pathology specifically associated with *Francisella* spp. infections have since been reported in tilapia from Taiwan, Latin America, the Midwest USA and the UK (Hsieh *et al*., 2006; Mauel *et al*., 2007; Soto *et al*., 2009b; Jeffery *et al*., 2010; Soto *et al*., 2011a). Similarities in host species, geographic range and clinical presentation suggest that the original cases associated with RLO infections were actually francisellosis (Birbeck *et al*., 2011). All sizes and age classes are affected and morbidity and mortality can reduce the biomass by 5% to 80% in affected populations (Mauel *et al*., 2007). It is evident that all cichlid species cultured for food or ornamental purposes are at risk of the disease (Hsieh *et al*., 2007) and that increased attention to biosecurity will be required to minimize future disease outbreaks (Jeffery *et al*., 2010).

Francisellosis was reported in Atlantic salmon parr maintained in freshwater cages in Lake Llanquihue, Chile in June 2006 (Birbeck *et al*., 2007; Bohle *et al*., 2009). Cumulative mortality ranged from 5% to 20% and granulomatous lesions occurred in moribund and dead fish. Analysis of 16S ribosomal RNA gene sequences showed the Chilean isolate to be 98% to 100% identical to *F. noatunensis* subsp. *noatunensis* from cod. The bacterium from the recent outbreak was similar to UA2, a previously unidentified *P. salmonis*-like organism recognized from farmed Atlantic and coho salmon and from rainbow trout in southern Chile (Cvitanich *et al*., 1995). Atlantic salmon in the earlier outbreak presented with granulomatous lesions in the liver.

Overall, cumulative mortality of 5% to 60% occurred among three-lined grunt ranging from 6 g to 510 g (Fukuda *et al*., 2002). The disease, which had been present in Japan since 1999, was subsequently confirmed as francisellosis (Kamaishi *et al*., 2005).

Francisellosis was associated with morbidity and low-level mortality among yearling hybrid striped bass (*Morone chrysops* × *M. saxatilis*), ranging from 10 g to 350 g, maintained in high-density freshwater recirculation systems (Ostland *et al*., 2006).

Diagnosis

Primary diagnosis of francisellosis is based on culture of the bacterium from specimens with typical clinical and pathological signs. Fish pathogenic *Francisella* spp. are fastidious and isolation requires L-cysteine and a source of iron in the culture medium. The bacterium grows optimally between 22° and 25°C with no growth at 37°C (Birbeck *et al*., 2011; Colquhoun and Duodo, 2011). Birbeck *et al*. (2011) summarize biochemical characteristics of *Francisella* spp. Amplification and sequencing of genomic DNA provides a confirmatory diagnosis (Ottem *et al*., 2006; Kulkarni *et al*. 2010; Soto *et al*., 2010b).

Clinical signs, gross and histopathological lesions

Clinical signs and gross lesions include discoloration of the skin with focal haemorrhage or petechiae, with or without elevated nodules, ulceration or scale loss, exophthalmia, emaciation, renomegaly, splenomegaly and serosanginous ascites (Olsen *et al*., 2006; Mikalsen *et al*., 2009). Widespread granuloma are associated with the surface of most internal organs and gills (Fig. 8.3). Histological changes include systemic granulomatous inflammation (Fig. 8.4) with extensive infiltration with fibrocytes and leukocytes. Granuloma are associated with a gradually developing focal to diffuse necrosis and necrotising vasculitis (Mauel *et al*., 2007).

Prevention and control

There are limited data on the efficacy of antibiotics against *F. noatuensis*. Oxytetracycline or florfenicol inhibit the growth of

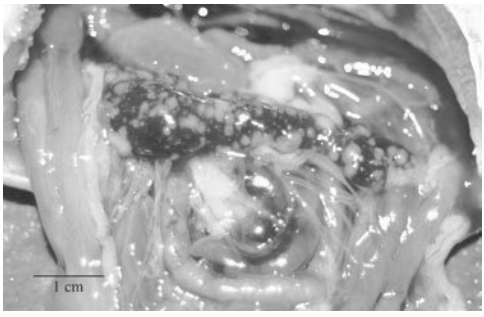


Fig. 8.3. Tilapia (*Oreochromis* sp.), enlarged spleen with numerous white nodules of variable sizes. Image courtesy of M. Manuel, Mississippi State University.

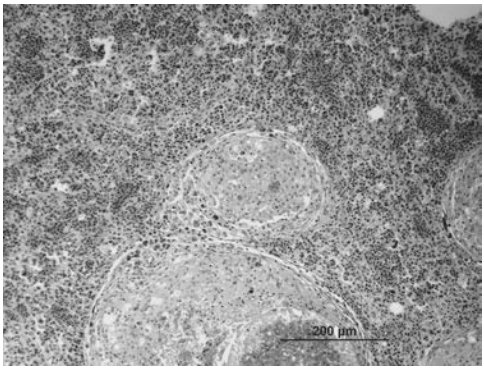


Fig. 8.4. Tilapia (*Oreochromis* sp.) spleen showing well-developed granuloma with necrosis in centre of lesions. H&E stain. Image courtesy of M. Manuel, Mississippi State University.

F. noatunensis subsp. *orientalis* isolated from tilapia and hybrid striped bass (Chern and Chao, 1994; Mauel *et al*., 2003; Ostland *et al*., 2006; Soto *et al*., 2010a; Soto *et al*., 2012). Treatments administered prior to or shortly after infection tended to be most efficacious. A cod-derived isolate of *Francisella* was sensitive to ceftazidime, tetracycline, gentamycin and ciprofloxacin (Ottem *et al*., 2006). Later, 30 cod isolates of *F. noatunensis* subsp. *noatunensis* were shown to be sensitive to flumequine, oxolinic acid and florfenicol but resistant to oxytetracycline and a combination of trimethoprim and sulfadiazine (Tribrissen) (Isachsen *et al*., 2012).

Vaccination may prove useful in preventing francisellosis; however, trials with killed *Francisella*-based bacterins conducted in cod in Norway have not been successful (Colquhoun and Duodo, 2011). Mutation of an intracellular growth locus gene rendered a strain of *F. noatunensis* subsp. *orientalis* avirulent in tilapia (Soto *et al*., 2009a). Bath vaccination with the avirulent mutant was shown to protect tilapia against virulent homologous challenge with the wild type strain (Soto *et al*., 2011b). This approach may prove useful in the development of a vaccine against francisellosis in cod.

Manipulation of water temperatures may provide some measure of control as, in contrast to the case in cod, even small decreases in temperature may exacerbate the disease in tilapia and hybrid striped bass (Ostland *et al*., 2006; Mauel *et al*., 2007). A commercial disinfectant was shown to be efficacious against an Atlantic salmon isolate of *F. noatunensis* in Chile (Muller *et al*., 2011).

Parvicapsula spp. (Parvicapsulosis)

Introduction

Myxozoa belonging to the genus *Parvicapsula* are coelozoic or histozoic parasites of the urinary or biliary systems of marine or anadromous fish (Lom and Dyková, 1992). The life cycle of *Parvicapsula* spp. is believed to be heteroxenous involving an invertebrate host (Køie *et al*., 2007). A total of 14 species have been described and some of these are pathogenic to their salmon hosts reared in seawater net pens. An unidentified *Parvicapsula* sp. was first reported in 1979 from moribund pen-reared coho salmon in Puget Sound in the state of Washington, USA (Hoffman, 1984). Infections with a similar parasite were subsequently observed in Chinook salmon (*Oncorhynchus tshawytscha*), masu salmon (*Oncorhynchus masou*), cutthroat trout (*Oncorhynchus clarkii*) and Atlantic salmon held in pens near the coho salmon in Washington (Johnstone, 1984) and at one site of pen-reared coho salmon in British Columbia (B.C.), Canada (Kent, 1998). Similar parasites were reported from wild Pacific cod (*Gadus macrocephalus*) collected near the site of the affected salmon in Washington (Johnstone, 1984) and from adult pink salmon (*Oncorhynchus gorbuscha*) spawning in the Quinsam River, B.C. (Jones *et al*., 2006).

The parasite in pink salmon was identified as a new species, *Parvicapsula kabatai*, and found to have an identical ribosomal RNA gene sequence to archival samples collected from the coho salmon in Puget Sound 20 years earlier (Jones *et al*., 2006). Meanwhile, the morphologically similar *P. pseudobranchicola* was described from dead and moribund pen-reared Atlantic salmon in Norway (Karlsbakk *et al*., 2002). Genetic evidence of *P. pseudobranchicola* infection was detected in pseudobranch of wild Atlantic salmon, sea trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*), suggesting these are natural hosts of the parasite (Jørgensen *et al*., 2011). A third species, *P. minibicornis*, has not been reported from farmed salmon despite having a high prevalence in nearby populations of wild Pacific salmon (Jones *et al*., 2003).

Impact(s) on fish production

In coho salmon, parvicapsulosis is detected as early as 8 to 10 weeks after fish are transferred to sea water (Johnstone, 1984). The prevalence is consistently higher and infections appear to persist longer among fish transferred to sea in November and December compared to those transferred in May; however, infections are not observed between August and December (Yasutake and Elliot, 2003). The prevalence is greatest in March and April and over 2 years ranges from 45% to 90%. Although the mortality among affected stock reaches 50% in some years, the extent to which parvicapsulosis contributes to the mortality is complicated by mixed infections with *Loma salmonae*, *Aeromonas salmonicida*, *Listonella anguillarum* or *Renibacterium salmoninarum* (Johnstone, 1984; Kent, 1998; Yasutake and Elliot, 2003). Infections with *P. pseudobranchicola* are associated with mortality that ranges from 2% to 40% among several salmon farms in Norway (Sterud *et al*., 2003; Nylund *et al*., 2005). There is no evidence that infections with bacteria or viruses contributed to the mortality (Sterud *et al*., 2003).

Diagnosis

Infections are diagnosed by direct observation of parasite spores in fresh wet mount preparations of affected tissues under phase contrast microscopy or in stained histological preparations. In both species the spores are approximately 12 μm in length with a characteristic curved shape and two apical polar capsules that are highly refractile under phase contrast illumination or prominent with histological staining. *P. kabatai* and *P. pseudobranchicola* affect and cause pathology in pseudobranch of coho and Atlantic salmon, respectively, although kidney infections also occur. Spores of the latter parasite have also been reported in the liver (Sterud *et al*., 2003). In contrast, there has been no evidence of *P. kabatai* infection in the pseudobranch of several hundred pink salmon, despite occasional heavy infections in the kidney, examined over nine years (S. Jones, unpublished observations).

Amplification of small subunit ribosomal DNA provides a rapid and sensitive means of detecting parasite DNA in a variety of tissues and is used to confirm diagnoses and to address questions of parasite epidemiology and phylogeny (Nylund *et al*., 2005; Jones *et al*., 2006; Jørgensen *et al*., 2011).

Clinical signs, gross and histopathological lesions

Coho salmon severely infected with *P. kabatai* may be darkly pigmented and lethargic (Kent, 1998). Associated mortality began at 9 weeks and peaked at 3% per week at 11 weeks post seawater transfer (Johnstone, 1984). Pseudobranchs are swollen with a mottled appearance ranging from grey to red with haemorrhagic foci. Occasional discrete whitish nodules occur in affected areas. Similarly, affected kidneys are swollen with a mottled appearance (Yasutake and Elliot, 2003). The interpretation of clinical signs and gross lesions must be tempered with the knowledge of concurrent infections. In coho salmon, microscopic lesions in affected pseudobranch included lamellar telangiectasis, focal haemorrhage and necrosis (Yasutake and Elliot, 2003). No histopathology was reported in other coho salmon tissues, however in the absence of bacterial or viral infections, infection in a juvenile (3.8 g) pink salmon was

associated with renal distension and granulomatous interstitial inflammation (Saksida *et al*., 2012).

Atlantic salmon infected with *P. pseudobranchicola* tend to swim near the surface and are lethargic. During later stages of the disease fish are darkly pigmented and display signs of blindness coincident with orbital haemorrhaging. Infected fish go off feed as indicated from empty gastrointestinal tracts (Karlsbakk *et al*., 2002). At necropsy, the pseudobranchs are melanotic to haemorrhagic and occasionally effaced with a caseous granulomatous layer (Karlsbakk *et al*., 2002). Necrosis results in loss of pseudobranch tissue (Fig. 8.5). Microscopic lesions in the pseudobranch and related connective tissues include a mononuclear inflammatory infiltrate and are remarkably similar to those caused by *P. kabatai* (Figs 8.6 and 8.7). Inflammation of the branchial filamentous epithelium and central vein is evident along with an increase in the number of circulating leukocytes. Renal melanomacrophage hyperplasia with increased accumulation of ferric iron in splenic macrophages is also evident (Sterud *et al*., 2003).

Prevention and control

Both *P. kabatai* and *P. pseudobranchicola* infect a range of wild hosts, which indicates natural cycles of the infections occur in western North America and western Norway, respectively. In neither case has the life cycle been completed experimentally and an annelid is the most likely invertebrate host (Bartholomew *et al*., 2006; Køie *et al*., 2007). An obvious but impractical control method will be to limit the establishment of netpen aquaculture in specific coastal areas enzootic for the *Parvicapsula* spp. No treatments for parvicapsuloses are available but efforts to treat other myxosporean infections of fish with fumagillin have been reported (Kent, 1998). Similarly, the coccidiostat nicarbazin is efficacious against *Kudoa thyrsites*,



Fig. 8.5. Atlantic salmon (*Salmo salar*) showing loss of tissue from centre of pseudobranch (black ring) resulting from infection with *Parvicapsula pseudobranchicola*. Image courtesy of T. Poppe, University of Oslo.

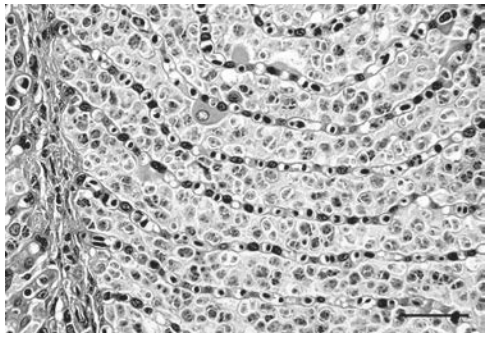


Fig. 8.6. Atlantic salmon (*Salmo salar*) showing *Parvicapsula pseudobranchicola* spores and plasmodia between parallel rows of pseudobranchial lamellae. H&E stain, bar is 50 μm. Image courtesy of T. Poppe, University of Oslo.

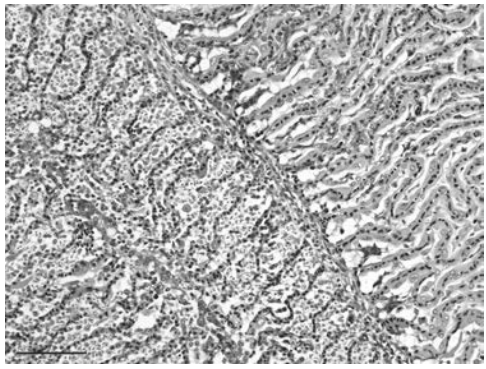


Fig. 8.7. Coho salmon (*Oncorhynchus kisutch*) showing pseudobranch infected with *Parvicapsula kabatai* (left) and apparently unaffected showing normal appearance (right). H&E stain, bar is 100 μm. Image courtesy of D. Elliott, Western Fisheries Research Center.

another histozoic myxosporean parasite of Atlantic salmon (Jones *et al*., 2012).

Pacific Salmon Jaundice

Introduction

Although jaundice or icterus is only sporadically observed in fish, there are a number of descriptions of this condition in salmonids and in a wide variety of other teleost species (Table 8.2). Outbreaks of jaundice have been observed in wild and cultured fish and in some cases are associated with high mortalities. Economic damage is also caused by the low growth rates of affected fish and by the loss of market value because of their unappealing colour.

Jaundice is caused by hyperbilirubinaemia, an excess of conjugated or unconjugated bilirubin (or both) which, in turn, produces a yellow or yellowish colour in different tissues. Typically, jaundice is detected externally in the skin of the belly and internally in the visceral fat because both tissues are usually white in healthy animals. Jaundice in fish has been classified grossly as pre-hepatic, hepatic and post-hepatic, according to the origin of the hepatobiliary system failure, as described in mammals. There are only a few studies about the biochemistry and metabolism of bile pigments of fish and these are limited to a few species (Cornelius, 1991; Goto *et al*., 2001; Ding and Xu, 2002). Nevertheless, it is clear that, in spite of general similarities, bilirubin metabolism of fish differs from that in mammals and, as could be expected, among fish species as well. Thus, although the serum of most fish is a pale yellow colour due to the presence of bilirubin, certain bony fish, such as eels of the order *Anguilliformes* and most members of the order *Scorpaeniformes*, have blue-green sera, due to the presence of biliverdin (Cornelius, 1991).

Most hyperbilirubinaemias in fish are preceded by an acute haemolytic crisis, i.e. prehepatic jaundice, which in some cases is also accompanied by a functional liver impairment. In turn, the aetiology of the haemolysis associated with the jaundice in fish is very wide and may include infectious agents, nutritional and toxic factors and genetic abnormalities. Bacterial and viral agents such as the erythrocytic inclusion body syndrome (EIBS) virus in Pacific salmon can be causative agents of haemolytic syndromes contributing to jaundice. Food containing oxidized polyunsaturated fatty acids, and vitamin E deficiencies are among the main nutritional causes of haemolysis that precede hyperbilirubinaemia. Also, toxins dissolved in the water contribute to haemolysis in fish and cause non-infectious hyperbilirubinaemia. For example, pollution in the River Don in Scotland derived from paper mill effluents and from an industrial area caused pigmented salmon syndrome in Atlantic salmon (Everall *et al*., 1992). Other chemicals that cause the same pathophysiological effect, but by parenteral exposure, are phenylhydrazine in yellowtail (*Seriola quinqueradiata*) and ß-naphthoflavone, resin acids and erythromycin in salmonids (Croce *et al*., 1995; Ito *et al*., 1999; Haukenes and Moffitt, 2002). Finally, acute haemolytic crises resulting in jaundice can also have a genetic origin in fish. For example, the congenital haemolytic anaemia, icteric liver and increased bilirubin concentrations observed in zebrafish (*Danio rerio*), are the result of a specific mutation in the gene that codes for protein 4.1, required to maintain erythrocyte deformability (Shafizadeh *et al*., 2002). These examples illustrate the importance of clinical pathology as a research and diagnostic tool for all diseases resulting from abnormal bile pigment metabolism in fish.

There are two diseases specifically related to jaundice in Pacific salmon in which icterus is the major, distinctive and consistent clinical sign. One affects Chinook salmon in Canada; in British Columbia, cultured Chinook salmon suffering a jaundice syndrome associated with mortalities have been observed for the past 7 years on the west coast of Vancouver Island. Fish exhibit mild to severe yellow discoloration of the skin, very pale gills and histopathological lesions in liver and kidney. Research to reveal the aetiology and the epidemiology of this disease is underway (Fisheries and Oceans Canada, 2011).

The second is coho salmon jaundice (infectious haemolytic anaemia of salmon) in

Table 8.2. Summary of diseases associated with jaundice in fish.

Species	Location (Wild/Cultured)	Disease	Primary type of increased bilirubin	Cause	Source
Anguilla japonica	Japan (Cultured)	Eel hyperbilirubinaemia	Conjugated in blood	Multifunctional liver impairment	Kawatzu and Sakai, 1980
Oncorhynchus mykiss	Finland (Cultured)	Unspecified	Conjugated in blood	Insufficient transcanalicular secretion in the liver caused by experimental exposure to resin acids	Mattsoff and Oikari, 1987
A. japonica	Japan (Cultured)	Eel hyperbilirubinaemia	Conjugated in blood	Intrahepatic disorder and secondarily by haemolysis	Endo *et al*., 1992
Salmo salar	Scotland (Wild)	Pigmented salmon syndrome	Unconjugated in blood	Haemolysis and liver damage caused by industrial effluents	Everall *et al*., 1992
Pagrus major	Japan (Cultured)	Red sea bream hyperbilirubinaemia	Unconjugated in blood	Unknown	Sakai *et al*., 1993
Clarias macrocephalus x C. gariepinus	Thailand (Cultured)	Catfish jaundice or yellow catfish disease	Conjugated in blood	Haemolysis caused by feeding fish with rancid chicken viscera	Pearson *et al*., 1994
O. kisutch	Japan, USA (Cultured)	Erythrocytic inclusion body syndrome	Total in blood	Haemolysis and a secretory disorder of bile pigments by EIBS virus	Sakai *et al*., 1994
Seriola quinqueradiata	Japan (Cultured)	Yellowtail jaundice	Unconjugated in blood	Haemolysis by lipid peroxidation and oxidative stress	Sakai *et al*., 1998
O. tshawytscha	USA (Cultured)	Unspecified	Undetermined	Erythromycin toxicity	Haukenes and Moffitt, 2002
Danio rerio	USA (Cultured)	Congenital anaemia	Total in organs	Haemolysis caused by protein 4.1 deficiency	Shafizadeh *et al*., 2002
O. kisutch	Chile (Cultured)	Infectious haemolytic anaemia of salmon	Total in blood	Haemolysis caused probably by an unknown virus and/or ISAV	Smith *et al*., 2006
C. betrachus x C. fuscusi	Taiwan (Cultured)	Haemolytic jaundice of hybrid catfish	Total in kidney and liver	Haemolysis probably caused by rancid food	Chang *et al*., 2008
C. gariepinus	Nigeria (Cultured)	Jaundice syndrome	Undetermined	Haemolysis caused by *Staphylococcus aureus*	Oladele *et al*., 2012

Chile. Since 1997, epizootics of infectious haemolytic anaemia of salmon (IHAS) have been associated with an icterus condition in coho salmon reared in seawater cages (Smith *et al*., 2002; Smith *et al*., 2006). The aetiology of this disease is not clear but experimental evidence indicates that it is caused by a transmissible infectious agent, most likely a virus. The disease can be severe with cumulative mortalities ranging from 1% to 30%. These figures are significant because, since 1994, Chile has been the largest global producer of coho salmon, exporting 385,325 t of this species in 2011 (SalmonChile, 2011).

Epidemiological, clinical and pathological features

Outbreaks of IHAS occur mainly at sea sites in the X and XI regions of southern Chile, usually with a higher incidence in the autumn. The earliest outbreaks begin 6 weeks after smolts are transferred to the sea and only coho salmon are affected. The disease is not observed in Atlantic salmon and rainbow trout stocked at the same or nearby sea-sites. Weekly cumulative mortality ranges from 1% to 5% and outbreaks last approximately 3 to 4 weeks. Affected fish have a yellowish colour in the skin of the abdominal region, at the base of the paired fins and in the periorbital tissues. The condition factor of affected fish is normal or high; fish in poor condition are rarely affected. Observations at necropsy include pale gills, ascites, abundant visceral fat with yellow colour, a light-brown liver with plethoric gall bladder, dark spleen, pale kidney and hydropericardium (Smith *et al*., 2006). Affected fish also have a severe clinical anaemia (Bustos, 1999; Smith *et al*., 2002; Godoy, 2004) consistent with haematocrits as low as 5% in moribund animals (J. Larenas, Santiago, Chile, 2012, personal communication).

Histopathological findings include a reduced number of erythrocytes within blood vessels and an absence of haemorrhage in all organs. In kidney and spleen, severe haemosiderosis accompanied with erythrophagocytosis occurs in kidney and spleen. Haemosiderin is in macrophages of the haematopoietic tissue and in the epithelial cells of the proximal tubules of the kidney (Fig. 8.8). In the liver, there is moderate to severe diffuse steatosis with occasional foci of acute necrosis. In some cases, cholestasis is also evident in liver. In some fish, a degeneration of myocytes, resembling the white muscle disease of mammals, is found in skeletal muscle and myocardium. The epicardium exhibits a great infiltration with adipose tissue with occasional foci of infiltrated mononuclear cells (Smith *et al*., 2006).

Experimental findings

IHAS was reproduced in naïve coho salmon following intraperitoneal inoculation with a 0.45 μm-filtered tissue homogenate obtained from a diseased coho salmon (Fig. 8.9). No evidence of the disease was observed in Atlantic salmon and rainbow trout injected with the same inoculum. Mortalities began at day 23 post inoculation (*p.i*.), and reached a cumulative value of 24% at day 27 *p.i*. In addition, IHAS occurred in non-inoculated cohabiting coho salmon, suggesting that this is a waterborne infection. Neither viruses, including ISAV, nor bacteria were detected in the inoculum or diseased fish. These results

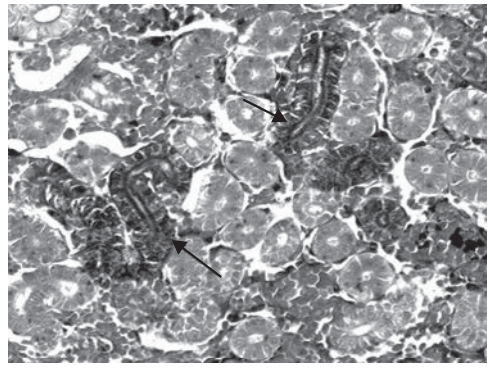


Fig. 8.8. Coho salmon (*Oncorhynchus kisutch*) with icterus collected from the field showing haemosiderosis in the epithelial cells (arrows) of the proximal tubules of a kidney. Prussian blue, ×200. Image courtesy of P. Smith, University of Chile.



Fig. 8.9. Coho salmon (*Oncorhynchus kisutch*) experimentally inoculated with a filtered homogenate (0.45 μm) exhibiting pallor in gills, heart and liver. Image courtesy of P. Smith, University of Chile.

suggest that a filterable agent, possibly an unidentified virus, is the cause of this disease.

Aetiology

It was initially believed that IHAS in coho salmon was caused by a strain of infectious salmon anaemia virus (ISAV) genetically related to the North American strains (Kibenge *et al.*, 2001). However, subsequent research on a number of outbreaks failed to isolate ISAV, and indeed to associate the aetiology of IHAS to any particular infectious agent (Bustos, 1999; Godoy, 2004; Smith *et al*., 2006). Furthermore, IHAS is reproduced in naïve coho salmon in the absence of ISAV (Smith *et al*., 2006). It is interesting to note that while IHAS and classical ISA both cause a severe anaemia, in the former the anaemia is due to haemolysis (Smith *et al*., 2006) while in the latter, it is mainly due to haemorrhage (Evensen *et al*., 1991; Mullins *et al*., 1998; Rimstad *et al*., 1999). More research is required to determine the epidemiological role of ISAV in IHAS in coho salmon. For example, it is possible that salmon in Chile suffer from clinically similar icteric syndromes caused by different aetiological agents, one of them being ISAV as reported by Kibenge *et al*. (2001). In conclusion, the available information suggests that the icterus condition in coho salmon is caused by one or more viruses but, Koch's postulates have not yet been fulfilled.

Enteromyxum spp.

Introduction

Infections with the intestinal myxosporean parasite *Enteromyxum leei* represent an increasingly significant impediment to the growth of netpen culture of European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) mainly in countries bordering the Mediterranean Sea (Diamant *et al*., 1994), but also in Japanese flounder (*Paralichthys olivaceus*) in Japan (Yasuda *et al*., 2005). Another species, *E. scophthalmi* is an important pathogen of farmed turbot (*Psetta maxima*) in Spanish waters (Branson *et al*., 1999; Palenzuela *et al*., 2002) whereas *E. fugu* is pathogenic in cultured tiger puffer (*Takifugu rubripes*) in Japan (Tun *et al*., 2002). These parasites are horizontally transmitted directly between fish hosts in the absence of an alternate invertebrate host (Diamant, 1997; Yasuda *et al*., 2002; Redondo *et al*., 2004; Yasuda *et al*., 2005; Estensoro *et al*., 2010), suggesting an epidemiological pattern more similar to bacteria and virus pathogens whose rate of transmission is greatly increased in the relatively dense host conditions found in aquaculture.

Impact on fish production

The rapid spread of infections in cage culture quickly leads to reduced productivity and in severe cases, mortality occurs. The impacts of infection will depend on species, age and the general condition of the host. Typically, infections are more prevalent and more severe in juvenile fish. However older, chronically infected fish, while tending to show fewer signs of severe disease, may be important sources of infection to more susceptible hosts in the same or nearby populations. Enteromyxosis renders the culture of sharp snout seabream (*Diplodus puntazzo*) unfeasible in some locations (Rigos and Katharios, 2010).

The expression of clinical signs of enteromyxoses is strongly temperature dependent although infections persist at suboptimal temperatures. In gilthead sea bream the permissive temperature range for disease caused by *E. leei* is 18°C to 22°C (Le Breton and Marques, 1995; Rigos *et al*., 1999), whereas in turbot, *E. scophthalmi* becomes virulent between 12°C and 18°C (Quiroga *et al*., 2006).

Diagnosis

Infection is diagnosed directly by the microscopic detection of mature spores in fresh or stained smears prepared from the intestinal mucosa. In some cases microscopic examination of histological preparations of intestine provides a reliable diagnostic tool. Either method may also reveal the presence of presporogonic parasite stages, which are more difficult to recognize by the untrained eye. Alternatively, polymerase chain reaction or *in situ* hybridization using parasite-specific oligonucleotide probes have been shown to be more sensitive diagnostic tools with the additional advantage that these tests confirm infections regardless of the stage of parasite development (Sitjà-Bobadilla and Palenzuela, 2012).

Clinical signs, gross and histopathological lesions

Clinical signs of enteromyxosis include poor appetite, weight loss and reduced feed conversion resulting in failure to meet growth expectations. The most frequent clinical sign is cachexia associated with muscle atrophy during chronic infections. In contrast, mortality associated with acute infections often occurs in the absence of clinical signs. Other signs may include generalized oedema and rectal prolapse, particularly in Japanese flounder and tiger puffer infected with *E. leei* and in turbot infected with *E. scophthalmi* (Sitjà-Bobadilla and Palenzuela, 2012). Gross signs reveal a deterioration of the intestine that becomes thin-walled, congested or haemorrhagic with mucus-like contents. A general pallor of other viscera and distension of the gall bladder are often noted. While the rapidity and severity of the histopathological response varies with host susceptibility, catarrhal enteritis is the most remarkable histopathological lesion and coincides with the distribution of the parasite, which ranges from over-dispersed in new infections to uniformly widespread when infections are well established. During *E. leei* infections in the susceptible sharp snout sea bream, a gradual loss of integrity of the gut mucosa progressed from localized vacuolation and necrosis of epithelial cells to complete detachment of epithelial cells from the submucosa (Álvarez-Pellitero *et al*., 2008). The intestinal lumen contains remnants of the epithelium and parasite stages. An inflammatory cellular infiltrate consisting of eosinophilic granulocytes (Fig. 8.10) was reported during infection in sharp snout sea bream (Álvarez-Pellitero *et al*., 2008). Macrophage-like cells were abundant in inflamed intestine of affected tiger puffer (Tun *et al*., 2002).

Prevention and control

A wide range of natural hosts in the Mediterranean Sea indicates that the risk of exposure to cultured fish will remain high. Treatment and preventative options are limited and

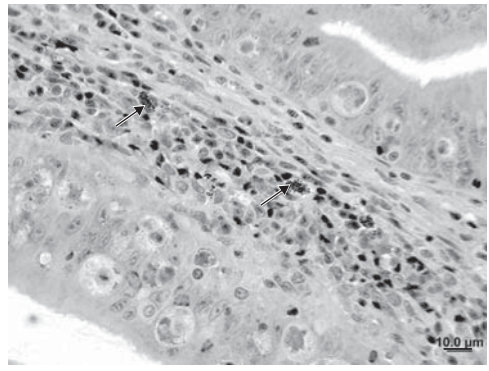


Fig. 8.10. Sharp snout sea bream (*Diplodus puntazzo*) showing eosinophilic granular cells (arrows) in the submucosa during infection with *Enteromyxum leei*. Semithin section from resin-embedded tissue. H&E stain. Image courtesy of A. Sitjà-Bobadilla, Instituto de Acuicultura de Torre de la Sal.

disease management continues to rely on early detection and removal of affected stock, disinfection and enhanced biosecurity. Limiting the mixing of age classes at a production site or among nearby sites may tend to improve the success of management actions. Land-based facilities have greater control over water quality and at these sites, ultraviolet irradiation, ozonation or filtration of incoming water, combined with temperature management may be feasible options to limit the introduction of infective stages and to minimize the development of clinical signs. In addition, protective immunity develops among sharp snout sea bream, turbot and red sea bream (*Pagrus major*) that have recovered from enteromyxoses (Golomazou *et al*., 2006; Sitjà-Bobadilla *et al*., 2007; Yanagida *et al*., 2008), raising the possibility of vaccine development.

Haemorrhagic Smolt Syndrome

Introduction

Anaemia, resulting from a reduction in number or size of circulating erythrocytes or from reduced haemoglobin, is a consequence of several infectious diseases of salmon. A previously undescribed haemorrhagic anaemia was reported in a stock of pre-smolt Atlantic salmon in Scotland (Rodger and Richards, 1998) and named haemorrhagic smolt syndrome (HSS). Subsequently, Atlantic salmon smolts at five farms in western and northern Norway were found to be affected with HSS (Nylund *et al*., 2003). As in the earlier case, signs of HSS were absent following transfer of affected stocks to sea. However Nylund *et al*. (2003) provided anecdotal evidence for the subclinical persistence of HSS among smolts transferred to sea water, with up to 90% morbidity. Injection with tissue homogenates prepared from HSS-affected salmon failed to induce clinical signs or mortality among naïve salmon and trout (Rodger and Richards, 1998; Nylund *et al*., 2003). However, Nylund *et al*. (2003) reported two types of viral-like particles in tissues of HSS-affected salmon: Type I was most likely the infectious pancreatic necrosis virus whereas the Type II particle was unidentified. In addition, a sequence obtained from one HSS-affected sample showed some similarity (90.4% to 95%) to known viruses of salmon belonging to the family *Togaviridae* (Nylund *et al*., 2003). While many signs of HSS and the presence of the viral-like particles suggest an infectious aetiology, the possibility that HSS results from an undefined nutritional deficiency or genetic disorder cannot be ruled out (Rodger and Richards, 1998).

Impact(s) on fish production

The scarcity of reports suggests a sporadic pattern of occurrence. However, given the low-level mortality, it is perhaps more likely that the disease, while of concern to the producer, is under-reported in the scientific literature. In the initial case, approximately 0.1% of the stock at the site died (Rodger and Richards, 1998). Similarly, mortality at affected sites in Norway was also low, on average less than 0.5% (Nylund *et al*., 2003). The persistence of morbidity in a significant part of the population may influence the overall health of the fish during and immediately after transfer to sea water. Similarly, because some signs can be confused with other haemorrhagic diseases, the occurrence of HSS may influence management actions.

Diagnosis

Primary diagnosis is based on the combined observation of total mortality less than 1% and haematocrit values less than 10% coincident with evidence of widespread haemorrhaging.

Clinical signs, gross and histopathological lesions

Morbid fish are lethargic, swim near the surface and appear dark in colour. The respiratory rate can be high and gill lamellae appear pale. Gross lesions include widespread petechiae and ecchymoses on the ventral

surface of the body, at the base of fins or on the inner surface of the operculum and throughout the abdominal viscera, heart, swim-bladder and musculature. The viscera also appear pale. Microscopic lesions demonstrated widespread occurrence of haemorrhage and necrosis in most tissues (Rodger and Richards, 1998; Nylund *et al*., 2003).

Prevention and control

The absence of an obvious aetiological agent limits options for treatment or vaccine development. Further research is required to identify infectious and non-infectious risk factors for HSS.

Rainbow Trout Gastroenteritis

Introduction

Since 1995, mortality associated with a severe catarrhal or haemorrhagic gastroenteritis affecting freshwater cultured rainbow trout has been observed in France, Spain, Italy, Croatia and the UK (Michel *et al*., 2002; Branson, 2003; Del-Pozo *et al*., 2009; Ronza *et al*., 2011). Mortalities most frequently occur during summer at a site in England and at farms in France and Spain; the onset of mortality occurs when water temperature exceeds 12°C and 16°C, respectively (Urdaci *et al*., 2001; Michel *et al*., 2002; Branson, 2003). A threshold of 9°C for an outbreak reported at a site in Scotland (Branson, 2003), requires confirmation. In the outbreak of rainbow trout gastroenteritis (RTGE) in England, there was an absence of infections commonly associated with mortality in farmed rainbow trout (Branson, 2003), however in other outbreaks, *A. salmonicida*, *Yersinia ruckeri* or *Tetracapsuloides bryosalmonae* have been identified (Michel *et al*., 2002; Del-Pozo *et al*., 2010c). Branson (2003) observed large, segmented filamentous bacteria (SFB) in histological preparations of the intestinal lumen. SFB had already been reported from the intestinal contents of trout collected in France and Spain that were affected with diarrhoea (Urdaci *et al*., 2001). The organisms were Gram-positive filamentous bacteria that formed spores within the distal segments and all attempts to propagate these organisms failed on a variety of bacteriological media. However, phylogenetic analysis of SFB ribosomal gene sequences showed that the trout specimens were most similar to filamentous bacteria from mice, rats and chickens, collectively referred to as *Candidatus arthromitus* (Urdaci *et al*., 2001). Although generally considered harmless in the vertebrate gut, SFB occur in approximately 85% of RTGE cases (Del-Pozo *et al*., 2010b). DNA consistent with SFB has been detected in the distal intestine of RTGE-free rainbow trout fry (Del-Pozo *et al*., 2010d). The uncertainty surrounding the role of SFB in the aetiology of RTGE is compounded by the inability to culture the organism. However, epidemiological data from the UK are consistent with a propagating pattern indicative of an infectious process (Del-Pozo *et al*., 2010a) while risk factor analyses support a mixed aetiology in which a variety of husbandry and environmental factors play a role (Del- Pozo *et al*., 2009, 2010a, b).

Impact(s) on fish production

An outbreak of RTGE is typically associated with daily mortalities ranging from 0.5% to 1.0%, and in exceptional cases up to 4%. Above the permissive temperature threshold, losses are greatest approximately 10–14 days after onset and may persist for up to 50 days (Del-Pozo *et al*., 2010a). The impact on production reflects the frequency of losses among fish ≥800 g, although smaller fish are also at risk. A direct effect on production is indicated by risk factor analysis, which links RTGE in the UK to intensive productivity (Del-Pozo *et al*., 2009).

Diagnosis

A diagnosis of RTGE is based on the case definition: 'a condition of rainbow trout, observed in (production) units with daily cumulative mortalities of 0.5% or more and present during the summer. Affected fish present a distended

abdomen externally, while internally their lower intestine is dilated, congested and oedematous, and has a yellow viscous content' (Del-Pozo *et al*., 2010c).

Clinical signs, gross and histopathological lesions

Lethargy, loss of appetite and altered behaviour, including slow, nervous or uncoordinated swimming, are evident among moribund stock (Michel *et al*., 2002). Affected fish may display patchy darkening of the skin along the flanks and void yellowish, mucoid faeces (Michel *et al*., 2002). The first sign of the disease on the farm is often the appearance of large quantities of the pale faecal casts (Branson, 2003). Reduced plasma electrolytes and an altered ratio of albumin to globulin are suggestive of a protein-losing enteropathy (Del-Pozo *et al*., 2010c). The most remarkable necropsy finding is acute enteritis which can be oedematous and haemorrhagic (Michel *et al*., 2002; Branson, 2003) or catarrhal (Ronza *et al*., 2011). The stomach, pyloric caeca and intestine are often enlarged and filled with a clear to straw-coloured liquid, which upon microscopic examination, is seen to contain SFB, often in abundance (Del-Pozo *et al*., 2010b). Microscopic examination of histological preparations revealed SFB in association with cells of the mucosa or in the lumen throughout the intestine (Michel *et al*., 2002; Branson, 2003; Del-Pozo *et al*., 2010b). Intact filamentous bacteria were more likely to be visualized or detected in the pyloric caeca than in the hindgut, possibly due to deterioration of the bacteria at the latter site (Del-Pozo *et al*., 2010d). Histopathological changes included detachment of enterocytes, with evidence of apoptosis (Ronza *et al*., 2011) and congestion of the adventitia and lamina propria. These changes occurred more frequently in the distal intestine compared with the pyloric caeca (Branson, 2003; Del-Pozo *et al*., 2010b). Michel *et al*. (2002) reported congestion in the pyloric stomach.

Prevention and control

Treating affected fish with dietary salt appears to be palliative possibly by compensating for the profound osmotic imbalance resulting from loss of intestinal mucosa. Additional husbandry measures to prevent or mitigate RTGE include reducing stock density, limiting mortalities due to predation or handling (i.e. reduction of stressors), reducing water temperature and enhancing biosecurity. The role of sound biosecurity practices is particularly important where one site is contiguous to another, but also applies to the movement of fish from RTGE-positive sites to more distant sites (Del-Pozo *et al*., 2010a). The effects of RTGE may be mitigated by a reduction or cessation of feed intake (Michel *et al*., 2002; Branson, 2003). Medicated feed may provide temporary benefit (Michel *et al*., 2002), and Branson (2003) indicated amoxycillin, oxytetracycline and potentiated sulfonamide are effective.

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9 Transmission of Infectious Agents between Wild and Farmed Fish

Sonja M. Saksida,[1]* Ian Gardner[2] and Michael L. Kent[3]
[1]*BC Centre for Aquatic Health Sciences, Campbell River, British Columbia, Canada;* [2]*Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada;* [3]*Oregon State University, Corvallis, Oregon*

Introduction

Cages used in farming fish are designed to contain cultured fish (with nets on the sides and bottom) while allowing for free water flow through the cages. This refreshment naturally maintains water quality by removing fish waste and debris from the pens and bringing in oxygenated water. Cage farms are widespread in coastal waters, but a farm typically occupies less than a hectare of coastal space (Dempster and Sanchez-Jerez, 2008). Although farms occupy small areas, they are highly attractive to wild fish as they provide both protection and food (in the form of excess feed, biofouling on cage structures, zooplankton and even aggregations of other fish species) to wild fish. This is no different than other man-made structures such as pontoons, wharfs, jetties, marinas, seawalls and even breakwaters, which change the ecology of local areas by attracting fish. Often referred to as artificial Fish Aggregating Devices (FAD) or artificial reefs, these structures provide fish with shelter and protection (Clynick *et al*., 2008). However, one unique concern with farmed fish is that cages bring wild and farmed fish in close contact, thereby providing heightened opportunities for transfer of infectious agents or pathogens.

Early studies suggested that fish assemblages around cages were minimal and in some cases consisted primarily of fish that likely escaped from the cages (Carss, 1990). This indicates issues with cage integrity and containment or alternatively a problem with the sampling method used (seine nets) (Dempster *et al*., 2002). With the introduction of new surveying methods such as SCUBA and underwater video camera systems a different story has emerged; though it has been suggested that even these methods likely underestimate the amount of benthic species aggregating around farms (Dempster *et al*., 2010).

Dempster *et al*. (2002, 2004) and Boyra *et al*. (2004) reported one to three orders of magnitude more wild pelagic fish immediately around fish farms in the Mediterranean Sea and the Canary Islands than even 50 m away, suggesting that they act as significant FADs. In Indonesia, Sudirman *et al*. (2009) reported significant aggregations of predominantly reef fish around cages with the biomass of wild fish outside the cages even higher than that of the fish contained in the cages. Though the magnitude of wild

* E-mail: sonja.saksida@cahs-bc.ca

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fish aggregation is similar in cold temperate farms, several studies from Norway suggest that the diversity of species is much lower (Dempster *et al*. 2009, 2010). Dempster *et al*. (2009) found that the total farm-associated abundances around salmon farms in Norway were five to ten times less than those reported around sea bass (*Dicentrarchus labra*), and sea bream (*Sparus aurata*) farms in the Mediterranean Sea. Farm-associated biomass however, was five to ten times greater in Norway than the Mediterranean because of the larger sizes of fish aggregating. Populations of fish around farms typically are stable with the populations residing for periods of weeks to months. Some economically important species such as salmon and tuna however, may only spend a brief period near farms on their journey to the feeding grounds in the deep ocean or back to the spawning grounds. For example, wild juvenile Atlantic salmon (*Salmo salar*) have been reported to migrate out of coastal waters in 2 days while brown trout spend most of their ocean stay in coastal waters (Pert and Middlemas, 2012).

There are significant differences in the construct of these assemblages among farms in different locations with no single taxa predominating at all the farms, suggesting that farms attract and concentrate fish from surrounding areas (Dempster *et al*., 2009). Reports indicate that the majority (>85%) of fish in these assemblages are adults (Dempster *et al*., 2002, 2004). Fernandez-Jover *et al*. (2009) reported that juvenile fish were also found around cage systems particularly in summer and autumn. In addition to the aggregation of fish outside and below the cages, some small wild fish may be able to enter pens through the mesh of the nets and may remain there for most of their lives cohabitating with the cultured fish in the cages.

As a consequence of the potential for close contact between wild and farmed fish, concerns have been expressed about cage culture and its possible impact. Concerns have included the possibility of introduction of exotic pathogens, the amplification of endemic pathogens and the chance of increasing prevalence of previously unidentified but potentially pathogenic agents (Colorni *et al*., 2002; Diamant *et al*., 2007; Walker and Winton, 2010; Kurath and Winton, 2011).

Pathogen Exposure between Wild and Farmed Populations

Farming of exotic species is common in both terrestrial farming and aquaculture. This has raised the concern of the potential risk of introducing exotic infectious agents in to the aquatic environment. In some cases, the movement of non-native fish species has led to the introduction of new infectious agents and diseases to a region. Examples include the introduction of *Mycobacterium marinum* and *Streptococcus iniae* into the Red Sea (Colorni *et al*., 2002; Diamant *et al*., 2007); furunculosis into many areas in Europe and North America (Bakke and Harris, 1998) and infectious haematopoietic necrosis virus (IHNV) to Asia and Europe (Traxler and Kent, 1992). Many of these events however, have been associated with deliberate release of fish into new waters to enhance river or lake or ocean fisheries, rather than being a result of aquaculture processes.

The contact between fish held in the cages and wild fish either cohabiting with the cultured population or migrating past the cages is likely much closer than occurs in other forms of farming. This potentially increases the risk of exposure to the infectious agents these distinct populations may carry. The most common and direct mode of transmission between wild and cultured fish is horizontal, without intermediate hosts or vectors. Although pathogens may be introduced by the cultured population, more often pathogens originate in wild populations and spillover (movement of a pathogen from its natural host to an alternative) into farmed populations (McVicar, 1997; Murray, 2009). The concern though, is that the pathogen may then be amplified or magnified in intensity in the farmed population, leading to disease outbreaks in farms and then spillback (movement of a pathogen from an alternate host back to the natural host) into the wild populations at higher intensity. Increased pathogen load is referred to as amplification and is a

common criticism of cage culture. Moreover, the aberrant accumulation of wild fish around cage systems may also enhance wild fish to wild fish transmission of pathogens that may not even exist in farmed fish.

The higher density at which cultured fish are reared would likely result in high loads of pathogens in the water in the farm vicinity, especially if fish health is not properly managed. As a result, there is potential that many fish could become infected and moribund from a pathogen. Amplification of a pathogen within a cultured population, however, cannot be an assumed outcome. This is particularly the case with parasites that require multiple hosts to complete their life cycles. For example, Mladineo *et al*. (2011) reported that tuna captured and grown to market size in cages (ranched) have lower parasite prevalence and abundance of parasites than their wild counterparts. Furthermore, studies have shown that farmed fish do not have similar macroparasites compared to wild fish assembling around the cages, and in fact have fewer parasites than the same species not assembling around farms (Fernandez-Jover *et al*., 2010; Dempster *et al*., 2011). This is likely a consequence of the differing diet that these groups are feeding upon, hence reducing or eliminating infection by trophically transmitted parasites.

Amplification is only a risk to susceptible wild fish populations if they are in the area when amplification occurs. For example, sea lice on farmed salmon in British Columbia can increase significantly in the autumn as a consequence of exposure to infested wild fish (spillover). The wild fish, however, already have considerably higher lice than the farmed fish and do not remain close to farms for an extended period of time (Beamish *et al*., 2006; Marty *et al*., 2010). In contrast, if farmed salmon have a heavy infestation of lice in the spring during the period of outmigration of juvenile salmon then there may be some risk posed to the out migration populations (Marty *et al*., 2010).

Pathogens passed from a parent to offspring (via vertical transmission) are some of the most problematic for the aquaculture industry, and are the most difficult to avoid. *Renibacterium salmoninarum*, the causative agent for Bacterial Kidney Disease (BKD) is an example. Though vertical transmission to wild fish populations is not a concern when cultured fish are contained within cages, it may occur with escapes or in situations where natural resource agencies or 'enhancement' agencies release juveniles to cohabit with their wild cohorts (Kurath and Winton, 2011).

From Exposure to Disease

Infectious agents commonly move between cultured and wild fish with four possible outcomes to the exposed individual/population: (i) they are resistant to the infection and therefore there is no effect; (ii) they are a 'dead-end' host or sink that is not able to maintain the infection without an external host; (iii) they are a spillover host and are able to transiently maintain the infection for a time but require periodic exposure from another source, or are a maintenance host; or (iv) they are able to maintain infection without further transmission from another species (Ryhan and Spraker 2010). Whether the outcome of the exposure is disease, however, is dependent on a myriad of environmental, infectious agent and host factors, which often interact in unforeseen ways.

Several studies have evaluated factors contributing to disease as a consequence of wild and farmed fish interactions (Hastein and Landstad, 1991; McVicar, 1997; Coutant, 1998; Hedrick, 1998; Olivier, 2002; Johansen *et al*., 2011). All authors agree that the complexity of many of these interactions makes identification or quantification of the contribution by any single factor to disease occurrence complicated. Moreover, the multifactorial nature of disease makes it difficult to conclusively determine cause-effect relationships (McVicar, 1997). The question, therefore, is not whether there is the likelihood of exposure to an infectious agent, as it is obvious that this is very likely, but rather the key question is whether this interaction leads to higher disease risks and rates in wild and cultured fish as a consequence of this interaction. The following sections in this chapter address this issue.

Much of the understanding of the ecology of diseases has been driven by the necessity to understand diseases of humans, crops,

farm animals and wildlife. Consequently, most epidemiological theory and management methods are based on medical or terrestrial animal or veterinary examples (McCallum *et al*., 2004). The basic principles, however, may not apply in the aquatic environment because of important differences including:

- Higher taxonomic diversity in the marine compared to the terrestrial environment;
- More unrestricted recruitment in the marine compared to the terrestrial environment;
- Differences in modes of pathogen (particularly parasite) transmission; and
- Differences in life histories between marine and terrestrial organisms.

Environment

In the aquatic environment, both the host and the infectious agent appear to be capable of dispersing large distances and as a consequence disease/epidemics can also spread at extremely rapid rates. For example, McCallum *et al*. (2003) reported herpes virus spread through wild pilchards (*Sardinops sagax neopilchardus*) in Australia at a rate of 10,000 km per year. In comparison, the most rapid terrestrial spread reported is that of the west Nile virus in birds in North America, which spread in excess of 1000 km per year.

In addition to dispersal, the aquatic environment significantly affects the growth, reproduction and survival of both host and pathogen and ultimately their ability to thrive or survive. All organisms, fish and infectious agents, have unique optimal environmental conditions (such as salinity, temperature, oxygen, water flow, circulation, turbidity, harmful phytoplankton). For example it is clear that salmon grow optimally at temperatures (< 18°C), *Leophtheirus salmonis* thrives in higher salinity waters > 27 ppt (Johnson and Albright, 1991; Tucker *et al*., 2000). IHNV is highly virulent at temperatures less than 15°C and less virulent at higher temperatures (LaPatra, 1998). Certain species even change their environmental requirements depending on life stage. For example, anadromous fish species have one lifestage in fresh water and another in salt water.

Understanding the optimal environmental conditions for the animal being reared in the cages is an essential requirement to maintain healthy fish in this environment. For species that have been cultured more extensively, there is a sound understanding of the environmental factors conducive to good growth and survival. In salmon farming this usually includes good water flow, moderate temperature profiles and good oxygen levels. Optimal rearing conditions are still being determined for more novel species.

The Host

Simply having an infectious agent transmitted to another host does not imply an effect. There is a large diversity of potential hosts in the marine environment with different life cycles and ecologies, which can live in significantly different environments. Unlike terrestrial animals where it is mainly adults or sub-adults that disperse, in the aquatic environment the larvae or eggs of many fish species play an important role in dispersion. Some species also have significant migration patterns (e.g. salmon, tuna) as sub-adults and adults. Others remain in a more localized area for their entire life (e.g. reef fish). Farmed fish in cages are more or less stationary, unless there is an escape.

As previous chapters have illustrated, disease associated with infectious agents often affects only a single family, genus or even one species of fish. For example, Atlantic salmon in British Columbia are 100× more sensitive to IHNV than sockeye salmon (*Oncorhynchus nerka*), whereas pink salmon (*O. gorbuscha*) appear to be completely resistant (Garth Traxler per communications). In contrast, IHNV was found in a single tubesnout (*Aulorhynchus flavidus*) associated with a farm where the caged population were experiencing an IHN outbreak with no clinical signs of disease (Kent *et al*., 1998). Atlantic salmon are more resistant to *Renibacterium salmoninarum* and *Loma salmonae* compared to *Oncorhynchus* species, whereas post mortem myoliquefaction associated with *Kudoa thyrsites* is a substantial economic problem in Atlantic salmon but not in farmed Chinook salmon reared in

British Columbia. It has however, been seen on occasion in farmed coho salmon. Clinical disease is observed in sea bream and sea bass associated with *Streptococcus iniae* infections, while wild fish sampled in the Red Sea were found to be infected but exhibited no clinical signs of disease (Colorni *et al*., 2002).

In some instances, only certain life stages of the host are susceptible to disease associated with an infectious agent. For example, sea lice (*Lepeophtheirus salmonis*) can be lethal on pink salmon at 0.7 g, but larger fish quickly develop resistance to infection (Jones *et al.,* 2006a, 2007, 2008; Jones and Hargreaves, 2007).

Infectious Agent

Characteristics of the infectious agent that contribute to disease development are: its infectivity (ability to infect an individual or population), virulence (ability to cause clinical disease) and pathogenicity (ability to cause serious disease or death). For example, the HPR4 strain is one of the most pathogenic variants of infectious salmon anaemia virus (ISAV), while the HPR0 strain is considered non-pathogenic. Some pathogens are infective only at certain stages (e.g. sea lice where only copepodid and motile stages are infective). Some pathogens have vegetative stages that allow them to persist in the environment for extended periods (e.g. *Aeromonas salmonicida*, which can cause furunculosis), while others are transmitted through ingestion of an infected food item or intermediate hosts. Juvenile anisakine nematodes are common in wild fish, but not in farmed fish because this trophically transmitted parasite requires ingestion of an intermediate host (Deardorff and Kent, 1989). Interestingly, Kent *et al*. (1991) reported a high prevalence of *Gilquinia squali* in farmed Chinook salmon. This infection is only contracted by ingestion of infected copepods or perhaps small fish acting as paratenic hosts.

Transmission of infectious agents from infected to susceptible hosts is strongly dependent on the environment and hence, the processes behind transmission of aquatic pathogens may be substantially different than those in terrestrial environments (McCallum *et al*., 2004). Overall there is no single mode of transmission for all infectious agents. In the aquatic environment, many infectious agents are transferred by direct interaction of infected and uninfected potential hosts. In this situation, as density of host population increases, so do the encounters and potential transmission of the infectious agent. This is referred to as density-dependent transmission (Murray, 2009). The transmission of *Aeromonas salmonicida* in salmon is an example of density-dependent transmission (Ogut *et al.,* 2004, 2005).

However, because water is such an effective medium for the transmission of infectious agents (McCallum *et al*., 2003; Kurath and Winton, 2011), direct contact between infected and susceptible hosts may not be necessary. In the aquatic environment, many pathogens are capable of surviving in the absence of a host for a long time, and can be carried by the water some distance. Infection can be transmitted to a new host exposed to pathogen-loaded water rather than through direct contact with the infected host. The concentration of infectious agents increases with increased shedding into the water, and the rates at which susceptible hosts become infected are also likely to increase. In this case transmission is dependent on the shedding rate of the infected host and the decay rate of the infectious agent in water. An example of this transmission is the spread of IPNV (Smith *et al*., 2000; Bebak-Williams *et al*., 2002; Murray, 2009). Sometimes infectious agents can be transmitted long distances by water movement. In this situation, local infection pressure may be independent of local pathogen production. Viral pathogens such as ISAV (McClure *et al*., 2005) and IHNV (Saksida, 2006) for example, have been observed to be hydrodynamically transported over many kilometres, as have sea lice (Murray and Gillibrand, 2006).

Certain infectious agents require intermediate hosts to complete their lifecycle or use vectors. In both situations, direct transmission between populations does not occur. Sometimes referred to as density-independent transmission, this is a function of the frequency of infected individuals in a population

rather than density (Rudolf and Antonovics, 2005; Murray 2009). This is an important mode of transmission for terrestrial diseases such as malaria and Lyme disease (McCallum *et al*., 2004), but it is less common in the aquatic environment. It has been suggested this could be attributed to lower search efficiency in the aquatic environment and the presence of fewer blood-borne pathogens (McCallum *et al*., 2004). This mode of transmission is used by some parasites of cage-reared fish, such as *K. thyrsites* and *Cardicola fosteri,* both of which require invertebrate alternative hosts to complete their life-cycle. One study indicated that ISAV may be transmitted by sea lice (Nylund *et al*., 1994) while Nylund *et al*., 2010 and 2011 reported that the sea louse probably is a vector for *Paranucleospora theridion* – as spores of this microsporidium have been found in both the sea louse copepodids as well as the host (Atlantic salmon).

Assessing and Managing Disease in Populations

Finfish cage culture has adopted and modified many principles and tools developed in more established terrestrial animal production to manage health, including biosecurity, husbandry and genetics. For example, the environment, density and nutrition are managed in order to meet specific physiological, biological and nutritional requirements for the species and even strain being reared. Aquaculturists are included in the considerable efforts being made through international organizations such as the World Organisation for Animal Health (OIE) and its member nations to reduce movement of infectious agents. With stricter international laws, risk of transmission of OIE-listed diseases is best controlled through improved surveillance and detection methods. Many countries have further restrictions in live fish movement to further protect their national and regional fish health status. Cage culture has been able to take advantage of antibiotic and other therapeutant development, disinfectant and vaccine development, advances in diagnostic tools and genetics – all elements that allow for better survival in more intensive rearing conditions. In addition, the implementation of biosecurity measures such as screening brood stock and eggs-only importation policies (Kent and Kieser, 2003) provide aquaculturists with tools to avoid pathogens that occur in wild fish. These elements together are designed to help reduce risks that have been proposed regarding cage culture. They specifically address potential impacts associated with spillover, by reducing amplification of infectious agents that are manageable.

Vaccines for locally important diseases have become essential tools for disease prevention in cage culture. For example, furunculosis and vibriosis were devastating to salmon cage culture in the 1980s and 1990s (McVicar, 1997). Following the introduction of effective vaccines, these conditions have become almost non-existent health issues (Lillehaug *et al*., 2006). Other activities such as routine monitoring and screening of fish and their environment have been incorporated into the management of fish in cages. Sick and dead fish are also removed from cages, and are examined to determine the cause of death. These activities provide critical information about the health of individuals and the population in the cages, thereby facilitating the development of informed courses of action. For infectious diseases, actions may include management or husbandry modifications (e.g. reduced handling, density modification, increased monitoring for pathogens and signs for disease, the inclusion of modified feed additives) or where appropriate, the use of available therapeutants. Fallowing of a cage farm or a group of farms in an area defined by a common water circulation model is being investigated and incorporated in some cage farming areas to reduce the potential of pathogen transmission between year classes and farms, respectively (St-Hilaire *et al*., 2002; Saksida, 2006). This mitigation also reduces pathogen load and disease in cage culture, thereby reducing the risk of direct transmission of infectious agents to wild fish populations. Risk of transmission from cage to wild can also be managed for parasites whose transmission involves an intermediate host. For example, the regular removal of dead fish from cages reduces the chance of

transmission of *K. thyrsites*. In this case, dead fish are removed before myxospores are released from the muscle during carcass decomposition into the environment to infect the invertebrate host. In this case the caged populations have the potential to act as parasite sinks, reducing the amount of parasite available to infect wild populations. However, transmission from wild to farmed fish can still occur.

As a consequence of the high connectivity of the aquatic environment, epidemics may be harder to control even with culling (McCallum *et al*., 2004). This depends, however, on both the host and pathogen. For example, Saksida (2006) reported that IHNV was eliminated from farmed populations following culling and fallowing during the 2001–2004 outbreak in British Columbia. Until 2012, there was no spillover of IHNV from the wild to the farmed population presenting a new genotype, different from any of the earlier outbreaks (K. Garver, personal communication). It is clear that actions taken to manage cage farms can affect disease processes, but this is highly dependent on the mode of transmission of the agent, the presence of an appropriate wild host and the environment.

In the last decade, several 'new' virus-linked diseases have been discovered in cage-cultured species (e.g. cardiomyopathy syndrome, heart and skeletal muscle inflammation, pancreas disease, jaundice syndrome). It is possible that something related to the activities linked to cage culture – husbandry (density), geographic location and site selection, genetics, nutrition may be making these animals more susceptible to the apparently novel viruses. Another possibility is that an environmental change may have occurred – making once undetectable viruses more virulent. Detection of these new conditions in cage-cultured fish may also reflect something also occurring in the wild population. Viruses are extremely abundant in aquatic systems and their abundance decreases with depth and distance from the shore. Currently, the best estimates range from approximately 3×10^6 viruses per ml in the deep sea to approximately 10^8 viruses per ml in productive coastal waters (Suttle, 2005). Because fish reared in cages are easier to collect and examine for disease, pathogens and infections are often reported for the first time in caged fish although they were previously present in wild fish. Therefore, farmed fish provide opportunities for researchers to better document pathogens and diseases in wild populations. They serve as sentinels because of the high frequency of monitoring for infectious agents. The distribution of pathogens in farming regions also provides possible clues as to the distribution of these pathogens in wild populations. There are several examples of this phenomenon. *Loma salmonae*, for example, was first documented in sea water in salmon farmed in the Pacific Ocean (Kent *et al*., 1989), but later was found to be common in both marine and freshwater wild salmon (Kent *et al*., 1998).

Wild populations are considered healthy because weak animals are removed through predation leading to natural selection (Bergh 2007; Ryhan and Spraker, 2010). However, all populations of wild and farmed fish can experience infections. Studying infectious disease however, is much easier with farmed than with wild fish. Often the emphasis or focus for research is on economically or culturally important species such as Pacific salmon on the west coast of North America or Atlantic salmon in Norway, Scotland and Ireland. This ignores the numerous other fish species that could be potential hosts. For example, most research initially conducted on the sea louse, *L. salmonis*, focused on monitoring strictly salmonid species. When Jones *et al*. (2006b) extended their surveillance program to include other species co-habitating with juvenile salmonids, it indicated that threespine sticklebacks (*Gasterosteus aculeatus*) also harboured large numbers of lice – suggesting that sticklebacks may act as alternate hosts.

In wild populations, disease is often detected only when morbidity or mortality is high or is catastrophic in nature. Detection is also more likely where disease occurs in a near shore environment with human inhabitants. Diseases that cause low-level mortality largely go unnoticed, especially in the marine environment where monitoring is more difficult due to issues around tracking. Moreover, it is well-recognized that many chronic diseases

cause mortality in wild animals by increasing susceptibility to predators (Holmes and Bethel, 1972), and this principle extends to fish (Lafferty and Morris, 1996). Thus the effects of these diseases are usually not observed in wild fish without detailed, focused collections and surveys. Data collected in surveys may not be effective in pinpointing what is killing fish because these are expensive to conduct and often only provide information at a single point in time. It could be argued that if the survey size was large enough then it would be possible to see a cross section of a population and observe those that are healthy and those that are not. Repeated cross-sectional studies however are rarely undertaken because it would be necessary to sample an excessive number of fish and really should be targeted to address an explicit question about a specific disease process with an estimated prevalence. For example, 298 healthy fish would need to be sampled to detect at least one infected fish with 95% confidence assuming a pathogen prevalence of 1% and a perfectly sensitive test. This sample size, however, can be substantially reduced using risk-based (targeted) sampling of dead fish where the pathogen might be present at higher prevalence.

Most often, wild fish disease and pathogen monitoring is therefore conducted to document the presence of pathogens rather than to assess their role in disease occurrence (Kent *et al*., 1998). Examination of fish that are captured and killed can provide indications as to which infectious agents are present, but this provides little information about what may be causing increased mortality in target populations. Histopathology can determine the extent and severity of microscopic changes in tissues and organs, but this requires a highly trained pathologist to assess the lesions and interpret as to whether the changes would be extensive enough to cause death (Saksida *et al*., 2012; Kent *et al*., 2013).

New tracking methods and monitoring devices are being developed such as acoustic monitoring tags that could provide opportunities to monitor individual fish, and possibly even populations over time. Currently, these devices are still in the experimental stages and would be very expensive to incorporate into a large-scale longitudinal monitoring programme. At present, almost all diagnostic tests routinely used to assess fish health require lethal sampling which obviously hinder any ability to follow individuals over time.

In recent years, the transmission of sea lice between wild-farmed fish has been heavily investigated and millions of dollars have been invested in this research. In fact at least 100 papers have been published on the subject over the last two decades. These studies are reviewed in the following section and the scientific understanding of this topic is presented. At this point, although there is evidence of bi-directional transmission between wild and farmed fish and the disease/health issues have been well-demonstrated in farmed fish, the disease and health implications in wild fish are still unclear.

Literature Review of Sea Lice Transmission Studies

Published research on *Lepeophtheirus salmonis,* also commonly referred to as salmon louse, has emanated from countries and regions with sizable salmon culture: Norway (Costelloe *et al*., 1995; Heuch and Mo, 2001; Bjørn and Finstad, 2002; Frazer, 2008; Costello, 2009); Ireland, England and Scotland (Costelloe *et al*., 1995; Tingley *et al*., 1997; MacKenzie *et al*., 1998; Butler, 2002; Revie *et al*., 2002a, b; Marshall, 2003; Costello, 2009); North America (Noakes *et al*., 2000; Carr and Whoriskey, 2004; Morton *et al*., 2004; Krkošek *et al*., 2005; Beamish *et al*., 2006; Krkošek *et al*., 2006; Krkošek *et al*., 2007; Frazer, 2008; Costello, 2009; Krkošek *et al*., 2009; Marty *et al*., 2010; Krkošek and Hilborn, 2011; Morton *et al*., 2011; Saksida *et al*., 2011) and Chile (Bravo, 2003; Zagmutt-Vergara, 2005).

Recent studies on sea (or salmon) lice can be broadly classified as: (i) development of molecular and analytical methods to facilitate identification of the source of sea lice infections and direction of transmission (e.g. wild to cultured fish or vice versa) (Todd *et al*., 1997, 2004; Shinn *et al*., 2000; Dixon *et al*., 2004); (ii) disease modelling (Heuch and Mo, 2001; Krkošek *et al*., 2007, 2009; Frazer, 2008);

(iii) spatial and/or temporal associations between cultured salmon and sea lice abundance in wild fish populations (Tingley *et al*., 1997; MacKenzie *et al*., 1998; Bjørn and Finstad, 2002; Carr and Whoriskey, 2004; Morton *et al*., 2004; Krkošek *et al*., 2005; Skilbrei *et al*., 2009; Welch *et al*., 2011; Price *et al*., 2011); (iv) wild fish survival (escapement) in relation to lice abundance among farmed fish situated along migratory routes (Marty *et al*. 2010; Krkošek and Hilborn, 2011; Morton *et al*., 2011); (v) survival of migratory fish with respect to exposure to cultured fish (Krkošek and Hilborn, 2011); and (vi) longitudinal studies investigating variation in sea lice abundance (Zagmutt-Vergara, 2005; Marty *et al*., 2010; Saksida *et al*., 2011) and the role of environmental conditions on wild fish survival (Beamish *et al*., 2006).

Shortcomings in the published literature on *L. salmonis* transmission

Evaluation of the published literature on *L. salmonis* transmission indicates limitations in areas such as: (i) sampling methods in wild fish populations; (ii) lack of appropriate comparison groups (controls); (iii) lack of information on 'normal' disease burden among wild and cultured populations; (iv) lack of information on variation in lice burdens; (v) reliance on spatial associations alone, often not considering temporal effects as causal evidence; (vi) presumption that disease transmission is primarily from cultured to wild fish; (vii) extrapolation of laboratory data to field studies; (viii) failure to consider the role of environmental conditions and their possible effects on disease propagation and transmission; (ix) basing conclusions on historical observations; (x) linking data from different studies to infer causal relationships; and (xi) drawing conclusions based on sparse or limited data.

Sampling methods

Sampling of wild populations presents numerous challenges that are usually absent among cultured fish stock. Sampling bias likely is present in the majority of published studies investigating diseases in wild fish, due to the logistical and practical difficulties of sampling wild fish. However, concerns about the impact of sampling bias have been largely ignored. Sampling bias may arise due to environmental, spatial and temporal factors, through differences in disease detection probabilities among cultured and wild populations, or during the sample collection process itself (Nagasawa, 1985; Tingley *et al*., 1997; Morton *et al*., 2004; Barlaup *et al*., 2013). The direction and magnitude of this introduced bias is unknown and has not been quantified.

Lack of appropriate comparison groups (controls)

Lack of appropriate study controls, which fail to consider relevant spatial, temporal and environmental factors, may bias results (Costelloe *et al*., 1995; Butler, 2002; Marshall, 2003; Morton *et al*., 2004). Although these factors have been considered in various studies, invariably one or more factors have been excluded during control selection. This is exemplified in the design implemented by Marshall (2003), which included controls for geographical and environmental variation, but failed to control for temporal effects. In contrast, Price *et al*. (2011) considered time and space when evaluating sea lice infection rates on wild juvenile sockeye salmon in areas with and without exposure to salmon farms in British Columbia and confirmed the origin of the sockeye with genetic analyses.

Lack of information on 'normal' lice burdens among wild populations

The lack of adequate background data defining 'normal' or endemic lice burdens in wild populations, in the absence of farmed fish populations, is a fundamental problem for the understanding of sea lice transmission dynamics. Presumptions about historical sea lice burdens (and the lack thereof), based on the recall of fishermen, long-time local residents and local biologists, have been used to support the presumed causal relationship between aquaculture and detrimental effects

on wild fish populations (McVicar, 1997). Although high lice burdens in wild fish are purportedly a new occurrence (based on recall of fishermen and first-nations), published empirical evidence from the same geographical area conflict with this view (White, 1940). High lice burdens in returning wild fish were reported in the 1940s, well before the advent of commercial fish farming, and were associated with large salmon returns (White, 1940).

Understanding variation in sea lice abundance

Information on the temporal variation of sea lice abundance is limited, but Revie *et al.* (2002a,b) demonstrated that wide variation in abundance occurs without any apparent explanation. Data on normal variation in sea-lice abundance among wild and cultured populations provide important baseline information when trying to draw conclusions about causal associations. Investigation into the factors associated with variation in sea lice abundance may provide insight into potential disease transmission factors.

Reliance on spatial relationships

Reliance on association between high lice prevalence on wild salmon in areas near farm sites as causal evidence that infections in wild fish came from farmed fish is a common theme among the sea lice literature (see for example, Tully *et al.*, 1999). Whereas this spatial relationship may provide evidence of potential causal pathways, it cannot provide definitive proof without additional evidence. Unrecorded extraneous factors, shared by both farm sites and wild fish, potentially may lead to spurious associations.

Presumption about the infectious source

Although published literature indicates that farmed fish originally acquire sea lice infections from wild fish, the presumption and focus of some disease transmission studies largely involves pathogen transfer from cultured to wild fish. Whereas favourable conditions for disease amplification are reported to exist among cultured fish (Morton *et al.*, 2004; Krkošek *et al.*, 2005, 2006), conclusive evidence of pathogen transfer from cultured to wild fish has not been documented. Without direct evidence of the source infection, causal inferences should be made cautiously and may be erroneous. Indeed, substantial lice burdens may occur in wild fish in waters with no fish farms (Beamish *et al.*, 2005; Krkošek *et al.*, 2005, 2007).

Extrapolating laboratory studies to field research

The inappropriate extrapolation of results from laboratory studies to field situations, in support of causal associations, has been reported in the literature (Beamish *et al.*, 2006). Most notably this was done with respect to mortality associated with sea lice burden (Morton *et al.*, 2004), but extrapolation of laboratory results as evidence of causal associations in the field may lead to spurious conclusions.

Failure to consider the influence of environmental conditions on disease propagation and transmission

Drawing causal conclusions based on sparse, or in the absence of environmental data, ignores the multifactorial nature of disease transmission, which may lead to erroneous assumptions about causal relationships. Beamish *et al.* (2006) noted that river discharge levels and subsequent effects of near-shore salinity may have significantly impacted survival of salmon smolts. The authors stated that the relationship between river discharge level and smolt survival indicates the importance of monitoring salinity levels during the course of any investigation. Inclusion of environmental data during the course of any investigation is important to capture the factors that are driving disease propagation and transmission.

Basing conclusions on historical observations

Historical observations have been used by various authors to support their views on disease transmission, namely transmission from farmed to wild fish. Some historical evidence used to demonstrate the historical absence of

disease among wild populations is based on the recall of fishermen, first-nations and biologists (Krkošek and Hilborn 2011). Using recall to document the absence of disease has been shown to be fraught with errors, as empirical evidence has contradicted the reported absence of historical disease in the same areas (White, 1940).

Linking data from different studies to infer causal relationships

Some published literature has made causal assumptions based on the findings of association between different studies or datasets. McVicar (1997) warns of the dangers of directly linking mathematical associations between different sets of numerical data with cause and effect, as correlations may have arisen by chance alone or through common external influencing factors.

Drawing conclusions based on sparse or limited data

Conclusions on causal relationships between farmed and wild fish, with respect to disease transmission, have been based on very limited or sparse data (Tingley *et al*., 1997; Carr and Whoriskey, 2004). Spatial data based on a few individuals, or on lice burdens estimated on only a few individuals cannot be used to justify causal relationships, as the data are highly susceptible to bias.

Failure to account for temporal effects

The majority of papers on *L. salmonis* are cross-sectional studies focused on finding associations between cultured and wild fish, with respect to sea lice burdens (Marshall, 2003; Morton *et al*., 2004), or on associations between wild fish survival estimates and lice abundance on marine farms associated with migration routes (Beamish *et al*., 2006; Krkošek and Hilborn, 2011; Morton *et al*., 2011). These cross-sectional studies do not account for temporal effects and do not take into account whether the causal factor preceded the study outcome. Revie *at al*. (2002b) demonstrated that factors such as lice abundance are highly variable over time, which calls into question any causal associations that are based on data with temporal variation. This is especially important for studies dealing with marine survival (escapement), where the study outcome is the result of factors acting over extended time periods.

Recommendations for future work assessing *L. salmonis* transmission

Future research into disease transmission between wild and cultured fish populations should take more of a multidisciplinary approach which includes the skills of disease modellers, ecologists, aquatic biologists, parasitologists and epidemiologists. Potential knowledge gaps that could be filled include:

Quantifying interactions and associated pathogen transfer between wild and cultured fish

Quantification of the frequency and duration of interactions between wild and farmed populations can help provide stronger evidence for the direction of *L. salmonis* transmission. Expanding the use of novel acoustic tagging methods to track wild fish movements (Carr and Whoriskey, 2004; Skilbrei *et al*., 2009; Welch *et al*., 2011) would allow for quantifying the level and duration of interaction between wild and farmed fish. The resulting data from such studies could be used to estimate the frequency of pathogen exposure and be incorporated into mathematical disease models.

To our knowledge, prior studies have not shown conclusive evidence of pathogen exchange. Molecular techniques and population genetic studies have failed to distinguish the origins of *L. salmonis* infections as being from farmed or wild fish (Todd *et al*., 1997, 2004; Dixon *et al*., 2004; Boulding *et al*., 2009, Messmer *et al*., 2011). Conventional nebulization ICPMS (Inductively Coupled Plasma Mass Spectrometry) involving the elemental analysis of the magnesium, vanadium and uranium, has the ability to distinguish between lice originating on cultured and wild salmon (Shinn *et al*., 2000) and thus has potential for use in field evaluation of *L. salmonis* transmission.

Quantifying nature and outcome of pathogen exposure

Quantifying the nature of pathogen exposure is an important component in understanding disease transmission. Based on data obtained from the previously mentioned acoustical tagging studies, pathogen exposure risk can be investigated through the use of sentinel cages of fish placed in appropriate spatial locations for realistic durations, to mimic the natural exposures fish may encounter. To adequately quantify pathogen exposure, studies would have to encompass the full spectrum of pathogen prevalence, infection management strategies and environmental conditions over long periods of time to capture any temporal effects.

Detailed environmental and ecological data

The work by Beamish *et al*. (2006) highlights the importance of considering environmental data when making causal associations. In the absence of environmental and ecological data, spurious causal associations may be made. Capturing environmental and ecological data during the study period avoids problems associated with making inferences based on unrelated datasets with differing temporal and spatial characteristics.

Longitudinal studies to inform disease models

Almost all published studies of *L. salmonis* transmission are cross-sectional in nature, thus limiting inferences about cause and effect, and explicitly the direction of transmission. Long-term longitudinal studies are required to ensure that spatio-temporal associations are correctly considered and measured, thus providing stronger evidence for causal relationships.

Further advancement of disease models, building on the work of Heuch and Mo (2001), Krkošek *et al*. (2007, 2009) and Frazer (2008), must incorporate new data, gained from previous research recommendations. These refined models will allow for exploration of potential impacts arising from changes in environmental parameters, population size and structure and management protocols. Sensitivity analysis will identify the critical factors that drive disease transmission.

Conclusions

There is considerable knowledge being gained regarding diseases in cultured fish, but there is still limited understanding of pathogen distribution and diseases in wild fish. Much research emphasis has been on 'bug hunting': listing pathogens/genomic signatures found in fish rather than studying diseases in populations. This documentation is a useful first step for understanding the host, and spatial and temporal distributions of pathogens. Simple pathogen discovery research, however, can lead to considerable misinterpretation about causes of diseases and mortalities in populations of concern. Understanding diseases in wild populations requires a holistic multidisciplinary approach, incorporating classical and new molecular pathogen detection methods, histopathology, epidemiology, and applying knowledge gained through genomic studies.

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Index

Note: Page numbers in **BOLD** refer to figures.

Aeromonas salmonicida 32–33, 295
  clinical signs 32–33
  diagnosis 32
  fish production impact 32
  prevention and control 33
Anisakis simplex 46
anti-nutrients 273–274
  clinical signs, gross and histopathological lesions 274
  fishmeal and fish oil replacement 273– 274
  natural/synthetic substances 273
  prevention and control 274
  protein sources 273
aquaculture wastes 275
Argulus sp. 232–234
  Channa micropeltes 232, **232**
  diagnosis 233
  pathology 233
  prevention and control 233–234

bacterial coldwater disease 77–80 *see also* *Flavobacterium psychrophilum*
  characterization 77–78
  coldwater disease (CWD) 77
  control, rainbow trout fry syndrome (RTFS) 77, 79–80
  diagnostic techniques 79
  posterior spinal compression, rainbow trout 78, **79**
  raceway systems 78
  salmonid-producing areas 78
bacterial diseases 26–37, 202–213
  coldwater fish in marine and brackish waters
    Aeromonas salmonicida 32–33
    antimicrobial veterinary medicinal products 26
    bacterial kidney disease (BKD) 35–37
    epitheliocystis 33–34
    franciselliosis 28–29
    marine environment 25
    Pseudomonas sp. 35
    salmonid rickettsial septicaemia 29–30
    salmonids 26
    tenacibaculosis *see* tenacibaculosis
    vibriosis *see* vibriosis
    water exchange rates 26
    winter ulcer 30–31
    yersiniosis 37
  Streptococcal septicaemia/meningoencephalitis 211–213
  warmwater fish in fresh water 203–211
    columnaris disease 209–211
    edwardsiellosis 207
    enteric septicaemia of catfish (ESC) 208–209
    motile *Aeromonas* septicaemia (MAS) 203–206
    Pseudomonas septicaemia 206–207
    stocking density, fish 202
  warmwater mariculture 159–165
    edwardsiellosis 161
    epitheliocystis 164–165
    lactic acid bacteria (LAB) 162–163
    mycobacteriosis 163–164
    photobacteriosis 160–161
    Tenacibaculum maritimum and flexibacters infections 161–162

bacterial diseases (*continued*)
vibriosis 159–160
zoonoses 165
bacterial kidney disease (BKD) 35–37, 80–82
fresh water, coldwater fish in
characterization 80–81
darkening, fish 81
diagnostic techniques 81–82
disease severity 81
ELISA OD values 82
freshwater and marine cage culture 81
histological characterization 81
prophylactic feeding 82
Renibacterium salmoninarum 80, 82
smoltification 80
vaccine 82
marine and brackish waters, coldwater fish in
clinical signs 36–37
diagnosis 36
fish production impact 35
morbidity and mortality 35
prevention and control 37
Renibacterium salmoninarum 36
bacterial pathogens diseases 71–82
coldwater disease 77–80
enteric redmouth (ERM) disease/yersiniosis 75–77
furunculosis 71–73
motile aeromonad septicaemia (MAS) 74–75
secondary impact, coldwater fish 82–83
BHI *see* brain heart infusion
BKD *see* bacterial kidney disease
brain heart infusion 163
branchiomycosis 213–214
diagnosis 214
pathology 213–214
prevention and control 214

cage culture 1–13
antiviral agent 2
aquaculture sustainability
ecological issues 13
economic viability 12
feed formulation 12
fish health and infectious diseases 11–12
global fish market 12
safeguards 11
self-monitoring and self-reporting 13
social licence from public perspective 11
Atlantic salmon 6, 9
cage-reared fish 9
carp and tilapia 5, 6
China's aquaculture production 3, **4**
consumer demand 9
crustacean and diadromous fish production 5
economic and social benefits
annual growth rates 10
aquaculture operations 9
fishermen and fish farmers, Asia 9, 10
operating costs 11
overcapitalization 10
production efficiencies 10
trade 11
fisheries and aquaculture 1
fresh fish market 9
freshwater production 7, **7**
marine, brackish and freshwater aquaculture 4, **5**
in marine/brackish waters 8, **8**
production trends
aquaculture 2
global trade and commerce 2
human consumption 3
stock enhancement programmes 2
World aquaculture production 3
salmon and trout 1
shrimp and tilapia 6
socio-economic benefits 1
cardiomyopathy syndrome (CMS) 23–24, 288–291
Atlantic salmon 23
clinical signs 24
diagnosis 24
fish production impact 24
prevention and control 24
sporadic diseases
causes 288
clinical signs, gross and histopathological lesions 289
diagnosis 289
economic impact 289
endomyocarditis 288
prevention and control 289–290
tissue homogenates 288
transmission 24
Carp Nephritis and Gill Necrosis (CNGV) 194–199
CCVD *see* channel catfish virus disease
ceratomyxosis 91–94
aquaculture 92
Ceratomyxa shasta 92
characterization 92
clinical signs 92–93
control/prevention 93–94
diagnostic techniques 93
myxosporean parasite 91
Ceratothoa gaudichaudii 45
channel catfish virus disease (CCVD) 201–202
acute infection 201
causative agent 201
clinical signs 201
diagnosis 202
haemorrhagic viraemia 201
prevention and control 202
water-borne transmission 202

Cichlidogyrus spp. 226–227
ciliates (Phylum Ciliophora) 219–220
climate change 275–276
coldwater fish in fresh water 60–98
Aeromonas salmonicida 61
Atlantic salmon 60
bacterial pathogens 71–82
fungal/psuedofungal pathogens 83–87
Henneguya salminicola 61
parasitic pathogens 87–94
rainbow trout cage culture 60, **61**
Renibacterium salmoninarum 61
resource enhancement and mitigation 60
viral pathogens 62–71
coldwater fish in marine and brackish waters
bacterial diseases 26–37, 202–213
cage culture 15
fungal-oomycetes diseases 37–39
parasitic diseases 217–229
treatment 15
vaccines 15
viral diseases 16–25
world production, salmon 15, **16**
coldwater fish in marine and fresh water 114–141
acute intoxications 124
behavioural indicators 116–117
chronic intoxications 124–125
coloration abnormalities, flatfish 140–141
gas bubble disease 120–122
harmful algal blooms (HABs) 117–119
harmful zooplankton - jellyfish 119–120
neoplasia 135–138
nutritional and feed related problems 125–133
oxygen deficiency 122
physical deformities 138–140
egg incubation 139
embryonic development 139–140
environmental and infectious stressors 139
farmed rainbow trout 138, **139**
internal malformations, soft tissues 140
juvenile stages 139
salmon aquaculture 139
shortened opercula 139
skeletal deformities 139
swim bladder 140
temperature stress 139
physicochemical parameters 120–125
predators 117
slurry intoxication 124, **124**
smolt failure, salmonids 114–115
stress 115–116
vaccination and treatment 133–135
water-borne irritants 122–124
columnaris disease 83, 209–211
causative agent 209
diagnosis 210
Flavobacterium columnare 83
gill disease 83, **83**
pathology 209–210
prevention and control 210–211
crustacean parasites 44–45
Cryptocotyle lingua 45

Dactylogyrus spp. 225–226
cyprinids 225
diagnosis 226
pathology 225
prevention and control 226
signs and pathology 226
Desmozoon lepeophterii 42
clinical signs 42
diagnosis 42
fish farming 42
prevention and control 42
dinoflagellates 223
Diplomonad flagellates 43–44
clinical signs 44
diagnosis 43–44
fish farming 43
prevention and control 44

edwardsiellosis 207
enteric redmouth (ERM) disease/yersiniosis 75–77
characterization 75
clinical signs 76
control 76–77
diagnostic techniques 76
infection sources 76
north-west US and Canada 75–76
salmonids and non-salmonid fish 76
Yersinia ruckeri 75
enteric septicaemia of catfish (ESC) 208–209
causative agent 208
diagnosis 209
pathology 208–209
prevention and control 209
Enteromyxum spp. 300–302
bacteria and virus pathogens 300
clinical signs 301
diagnosis 301
fish production impact 300
macrophage-like cells 301
prevention and control 301–302
sharp snout sea bream 301, **301**
environmental and anthropogenic factors 255–263
adverse effects 258
agriculture industry/antifouling treatments 256
algal blooms 260–261
algicides 259

environmental and anthropogenic factors (*continued*)
ammonia concentration 257
antifouling treatments 258
biological cycle 258
contaminant concentration 258
dissolved oxygen 256–257
fish physiology 255
fouling and antifouling treatments 259–260
genetic selection programmes 259
hatchery and nursery phases 255
human landbased and coastal activities 257
noise 262–263
phagocytic activity 258
rearing system 258
vaccination procedures 261–262
water temperature 256
epitheliocystis 33–34
Candidatus clavochlamydia 33
clinical signs 33
diagnosis 33–34
fish production impact 33
gram-negative cocci/coccobacilli 33
prevention and control 34
warmwater mariculture 164–165
epizootic ulcerative syndrome (EUS) 38–39, 214–217
causative agents 214–215
EUS-associated bacteria 215
EUS-associated pseudofungi 215
EUS-associated virus 214–215
coldwater fish, marine and brackish waters
clinical signs 39
diagnosis 38–39
prevention and control 39
diagnosis 216–217
pathology 215–216
prevention and control 217
ESC *see* enteric septicaemia of catfish
EUS *see* epizootic ulcerative syndrome
EUS-associated bacteria
causative agents 215
pathology 216
EUS-associated pseudofungi
causative agents 215
pathology 216
EUS-associated virus
causative agents 214–215
pathology 215–216
Exophiala sp. 37–38
cerebral lesions 37
clinical signs 38
diagnosis 38
fish production impact 38

fish vibriosis
cytochromoxidase enzyme test 159
facultative pathogens 159
global warming 159
haemorrhagic septicaemia 160
taxonomy 160
treatment, medicated feed 160
warmwater mariculture 159–160
flagellates 42–43
clinical signs 43
Cryptobia salmositica 42–43
diagnosis 43
fish farming 43
prevention and control 43
Flavobacterium psychrophilum 77, 78, **79**
fouling and antifouling treatments 259–260
antifouling strategies 260
cage nets 259, **259**
chemical antifoulants 260
offshore aquaculture 259
organic booster biocides 260
water exchange restriction 259
Francisella noatunensis (francisellosis) 292–294
chronic granulomatous disease 292
clinical signs and gross lesions 293
cod aquaculture industry 292
cumulative mortality 293
direct transmission 292
phylogenetic affinity 292
prevention and control 293–294
primary diagnosis 293
rickettsialike organisms (RLOs) 293
viral-like particles 292
water temperatures 292
franciselliosis 28–29
clinical signs 29
diagnosis 28–29
fish production 28
infections 28
prevention and control 29
fungal-oomycetes diseases
EUS *see* epizootic ulcerative syndrome
Exophiala sp. 37–38
fungal/psuedofungal pathogens 84–87
microsporidiosis 85–87
Saprolegniasis 83–85
furunculosis 71–73
Aeromonas salmonicida 71
characterization 71
clinical signs 72, **72**
diagnostic techniques 72–73
farmed and wild fish stocks 72
fish vaccines 73
fresh and marine environments 71
immunostimulants 73
oxytetracycline (OT) 73
transmission mode 71–72
treatment, infected fish 73

gas bubble disease 120–122
clinical signs, gross and histopathological lesions 121–122
diagnosis 122
gas filled dermal vesicles 121, **121**
gas saturations 121
prevention and control 122
grass carp reovirus (GCRV) disease 199–201
causative agent 199
diagnosis 200–201
pathology 199–200
prevention and control 201
gyrodactylosis 95–96
clinical signs 96
control 96
diagnostic techniques 96
infected salmonids 95
monogenean ectoparasite 95
Norwegian rivers 96
rainbow trout 95
russian-doll formation 95
Gyrodactylus spp. 229

HABs *see* Harmful algal blooms
haemorrhagic smolt syndrome (HSS) 302–303
anaemia 302
clinical signs, gross and histopathological lesions 302–303
fish production impacts 302
prevention and control 303
primary diagnosis 302
harmful algal blooms (HABs)
coldwater fish 117–119
clinical signs, gross and histopathological lesions 118
control and prevention 119
cyanobacterias 118–119
diatoms 118
dictyochophytes 118
dinoflagellates 118
farmed Atlantic salmon *(Salmo salar)* 117, **118**
gas-bubble trauma 117
high mortalities and economic losses 117
oxygen depletion, water 117
red tides 117
risk factors 119
rraphidophytes 118
warmwater fish 260–261
cage site selection 261
ecological, social and economic consequences 260
fibre optical sensors 261
fish death 261
jellyfish blooms 261
offshore aquaculture 261
oxygen depletion 260
toxin producers 260
heart and skeletal muscle inflammation (HSMI) 24–25
clinical signs 24–25
diagnosis 24
fish production impact 24
prevention and control 25
Helminths diseases 229–230
acanthocephalans 229, 230
cestodes 230
nematodes 230
trematodes 230
Henneguya salminicola 94
clinical signs 94
description 94
diagnostic techniques 94
mortality 94
prevention/control 94
spores 94
HSS *see* haemorrhagic smolt syndrome

ichthyobodosis 97–98
characterization 97
chemical treatments 97
coldwater fish 97
Ichthyobodo necator and *Ichthyobodo salmonis* 97
light micrograph 97, **97, 98**
ichthyophthiriosis 221, 223
Ichthyophthirius multifiliis 221–223
antiparasitic chemicals 222
diagnosis 222
disease control 222
malachite green 222
pathology 221–222
potassium permanganate 222
prevention and control 222–223
recombinant protein vaccine 223
Ich/white spot disease *see Ichthyophthirius multifiliis*
IHAS *see* infectious haemolytic anaemia of salmon
IHN *see* infectious haematopoietic necrosis
inbreeding 274–275
infectious haematopoietic necrosis (IHN) 16–17, 64–65
clinical signs 17
coldwater fish in fresh water
characterization 64
clinical signs 65
control method 65
diagnostic techniques 65
M genogroup 65
Pacific salmon aquaculture 64
salmonid fish, North America 64
U/L genogroups 65

infectious haematopoietic necrosis (IHN) (*continued*)
diagnosis 17
epizootics 16
fish production impact 17
prevention and control 17
reservoirs and vectors 17
infectious haemolytic anaemia of salmon (IHAS) 299
infectious pancreatic necrosis (IPN) 19–20
aquabirnaviruses 19
clinical signs 19–20
diagnosis 19
fish production impact 19
prevention and control 20
infectious pancreatic necrosis virus (IPNV) 67–68
avoidance 68
characterization 67
clinical signs 67
diagnostic techniques 68
rainbow and brook trout 67
infectious salmon anaemia (ISA) 20–22
apathogenic strains 21
clinical signs 21–22
diagnosis 21
fish production impact 21
ISAV strains 21
isolation 20–21
prevention and control 22
salmon industry 20
vertical transmission 21
IPNV *see* infectious pancreatic necrosis virus

jellyfish 119–120
clinical signs, gross and histopathological lesions 120
control and prevention 120
fish losses 119
gastrointestinal tract 119
global warming 119–120
risk factors 120
skin damage/ulceration 119

Koi herpesvirus disease (KHVD) 194–199
causative agent 195
diagnosis 197–198
mass mortalities 194
pathology 195–197
acute and reactivation phases 197
hyperplasia 195
immunofluorescence 196
Koi carp, whitish grey necrotic gill tissue 195, **195**
secondary gill lamellae fusion 195
semi-quantitative PCR 196
strain differences 197
temperature 196
vacuolation 196
prevention and control 198–199
sequence analysis 195

lactic acid bacteria (LAB) 162–163
bacteriological isolations 163
clinical signs 163
ecological niches 162
experimental vaccine 163
gram-positive chain-forming bacteria 162
Mediterranean coast transmission 162
lactococcoses 162–163
Lepeophtheirus salmonis transmission 321–324
adequate background data lacking 321–322
appropriate study control lacking 321
causal relationships 323
disease propagation and transmission 322
environmental and ecological data 324
extrapolating laboratory studies 322
failure, temporal effects 323
historical observations 322–323
interactions and pathogen transfer 323
longitudinal studies, disease models 324
nature and outcome, pathogen exposure 324
presumption, infectious source 322
reliance, spatial relationships 322
sampling methods 321
variation, sea lice abundance 322
Lernaea spp. 231–232
diagnosis 232
pathology 231–232
prevention and control 232
Loma. salmonae 41–42
clinical signs 42
Desmozoon lepeophterii 42
diagnosis 42
fish farming 41–42
prevention and control 42
lymphocystis disease (LCD) 156–157
basophilic cytoplasmic inclusions 156
fibroblastic cells 156
fish surviving infection 157
juvenile disease 157
mortalities 156
secondary skin bacterial infection 157

MAS *see* motile *Aeromonas* septicaemia
Metazoa 44
metazoan macroparasites (flatworms, flukes and 'sea lice') 172–178
chemotherapeutants 177
circulatory and respiratory systems 172
Copepoda 176–177
Cymothoids 177

digeneans 175–176
Gnathia spp. 178
Isopoda 177
metazoan marine parasites 173
monogeneans 173
Monopisthocotylea 173–174
parasitic crustacea 176
Polylabroides multispinosus 175
Polyopisthocotylea 174
'sea lice' 173
Sparicotyle chrysophrii 174–175
turbellarians 176
metazoan microparasites (myxozoans) 168–172
Ceratomyxa spp. 169–170
enteromyxum 168–169
functional cellular specialization 168
Henneguya pagri 171–172
Kudoa sp. 171
Myxobolus sp. 169
Polysporoplasma sparis 169
Sphaerospora spp. 170–171
Microsporidia
Desmozoon lepeophterii 42
Loma. salmonae 41–42
microsporidiosis 85–87
characterization 85–86
clinical signs 86
diagnostic techniques 86–87
fish mortality 86
freshwater culture 85
host specificity and tissue tropism 86
intracellular parasites 85
treatment 87
monogeneans 224–225
motile *Aeromonas* septicaemia (MAS) 74–75, 203–206
Aeromonas hydrophila 74
characterization 74
clinical signs 74
control approach 75
diagnostic techniques 75
salmonids 74
warmwater fish, fresh water
A. hydrophila infection 203
causative agent 203
clinical signs 203
diagnosis 205
DNA hybridization 205
epizootics 204
extracellular products (ECP) 204
inflammatory response 204
motile aeromonads 204
prevention and control 205–206
pulse-field gel electrophoresis (PFGE) 205
siderophore production 204–205
virulent and non-virulent strains 205
mycobacteriosis 34–35
clinical signs 35
diagnosis 34–35
fish production impact 34
prevention and control 35
warmwater mariculture 163–164
myxosporeans 218–219
diagnosis 219
Myxobolus koi 218
Myxosoma sp. 218
pathology 219
plasmodia and spores 218
prevention and control 219
Sutchi catfish *(P. hypophthalmus)* 218
Thelohanellus sp. 218

neoplasias 272–273
chemical carcinogens 135–136
coldwater finfish
endocrine system 138
gastrointestinal tract and swimbladder 138
gills and pseudobranchs 137
haematopoietic tissue 138
kidney 137–138
liver 138
reproductive system 138
skin 136
warmwater fish
clinical signs, gross and histopathological lesions 272–273
diagnosis 272
fish production impacts 272
prevention and control 273
non-infectious disorders
coldwater fish *see* coldwater fish in marine and fresh water
warmwater fish
anti-nutrients 273–274
aquaculture sector 254
aquaculture wastes 275
climate change 275–276
commercial feeds 255
disease susceptibility 255
environmental and anthropogenic related problems 255–263
floating sea cages 254
inbreeding 274–275
inbreeding/climate change 255
neoplasia 272–273
nutritional imbalance 263–267
offshore cage technology 254
oxygen deficits and cage fouling 254
skeletal abnormalities 269–272
stress 267–269

nutritional and feed related problems 125–133
carbohydrates 126
chemical composition, water 125
clinical signs 125
lipids, including rancidity 126–128
carnivorous fish 126
clinical signs, gross and histopathological lesions 127–128
exposure to sunlight 126
food storage 126
growth and weight gain 126
prevention and control 128
minerals
calcium 131
deficiencies 130
lithiasis 131
nephrocalcinosis 131, **132**
selenium (Se) 130
toxicity 131
proteins 125–126
starvation 125
supplementation 125
toxic organic components 126–128
mycotoxins 131–133
soybean induced enteritis 133
vitamins
vitamin A (retinol) 128
vitamin C (ascorbic acid) 129–130
vitamin E (tocopherol) 128–129
water supply 125
nutritional imbalance 263–267
clinical deficiencies 263
clinical description 264
diagnosis 264
diet formulation and manufacturing 263
fish production 264
lipid deficiency 263
lipid peroxidation 265
macronutrient deficiency 263
oxygen carrying capacity 265
prevention and control 265
winter syndrome (WS) 265–267

oxygen deficiency 122

Pacific salmon jaundice 297–300
aetiology and epidemiology 297
cumulative mortalities 299
diseases, fish 297, 298
epidemiological, clinical and pathological features 299
haemolytic syndromes 297
haemosiderin 299, **300**
hyperbilirubinaemias, fish 297
infectious haemolytic anaemia of salmon (IHAS) 299, **300**
jaundice 297
parenteral exposure 297
parasitic arthropod diseases 230–234
Alitropus sp. 231, **231**
aquaculture systems 231
Argulus sp. 232–234
cage-culture systems 231
fish importation 230
Lernaea sp. 231–232
warm-water aquaculture 230
parasitic diseases 217–229
coldwater fish, marine and brackish waters
crustaceans 44–45
digenetic trematodes 45
nematodes 46
pennellidae 45
protista and metazoa 39–44
warmwater fish, fresh water
Cichlidogyrus sp. 226–227
ciliates (Phylum Ciliophora) 219–220
Dactylogyrus sp. 225–226
dinoflagellates 223
Gyrodactylus sp. 229
ich/white spot disease 221–223
monogeneans 224–225
myxosporeans 218–219
pathogenic trichodinids 220–221
protistan parasites 217–218
Pseudodactylogyroides marmoratae 227–228
Thaparocleidus sp. 228
Trianchoratus and *Sundanonchus* infections 227
velvet/rust disease 223–224
warmwater mariculture 165–178
The protistan ectoparasites 165–166
protistan endoparasites 166–168
parasitic pathogens 87–94
ceratomyxosis 91–94
gyrodactylosis 95–96
Henneguya salminicola 94
ichthyobodosis 97–98
PKD *see* proliferative kidney disease
whirling disease 88–91
Parvicapsula spp. (parvicapsulosis) 294–297
clinical signs, gross and histopathological lesions 295–296
diagnosis 295
fish production impacts 295
myxozoa 294
penreared coho salmon 294
pink salmon 295
prevention and control 296–297
PBDEs *see* polybrominated diphenyl ethers
PCBs *see* polychlorinated biphenyls
photobacteriosis 160–161

agglutination test kit 160
antibiogram 161
bipolar staining 160
identification, bacterium 160
morphological and biochemical features 160
splenomegaly 161
piscine myocarditis virus (PMCV) 288
piscine reovirus (PRV)
aetiology and pathogenesis 290–291
diagnostic ruleouts 291
fish production impacts 291
histopathological lesions 290, 291
pathological condition 290
prevention and control 291–292
primary diagnosis 291
supernatant and cell lysates 291
transmission experiments 290
viral replication and development 291
visible signs 291
PKD *see* proliferative kidney disease
PMCV *see* piscine myocarditis virus
polybrominated diphenyl ethers (PBDEs) 258
polychlorinated biphenyls (PCBs) 258
proliferative kidney disease (PKD) 87–88
characterization 87
clinical signs 87–88
control methods 88
diagnostic techniques 88
extrasporogonic stage 87
hatchery-reared salmonids 87
Myxozoa 87
protista 40–41
AGD 40
clinical signs 40
diagnosis 40
fish production 40
Ichthyophonus hoferi 41
phylogenetic analysis 40
prevention and control 40–41
protistan endoparasites 166–168
apicomplexans 167–168
ectoparasites 166
metazoan macroparasites (flatworms, flukes and 'sea lice') 172–178
metazoan microparasites 168–172
microsporidians 167
protistan parasites 217–218
Pseudodactylogyroides marmoratae 227–228, **228**
pseudofungal diseases 213–217
branchiomycosis 213–214
EUS 214–217
saprolegniasis 213
Pseudomonas sp.
clinical signs 35
diagnosis 35
fish production 35
prevention and control 35
septicaemia 206–207
causative agent 206
diagnosis 206
pathology 206
prevention and control 207

rainbow trout gastroenteritis (RTGE) 303–304
clinical signs, gross and histopathological lesions 304
diagnosis 303–304
fish production impacts 303–304
haemorrhagic gastroenteritis 303
husbandry and environmental factors 303
prevention and control 304
red sea bream iridoviral disease (RSID) 157–158
diagnosis 157
formalin-fixed and DNA antigens 157–158
transmission 157
rickettsia-like organisms (RLOs) 293
RSID *see* red sea bream iridoviral disease
RTGE *see* rainbow trout gastroenteritis

salmonid alphavirus (SAV) infections 22–23
Atlantic salmon 22
clinical signs 22–23
diagnosis 22
fish production impact 22
prevention and control 23
salmonid herpes virus infections 69–71
characterization 69–70
clinical signs 70
control 70–71
description 69
diagnostic techniques 70
SalHV-1, SalHV-2 and SalHV-3 70
salmonid rickettsial septicaemia 82–83
coldwater fish in fresh water
coho salmon and rainbow trout 83
Piscirickettsia salmonis 82
coldwater fish in marine and brackish waters
clinical signs 30
diagnosis 30
fish production 30
prevention and control 30
rainbow trout and Chinook salmon 29
sporadic cases 29
saprolegniasis 83–85
characterization 83–84
chemical prophylactics 84
clinical signs 84
control 85
diagnostic techniques 84–85
environmental conditions 83
salmon and catfish 84
temperature range 83

saprolegniasis 213
sea lice
caligid-parasites 45
clinical signs 45
diagnosis 45
fish farming 45
in marine and brackish water 44
prevention and control 45
sea lice transmission 320–324
disease modelling 320
Lepeophtheirus salmonis transmission 321–324
molecular and analytical methods 320
sea (or salmon) lice 320
spatial/temporal associations 321
wild fish survival (escapement) 321
'Sentinel Method' 198
skeletal abnormalities
axial deformities 269–271
aquaculture products 270
body morphology 270
deformities in meagre *(Argyrosomus regius)* 270, **271**
Juvenile specimens of white sea bream *(Diplodus sargus)* 270, **270**
Saddleback syndrome 270
types, vertebral 269–270
gillcover anomalies 271–272
morphoanatomical abnormalities 269
sleeping disease 68–69
characterization 68
clinical signs 69
cultured rainbow trout 68–69
diagnostic techniques 69
salmonid alphaviruses (SAVs) 68
temperature 69
smolt failure, 114–115
captivity 115
clinical signs, gross and histopathological lesions 115
control and prevention 115
season- related smolts 115
smoltification 114
temperature and photoperiod conditions 115
sporadic diseases and disorders
aquaculture husbandry practices 288
diagnostic approaches 288
Enteromyxum spp. 300–302
enzootic 288
Francisella noatunensis (francisellosis) 292–294
haemorrhagic smolt syndrome (HSS) 302–303
host resistance 288
management and containment activities 288
massive research efforts 287
Pacific Salmon Jaundice 297–300
Parvicapsula spp. (parvicapsulosis) 294–297
pathogen transmission 288
PMCV *see* cardiomyopathy syndrome
PRV *see* piscine reovirus
rainbow trout gastroenteritis (RTGE) 303–304
transitions, coastal ecosystems 287
viral haemorrhagic septicaemia virus (VHSV) 287
spring viraemia of carp (SVC) 65–67
characterization 66
clinical signs 66
cold environments 66
cool- to warm-water species 66
diagnostic techniques 66
prevention 66–67
rhabdovirus 65–66
virulence 66
streptococcal septicaemia/meningoencephalitis 211–213
causative agent 211
diagnosis 212
pathology 211–212
prevention and control 212–213
streptococcoses 162–163
stress
coldwater fish (marine and fresh water) 115–116
warmwater fish 267–269
adverse weather conditions 268
biomarkers 267–268
chronic 267
fish production impacts 267
metabolic pathways 267
neuroendocrine reaction 267
organosomatic indexes 267
prevention and control 269
secondary stress response 267
severity and duration 267
signs, histopathological lesions and immune parameters 268, 269
suboptimal culture conditions 268
Sundanonchus infections 22
SVC *see* spring viraemia of carp

tenacibaculosis 31–32
clinical signs 31–32
diagnosis 31
fish production impact 31
prevention and control 32
Thaparocleidus sp. 228, **228**
Trianchoratus infections 227
trichodinid diseases 220–221
diagnosis 220–221, **221**
fish mortality, hatcheries 220
pathological effects 220
prevention and control 221
tryptic soy agar (TSA) 163

vaccination procedures 261–262
fish/antibiotic bath exposure 262
fish disease/mortality 261–262
intraperitoneal injection 262
therapeutic/prophylactic treatment 262
vaccine-induced side effects 262
vaccines
chemical treatments 134–135
clinical signs, gross and histopathological lesions 133–134
velvet/rust disease 223–224
diagnosis 223–224
histopathological changes 223
Piscinodinium pillulare 223
prevention and control 224
VHS *see* viral haemorrhagic septicaemia
vibriosis 26–28, 159–160
clinical signs 28
cold-water 27
diagnosis 27
fish production impact 27
Pacific salmon 27
Vibrio anguillarum 26
viral diseases 16–25
CMS *see* cardiomyopathy syndrome
heart and skeletal muscle inflammation (HSMI) 24–25
infectious haematopoietic necrosis (IHN) 16–17
infectious pancreatic necrosis (IPN) 19–20
infectious salmon anaemia (ISA) 20–22
salmonid alphaviruses (SAV) 22–23
viral encephalopathy and retinopathy (VER) 25
viral haemorrhagic septicaemia (VHS) 17–19
warmwater mariculture 156–159
lymphocystis disease (LCD) 156–157
red sea bream iridoviral disease (RSID) 157–158
viral encephalopathy and retinopathy (VER) 158–159
viral encephalopathy and retinopathy (VER)
coldwater fish, marine and brackish waters
clinical signs 25
diagnosis 25
fish production impact 25
prevention and control 25
warmwater mariculture 158–159
clinical disease 158
experimental vaccines 159
neurological signs 158
neurotropic viruses 158
nonlethal molecular diagnostic methods 159
pathogenesis 158
Reverse transcription polymerase chain reaction (**RT-PCR**) 158
vertical transmission 159
viral haemorrhagic septicaemia (VHS) 17–19, 62–64
coldwater fish, fresh water
characterization 62
clinical signs 63
diagnostic techniques 63
impacts, aquaculture 62–63
prevention/eradication 63–64
coldwater fish, marine and brackish waters
clinical signs 18, **19**
diagnosis 18
diseased sea-reared rainbow trout 18
fish production impact 18
genotype Id strains 17
isolation 17
prevention and control 18–19
viral infections 194–202
channel catfish virus disease (CCVD) 201–202
grass carp reovirus (GCRV) disease 199–201
Koi Herpesvirus Disease (KHVD) 194–199
taxonomic classification and physical characteristics 201
viral pathogens diseases 62–71
infectious haematopoietic necrosis (IHN) 64–65
IPNV *see* infectious pancreatic necrosis virus
salmonid herpes 69–71
sleeping disease 68–69
spring viraemia of carp (SCV) 65–67
VHS *see* viral haemorrhagic septicaemia

warmwater fish in fresh water 193–235
bacterial diseases 202–213
cage culture 193
diseases, parasitic arthropods 230–234
drugs and chemicals 235
epizootics 234
feral and cultured fish 193
fish-borne zoonotic diseases 193
helminths diseases 229–230
macrorestriction analysis 235
parasitic diseases 217–229
pseudofungal diseases 213–217
viral infections 194–202
warmwater mariculture 155–178
'artificial islands' 156
bacterial diseases 159–165
cage environment 155
parasitic diseases 165–178
physico-chemical parameters 155
public environmental awareness 155
viral diseases 156–159
water-borne irritants 122–124
ammonia 122–123
nitrite 123–124

whirling disease 88–91
cage culture operation 89
Celsius temperature units (CTUs) 89
characteristic black tail 88, **88**
clinical signs 90
control 91
diagnostic techniques 91
life cycle, *Myxobolus cerebralis* 89, **90**
Myxobolus cerebralis 88
rainbow trout 90
triactinomyxon (TAM) stage 88
wild *vs.* farmed fish 313–324
aquatic environment 316
bi-directional transmission 320
cage farms 313
cause-effect relationships 315–316
chronic diseases 319–320
diagnostic tools and genetics 318
ecology, diseases 315–316
endemic pathogen amplification 314
environmental change 319
epidemics 319
epidemiological theory 316
fish aggregating devices (FAD) 313
farm-associated biomass 314
fish aggregation 313–314
fish assemblages 313
furunculosis and vibriosis 318
host 316–317
infectious agent 317–318
characteristics 317
density-dependent transmission 317–318
malaria and Lyme disease 318
transmission 317
low-level mortality 319
management/husbandry modifications 318
management methods 316
Office International des Epizooties (OIE)-listed diseases 318
pathogen exposure 314–315
pathogen transmission 318
risk-based (targeted) sampling 319–320
sampling method 313
sea lice transmission studies 320–324
winter disease (WS) 265–267
causes 265
diagnosis 265–266
fish production 265
gross and histopathological lesions 266
opportunistic pathogens 265
prevention and control 266–267
winter ulcer disease
clinical signs 31
diagnosis 31
fish production impact 30–31
Moritella viscosa 30
prevention and control 31
WS *see* winter disease

yersiniosis 37

zoonoses 165