# Diseases of Carp and Other Cyprinid Fishes

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# Preface

Cyprinids are the major group of freshwater fish that have a global significance as a source of food, for sport, for their ornamental appeal, and as experimental models for research. It is therefore surprising that there has not, until now, been any book published that deals solely with cyprinid diseases. This contrasts with the relatively vast amount of literature on diseases associated with salmonids. This book attempts to redress this imbalance. The book considers both infectious diseases, i.e. transmissible between fish, and noninfectious diseases, e.g. nutritional disorders and tumours, etc. in a series of chapters.

Although each of these chapters can be read in isolation there is inevitably much cross-referencing and the reader is strongly encouraged to consult other sections where appropriate. Given the great interest in cyprinids, ranging from the angler and novice aquarist to the research scientist who uses fish such as carp and zebra danio in experimental systems, the authors have chosen to write in a style that we hope is helpful to all interested parties. This has meant that wherever possible generic names for fish species have been avoided in the text, although they can been found in a list at the end of the book.

Inevitably some areas of the book contain technical and scientific terminology and the readers' attention is drawn to the glossary of terms.

The interactions between humans and cyprinid species have meant that disease is of major importance to all systems where cyprinids are held in captivity and in the semiwild stocks that are exploited for sport. From the angler fishing a pool, the ornamental hobbyist looking after a pond or aquarium, the scientist carrying out research studies to the farmer producing cyprinids for restocking or for food, all will inevitably encounter diseased fish. It is hoped that the contents of this book are presented in a digestible format and will help in the understanding of the disease problems.

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# Chapter 1
# Introduction

## 1.1 GEOGRAPHICAL DISTRIBUTION

The Cyprinidae is one of the largest families of teleosts in the world comprising at least 1700 species and over 200 genera (Plates 1.1, 1.2, 1.3, 1.4). Natural populations of cyprinids are widely distributed in most freshwater rivers, lakes and ponds in Eurasia, Africa, Japan, East Indian Islands and in North America at latitudes greater than 40°N. They can thus tolerate ranges of temperature from near freezing to 40°C. Cyprinids are also found in some river estuaries and other waters of low salinity, for example the Baltic Sea and Gulf of Bothnia. Their natural habitats can range from raging torrents in the mountainous areas of Asia to stagnant, weedy, muddy farm ponds in rural England.

Humans have also artificially spread cyprinids from their natural habitats. The most obvious example of this unnatural transference is the introduction of the common carp into Europe. Carp originally were a tropical and subtropical fish, yet for many years, they have been cultured on a large scale in colder areas. It is interesting to note that the first recording of carp being present in the United Kingdom was attributed possibly to Dame Juliana Berners, Prioress of Sopwell Nunnery (Fig. 1.1), Hertfordshire, England in *Treatyse of Fysshynge with an Angle* published in around 1496 (Fig. 1.2).

**Fig. 1.1** Remains of the Sopwell Nunnery, Hertfordshire, England.


**Fig. 1.2** The fisherman from the book *Treatyse of Fysshynge with an Angle* written by Dame Juliana Berners, Prioress of Sopwell Nunnery, Hertfordshire, England, published in around 1496.

Cyprinids have been introduced into new habitats for many reasons including:

- food stocks for migrating human populations, religious groups or invading forces, e.g. monastic ponds;
- as an important species for aquaculture, e.g. common carp, silver carp, grass carp and other large carp were imported into Russia as food fish;
- as familiar species for the sporting interests of pioneers, e.g. common carp, roach and tench;
- as ornamental animals, e.g. koi, orfe, goldfish, and numerous small tropical species for aquaria;
- as bait-fish by anglers, e.g. fathead minnow and goldfish;
- as a research animal or for toxicological studies, e.g. common carp, zebra danio, goldfish and fathead minnow;
- as escapees from fish farms, fisheries and other forms of culture, e.g. goldfish, koi and common carp;
- for biological control of water weeds, e.g. grass carp, silver carp, bighead carp and black carp.

In some countries the introduction of cyprinids has become problematic resulting in:

- massive over-population;
- competition with native fish species and other aquatic animals;
- general environmental damage, and
- the transmission of pathogens.

For example, common carp have been designated as undesirable species in some countries including the United States of America and Australia and unfortunately the control or eradication of these unwanted fish has proved difficult, if not impossible. In the case of ornamental koi and goldfish, these have been released into many freshwaters around the world, including Australia where cyprinids are not naturally found. In some regions these introductions have had deleterious effects on the native fish fauna.

Of particular relevance to this book is that this uncontrolled dispersion of cyprinids has led to the spread of pathogens, e.g. in the UK the tapeworms *Bothriocephalus acheilognathi*, *Khawia sinensis* and the virus, *Rhabdovirus carpio*, which is the causative agent of spring viraemia of carp. Atypical strains of the bacterial pathogen, *Aeromonas salmonicida*, have allegedly been introduced along with goldfish into Australia, and there is unconfirmed evidence that the import to France from North America of cyprinids designated for bait-fish led to the introduction there of the salmonid pathogen, infectious haematopoietic necrosis virus (IHNv).

## 1.2 ECONOMIC IMPORTANCE OF CYPRINIDS

It is difficult to value cyprinids in monetary terms. However, over ten years ago, it was estimated that worldwide commercial yields of cyprinids was about 800 000 tonnes. Of that figure, half were made up from wild stocks inhabiting natural water courses such as rivers, ponds, lakes and reservoirs (Fig. 1.3). The other half were raised in some form of aquaculture system (Fig. 1.4). The world catch for carp annually exceeds 200 000 tonnes. In 1990 the Food and Agriculture Organization (FAO: Fisheries Circular No. 815 Revision 2) revealed that the annual production of freshwater fish was in excess of 5 000 000 tonnes. Of this, 4 589 052 tonnes were carp and other cyprinids, 229 140 were tilapia and other cichlids and 238 169 were salmonids. Over half the production of cyprinids occurred in Asia.

In Europe, the main species taken from rivers are bream and roach, whilst in commercial situations the main species are common carp and crucian carp. Cyprinids are an important part of the economy in many countries such as central, eastern and southern Europe and the countries that made up the former USSR. The input that cyprinids have on the economy can vary.

**Fig. 1.3** Netting of cyprinids in the UK.

**Fig. 1.4** Carp pond in Israel covered with polythene to increase temperature and exclude predators. Note paddle aerator in foreground.

According to the FAO, in 1990 the primary marketable cyprinids for Hungary were common carp, asp, barbel, tench, bighead carp, grass carp and silver carp. Less important marketable cyprinids included common bream, white bream, silver crucian carp, orfe, chub, roach, bleak, nase, rudd and vimba. In 1987, a total of 16 789 tonnes of cyprinids were marketed of which 11 406 tonnes consisted of common carp.

The situation is different in Ireland, a country that has cyprinid populations, but where they are not commercially exploited for food. However, they have an economic value because their presence brings in considerable money to the country through the tourist industry's marketing policy on sport fishing.

Several countries have established tourist industries as they realise that wild cyprinids have considerable value and can be exploited.

Coarse fish (nonfreshwater game fish) holiday packages are marketed in Ireland, Spain, France, Denmark and the UK.

There are even specialist holidays in Northern India catering for anglers searching for the excitement of catching the golden mahseer. This is one of the world's largest cyprinids, which can reach a length of 2.75 m and a weight of over 150 kg.

In the UK, there are approximately 3 million domestic anglers who act either individually or are organised into clubs or specialist groups. National licences to fish any inland water have to be purchased in addition to those obtained from the riparian owner or appropriate authority. There are regular competitions (matches) organised which can have substantial cash prizes, large enough for some anglers to make match fishing their sole occupation. The support industries of these activities can generate a large amount of revenue, e.g. publishing, clothing and travel. The total UK industry was estimated to be worth £2.2bn in 1994.

It is, however, in Asia where cyprinids have the greatest impact on both the economy of villages and the country. In China systems of polyculture of major Chinese carps (bighead carp, grass carp, silver carp and black carp) have been practised for thousands of years. These species comprise bottom feeders, midwater feeders and surface feeders and therefore do not greatly overlap, allowing all food sources to be utilised economically.

Among ornamental cyprinids, koi and goldfish are cultured in large numbers of fish farms to supply the international aquarium and pond trade. Top quality koi, as determined by size and colour patterns, may have a retail value exceeding £10 000 per fish.

From the above examples, it is clearly impossible to establish the total numbers or monetary value of cyprinids used by humans because, besides the commercial fisheries, not all catches of cyprinids are recorded in annual totals by fishermen. It is evident however that cyprinids are of considerable economic importance and that the current problems of pollution, water transfers and future predictions of the detrimental effects of global warming on inland waters are of extreme importance.

Whether cyprinids are exploited by commercially catching wild stocks or from fish raised in aquaculture, there has to be some system of management. Even sport fishing demands fishery management. For wild fish, the most important considerations are sustainability of stocks and sufficient recruitment of the economically desired species at the right size for consumption or sport. For fish raised in culture, there has also to be a continued production of marketable stock.

One major problem associated with any fish system is the risk of introducing diseases either via the fish or their transport water. Disease transmission between sites is therefore a serious concern and in the case of transfers over long distances such as between countries or between climatically different regions, then there is a greater risk of introducing an exotic disease. In Europe, there are now European Union laws to prevent the indiscriminate movements of fish between

European countries and into Europe from third countries without appropriate health certification and import licences.

The trauma of being caught, maintained in holding nets, being handled and transported, stresses the fish and lowers their immunity, rendering them vulnerable to latent or opportunistic pathogens. The immunosuppressed fish may develop a disease during transportation and can transmit the pathogen to any fish inhabiting the waters to which they are stocked. Alternatively, the transported fish may become diseased if exposed to pathogens in their new environment to which they have no protective immunity.

Even when fish are transferred from presumptively 'disease-free' sources, although rigorous testing is carried out, complete assurance that the fish are disease-free cannot be given. Some pathogens are extremely difficult to detect in 'carrier' fish and some pathogens may go undetected because clinical disease has not yet manifested. In such situations new diseases could emerge some years after the introduction when conditions become more favourable to the pathogen. Likewise, parasites are not always recorded on veterinary health checks because they are 'common' in that area and do not cause overt disease. However, when transferred along with their hosts, they can become problematic in native fish. For example, the skin fluke *Gyrodactylus salaris* has become problematic for wild Atlantic salmon in some rivers in Norway, because of introductions of salmon from Sweden where this parasite has a benign association with the native stocks. The problem of disease introduction is becoming more acute with the increase in movement of cyprinids worldwide and the occurrence of drug resistance in several pathogens, e.g. in some ornamental cold-water cyprinids there are antibiotic resistant strains of *Aeromonas* spp. being isolated in freshly introduced fish. Further disease problems arise because nonindigenous species are being transferred into countries where there are different water temperatures, poor water quality, insufficient natural food and incompatible fish stocks present.

To protect native fish species from exposure to exotic diseases and parasites, it is now a legal requirement in some countries, including the UK, to obtain a licence to keep or release a variety of nonindigenous fish species. Cyprinids that require licensing include silver carp, clicker barb (Fig. 1.5), toxostome or French nase, schneider, asp, bighead carp, bitterling, blageon, blue bream, Chinese black or snail-eating carp, Danubian bleak, grass carp, Mediterranean barbel and vimba. Illegal introductions or the absence of an appropriate licence can lead to prosecution of the dealer and/or purchaser. Nor is the danger only from illegal imports: grass carp were legally imported into England for controlling aquatic weeds, but these still require a licence.

**Fig. 1.5** Clicker barb.

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# Chapter 2
# Cyprinid Biology

An important aspect of understanding the pathological response induced by infection, environmental factors or abnormal biological functioning is an awareness of the normal structure of the biological organ or system involved. The aim of this chapter is to provide a brief overview of cyprinid biology, in particular those systems that are prone to pathological changes following external or internal insults. It is not intended to give an extensive description of the biology of fishes, rather to provide the reader with a general description of the structure and function of cyprinid organs so that the ramifications of pathological changes described in the following chapters can be appreciated.

## 2.1 WATER: THE CONTROLLING FACTOR

Given the diversity of fish habitats and adaptations it is somewhat surprising that the majority of cyprinid fish have adopted an easily recognisable form, that is usually taken as the archetypal fish design. The reason for this is the chemical and physical constraints imposed by water. Such factors as density, compressibility and chemical properties are of fundamental importance. The fact that water is approximately 800 times denser than air results in a reduction of the effects of gravity on the fish body.

Fish therefore can achieve nearly neutral buoyancy, which means that muscular effort can be directed to providing thrust. This is an important consideration since the high density and incompressibility of water means that movement through water is more difficult than through air. Cyprinids, like the majority of other fish, solve this problem by having streamlined bodies and a high proportion of muscle. The incompressibility of water also creates turbulence and increases drag. This may, however, not be completely deleterious to the fish for they exploit these water properties; small amounts of water displacement are registered by the extremely sensitive lateral line system, a sensory array located on the lateral aspects of the fish.

These properties of water also have another important aspect as sound can be carried further and faster in water (1433 m/s) than in air (355 m/s). As a consequence fish have very good hearing even though they lack any form of external ear. These structures are not required as fish tissue is approximately the same density as water and is therefore transparent to sound waves. This also means that internal structures

such as the calcified ear bones, the otoliths, and the swimbladder have a role in the auditory system.

Just as important to the fish are the chemical properties of water, in particular water's role as an almost universal solvent containing organic compounds, salts and a mixture of gases, many of which are required to sustain life. Of the gases the most important is oxygen, which when compared to air occurs in water in extremely small amounts. For example, 1 L of water can contain up to 8 mL of oxygen whilst 1 L of air contains 210 mL of oxygen. There are of course variations in these amounts; eutrophic water sources can contain less than 8 mL of oxygen per litre.

In any aquatic situation the amount of oxygen present can place severe limitations on fish metabolism, activity and survival. Some fish have made attempts to alleviate this problem by breathing air for short periods. In order to extract oxygen from the water the fish requires an extensive surface area over which oxygen can be taken up. The region should also contain an adequate supply of blood and thus fish expose a large, highly vascularised gill surface to the water. Such an organ design also has other advantages and allows the fish to eliminate waste products, e.g. carbon dioxide, ammonia, salts and heat from this large surface area. This basic design, however, does have disadvantages since a large area of exposed gill is particularly prone to adverse effects from harmful water contaminants such as pollutants and is an ideal location either for entry of viral and bacterial pathogens into the body or as a location for parasitic infections.

## 2.2 BASIC EXTERNAL ANATOMY

Although life within an aquatic environment can put severe constraints on the basic body design of fish, the cyprinidae have generally adopted the classic fish body plan: a fusiform to moderately deep body, conspicuous scales, abdominal pelvic fins, with a head that contains large eyes and a small terminal or subterminal mouth. Their size varies from the smallest freshwater fish known, the *Danionella translucida*, approximately 10 mm in length, to some of the huge intensively reared major Indian carp, which reach 2–3 m in length and the endangered Colorado squawfish, which has been reported to be up to 2 m in length and 45 kg in weight.

The external covering of a fish forms an ideal location for the growth of organisms being in intimate contact with the external medium. Although many of the skin colonisers are commensals and do little harm others can, particularly when present in large numbers or when the fish is under some other severe environmental threat, cause severe pathological problems and induce abnormalities in the external layer, which breaches its protection. The colour of the external skin surface reflects camouflage, gender differences and breeding displays, which may be affected by pathological changes that affect the outward appearance of the fish. Scales, which cover the external surfaces of the

majority of fish, vary considerably in size between cyprinid species. For example, in common carp, they can reach 5 cm in diameter. Scales evolved independently in the cartilaginous and bony fish, the latter of which includes the cyprinidae. Whilst the former contain the tooth-like placoid scales, those of bony fish, and in particular the cyprinidae, are round, flat, thin cycloid scales located in the dermis of the fish and overlaid by a thin epidermis that contains mucous glands. The mucus produced by these glands is known to have protective functions. For example, (a) its secretion forms a slippery film over the body surface, reducing resistance to the water when the fish is swimming, (b) it protects the body surface by forming a barrier to bacterial and fungal pathogens and is considered to have antimicrobial properties, (c) it protects the body surface from abrasions and (d) it plays a part in the osmoregulatory process.

The scales can easily be removed from the fish without causing great stress, and in temperate fish serve as a useful mechanism for ageing the animal as the growth and periods of adverse stress, e.g. spawning, are reflected in the normal growth patterns expressed in the scale (Fig. 2.1). Scales, usually considered as a characteristic of fish, can be reduced in number, e.g. the mirror carp, and are absent from certain areas of the body, i.e. the fins.

The fin position, size and shape are closely related to the rest of the body form and the behaviour of the species. Most cyprinids have seven fins. The paired fins (pectoral, pelvic) and the unpaired fins (dorsal, anal and caudal) act as an integrated system that simultaneously manoeuvres, stabilises and propels the fish. Each are controlled by a set of muscles, the anatomical details of which will not be expanded here. Each fin regardless of its position on the body is supported internally by soft fin rays in the majority of cyprinids, although in some notable exceptions, e.g. carp and goldfish, rays have been modified into spines. The fact that fins project from the body surface and are constantly in

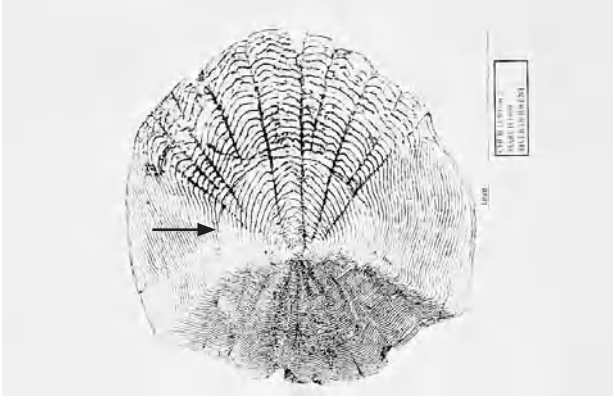

**Fig. 2.1** Cyprinid scale showing growth rings (arrowed). In this case the fish was 2 years old. Magnification × 10 (courtesy of the Environment Agency, UK).

the presence of water makes them particularly prone to infection or attack.

The skin may also have a respiratory function. In some fish species aquatic cutaneous respiration can take place. In larval stages in particular, the cutaneous surface can account for a significant component of the respiratory area and may supply oxygen to internal organs. In adult stages however, oxygen taken up through the skin is only utilised locally. In most adult cyprinids the major gaseous exchange tissue is the gills which are located under the opercula on either side of the head.

The gills consist of bony and cartilaginous stiffened arches (Plate 2.1), four on either side of the head. The medio-anterior edge is usually equipped with gill rakers which function as filters for food particles. Arising from each gill arch are filaments from which numerous lamellae protrude from both sides. These lamellae are the major site of gaseous exchange and are made up of external thin epithelial cells and supportive pillar cells that facilitate blood flow through the gill. The thickness of these lamellae varies depending upon the activity of the fish species. Roach, for example, have approximately 12 lamellae per mm which are 12 µm thick. The distance between the water and the blood is 2 µm. In addition, gaseous exchange is facilitated by the adoption of a counter-current system in which blood flow is in the opposite direction to water flow. The latter is maintained by a series of coordinated pressure changes in the mouth and opercular cavities. Gills also contain mucus-producing cells and cells which excrete ammonia and excess salts. The position of the gills, their delicate structure and intimate contact with the water makes them particularly vulnerable to changes in environmental conditions. Their large surface area and rich supply of blood also make them the favoured sites of viral, bacterial and parasitic infections.

The body of the fish comprises a series of integrating systems (see Fig. 12.3) that together maintain the correct physiological homeostasis of the animal.

## 2.3 BASIC INTERNAL ANATOMY

### 2.3.1 Skeletal and muscular systems

In cyprinids, as in all bony fish, the skeleton is comprised primarily of bone and consists of three main sections: the vertebral column, the skull and the appendicular skeleton. The proportion and form of each varies depending upon the species and results in the array of different body forms. Although all bones could be considered to have developed into specialised shapes adapted for an appropriate role some have been modified to an extreme. For example, the otoliths are used as part as the sensory system, perhaps equivalent to the human ear bones. These bones may be used to age the fish (Fig. 2.2).

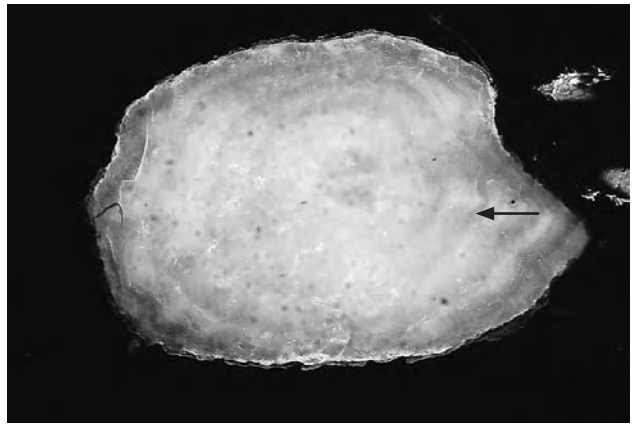

**Fig. 2.2** Split otolith of a roach showing growth pattern and rings (arrowed). This fish was 5+ years old. Magnification ×25 (courtesy of the Environment Agency, UK).

Associated with the skeletal structures are numerous muscles that comprise the majority of the body mass. Within the main trunk of the fish these muscles are arranged vertically into 'W' shaped blocks called myomeres (myotomes) and form the main propulsive power. Fish muscles on closer inspection can be seen to comprise several different types which vary in colour according to the amount of respiratory pigment (haemoglobin) present. Red muscle has numerous capillaries per cubic millimetre and is used to sustain levels of low activity swimming, hence it is also known as 'slow' muscle. In contrast, white (fast) muscle has a poor supply of blood and is used to give short bursts of rapid swimming usually resulting in anaerobic metabolism. Pink muscle may also occur and acts as an intermediate to the role of red and white muscle. The proportion of each muscle type varies between fish species depending upon behaviour and activity, e.g. in carp the pink and white muscles are recruited for moderate increases in swimming speed. The skeletal and the associated muscles are involved in several disease states. These can arise from developmental abnormalities resulting from, for example, genetic alterations, environmental effects or infection of pathogens.

### 2.3.2 Digestive system

Fish can be classified on the basis of their feeding habits. Whether a fish is a detritivore (feeding on detritus), herbivore (consuming plant material), carnivore (feeding on other animals) or omnivore (unspecialised plant and animal feeder) is reflected in the construction of the alimentary tract and it is therefore difficult to generalise about the typical gut of a cyprinid fish. The basic gut system does, however, follow a general pattern. The mouth, or buccal-pharyngeal cavity, is specifically adapted for food seizure and ingested food passes quickly down the gullet or oesophagus to the stomach. The basic morphological differ-

ences in cyprinids start at the mouth. In some species such as common carp, common bream or gudgeon, which are benthic feeders, the lips are able to protrude and are ventrally situated to enable them to suck small invertebrates, e.g. dipteran larvae, molluscs, etc., from the sediment or off vegetation. Pelagic feeders include the asp and bleak. In those species, the lips are fairly firm and the bottom lip extends slightly to enable surface feeding. Cyprinids such as roach, dace and chub are generally considered to have somewhat firmer lips. However, although macrophytes and filamentous algae form much of their diets, they also consume zooplankton, insect larvae and molluscs. In fact, these fish are not true herbivores, but rather omnivores. There is considerable overlap in food intake by cyprinids. For example, the chub includes small fish and crayfish in its diet and dietary preferences can also vary according to the stage of development.

Cyprinids are characterised by having no teeth in their jaws, but have teeth present on the pharyngeal bones, just behind the gill chamber. These grind food against a horny pad, the pharyngeal pad, situated in the roof of the pharynx. The pharyngeal teeth may be used as a diagnostic feature to distinguish between closely related cyprinids. In addition, gill rakers protrude from each gill arch and are specialised for different modes of feeding. Fish can taste food. Those, such as bream, which feed by rooting in the mud at the bottom of the water, tend to have more taste buds than fish which feed in mid water, such as bleak. Barbels, which are finger-like appendages adjacent to the mouth, are present on a number of cyprinids including common carp but not crucian carp, goldfish or roach. They are used as tactile and taste organs. Further down the alimentary tract a specialised area produces a variety of digestive enzymes, e.g. pepsin in an acid environment of pH 2.0. Further breakdown of the food occurs in the intestine where absorption of nutrients also occurs. Indigestible fragments pass out in the form of faeces. Also associated with the gut are two important organs, the liver and the pancreas. The liver is a large conspicuous brown-pink organ situated at the anterior end of the body cavity and serves an important role in the metabolism of proteins, carbohydrates and lipids. Between its lobes is located a small greenish sac, the gall bladder, which contains bile that is secreted into the intestine via a duct, the bile duct, and facilitates the breakdown of food.

The other important organ, the pancreas, is less noticeable than the liver being cream/white in colour, diffuse and located in the mesenteric membranes surrounding the intestine and liver. The pancreas has two important secretory functions termed exocrine and endocrine. The former is associated with the production of enzymes such as trypsin, which are passed to the gut to aid food digestion. In the latter the hormone insulin is produced which controls sugar and protein metabolism and prevents the fish becoming diabetic. All the organs associated with digestion are particularly prone to disease. The gut is used as an access route or site of development for many pathogens and the liver, with its good blood supply, is also a site of infection.

Linked to the digestive system are the nutritional requirements of the fish. Most of our knowledge on dietary requirements of fish has been obtained from economically important cultured species including the salmonids and several cyprinid species. These studies have revealed that dietary proteins, lipids and carbohydrates are required in correct proportions as are small quantities of vitamins and minerals. Deficiencies in either quantitative and/or qualitative aspects of the food supply can lead to stunting of growth and nutritional deficiency diseases.

### 2.3.3 Excretory system

The breakdown of food not only results in the production of energy and molecular building blocks for growth but also produces waste products of metabolism that have to be excreted from the body. These waste molecules are processed in different ways. Water is either conserved or excreted depending upon the environment. In marine fish, water tends to be retained whilst in the case of cyprinids that are mostly freshwater fishes, it is lost. The respiratory waste product carbon dioxide interacts with the bicarbonate equilibrium of the blood and is eventually lost from the body via the gills. The digestion of protein produces nitrogenous compounds. In the case of fish this is toxic ammonia; fish are therefore said to be ammoniotelic. This is in contrast to mammals, which produce mainly urea, and are therefore known as ureotelic. The advantage of producing ammonia instead of urea as a waste product is that the former maintains ion exchange and conserves energy. Carp and goldfish excrete up to 10 times more nitrogen through their gills than they do via the kidney. The kidneys of fish do, however, serve an important excretory function and are therefore very versatile organs, having also immunological and endocrine roles.

The excretory component of the kidney is a long dark reddish structure located on the dorsal aspect of the body cavity. It filters the blood through a sieve-like mechanism, the glomerulus, which comprises a series of capillaries and part of the excretory tubules, the Bowman's capsule. This area is connected to the rest of the tubule called the nephron, which empties via paired ureters into the bladder. In freshwater fish the excretory tubule comprises the first and second proximal segments. In the first segment, protein, amino acids, glucose and ions, e.g. sodium and chloride, are reabsorbed whilst in the latter, the largest segment of the nephron, both reabsorptive and secretory roles exist. The association of the kidney with a rich blood supply together with its filtration role means that this organ is one of the major sites of infection in the body.

### 2.3.4 Reproductive system and life cycle

The gonads of cyprinids, testes in males and ovaries in females, are typically paired and suspended by mesenteries from the dorsal aspect of the body cavity close to the kidney. Their appearance changes dras-

tically during the fish's development and annually particularly during the spawning season. Both testes and ovaries are usually small in juvenile fish and adults out of spawning. During the spawning season however, the testes, which are smooth, white structures, increase in size and can account for up to 12% of the body mass. In contrast, the ovaries are yellowish in colour, swell with eggs and have a granular appearance, and may be up to 70% of the fish's weight. Development of the eggs and sperm, termed gametogenesis, occurs prior to the spawning season. Within the testes spermatogonia develop through spermatocyte and spermatid stages to the mature spermatozoa. These with other reproductive secretions form the semen or milt. In the ovaries oogonia transform into mature ova, through the secondary oogonia and oocyte stages. If spawning fails to occur and the eggs are not released then they may be reabsorbed. Gametogenesis and gonadal development are controlled by a series of hormones that may themselves be affected by a number of environmental or physiological stimuli. Initially gonadotropin-releasing hormones in the brain affect the pituitary gland located on the ventral aspect of the brain. Within this gland are gonadotrophs (cells that produce gonadotropins), which affect the gonads. The latter produce oestrogen or testosterone which prevent excessive production of the gonadotropins. Reproduction is therefore a complex interaction between gamete and organ development and hormonal balance and as such any disease that affects any of these, either directly or indirectly, can lead to death or the impediment of reproductive potential, and thus a reduction in fish numbers.

Few people are aware that a number of cyprinids migrate. This lack of knowledge reflects the paucity of literature on the subject compared with the wealth of information published on migratory salmonid behaviour. Upstream migrations by cyprinids are normally associated with spawning, when the fish need certain environmental conditions. As young fry hatch, they move back downstream aided by currents and are thus spread throughout the water course. There are reports in Russian literature that *Rutilus frisii* (a species of roach) and the Aral Sea barbel make migrations as far as 1000 km. Prevention of migration occurs by building weirs and dams and water abstraction, and while passes and ladders are constructed at some obstructions, they are mostly designed for salmonids. The fact that some cyprinids make migrations is important when assessing the spread of diseases and parasites in a water catchment area. In fact, there is little hope of stopping the spread of infection in natural riverine situations unless movements of fish can be prevented.

In cyprinids fertilisation is external and the fertilised egg undergoes a series of developmental stages to transform into the adult. Initially the egg divides into several cells (cleavage phase) which are reorganised into tissues and organs (embryonic phase). Eventually the embryo becomes free of its egg membranes although it still relies on yolk reserves, in the form of a yolk sac, for food. When this reliance is lost the fish has the ability to capture its own food and thus a larval stage is formed. In cyprinids, the larval stage is fairly brief, and the

larvae spend much of their time within the relative security of heavy cover, e.g. amongst aquatic plants. The transformation of the larval stage into the juvenile entails further organ restructuring and the development of formed fins. Once the gonads are mature the fish is an adult. Such a developmental process is obviously fraught with dangers and there is considerable loss of individuals by predation. In addition, several factors, either intrinsic, e.g. genetic makeup or extrinsic, e.g. pollution, may affect development that leads to abnormalities that may cause death or seriously affect biological functions.

### 2.3.5 Endocrine system

The discussion of nutrition and reproduction introduced the concept of the control of physiological processes being mediated by chemical signals, e.g. insulin, gonadotropin, oestrogen. These chemical messages, called hormones, are produced by tissues or glands, which together comprise the endocrine system. The most important endocrine gland is the pituitary, which as highlighted by its role in reproductive physiology, could be considered as the conductor of the endocrine system. Its numerous functions include the control of growth, stress responses, and colour. In addition, scattered around the body of the fish are a number of other specialised hormone-producing organs. The interrenals, equivalent to the adrenal glands in mammals, are located at the anterior end of the kidney, the pronephros, and are associated with the production of the so-called 'fight or flight' hormones, adrenaline and noradrenaline. In addition, within this area of the kidney is found the chromaffin tissue, which is also associated with a stress response, being the site of the production of the stress hormones, the corticosteroids. An increase in these hormones may be linked to several disease states. Other endocrine glands include the thyroid, which is located against the anterior gut and produces growth hormone; the corpuscles of Stannius, embedded in the kidney, that are involved in calcium uptake by the gills; the urophysis, a swelling near the end of the spinal cord, and the pineal gland (the so-called 'third eye') which are associated with light sensitivity and pigment control.

### 2.3.6 Nervous and sensory systems

In addition to hormonal control, fish possess a nervous system like all other vertebrates. The nervous system comprises the brain, spinal column and peripheral nerves. The structural composition of the brain partly reflects the behaviour of the fish species, particularly regarding those areas of the brain responsible for the senses. The area responsible for smell, the so-called olfactory region, is located at the front of the brain, is usually highly developed and is connected directly to the nostrils. Immediately behind the olfactory region are two large rounded structures, the optic lobes. These connect the optic nerves to the eye. This latter organ comprises an outer casing of scleral cartilage, the inner border of which consists of the nutritive choroid layer and the

light-sensitive retina. The retina consists of both densely packed rods and cones, the former being associated with the definition of lightness and darkness especially in dim light, and the latter providing high resolution and colour usually in bright conditions. The lens of the eye protrudes through the opening in the anteriorly positioned iris, causing the eye to bulge from the body surface. The lens is spherical, giving the fish a considerable arc of vision both in front and behind. In a healthy fish the lens is completely clear; however, in certain disease conditions, e.g. infection and nutritional disorders, the lens becomes cloudy and blindness may result. Immediately behind the optic lobes is the cerebellum, comparable to the large cerebral hemispheres in humans. This area in fish is much smaller in proportion to its human counterpart, but it may be responsible for what may be considered as 'thinking'. The spinal cord extends from the back of the brain and passes down the middle of the vertebral column. Nerves extend from the cord and insert in tissues and organs throughout the body.

### 2.3.7 Cardiovascular system and blood

The circulatory system in fish, as in all other vertebrates, is a closed system consisting of a series of tubes, i.e. arteries, veins, capillaries and a pump, the heart. The latter is a highly muscular organ located in the ventral anterior body cavity, immediately behind the opercular cavity and gills. The heart is a much simpler structure than in humans and consists of basically two chambers, the very muscular, triangular, red ventricle and the atrium. The blood enters the former chamber which contracts and forces the blood under great pressure into the bulbus arteriosus. The elastic nature of this component converts the surge in pressure into a more pressure-balanced movement and thus prevents damage to, in particular, the capillaries of the gill. Blood exiting the gills and entering the dorsal aorta is therefore not only oxygenated but under reduced pressure. From the dorsal aorta arteries extend and supply blood to areas of the body, e.g. mesenteric arteries to the gut, carotid artery to the head, and caudal artery to the tail. Each organ also has an appropriately named venous counterpart, which passes deoxygenated blood back to the heart via the vena cava. The passage of blood is however not direct. Blood from the tail, in the caudal vein, passes through the kidneys into the posterior vena cava, which also receives the blood supply from the gut via the hepatic portal vein, liver and hepatic vein. The jugular vein carries blood from the head back to the heart.

Fish blood, like that of all other vertebrates, consists of red blood corpuscles (erythrocytes) and white blood cells (leucocytes) suspended in plasma (Plate 2.2). In cyprinids as in other teleosts, erythrocytes are produced in the red pulp of the spleen. Red blood cells are the most abundant cells in fish blood, up to 4 million per $mm^3$. Fish red blood cells are nucleated, unlike those of birds and mammals. The numbers of red blood cells may alter in response to infection, but may also vary according to season, physiological status and environmental

conditions and they are therefore not an accurate indicator of fish health status. Leucocytes, which are less abundant (approximately 150 000 per $mm^3$) than erythrocytes in fish blood, occur as several different types. Their relatively low numbers do not however reflect their biological significance for they serve key roles in the clotting mechanism of the blood and in the immune responses. The total number of leucocytes and percentages of each type within the blood can vary widely depending upon the physiological and pathological status of the animal. Circulating leucocytes can also vary depending on season and the presence of infection. Unfortunately, the change in total and differential leucocyte counts is very variable and not specific so its use as a diagnostic tool for disease is limited.

### 2.3.8 Immune system

In addition to the natural or innate protection afforded by several physical barriers such as skin and chemical barriers such as stomach acidity, fish, like all other vertebrates, have an immune system. This includes organs, cellular (leucocytes) and serum components that together play a vital role in disease processes. The immune response to infection can vary and the intensity of the response evoked can be influenced by several factors, e.g. the genetic make-up of host and pathogen, the fish's age, season, environmental conditions including the presence of pollution, and stress. However, a knowledge of the interaction between the immune response and the pathogen is of importance in understanding not only the pathological response evoked but also in the development of vaccines. Lack of space precludes a detailed examination of the immune system and the reader's attention is directed to the further reading at the end of this chapter.

Cyprinid fish, like the majority of teleosts, lack the bone marrow and lymph node component of the immune system present in mammals. However, as in their mammalian counterparts, the organs involved in the immune response can be categorised as primary and secondary lymphoid organs. The former are the producers of the cellular components of the immune response and the latter provide the correct physiological environment to enable the immune factors to interact with foreign molecules, i.e. antigens. The major primary lymphoid organ in fish is the thymus, which is a superficial, paired organ located below the epithelium in the branchial cavity near the gills. It is usually the first organ to become lymphoid and although the time at which it does so is dependent on species it usually occurs around hatching, e.g. in carp 2 days post-hatch. The thymus contains several types of leucocyte, e.g. macrophages, myoid, granulocytes and, importantly, lymphocytes. Some of the latter have surface antibodies and thus may be equivalent to mammalian plasma cells. The kidney is also an important lymphoid organ and consists of two distinct areas: the anterior, cephalic or head kidney sometimes called the pronephros and the middle and posterior trunk kidney. Although both have some immune capacity it is the head kidney in which the renal function has been lost and an immunological

function occurs. This area of the kidney serves both as a primary and secondary lymphoid organ. Some authorities have suggested that the kidney resembles the mammalian lymph node and is associated with antibody production, but it also houses differentiating blood cells and thus is similar to the mammalian primary lymphoid organ, the bone marrow. The leucocyte content of the head kidney is similar to that of the thymus although there are quantitative differences. In addition, specialised macrophages containing dark pigment, i.e. melanin, haemosiderin, lipofuscin – the so-called melanomacrophages – also occur. Such cells also occur in the major secondary lymphoid organ, the spleen. This organ is the last to become lymphoid during development and usually develops an immune capacity about the time of the first feed. It is a large, elongated dark-red organ situated adjacent to the gut. It has a complex structure comprising red pulp associated with a high density of erythrocytes and white pulp containing several leucocyte types and having an immune capacity. Usually in fish that have an abundant lymphohaemopoietic tissue in the kidney the splenic lymphoid tissue is poorly developed. The immune capability of the spleen does, however, mean that its leucocytic component and the organ's size can be affected by infection and pathological process. One organ of the fish that is particularly prone to infections is the alimentary tract. Perhaps because of this susceptibility the gut wall contains diffuse accumulations of lymphoid tissue, the so-called GALT (gut associated lymphoid tissue). This tissue contains several leucocyte cell types including lymphocytes, plasma cells, granulocytes, and macrophages. This immune capacity means that antibodies have been found within the gut mucus thus making the oral route for vaccinations feasible.

One feature that all these immunocompetent organs have in common is the presence of several leucocyte types. Such cells play an important role within the inflammatory and pathological responses. It is only relatively recently that there has been some agreement on the terminology of fish leucocytes. The initial problem arose for workers trying to make analogies with mammalian leucocytes based on morphology. Research on functional aspects has now clarified the role of some fish leucocytes.

Monocytes comprise a small percentage of the blood leucocyte population but can increase in number during infection. Within tissues they mature to form macrophages which represent the main phagocytic cell in the pathological response. Combating of infections by these cells involves a series of killing mechanisms, e.g. enzymes, toxic oxygen radicals. Granulocytes, as the name implies, are leucocytes that contain conspicuous granules within their cytoplasm. Although there is still some confusion regarding terminology in some cyprinid species three basic types occur: basophils, eosinophils and neutrophils which relate to their staining characteristics in light microscopy, i.e. basic, acid (e.g. eosin) and neutral stains respectively. Such cells also contain a lobed nucleus. In addition to the former granulocytes another has been found in some cyprinid species. It is usually termed a rodlet cell due to the presence of elongate granules. Controversy exists as to the origin of this particular granulocyte. Whilst some authorities

consider it to be a component of the leucocyte population others suggest it may be of parasitic origin. Thrombocytes are oval spiked cells, which in blood smears can appear as lone nuclei due to the high nuclear/cytoplasmic ratio. Such cells are an important component of the clotting mechanism. The final major component of the leucocyte population are the lymphocytes which can vary in size (4.5–12 µm) and are dominated by the nucleus surrounded by a small amount of basophilic cytoplasm. Based on functional grounds there are two main groups of lymphocyte, the T lymphocyte and the B lymphocyte. In fish, as in mammals, there is evidence that the former consists of different subsets which serve a variety of immune functions including suppression of the response, cytotoxic activity and a helper role assisting the B lymphocyte to produce antibody which binds to the foreign substance, the antigen, which stimulated its formation. Antibody is just one of a series of immunochemicals which either co-ordinate the immune response, e.g. interleukins, or actually attack pathogens, e.g. complement, acute phase proteins.

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*Diseases of Carp and Other Cyprinid Fishes*
D. Hoole, D. Bucke, P. Burgess, I. Wellby


# Chapter 3
# Disease Symptoms

In the case of large scale aquaculture practices, signs of disease are usually only reported when one or more dead and moribund fish are noticed in the water. In the case of fisheries and fish farms that are well managed, routine health checks are made on the fish stocks. The fishery owner or manager is thus quick to take action in situations where the stock displays signs that may indicate ill-health, for example a reduction in food intake and/or unusual behaviour in his fish, such as abnormal swimming, listlessness and congregation at the outlets of ponds. Small numbers of dead fish are usually observed immediately. However, in cases where health surveillance is poor, or in open water situations where the stock may be difficult to monitor from land, it is often too late to alleviate the problem or apply treatments. When that situation occurs, it may be more profitable or even necessary to completely clear the site of fish, disinfect all the ponds, surrounding areas, facilities and equipment and start restocking with approved 'disease-free' stock.

The situation is less complicated when cyprinids are held under aquarium conditions, since opportunities for early disease diagnosis are generally much greater. This is because the high clarity of the water together with the ability to see the fish from all sides rather than just from above facilitates easy monitoring for any clinical or behavioural symptoms of disease.

The health status of fish kept in a range of managed systems, e.g. farms or aquaria, can be monitored by studying the behaviour of the fish. If there are fish showing unusual signs, a few moribund fish should be sacrificed and examined on site for gross abnormalities. However, in situations where each fish is of high monetary value, such as koi, or of sentimental value, such as pet goldfish, then sacrificial sampling may not be desirable.

With a little experience, it is often possible to recognise clinical signs of disease and consult expert advice where appropriate. If an infectious agent is causing the disease, it is important to isolate and identify it as quickly as possible. There are some rapid diagnostic tests available that can be used to identify certain fish pathogens. In the case of aquarium systems, the often low monetary value of the stock means that a veterinary surgeon or other fish health expert is often not consulted. Instead, many aquarists attempt to make their own diagnosis, often incorrectly, sometimes resorting to unorthodox medication as the basis for treatment.

Of course, problems arise when clinical signs are nonspecific and pathogens are either not isolated, or several pathogens are found to be present. This frequently happens with mortalities in wild fish because the problems are invariably multifactorial and can include a number of natural and man-made events. In those instances, other aetiologies such as nutrition or adverse water quality have to be investigated as primary causes. The debilitated fish may be affected by 'secondary' pathogens.

Whatever the situation, every attempt should be made to identify the cause of mortalities in fish, even in wild stocks. Certain diseases are designated notifiable under national or international legislation. In those instances, movement controls of fish and other materials may be applied to prevent the spread of disease to other waters.

## 3.1 BEHAVIOURAL SYMPTOMS OF DISEASE

The onset of abnormal behaviour in one or more fish is often the first visible sign of a health problem. For this reason, some fish culturists routinely monitor one easily observed behavioural indicator of the stock's health: the feeding response.

Table 3.1 lists some of the common behavioural symptoms of ill-health, including respiratory activity (e.g. opercular beat rate) and changes to the general body pigmentation (darkening/pallor). It is important to first eliminate possible normal causes for seemingly aberrant behaviour (see the comments column in the table) before linking the behaviour with a health problem. The aquaculturist must therefore be well acquainted with the normal behavioural repertoire, including spawning behaviour, of the species in question. Care must be taken, however, since what is perfectly normal behaviour for one species may be a sign of disease in another. For example, it is quite normal for some cyprinids, such as the zebra danio, to remain within the mid- to upper-water column for much of their lives. However, such swimming activity in predominantly benthic cyprinids, such as *Rhinogobio* spp., could be indicative of respiratory distress forcing the fish to rise to the more richly oxygenated water surface.

The overall activity and respiratory rates of fish tend to increase with water temperature within the physiological limits for the species in question. This is because fish are ectothermic ('cold blooded') animals whose metabolic rate is largely driven by the temperature of their surroundings. In temperate cyprinids especially, water temperature can greatly affect their appetite, to the extent that some fish may stop feeding altogether during the depth of winter. A rise in water temperature has a dual effect on fish respiration. It increases the fish's metabolic activity, including feeding rate, and hence its oxygen demand, but at the same time the warmer water carries less oxygen. The net effect is that fish may experience respiratory stress when exposed to abnormally high temperatures for the species.

## 3.2 GENERAL EXTERNAL VISUAL SIGNS

The following signs may be observed from visual observations of the fish's body surface. It is important to document these, as they will assist the pathologist or veterinarian when making a diagnosis.

### 3.2.1 Colour changes

Abnormal coloration in cyprinids is occasionally reported, however, this may not necessarily imply a health problem. This can arise, for example, where colour varieties of carp, e.g. koi have been introduced into open waters and allowed to breed with wild carp as has occurred in UK fisheries. Colour changes can be associated with disease and/or stress status, for example darkening or pallor in zebra danios and other species.

### 3.2.2 Skeletal deformities

Skeletal deformities (Plate 3.1) such as 'pug-head', scoliosis, lordosis and gill-cover shortening occasionally occur in fish populations and some workers consider that pollutants are responsible, possibly when the eggs or fry come into contact with chemical contaminants or by maternal transfer to the eggs. Traumatic shock caused by, for example, vibrations and changes in extremes of temperature can have effects on embryonic development, resulting in skeletal deformities. At a later stage, parasitic infections have been reported to be the cause of skeletal deformities in fry and juvenile fish. Gill-cover shortening is sometimes associated with infections of filamentous bacteria (*Flexibacter* spp.). In most instances the majority of deformed fish probably die at an early age as few adult deformed fish are reported. In general, skeletal deformities constitute a 'grey area' of understanding and in those cases a genetic aetiology is often proposed, mostly without any scientific proof.

### 3.2.3 Abdominal swellings

Swollen abdomens can occur as a result of gonadal development particularly prior to and during the spawning season. Such swellings are of course part of the normal appearance of the fish during certain times of the year. However, there are times when swollen abdomens are the result of fluid accumulation in the abdominal cavity, a condition known commonly as 'dropsy'. This condition is usually attributed to bacterial infections, possibly opportunistic aeromonads or pseudomonads, causing secondary infections in fish that are already debilitated by the spring viraemia of carp virus (SVC). Abdominal dropsy, however, has also been recorded in carp and other cyprinids with no association with the SVC virus. Dropsy is often accompanied by raised scales and exophthalmia.

**Table 3.1** Behavioural symptoms of disease and possible causes.

| Symptoms | Examples of possible causes | Comments |
|---|---|---|
| **Body colour** | | |
| Darkening or lightening (pallor) | Stress* | Need to rule out normal changes in pigmentation, such as camouflage response to light/dark surroundings |
| **Appetite** | | |
| Poor feeding response/refusal to eat | Disease or general stress*<br>Chronic (sublethal) hypoxia | Feeding activity may be influenced by water temperature |
| **Overall activity** | | |
| Sluggish/lethargic | Various debilitating diseases<br>Hypothermia or hyperthermia | Overall activity of fish may increase with water temperature |
| Hyperactive | Hyperthermia<br>Acidosis<br>Response to certain parasites and toxins | |
| Comatose | Near-death (various underlying causes)<br>Hypothermia<br>Response to deep anaesthesia | |
| **Respiratory activity** | | Respiratory activity will increase with water temperature |
| Increased | Hyperthermia<br>Low dissolved oxygen level<br>Nitrite poisoning<br>Ammonia poisoning<br>Damage to gills by pathogens or parasites<br>Chemical damage to gills (e.g. due to chlorine) | |
| Decreased | Hypothermia<br>Response to anaesthesia | |

| | | |
|---|---|---|
| **Swimming behaviour** | | Usually coincides with increased respiratory activity. |
| Remain at surface. Gulping near the air–water interface (also known as 'piping') | Hyperthermia<br>Low dissolved oxygen level<br>Nitrite poisoning<br>Ammonia poisoning<br>Damage to gills by pathogens or parasites<br>Chemical damage to gills (e.g. due to chlorine) | Some species are naturally surface dwellers |
| Uncontrolled, uncoordinated | Infections affecting the central nervous system<br>Neurological damage due to ammonia toxicity | |
| Sinks to substrate or floats at surface | Swim-bladder derangement (due to infection, tumour, or injury) | Certain stumpy-bodied goldfish varieties are predisposed to swim-bladder problems |
| Stationary in the water with pronounced sideways body undulation ('shimmying') | Response to certain protozoa diseases (e.g. *Ichthyophthirius*)<br>Hypothermia<br>Sudden change in water parameters ('chemical shock') | |
| **Fish repeatedly rubs itself** | | |
| Fish rubs its flanks against solid objects (e.g. rocks) – known as 'flashing' | Skin parasites<br>Response to chemical irritants | |
| **Shyness** | | Egg guarders (e.g. fathead minnows) may remain hidden with their clutch |
| Hides away | Aggression by other fish<br>Disease or injury<br>Avoidance response to excessively bright lighting (for the species concerned) | |

*Stress: stressors may be acute or chronic, and be caused by factors such as: adverse environmental conditions; aggression by con-specifics or other fish; various infectious and noninfectious diseases; adverse reaction to chemical disease remedies; excess handling; anaesthetics.

Lateral swellings in cyprinids can be associated with parasitic infections such as ligulosis, caused by the presence of plerocercoids of the cestode, *Ligula intestinalis*, which lives within the body cavity of several cyprinid species (Plates 5.32, 5.33). Myxosporeans have also been reported to cause abdominal swellings, e.g. *Sphaerospora* spp. in the renal tissues. Sometimes these latter protozoans affect both kidneys resulting in bilateral swellings. They are commonly recorded in crucian carp, goldfish and their hybrids. Tumours in the abdomen or peritoneal muscle also can show up as abdominal swellings (Fig. 6.6). In goldfish, for example, a condition known as polycystic kidney may cause a gross distension of the abdominal region (Plate 5.13). Swelling of the abdomen is a common feature in photographs of large carp and tench shown in the angling press. These conditions could be due to abnormal amounts of fat within the peritoneal cavity due to the fish feeding on nutritionally imbalanced artificial baits, or to enlarged, mature gonads prior to spawning.

### 3.2.4 Emaciation

Emaciation in cyprinids (Plate 3.2) can be the direct result of malnutrition and can also occur following the onset of infectious diseases. The clinical signs are sunken eyes, concave abdomens and soft musculature. These signs are sometimes associated with spring viraemia in carp (SVC), especially when the fish show erratic swimming movements (ataxia). This type of the SVC disease is known appropriately as the 'nervous form'. In addition, certain bacterial and parasitic infections may cause the fish to become emaciated.

### 3.2.5 Abnormal swimming activity

Irregular swimming behaviour might involve fish 'flashing', i.e. swimming erratically through aquatic vegetation or rubbing themselves against underwater structures, such as rocks. This behaviour may be due to heavy infestations of skin parasites, e.g. *Ichthyobodo necatrix, Trichodina* spp., *Chilodonella* spp., *Ichthyophthirius multifiliis.* The possibility that such behaviour is an irritation or 'escape reaction' to adverse environmental conditions rather than to parasites, must be considered, especially under intensive enclosed systems where water quality can quickly deteriorate.

In other instances, some cyprinids, especially carp and orfe, are seen to leap out of the water. It is suggested that such behaviour is because the fish are trying to dislodge leeches or fish-lice which cause irritations although it can also reflect an escape response to adverse environmental conditions, such as low oxygen or pollutants.

### 3.2.6 The skin

**Histological features and functions**

As in all teleosts, the skin of cyprinids is composed of two major layers.

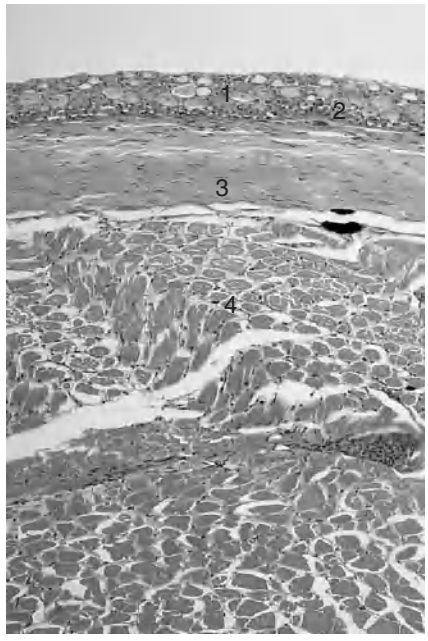


**Fig. 3.1** Section of the skin of carp showing (1) epidermis; (2) pigment cells; (3) dermis; (4) skeletal muscle. Stained with haematoxylin and eosin. Magnification × 150.

The outer layer or epidermis covers the entire body and the underlying layer or dermis contains sensory receptors, scales and mucous glands (Fig. 3.1).

**The epidermis**

This is made up of several layers of epithelial cells, which adjoin the basement membrane. Amongst the epithelial cells are mucous cells, club cells, free migratory lymphocytes, eosinophils and sometimes macrophages. The epithelial cells are of the squamous type, being produced by active cell division from the basement membrane, with the outer cells becoming flattened and slough off. The mucous cells also originate from the basement membrane, but differentiate as they approach the surface region. These cells become enlarged with mucus, which is eventually secreted. The mucus is composed of mucopolysaccharide, showing a positive staining for neutral mucins using the periodic acid–Schiff reaction (PAS) and for acid mucins with the alcian blue staining reaction. Club cells also differentiate from the basement membrane and most likely secrete pheromones and alarm substances in cyprinids. Free migratory cells such as lymphocytes and macrophages present in the epidermis play a part in the immune response and are important in the disease process.

At spawning times, some cyprinids, e.g. common bream, goldfish and fathead minnows, exhibit a covering of small, white nodules on the dorsal anterior (head) region. These nodules are known as spawning

tubercles (Plate 3.3) and are not a pathological condition, as some laypeople believe.

**The dermis**

This layer consists mainly of collagenous connective tissue amongst which pigment cells, chromatophores, occur. These cells provide colour to the fish and consist of melanophores, erythrophores, xanthophores, leucophores and iridophores.

Scales are also present in the dermis and consist of outer osseous (bony) material and inner fibrous material. The scales of most cyprinids are approximately rounded and lie in the loose, outer layers of the dermis. The scales slant upwards in a tile arrangement, slightly overlapping each other. Scales do not protrude, but are covered by the epidermis, which contains pigment cells and mucus secreting cells. As scales grow, they form annual growth rings, which can be used to age fish.

**Visual examination**

Fish should be thoroughly examined all over, especially the fins and adjacent areas and under the gill covers. Abnormalities found could include haemorrhages, scale loss, ulcerations, epidermal growths, tumours and the presence of parasites. More detailed examination requires skin scrapings and observations under a microscope at ×100 and ×400 magnifications. Also, affected fish might show a grey film over the skin. This is mucus exudate, which the fish has secreted as a defence mechanism against parasites. Examination of the external surface of the fish may reveal the presence of raised or lost scales and several types of injury or inflammatory responses. Some of these may result from damage inflicted by external parasites, fighting, predators or spawning behaviour. Whilst such sites can form the foci of infection by secondary pathogens, several abnormalities on the surface of fish, e.g. ulcers, are a feature of infection of primary viral or bacterial pathogens, e.g. atypical *Aeromonas salmonicida.*

A brief description of some common epidermal changes are given below.

Haemorrhages

Haemorrhages on the skin surface (Plate 3.4) can be caused by abrasions incurred when the fish are captured. Such artefacts should be reported in the clinical notes, as the pathologist needs to be able to differentiate them from disease signs. Haemorrhages on the skin surface are usually seen as small red spots or petechiae, which can be the result of bacterial infection or the attachment of ectoparasites. Also, they can be caused by leaching of capillary blood cells under the skin because of septicaemia. Some nutritional deficiency disorders may also result in skin haemorrhaging.

Scale loss

Scale loss (Plate 3.5) and scale erection (lepidorthosis) may be associ-

ated with infection, for example renal sphaerosporosis, a myxosporean disease, and the condition known as dropsy where the scales may protrude outwards, giving a serrated edge (pine-cone effect) to the fish's body contour. Of course, traumatic injuries caused for example by handling and netting (especially fish held in overcrowded 'keep-nets') can also result in scale loss. The mucus secreted from cells in the epidermis acts as a bactericide in fish and when displaced, e.g. because of scale loss, can render the skin prone to entry by bacteria, fungi and other organisms. Scale loss may also be due to attack or aggression by predators and other fish.

### Skin ulcerations

Skin ulcerations (Plate 3.6) are common in diseased cyprinids, e.g. carp erythrodermatitis and roach ulcer disease. In fully scaled fish, the ulcers can be small to large irregular shapes, and usually appear as shallow lesions. In fish which have few scales, including varieties of mirror or common carp, skin ulcerations begin as haemorrhagic petechiae. In carp, the ulcers are often spherical, with red necrotic centres and white peripheries. Histological examination reveals loss of epidermal epithelia, inflammation and necrosis. Occasionally, there are Gram-negative bacteria present and in chronic cases, fungal hyphae. When skin ulcers undergo healing and repair they become pigmented with melanin.

### Blackspot disease

Black spots present in the epidermis are the result of the host's response to certain parasites. The black spots are in fact accumulations of melanocytes. In histological sections, the metacercaria of digenean parasites, e.g. *Posthodiplostomum* (syn. *Neasus cuticola*) and *Apophallus*, can be seen surrounded by the pigment cells (Plate 5.26).

### Epidermal growths and tumours

Epidermal hyperplasia, seen as slightly raised, white, waxy coatings on the skin surface, are proliferations of epithelial cells (Plate 3.7). Some authorities consider epidermal hyperplasia to be a precursor of epidermal papilloma described below. Epidermal hyperplasia is fairly common in carp but less so in other cyprinids, and is probably caused by a herpes-like virus. This condition disfigures the fish but does not usually result in mortality.

Papillomas are essentially similar to epidermal hyperplasias, but they are more 'wart-like', having a connective tissue support and blood system. Possibly, the cause is also viral. Basal cell tumours are growths deriving from the basal cells, and have been recorded in cyprinids. Fibromas and lipomas, which have frequently been observed in the dermal connective tissues of cyprinids, are idiopathic lesions. Other tumours of the epidermis and dermis include epitheliomas and neurilemmoma; the latter involve peripheral nerve (Schwann) cells.

### 3.2.7 The gills

The gills are important organs for examination in disease diagnosis, because of their direct contact with the environment, which means they are sensitive to a number of irritants, parasites and pollutants present in the water.

**Histology**
The respiratory components of the gill are the filaments or primary lamellae and the plate-like projections that protrude from the filaments, the secondary lamellae (Plate 3.8a). At the centre of the filaments are bony or cartilaginous plates which provide support to the structure. Between the supporting structure is a layer of connective tissue that contains eosinophilic cells and blood vessels. The secondary lamellae consist of two surfaces held together by supporting pillar cells between which blood cells circulate. The cells that cover this fragile tissue make up the respiratory epithelium. The respiratory cells in healthy fish comprise only two or three layers of flattened epithelial cells and sit on a basement membrane. Amongst the epithelial cells are goblet cells that produce mucus and chloride cells, which are important in osmoregulation.

**Histopathology**
The most common pathological change observed in cyprinid gills is respiratory cell hyperplasia (Plate 3.8b). This hyperplasia can be the result of chemical stimuli from pollutants, parasitic infection and other forms of environmental stress, e.g. low pH. The number of mucous cells also increases in response to parasitic infection and deteriorating water quality. These cells discharge their mucus material onto the epithelial surface in an attempt to protect the gill tissue. Acute changes in gill tissues include fusion of lamellae and cell pycnosis. In chronic cases, there will be necrosis, cell desquamation, oedema, and marked degrees of infiltration by eosinophilic granular cells and other leucocyte types. These conditions all reduce the efficacy for gaseous diffusion and other gill functions, which can be fatal.

## 3.3 INTERNAL SIGNS OF DISEASE

### 3.3.1 The digestive tract

**Histology**
The gastrointestinal tract of cyprinids obviously varies to some extent because the family comprises herbivorous, pelagic, benthic and other feeders. The tract extends from the mouth to the anus and basically comprises the oral cavity, pharynx, oesophagus, intestine (which forms an intestinal bulb instead of a stomach) and then continues, often in several coils, as intestine to the anus. The intestine as viewed in transverse section shows a lumen enclosed by folds of tissue made

up of a mucosal epithelium supported by a lamina propria, and an adjacent cellular/connective zone. These tissues are bounded by two layers of smooth muscle, a serosal layer of connective tissue and blood vessels (Plate 3.9a).

**Clinical signs**
Pathological signs at the oral cavity include ulcerations and papillomas. The establishment of sport fisheries for carp and other cyprinids has unfortunately led to an increase in hook damage caused by poor handling (Plate 3.10). These hooking lesions, which can become necrotic and infected with viruses, bacteria and fungi, can heal, but may cause permanent disfigurement to the mouth, making feeding difficult, and badly damaged fish may eventually die from starvation. Protozoan and metazoan parasites are often found within the gut and are associated with haemorrhages and excess mucus production.

**Histopathology**
The intestinal tract is an important organ in relation to disease. Many pathogens and parasites are able to gain entry to the gut via the oral route, notably via contaminated food items. With infection there is an invasion of the layers by lymphocytes. Subsequently, inflammation occurs and the condition progresses to degeneration, cell desquamation and mucus secretion into the lumen. Necrosis then spreads throughout the lamina propria and smooth muscle tissues (Plate 3.9b). The sensitive mucosal epithelium can incur localised inflammation and ulceration associated with gut pathogens, other gut parasites and ingested foreign bodies such as wood and stones. In severe cases of gut degeneration the intestinal absorptive functions cease and the fish starves.

### 3.3.2 The liver

**Histology**
The liver (Plate 3.11a) is an important digestive gland originating from intestinal cells at the embryonic stage. The liver in cyprinids is mostly multilobulated and somewhat diffuse, intertwining between the mesenteries of the intestine. It is surrounded by a thin mesentery. The lobules or parenchyma consists of cords of hepatic cells surrounding a triad of vessels, which link to the hepatic artery, portal vein and biliary duct. As the blood vessels diminish in size, they are termed sinusoids and the bile ducts are said to be the centre of the lobules. Other cells in the liver include endothelial cells, fat cells, Kupffer cells and fibroblasts. Blood cells will be present in the vessels and there are a few lymphocytes surrounding the vessels even in normal liver. The hepatic cells store lipid, glycogen and, at certain times of the year, vitellinogen. Normally, there will be some degree of cytoplasmic vacuolation in histological sections because of these storage products. Postspawned fish will demonstrate hepatic depletion or atrophy.

**Histopathology**
Pathological signs in the liver include colour changes, mottling and haemorrhages.

Histological examination in those cases reveal cloudy swelling of hepatocytes, uptake of hyaline material, fatty accumulation, the presence of melanomacrophage centres, sinusoidal congestion and leaching of blood into the surrounding tissue (peliosis). Inflammation, karyorrhexis, pycnosis and eventually necrosis are often associated with toxic effects or infectious disease (Plate 3.11b). Fatty degeneration may be due to metabolic problems, but also could be associated with contamination by pollutants such as polyanhydrous hydrocarbons. Hepatic tumours are rarely recorded in cyprinids, but if they do occur, they are usually benign and regarded as adenomas. Proliferation of bile ducts is termed cholangioma. The cause of this is unknown.

### 3.3.3 The pancreas

The pancreas is a diffuse cream coloured organ, closely associated with the liver and intestinal mesenteries, which has two functions, an exocrine function and an endocrine or hormonal function.

**Histology**
In some cyprinids, e.g. common carp, the exocrine pancreas follows the main hepatic veins throughout the liver parenchyma. When this occurs, the two organs have the combined name of 'hepatopancreas'. Exocrine cells are arranged in acini; they have a basophilic nucleus and basophilic cytoplasm with strikingly eosinophilic zymogen granules.

**Histopathology**
Pathological changes include atrophy and necrosis, which is commonly seen in association with septicaemia. Pycnosis and karyorrhexis of exocrine cells are features of the viral disease, spring viraemia in carp.

### 3.3.4 The kidney

The kidneys are paired organs and in cyprinids each consists of two parts, the body kidney or mesonephros and the head kidney or pronephros. The former is the functional excretory part of the kidney whilst the latter has a lymphoid or haematopoietic role.

**Histology**
The mesonephros is made up of nephrons and interstitial tissue (Plate 3.12a). The nephron is basically divided into the glomerulus, renal tubules (proximal and distal) and collecting ducts, which lead into the ureter and the urinary bladder. The interstitial tissue is made up of reticular cells, capillaries, blast cells (undifferentiated blood cells) and mature blood cells. The reticular cells can frequently be seen

to phagocytose brown pigmented material, which is either melanin or lipofuscin. Large eosinophilic vesicles are seen in kidney tissues of some cyprinids, e.g. common carp and goldfish. These are ectopic thyroid vesicles (Plate 3.12b), and their occurrence and numbers depend on the maturation stage of the fish. The pronephros or haematopoietic part of the kidney is highly active with mitoses frequently observed in the lymphoid and haematopoietic cells. There are also melanomacrophage cells, which are often grouped together forming melanomacrophage centres (MMCs).

**Histopathology**

In cyprinids, proliferation of the interstitial tissue with basophils and eosinophils sometimes accompanied by dilated capillaries, pycnosis and necrosis is probably the most common condition observed. These are nonspecific changes possibly related to infections, especially those caused by bacteria and protozoans. In cases of spring viraemia in carp, marked interstitial cell necrosis is a common feature. Myxosporeans are commonly seen in kidneys. For example, *Sphaerospora* spp. infections can cause grossly dilated tubules and collecting ducts. In some instances, the ducts contain various stages of these organisms, with necrotic and calcified material appearing as multiple cysts (polycystic) or luminal precipitation, the latter being observed in fish that have been exposed to algal toxins. Other common changes observed in kidney tissues may be the result of protozoan or bacterial infections, nutritional deficiencies and environmental effects of poor water quality. These include inflammation and hyaline deposits (protein material) in the glomerulus and nephrons (glomerulonephritis), inflammation of the interstitial tissue (nephritis), deposits of calcium-like material in the tubule lumens (nephrocalcinosis) (Plate 3.12c) and cysts and granulomas, probably the result of protozoan or bacterial infections. Cytoplasmic vacuolation of nephron epithelial cells probably results from excess glycogen or lipid materials deposited due to metabolic disorders. Increased MMCs containing melanin and other 'lipo-pigments' are common in diseased and older fish. These pigments are sometimes referred to as 'wear and tear' material. Neoplasias such as adenomas, lymphomas and nephroblastomas have been recorded in cyprinid kidneys. The causes are unknown.

### 3.3.5 The gonads

The gonads are the functional reproductive organs. The testes are the male paired organs, which become enlarged and creamy white when the fish reaches maturity. They are lobulated; each lobule is composed of spermatogonia at various stages of spermatogenesis, Sertoli cells and connective tissue (Fig. 3.2). Histopathological changes are usually inflammation and necrosis associated with protozoan infections, especially *Pleistophora* spp.

Of more recent concern is a form of hermaphroditism, which is becoming common in fish, especially certain cyprinids. The lobules of

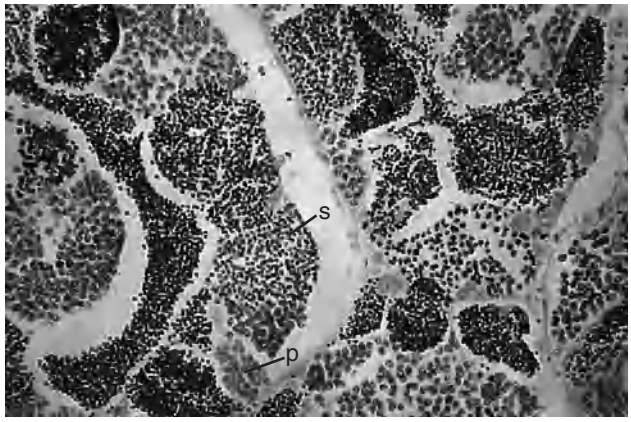


**Fig. 3.2** Roach testes showing various stages of maturation of spermatozoa including primary spermatocyte (p) and spermatids (s). Stained with haematoxylin and eosin. Magnification × 180 (courtesy of Professor Chris Arme).

affected fish contain mainly spermatogonia, but in addition they contain few to moderate numbers of oocytes. These oocytes are usually observed at early stages of oogenesis (see Plate 7.2), although later stages have been reported. The cause is said to be exposure to effluents containing oestrogen mimicking chemicals such as polyanhydrous hydrocarbons.

The ovaries are the female paired organs, which appear pinkish but sometimes take on a greenish hue when mature. The functional part of the ovary is composed of ovarian follicles, which contain oocytes at various stages of oogenesis (Fig. 3.3). Histopathological changes occasionally observed include inflammation and necrosis. These changes may be associated with cysts of microsporean or bacterial infections. Atresia is a stage when ripe oocytes have not been liberated and are undergoing resorption.

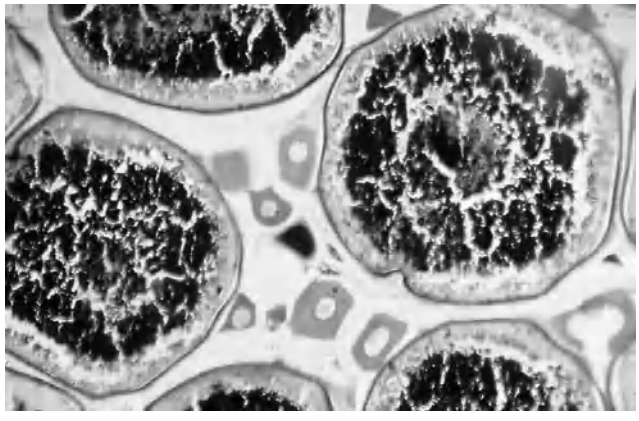

**Fig. 3.3** Ovary of a roach showing large yolky oocytes. Stained with haematoxylin and eosin. Magnification × 60 (courtesy of Professor Chris Arme).

### 3.3.6 The thyroid gland

This endocrine gland is composed of follicles situated on the ventral aspect adjacent to the branchial artery. In goldfish and common carp, thyroid follicles are also found in the kidneys, spleen and sometimes the heart (Plate 3.12b). The follicles consist of colloid, which stains red with eosin, surrounded by a wall of cuboidal epithelial cells. Histopathological changes are recognised as tumours, but are rarely diagnosed as adenomas and more often hyperplasias of the thyroid epithelial cells. Adenoma and hyperplasia of the thyroid tissue are conditions that are difficult to differentiate (see Plate 6.5).

### 3.3.7 Skeletal or bony tissues

The skeleton is made up of osseous and cartilaginous tissue, which are attached to ligaments and tendons. The osseous or bony tissue in cyprinids is composed of osteocytes, which are flat spindle-shaped cells located in the bone lacunae and bone matrix, composed of collagen, osseomucoids, osseoalbumoids and minerals. The bone tissue is surrounded by a periosteum, which is made up of connective fibrillar tissue and other cellular elements. Bone tissue stains positive with the PAS reaction.

Cartilaginous tissue is composed of chondrocytes and cartilage matrix. This in turn is made up of chondromucoids, chondroitin sulphate and chondroalbumoids. Cartilage stains basophilic with haematoxylin and is alcian blue positive. Diseases of the skeletal system include deformities, e.g. scoliosis, lordosis, gill-cover shortening, fin ray aberrations and osteoma (see Plates 3.1, 6.1 and 6.2). Skeletal diseases may be the result of pathogens, nutritional pathologies, genetic or developmental abnormalities, or adverse environmental conditions. Injuries or very rough handling may also cause skeletal damage, notably a broken backbone. The technique of electro-fishing has been known to damage or break the backbone of large fish.

### 3.3.8 Smooth muscle tissues

Smooth or involuntary muscle is found in the walls of blood vessels, the digestive tract, bile and pancreatic ducts. It comprises long spindle-shaped fibres which are nonstriated and supplied with blood vessels and nerve endings. The muscle fibres are strong and flexible and can perform involuntary contracting movements. For example, in the digestive tract there are two layers of smooth muscle. One runs longitudinally along the length of the tract and the other circumscribes the tract. Thus a strong but flexible 'belt' protects the digestive tract when it is full of food. Pathological changes include inflammation and necrosis in which parasites and bacterial infections are most likely to play a part.

### 3.3.9 The striated or skeletal muscle

Striated muscle forms both the skeletal muscle and heart muscle. For example, the flesh or edible part of the fish is made up of myomeres arranged side by side along both sides of the body. The myomeres are separated by collagenous septa. There are two types of striated muscle, white and red muscle. Pathological changes include necrosis (myopathy), inflammation and hyaline degeneration (Fig. 4.3). Carp affected with Sekoke disease, a form of diabetes, and vitamin deficiencies, are reported to show muscle myopathy. Tumours of skeletal muscle, e.g. rhabdomyoma, have been recorded in cyprinids.

### 3.3.10 The swimbladder

The swimbladder forms from an outgrowth of the alimentary canal through which air enters, regulated by water pressure. In carp this organ is in two parts, formed by a constricted connective tissue band. In histological section, at first glance, it could easily be mistaken for part of the alimentary tract. However, it is a much simpler structure, comprised of two main layers. The inner layer is made up of a simple columnar epithelium and the underlying tissue is made up of connective tissue, including collagen and elastic fibres. Blood vessels are present and sometimes the organ takes on a reddish hue. Post mortem examination can reveal that the organ becomes filled with foul smelling fluid containing masses of bacterial cells. In these cases, the fish appear listless, with heads pointing downwards, and over a period of time, affected fish die. In some ornamental varieties of goldfish, particularly stumpy bodied forms, the swim bladder is prone to derangement, causing buoyancy problems in the fish. Common swimbladder problems, occurring mostly in carp, are hyperplasia and inflammation; both conditions are frequently associated with parasitic infections (Fig. 3.4).

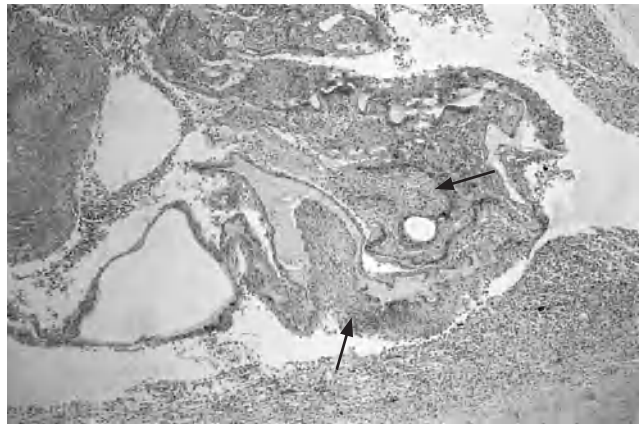

**Fig. 3.4** Inflammation of swimbladder. Note marked degrees of distortion, epithelial hyperplasia of the gas gland (arrowed), associated with myxosporean (*Sphaerospora* sp.) infection. Stained with haematoxylin and eosin. Magnification ×100.

### 3.3.11 The heart

The heart, which occurs ventrally, anterior to the peritoneal cavity, consists of an atrium (auricle), a ventricle and a bulbus arteriosus.

**Histology**
The atrium is thin and contains few muscle fibres, whereas the ventricle is much thicker and is made up of the main cardiac muscle which forms the inner myocardium and the outer epicardium. Each cardiac muscle fibre is a single cell with its own nucleus, which is joined by discs to other fibres. The fibres are bifurcated, and joined together by special structures called intercalated discs to form a mesh-like structure (Plate 3.13a). The heart muscles are connected to the vagus nerve and produce rhythmical contractions. The bulbus arteriosus is a thick-walled part of the heart made up of elastic connective tissue and leads into the ventral aorta. A thin layer of connective tissue, which is rich in small blood vessels, surrounds the whole heart. This is called the pericardium.

**Histopathology**
Pathological changes include infiltration by lymphocytes and other leucocytes, increased connective tissue, necrosis and fatty degeneration (Plate 3.13b). The terminology for this condition is myocarditis, epicarditis or pericarditis, depending on which area of heart tissue is involved. In cultured carp, it is sometimes noticeable that there are large numbers of fat cells surrounding the heart and other visceral organs. It is possible that this is not pathological, but is associated with certain fatty diets.

### 3.3.12 Blood vessels and cells

Blood vessels consist of layers of smooth muscle, connective tissue fibres and an inner lining of endothelial cells. The connective tissue contains collagen and elastic fibres. Arteries are thicker than veins whilst capillaries are much simpler, with an endothelium, basement membrane and pericytes. All fish have nucleated blood cells, which distinguish them from higher vertebrates. Blood cells are suspended in a fluid called plasma, which is composed of water plus various proteins, carbohydrates, lipid and ions. Red blood cells (erythrocytes) carry oxygen and carbon dioxide attached to a pigment, haemoglobin. White blood cells (leucocytes) are defence cells and comprise granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes are differentiated into: neutrophils, which are primary defence cells rapidly moving into acute stage lesions where they ingest bacteria; basophils, again present at sites of acute lesions; and eosinophils, which contain eosinophilic staining cytoplasmic granules. Although eosinophils are commonly present in cyprinids, their function is unclear. Monocytes differentiate into the macrophages, which are commonly seen at sites of chronic stages of lesions, ingesting bacteria and dead cellular matter.

They also ingest (phagocytose) lipopigmented materials such as melanin, lipofuscin and ceroid, e.g. 'wear and tear pigment'. Lymphocytes are immune response cells.

**Histopathology**
In pathological conditions, lymphocytes decrease at acute stages of inflammation and increase at chronic stages of disease. In stressed fish lymphocytes may be reduced in number and activity.

Thrombocytes, which are the platelet cells of fish and take part in the blood clotting mechanism, occur at lesion sites, where they form a network of material called fibrin. This can be seen in section when special stains are applied. In cases of severe nitrite poisoning, the blood may appear brownish, due to nitrite-induced transformation of haemoglobin into methaemoglobin.

### 3.3.13 The spleen

In cyprinids, the spleen, which is present in the visceral mass, is often divided into two or three separated portions. The surface of the organ is covered with a fibrous capsule.

**Histology**
The internal structure basically consists of two areas, white pulp and red pulp. The white pulp contains the germinal centres, which produce leucocytes, and thus have a lymphoid function and are important in the immune system in fish. The red pulp consists of ground substance, in which blood vessels of varying size are present. The ends of the smaller vessels or arterioles are surrounded by reticuloendothelial cells called ellipsoids. Macrophages, often containing pigmented materials, accumulate in these areas, and are called melanomacrophage centres.

**Histopathology**
Histological sections of spleen are not always easy to interpret because they are often congested with effete blood cells. The problem is that unless fish have been exsanguinated, i.e. bled out at sacrificial stage, erythrocytes accumulate in the spleen causing hypertrophy. However, common signs of disease are karyorrhexis, pycnosis and necrosis. Also the reticuloendothelial cells phagocytose necrotic cellular materials and bacteria. In addition, the size of the spleen may be affected by infection. This may reflect an increase in the immune activity of the organ which leads to an increase in its size, i.e. splenomegaly. This can be observed in roach infected with the tapeworm *Ligula intestinalis*.

### 3.3.14 The thymus

The thymus is a paired organ, situated at the back of the pharynx near the gills. It is composed of an epithelial reticular cell framework containing lymphocytes (thymocytes), which function to distinguish for-

eign materials (e.g. pathogens) from host cells. The organ therefore plays an important part in the immune function of fish.

### 3.3.15 The brain

The brain and central nervous system of cyprinids is often overlooked in diagnostic investigations. This is principally because there is little information in the literature regarding its histopathological changes. Nevertheless, a brief introduction to its normal histology might encourage workers to include it in their disease investigations.

**Histology**
In histological section, the brain comprises grey matter, which includes nerve cells and Purkinje's cells, and the white matter, which contains masses of myelinated nerve fibres. There is a good network of blood vessels throughout the tissue. Special silver impregnation or other staining techniques applied to histological sections demonstrates the various nerve cell types, their processes and the fibres. The brain is encased in a connective tissue sheath, which contains fibres and blood vessels.

**Histopathology**
Common pathological changes include: nerve cell atrophy, pycnosis, necrosis, reduction or lack of Nissl granules (chromatolysis), demyelination of the myelin sheath covering the nerves and microglial infiltration (microglia are of mesodermal origin and form foci at lesion sites). Also, inflammation of the meningeal connective tissue sheath is noticeable after trauma and infection.

### 3.3.16 The spinal cord

The spinal cord extends from the medulla oblongata. Transverse section reveals a central grey matter with many nerve cells, surrounded by white matter, which contains the myelinated nerves. Also seen is a central canal, the spinal canal, which is filled with spinal fluid. Fibrous connective tissues and blood vessels surround the spinal cord. Pathological changes involve necrosis and inflammation, which in some cases are caused by protozoans in the meningeal connective tissue.

### 3.3.17 Peripheral nerves

Peripheral nerves extend out from the brain and spinal cord and are made up of nerve ganglia and nerve fibres. They lead to various organs and tissues of the body. Pathological changes include necrosis and pycnosis of nerve cells. Goldfish and possibly other cyprinids are known to develop tumours affecting the myelin sheath of the nerves, i.e. neurilemmomas and neurofibromas.

### 3.3.18 The eye

The structure of the eye is shown in Fig. 3.5.

**Histology**
The anterior part of the eye is the cornea, which consists of a layer of corneal epithelium over a corneal stroma, comprising connective tissue fibres and an inner layer of corneal endothelium. The iris is a structure that projects over the anterior surface of the lens with its free edge outlining the pupil. The lens, which is bound by a capsule, is a crystalline structure of fibres, which are modified epithelial cells. Accommodation of the lens is achieved by flexations of the lens muscle. The posterior wall of the eye is composed of three layers: the sclera, a thick connective tissue structure with some cartilage; the choroid, composed of connective tissue and many blood vessels; the innermost layer is the retina. The retina is composed of ten layers, which include pigment cells, rods and cones and ganglia. These latter cells are linked to nerve cells and ultimately, the optic nerve and the brain. Although a few cyprinids are naturally blind, e.g. the cave form of *Garra barreimiae* and the cave barb, deterioration of vision in most cyprinids can lead to problems.

**Histopathology**
Pathological conditions include exophthalmia, corneal and lens cataracts and retinal changes. Exophthalmia or 'pop-eye' (Plate 3.14) can be the result of oedema forming around the outer layer, the sclera, usually caused by trauma from injury or bacterial or parasitic infections. Exophthalmia often occurs in cases of dropsy. Corneal opacity and damage can be caused by pollution, i.e. chlorine, and injuries, and is sometimes associated with secondary bacterial or fungal infections. Epidermal hyperplasia has been seen to spread over the corneal epi-

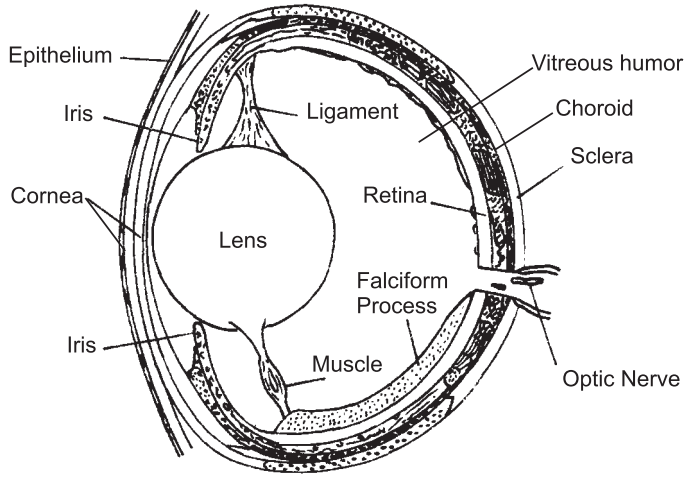


**Fig. 3.5** Diagram of a section through the eye of a fish (based on Lagler *et al*. 1963).

thelium. Lenticular cataracts can occur at times in fish with infections of *Diplostomum* spp. (see Plates 5.23–5.25). However, in cyprinids, the flukes appear to reside within the crystalline lens in the early stages, but over time, especially in roach, the lens disappears and the cornea takes on a pycnotic appearance. Fish that are exposed to highly gas-supersaturated waters, notably nitrogen gas supersaturation, may develop gas bubbles in the eyes, which can lead to blindness.

Nutritional deficiencies have been reported to cause lenticular cataracts. The effects of certain vitamin deficiencies on the eye has been shown experimentally in carp, e.g. Vitamin A and E deficiencies have been linked with exophthalmia. In Japan, Sekoke disease has been reported in carp. Effects on the eyes included dilated blood capillaries and retinal degeneration.

### 3.3.19 The olfactory organ

The olfactory pouch or nares are paired structures on the dorsal region of the head. In section, there are lamella-like structures, which are formed in a rosette. The surface of the lamella is an epithelium of olfactory cells, supporting cells, mucous cells and basal cells. The olfactory cells are really neurones that are linked to the olfactory bulb. Pathological changes in the olfactory lamella are reported to be due to exposure to pollutants and infestation by certain parasites, e.g. *Paraergasilus longidigitus* (see Fig. 5.34).

### 3.3.20 The lateral line system

The lateral line is on the surface of the body, usually seen along each flank of the fish.

The receptors are chemical and tactile organs composed of neuromasts, which are linked to the peripheral nerves. Pathological changes in the lateral line system in cyprinids are not common, however, occasional parasitic infection with *Echinochasmus perfoliatus* have been reported (see Fig. 5.36).

### 3.3.21 Taste buds

The taste buds are mostly situated in the epithelium of the lips, the oral mucosa and the gill arches. They are composed of sensory cells, with sensory hairs. Pathological changes in taste buds have involved inflammation and necrosis. No cause has been identified, but for bottom feeding fish, chemical contaminants in the sediments should be considered.

### 3.3.22 The barbels

These finger-like appendages are composed of a dermis and an epidermis. In the epidermis, there are numerous taste buds and nerve endings, which are linked to nerve bundles of the peripheral nerves and

ultimately the brain. Pathological changes in barbels were reported in the barbel from certain UK rivers in the early 1990s. The signs included 'splitting', deformities, and epithelial hyperplasia. The cause has never been identified, but pollutants have been suggested as one possibility. The rivers were, however, subjected to heavy angling pressure and trauma could have been the reason.

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 4
# Infectious Diseases – Viruses, Bacteria and Fungi

## 4.1 VIRAL PATHOGENS AND DISEASES

Viruses are minute entities, e.g. between about 10 and 500 nm, which carry their genetic information in one type of nucleic acid, either RNA or DNA. The genetic material is usually surrounded by a protein coat that is characteristic for different viruses (Fig. 4.1). Viruses are unable to multiply outside a host cell, as they require the host cellular components to synthesise their genetic material; they are therefore considered obligate intracellular parasites.

Virology is the study of viruses and there are many disease conditions where a virus aetiology is suspected, but actual isolation of the viral agent, even in some human diseases, has proven difficult. The problem in identification relates largely to their very small size, which is below the resolution of the light microscope. This means that there are diseases being identified that are suspected of having a viral aetiology without a definitive causal agent. To put this into perspective, it was not until the 1960s that any of the fish viruses were artificially propagated from diseased fish by the pioneer fish virologist, Ken Wolf.

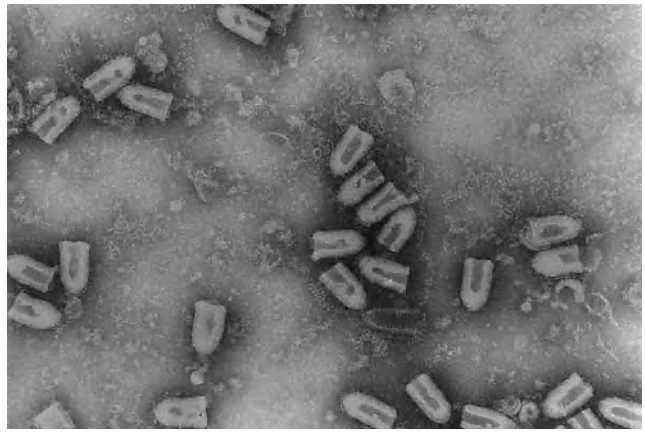

**Fig. 4.1** Electron microscope picture of virus particles of *Rhabdovirus carpio*. Magnification × 47 000 (courtesy of Peter Dixon).

Although many viruses have been isolated from the carp family so far few have been shown to cause significant disease. New viruses continue to be regularly recorded from cyprinids, and other fish species, though most are considered to be relatively benign.

Viral diseases currently regarded as of economic importance in the cultivation of cyprinids are spring viraemia of carp (SVC) and grass carp haemorrhagic disease (GCHD). Another disease, possibly of less significance but, nevertheless, well known to fishery workers is fish pox (or carp pox). Recently, concern has been shown about diseases caused by herpes-like viruses and non-SVC rhabdoviruses.

### 4.1.1 Spring viraemia of carp (SVC)

Spring viraemia of carp has been a disease problem known for some time. The long history of research into the aetiology of spring viraemia hints to the difficulty in studying and elucidating viral diseases. To the early scientists and veterinarians SVC was known as 'Infectious Dropsy of Carp' (IDC). From the 1920s until the 1960s another fish pathologist of note, Wilheim Schäperclaus, made many investigative studies into this disease. In his early research, he was convinced that IDC, or 'Infecktiöse Bauchwassersucht des Karpfens' as he described the disease, was an uncomplicated bacteremia, caused by a nonmotile bacterium, *Aeromonas punctata*. In later years, he decided that the aetiology of the disease might be more complicated. His research into the transmission of the disease revealed that, when he injected bacteria-free isolates from carp, infected with the typical dropsy condition, into naïve carp, the dropsical condition appeared in those fish. However, he was not convinced that a virus was the primary cause of the disease. He maintained that *A. punctata* or some other species of bacteria needed to be present, and it was this in combination with a virus that caused the disease state in the fish.

In 1971, Nikola Fijan and colleagues isolated a virus from infected carp and proved the primarily viral character of the aetiology of the disease. Fijan named the virus *Rhabdovirus carpio* and this has been accepted as the significant aetiological agent of SVC. In further research, Fijan considered that the viral form of the disease was the acute form and the chronic form of the disease, where carp exhibit skin ulcers, he named 'carp erythrodermatitis' (CE). Most scientists now agree that SVC and CE are distinctly separate diseases and CE will therefore be described in the section on bacterial diseases. However, this distinction is not totally convincing, as SVC virus can on some occasions be isolated from carp with clinical signs of CE. There is no reason why there should not be a disease in fish with dual bacterial and viral aetiologies. It certainly happens with some human diseases. Until fairly recently, it was considered that another disease of carp, 'swim bladder inflammation disease' was a separate disease caused by a similar virus to SVCv. It is now known that 'swim bladder inflammation disease' can

have either viral, bacterial or protozoan aetiologies, but the viral form is associated with *Rhabdovirus carpio* (SVCv).

**Occurrence**

The disease appears to be confined to Europe and has been reported from the following countries: Austria, Belarus, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Georgia, Hungary, Italy, Lithuania, Moldova, Montenegro, the Netherlands, Poland, the Russian Federation, Romania, Serbia, Slovakia, Spain, Ukraine and the United Kingdom. In fact the disease has occurred in all European countries where the cultivation of carp is practised. Carp and other cyprinids elsewhere in the world were, until recently, considered to be free from SVC. However, in the late 1990s SVC was confirmed in a consignment of goldfish and koi imported into the UK from China.

Spring viraemia of carp is an acute, systemic viral disease of primarily the common carp, especially fish in their first and second years, and almost certainly is exacerbated in intensive culture conditions where losses are usually high. The disease also affects other cyprinids with varying, but lesser, degrees of virulence, including crucian carp, goldfish, bream, tench, barbel, roach, grass carp, silver carp as well as some noncyprinid species such as the European Wels catfish.

In many cases the disease predominantly occurs in cultivation systems. However, it also occurs in wild fisheries particularly those that have been artificially manipulated by introducing stocks of carp. In these situations, which usually comprise high stocking densities, considerable mortalities and consequent economic loss have arisen from SVC infections. In Europe this has led to SVC being included as the only cyprinid disease in the legislative controls, i.e. in the UK it is a notifiable disease and is listed as a Category 3 disease by the European Union Council Directive (91/67/EEC) concerning animal health conditions governing the marketing of aquaculture animals and products.

**Clinical signs**

The clinical signs of SVC include: lethargy, enteritis, peritonitis, oedema, exophthalmia, thickening of the swim bladder and petechial haemorrhages in the internal organs, skin and muscle (Plate 4.1). Many scientists regard distension of the abdomen as the main feature of the disease. However, other clinical signs can include emaciation and erratic swimming movements, the latter denoting ataxia.

Mortalities are usually fairly low at first, but level out to steady daily losses, depending on the environmental conditions, e.g. low water temperature and level of secondary infections. Over-wintering fish that are in poor condition in early spring are particularly susceptible to SVC. Spring viraemia of carp occurs in the spring in rising water temperatures up to 15°C. It is possible to isolate the virus from fish showing clinical signs even when the water temperature rises above 15°C, but in those cases the actual disease may be due to other pathogens and the virus isolated as an incidental event. All age groups of carp are susceptible, but it is usually fish experiencing their first or possibly

their second spring that are likely to be affected. There is a theory that carp succumb to the disease following over-wintering because these fish originated from tropical or subtropical environments and have not yet acclimatised to cold temperatures. Some authorities have suggested that transferring fish from ponds at the beginning of spring can induce outbreaks of the disease. There is evidence that carp cannot produce specific antibodies at temperatures lower than 10°C. The fish's reduced immune capability at low temperature may therefore enable the virus to manifest and cause disease. These temperature factors could explain why this virus disease has not yet been recorded in Israel, an area that would be expected to have been infected in the past, because of the considerable carp aquaculture in this country.

**Diagnosis**

The only reliable method of diagnosis at present is by cell culture techniques. Briefly, this involves inoculating established fish-cell lines with samples from kidneys, spleens and brain taken from fish exhibiting clinical signs of the disease. This tissue culture method can take between 2 and 20 days. There are immunodiagnostic methods, e.g. immunofluorescence and enzyme linked immunosorbent assay (ELISA) developed for SVC diagnosis, however, these rapid diagnostic techniques should still be performed in conjunction with cell culture techniques for verification. Histological examination of internal organs is rather nonspecific, but necrosis of liver, spleen, kidney and intestine are common features of the disease and along with clinical signs, might warrant a presumptive diagnosis to be made on suspicion of the disease (Plate 4.2).

A number of scientists from the former USSR linked the presence of eosinophilic inclusions in the Purkinje cells of carp as a diagnostic feature of the disease. The Purkinje cells are types of neurone cells situated in the cortex of the brain, and the inclusions would presumably be the sites of virus infection. Their presence could well be significant, as in some other viral diseases in mammals, e.g. Aujeskey disease in pigs, where nerve cell inclusions coincide with locomotor dysfunction in those animals.

**Prevention and treatment**

Prevention is only effective where there are no introductions of infected fish into new areas. The mode of transmission of the disease appears to be horizontal, with the possibility of transmission by leeches and other ectoparasites as well as piscivorous birds. Obviously, the spread of this disease has been greatly increased by the movements of fish as part of the worldwide trade in carp.

Viral diseases are impossible to control by chemotherapeutic methods, therefore a prophylactic approach, by the vaccination of young fish, would be highly desirable. Unfortunately, vaccination methods for SVC are still very much at the experimental stages despite numerous trials. Only live SVC vaccines have so far proved to be successful and such methods are not allowed in EU countries.

### 4.1.2 Grass carp haemorrhagic disease (GCHD)

Recently, a group of viruses, known as Reoviruses and the subfamily, the Aquareoviruses, have been described in fish and amphibia. Aquareoviruses have been isolated from a small number of cyprinid species, but there is very little information available on their pathogenicity. The viruses have been isolated from golden shiners, tench, chub, grass carp, black carp and common carp.

Studies in China have shown that a haemorrhagic condition in cyprinids is associated with a reovirus when it coexists with a second virus. The coexisting virus is tentatively classified as a picornavirus, and the disease is grass carp haemorrhagic disease (GCHD). Although disease occurs only when the two viruses are present, researchers have not yet demonstrated conclusively which virus is the predominant pathogen.

**Occurrence and host range**
Reoviruses have been reported from the USA (California) in cyprinids (golden shiners), but clinical disease has not been observed. However, GCHD is a major disease of economic importance in China. The main species affected are grass carp.

**Clinical signs and economic importance**
There are no obvious signs of GCHD. One- and two-year-old fish are most susceptible, and mortalities in China can reach 80%, which is significant for a country producing over a million tonnes annually.

### 4.1.3 Fish pox

Fish pox is a fairly common hyperplastic/papillomatous condition, occurring on the skin of many cyprinids and other fish species. It has been recorded in ornamental cyprinids as well as in natural stocks of common carp in the UK. It has also been reported in roach, bream, barbel, tench and chub. Mortalities are very rare although extreme infections like the one in Plate 3.7 have been recorded. However, the disease can still be economically significant in that the lesions on the skin of fish are very obvious and can deter customers, whether the fish are destined for consumption or for ornamental purposes. This disease can therefore be a considerable problem for the ornamental trade. Some workers have described carp pox as having a debilitating effect, subjecting the fish to secondary infections and death.

Historically, the disease was described as 'Karpfenpocke' (carp pox), in the writings of Conrad Gessner (*Historiæ Animalium Liber IIII, qui est Piscium et Aquatilium Animantium Natura*. Zurich: Tiguri. 1558) and is one of the oldest fish diseases to be documented. At the beginning of the 20th century, various causes for its occurrence were suggested. Some writers considered protozoan parasites were involved, because numbers of ciliates have often been observed on the skin surface of affected fish. More recently, the disease was suspected to be

caused by a virus, because virus-like bodies were identified with the aid of the electron microscope. Many authorities now accept this viral aetiology, and have even gone as far as classifying the virus as a herpes-type. However, others still question this and consider that an environmental factor may be involved.

**Clinical signs**
The clinical signs of fish pox are identified as smooth, opaque, sometimes white, raised areas on the skin surface. The lesions resemble drops of wax from a white candle dripped onto the skin of the fish (Plate 4.3). The lesions first manifest as individual, 1–2 mm diameter patches that, in extreme cases, may almost completely cover the body surface, including the fins. They are the result of hyperplasia of the epithelial cells of the epidermis. Sometimes the lesions progress from a simple hyperplasia to a papillomatous stage. Fish pox appears to be a seasonal condition, with the lesions reducing in number and severity in the summer months. At this time the normal epidermis usually regenerates under the lesion.

**Occurrence**
The disease occurs during the colder winter period, and is widespread in most countries where cyprinids are found, and where temperatures fall below 14°C in the winter.

**Diagnosis**
Diagnosis is easily made from external clinical signs. Histological examination of affected skin will demonstrate classical signs of epidermal epithelial cell hyperplasia and loss of mucous cells in the lesion area (Plate 4.4). Electron-microscopical examination of affected tissue has revealed virus-like particles. Such studies support the concept that this disease is attributable to a 'herpes virus'. To date the virus has not been isolated on cell culture.

**Prevention and treatment**
The only effective control is achieved by preventing the introduction of affected fish. There are no known treatments, however, raising the temperature of the water to above 15°C will usually cause reduction of the lesions, to be replaced by unmarked epidermis.

### 4.1.4 Emerging viral diseases

**Herpes-virus**
In 1998 there were mass mortalities in koi in Israel. Since then, deaths occurring in koi have been reported in Europe and the USA. Scientists in both continents have independently isolated a herpes-like virus from diseased koi associated with these recent outbreaks of disease. At the time of writing, the pathogenesis of the epizootic has not been elucidated. Israel exports large numbers of koi and other ornamental

cyprinids around the world. However, several other countries also export koi into Europe and the USA.

Clinical signs

The disease in all instances appears to be restricted to carp. In Israel where the fish were raised in earthen ponds, the disease was extremely contagious and reached 90% prevalence. The fish appeared lethargic, swimming with uncoordinated erratic movements. Gross disease signs were inconsistent, although focal necrosis of gill tissue, superficial haemorrhages and increased mucus secretion were the main changes. Internal signs include petechial haemorrhages on the liver.

Occurrence

Similar outbreaks of disease occurred in koi in the USA, Germany and the UK. The disease occurred in water temperatures between 17 and 23°C. Other fish species, including goldfish, held in the same waters as the carp were not affected. Koi imported from other countries have shown no signs of infection.

Diagnosis

Early investigations indicated that the aetiological agent was viral. Recent investigations independently have identified a herpes virus in gill, kidney and brain tissues examined using transmission electron microscopy. The virus, which has the characteristics of a herpes virus, was grown on cell tissue cultures. Transmission experiments have been successful with the virus isolated from the clinically infected fish.

Treatment and control

There are no current treatments for this disease. Eradication by clearing infected sites of fish followed by disinfection appears to be successful. There have been suggestions that this disease could have more far-reaching problems and might affect wild carp and other cyprinids. At the time of writing, there is no legislation to restrict imports of carp from infected areas. However, it would be prudent to act with caution and the best advice would be not to import carp from infected countries until more is known about the disease.

**Non-SVC rhabdovirus**

About 10 rhabdoviruses that are genetically distinct, but serologically similar to SVCv, have in the past decade been isolated from cyprinids and other fish. Additionally, pike fry rhabdovirus (PFRv), which has some chemical similarities to SVCv, has also been obtained on occasions from cyprinids. Some of these viruses have been isolated from fish that showed clinical signs similar to SVC disease (Plate 4.5).

Clinical signs

Haemorrhaging at the base of fins and along the flanks, ascites and petechial haemorrhages in the musculature, visceral organs and adipose

tissue have been described in carp, bream, tench, crucian carp and roach.

Occurrence
Isolations of 'SVCv-like' rhabdoviruses have been obtained from cyprinids in Germany, Northern Ireland and England over the past ten years.

Diagnosis
Understanding of the various rhabdoviruses is still evolving and regarded as a new area of research for virologists. However, in simple terms, to differentiate the different rhabdovirus isolates, advanced diagnostic techniques, including nucleotide sequence analysis of the viral RNA using polymerase chain reaction (PCR) techniques, have had to be used. Results have shown that some of the 'cyprinid' rhabdoviruses are genetically distinct from SVCv and PFRv, but they were serologically related to both viruses and cause a disease similar to SVC disease. These rhabdoviruses are currently being reclassified. It is very probable that more 'SVC-like' rhabdoviruses will come to light as improved diagnostic methods come into general use.

Prevention and control
Apart from not introducing infected stock into new areas all methods of control are similar to those for SVC disease.

## 4.2 BACTERIAL PATHOGENS AND DISEASES

Bacteria are unicellular organisms that lack any membrane bound organelles, such as a nucleus. Their DNA is in the form of a single circular molecule and under the right conditions they have a massive reproductive potential. Bacterial diseases have a significant impact on cyprinids, and can equally affect both wild and cultured cyprinids in situations where stress and crowding will favour transmission. Some cyprinid fish establish favourable transmission conditions for pathogens by forming into vast shoals, especially at spawning times.

Some diseases are the result of obligate bacterial pathogens. However, many potentially pathogenic bacteria of cyprinids normally exist in a commensal association with the host or free in the environment. Both these types of bacteria become pathogenic when the fish is immunocompromised by some form of stressor. Bacteria can also cause problems in combination with a primary pathogen, or become virulent after a primary pathogen has debilitated the fish, i.e. in these situations they are secondary pathogens. In many such instances, the underlying cause may be masked by the secondary bacterial infection, or is difficult to identify. Unless the initial cause can be established then a considerable amount of time and money can be wasted using ineffective control measures.

Bacteria that are significant pathogens of cyprinids are predominantly the 'Gram-negative' types.

### 4.2.1 *Flexibacter*

*Flexibacter columnaris* and *F. psychrophila* are the most frequently isolated species of this genus in cyprinids and are commonly referred to as gliding bacteria.

Infection caused by *Flexibacter* is frequently termed 'Columnaris disease', and was first reported in wild cyprinids in the UK in the mid-1960s. At that time, there had been an epizootic amongst populations of roach and bream occurring in waters mainly in the English Midlands. The infected fish exhibited acute epidermal ulcerations and mortalities were at first high, but gradually the disease disappeared and there has been no recurrence. At the same time as the epizootic in cyprinids, there was another epizootic occurring in salmon in rivers of the British Isles as well as elsewhere in northern Europe. At first, some authorities considered the salmon problem, ulcerative dermal necrosis (UDN), was also caused by *Flexibacter* sp., and it was named 'columnaris disease'. That was later disproved.

However, the salmon disease attracted considerable attention, certainly much more than the epizootic in wild cyprinids. Columnaris disease was first described in cultivated carp in northern Europe in the 1970s. Investigations into these instances revealed that the disease particularly affected the gills of carp and goldfish held in earthen ponds. Research into the pathogenesis of the disease showed that the causative organism, *F. columnaris*, was a secondary pathogen particularly affecting stressed fish, which might be the host to several pathogens.

It is important to remember this point, because misdiagnosis of columnaris disease in carp is probably very common. The problem is that another common disease of carp, carp erythrodermatitis (CE), has similar clinical changes. CE is primarily associated with aeromonad organisms, but it is not unusual to isolate *Flexibacter* sp. from fish with that disease.

Aquarists often refer to *F. columnaris* disease as 'fin rot' or 'cotton wool disease', the latter name reflecting its physical resemblance to fine strands of cotton wool, which may lead inexperienced aquarists to misdiagnose the disease as a fungal infection. Tropical cyprinids may be at greater risk of mortality from this disease because the bacterium is generally more pathogenic at higher water temperatures.

**Occurrence**

Columnaris disease is widespread throughout the world, primarily affecting the gill tissues of fish, where the filamentous bacteria live amongst the detritus and mucus between the primary and secondary lamellae. The organisms, which primarily occur in cyprinids at above 15°C, appear to gain entrance into the fish by being surrounded by fusions of epithelial cells, 'clubbing' and then entering other cells either via phagocytosis by the host cells or by their own efforts. The

organisms are also frequently present at sites of epidermal ulcerations or injuries. The disease occurs more often when water temperatures are elevated and environmental conditions are poor.

**Clinical signs and diagnosis**

Lesions may be observed on the head, body flanks, fins or gills (Plate 4.6). They may appear as small grey-white spots to deep, open, haemorrhagic ulcers. These types of lesions are however common with a wide variety of different diseases. The gill tissues are frequently affected and in those cases the condition is termed 'bacterial gill disease' (BGD) (Fig. 4.2). Where fin damage occurs, it is often the caudal fin that is affected, and this may become ragged as the infection destroys the membranes between the fin rays. Presumptive diagnosis of columnaris disease can be made by examining tissue scrapings that are mixed with a little water and observed with a light microscope. Masses of long, thin, gliding bacterial rods may be present. For confirmatory diagnosis, it is necessary to isolate and identify the causative organism, *F. columnaris*.

**Prevention and treatment**

Prevention by avoidance of overcrowding, and not handling or netting during times of elevated water temperatures, is of utmost importance. The exact causative agent needs to be identified and a suitable antibiotic identified. Some water-borne chemicals such as benzalkonium chloride (BKC) are effective. In the case of highly prized fish, e.g. some ornamentals, antibiotics may be effective, and the early stages of the infection can sometimes be treated with an antibacterial bath, e.g. copper sulphate. Some scientists believe that, in comparison with some other fish species such as salmonids, carp affected with BGD are able to survive the loss of a larger part of the gill and, in some instances, the gill is able to regenerate sufficiently for recovery.

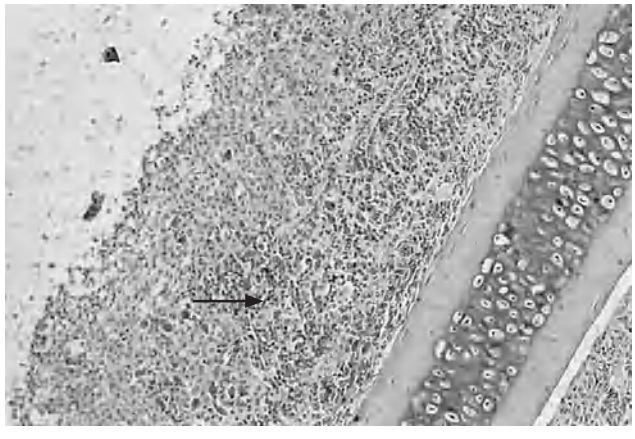

**Fig. 4.2** Carp gill infected with *Flexibacter*. Note the degeneration of individual lamellae, epithelial hyperplasia and signs of inflammation (arrowed). Stained with haematoxylin and eosin. Magnification × 150.

### 4.2.2 Aeromonads and pseudomonads

Pioneers in fish pathology considered motile forms of aeromonads and pseudomonads to be the main cause of the disease, 'infectious dropsy in carp' (IDC), in Europe. The principal organisms involved were *Aeromonas hydrophila* and *Pseudomonas fluorescens*. However, as described previously, IDC or spring viraemia in carp (SVC) as the disease is now called, has now been demonstrated to have a primary viral aetiology. Frequently however, secondary pathogens such as *A. hydrophila*, *A. punctata* and *P. fluorescens* are isolated from diseased carp and in many instances their activities may have led to clinical disease and mortalities.

Many authors have identified fish species that are susceptible to all types and strains of motile aeromonads, but it is quite likely that most septicaemic forms of diseases in cyprinids are the result of aeromonad infection. In the case of the nonmotile aeromonads, *Aeromonas salmonicida* subsp. *achromogenes* (nova), subsp. *masoucida*, subsp. *smithia*, these are frequently associated with acute epidermal ulcerations in many species of fish (Plate 4.7). These subspecies of *A. salmonicida* are known as atypical strains. For example in cyprinids, the familiar ulcerative disease in carp and goldfish may be caused by *A. salmonicida* subsp. *achromogenes*. In Europe, infection by this group of bacteria in cyprinids is widely known as 'summer ulcer disease'. However, if only carp are found to be infected and exhibit typical skin ulcerations, the disease is sometimes referred to as 'carp erythrodermatitis' (CE) (Plate 4.8). Whereas, the typical form of *A. salmonicida*, *A. salmonicida* subsp. *salmonicida*, which primarily affects salmonids, causes the disease termed 'furunculosis'. This terminology should not be used when dealing with infections caused by atypical forms of the bacteria. However, atypical forms of *A. salmonicida* are also pathogenic to salmonids. To make the picture more complicated, there are occasions when any form of *A. salmonicida* can be pathogenic to a wide variety of fish species, e.g. dace, tench, common carp (including ornamental varieties), goldfish, silver bream, common bream, roach, rudd, fathead minnow, common minnow and chub.

**Clinical signs and diagnosis**

These organisms can be responsible for septicaemic disease in cyprinids, and the changes include haemorrhaging and surface ulcers of the skin, protruding scales, scale loss, exophthalmia, abdominal swelling and petechial haemorrhages on the gills. Internal examination may reveal varying amounts of pinkish-red fluid liberated from the abdomen when the fish is incised. Frequently there are haemorrhages and reddening of the gastro-intestinal tract, enlargement of the spleen and mottling of the liver. Carp erythrodermatitis is categorised separately from bacterial septicaemia in cyprinids, although there is no doubt that on some occasions the two diseases overlap. The term carp erythrodermatitis appears to be reserved for carp and is identified by its clinical feature of clear, often deep surface ulcers. It is easier to

recognise in fish with few or no scales (mirror carp). However, it occurs in all carp variants including koi and also in other cyprinids including ornamental varieties. In scaled fish, the rims of the ulcers are more often jagged instead of rounded. In some instances, the ulcers can become overgrown with the fungus, *Saprolegnia* spp. (Plate 4.10). In histological sections, the lesion site reveals a loss of the epidermal epithelium, with inflammation and necrosis extending through the dermis and in some cases into the musculature (Fig. 4.3). When the muscle tissue is affected, it becomes haemorrhagic and liquefied and colonies of bacteria can usually be seen in histological sections.

**Occurrence**

Bacterial septicaemias and CE in cyprinids are both frequently associated with poor environmental conditions such as overcrowding, pollution, low oxygen levels and raised water temperatures. In fact, any combination of the above can lead to septicaemic disease in temperate cyprinids. Handling stress, warm weather and raised water temperatures (above 12°C) are common predisposing factors in epizootics associated with many bacterial diseases in cyprinids. Mortalities are generally low, perhaps up to 25%, but this figure depends on the population density and water conditions. Injuries, such as those caused by predators or angling, cause wounds that are ideal for aeromonad infections.

In terms of geographical distribution, diseases caused by motile aeromonads and pseudomonads are found in both farmed and wild cyprinids, worldwide. Diseases in farmed carp caused by atypical aeromonads have been reported more conservatively for example in Australia, Denmark, Germany, Hungary, the Netherlands, the UK and

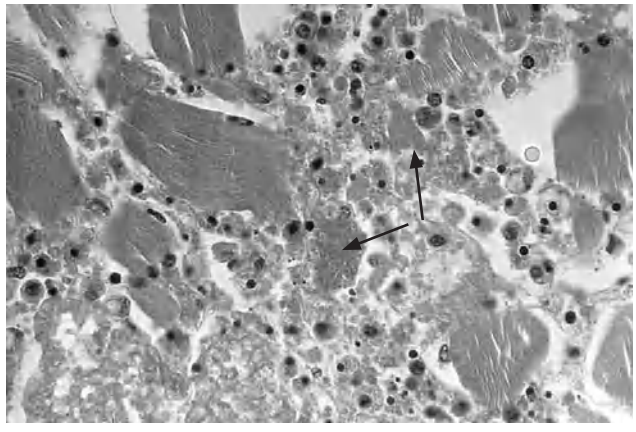

**Fig. 4.3** Section of skeletal muscle of common carp. Note the muscle degeneration, necrotic cells, and clump of bacterial cells (arrowed). This is typical for an acute infection by *Aeromonas salmonicida* var. *achromogenes*, giving rise to the condition known as carp erythrodermatitis. Stained with haematoxylin and eosin. Magnification × 800.

the former Yugoslavia. Atypical aeromonads also occur in farmed goldfish, in those same countries, as well as Italy, Japan, Singapore and the USA. There are published reports of atypical *A. salmonicida* subsp. *achromogenes* associated with infected wild cyprinid species in Europe, Australia and Canada. It is likely that the distribution of atypical *A. salmonicida* infections in cyprinids is far wider than listed above, but not all cases of disease in wild fish are investigated in depth. This is partly because some authorities assign lower research funding priorities to cyprinids as compared to salmonids.

**Prevention and treatment**

Prevention of aeromonad and pseudomonad diseases, like the majority of bacterial diseases in cyprinid populations, is made difficult because of the huge amount of movements of fish that take place for the ornamental industry and for sport fishing and aquaculture purposes. Some of these movements can be monitored, when health checks and movement controls can be administered. However, there are many situations in the world where it is not yet possible to control movements of fish, and many unauthorised movements take place, thus exacerbating the spread of diseases. In an ideal situation, the movement of fish stocks without health checks should not take place.

Treatments can be administered either for prophylactic control or chemotherapeutic measure, however, antibiotic sensitivity tests must be performed before any antibiotics are administered. Many of the diseases discussed above have developed considerable antibiotic resistance. Treatments can also include general antibacterial compounds. Protection for CE can be achieved using commercially available modified furunculosis vaccines.

### 4.2.3 Mycobacteriosis

Mycobacteriosis is a widespread, but infrequently reported problem in wild and farmed cyprinids. Although fairly common in small tropical aquarium species, the disease is not so common in larger temperate cyprinids particularly those destined for the ornamental trade, e.g. goldfish and common carp.

Two species occur in fish – *Mycobacterium marinum* and *M. fortuitum*. Aquarists often refer to the disease as fish tuberculosis (TB), because the bacteria belong to the same genus as the one causing human tuberculosis (*M. tuberculosis*). Note that this disease is of zoonotic importance.

In carp, the disease apparently takes on the cutaneous form, rather than the visceral form usually associated with warm water aquarium fish. Infected fish appear emaciated, stunted and show loss of appetite. Areas of light grey discoloration are present on the body surface and can vary from focal areas of scale loss to open ulcers. Goldfish and other ornamental cyprinids that are kept near, or in the same water systems as ornamental warm water fish species, e.g. livebearers of the

family Poeciliidae, are occasionally diagnosed with the disease. However, as the disease does not always manifest itself as a clinical problem or always lead to mortalities, it is probable that many cases go undiagnosed. High water temperature would appear to be necessary for infection. It is thought that fish acquire infection mostly via the oral route, by consuming detritus that contains bacteria shed from the ulcers of infected fish, or by foraging on infected corpses.

**Diagnosis**
Clinical symptoms are vague, and confirmation requires examination of internal organs for the presence of numerous pale granulomatous nodules. Granulomas can result from other pathogenic diseases, however, if they contain mycobacteria then the bacteria will stain positive with the acid-fast stain of Ziehl–Neelsen (Plate 4.9).

**Prevention and treatment**
In the case of temperate cyprinids, prevention is by not cohabiting the stock with warm water ornamental fish species. The prompt removal of corpses and dying fish, where feasible, will help reduce transmission. There is no significantly effective treatment available, but some success has been achieved with ornamentals using multidrug antibiotic therapy.

### 4.2.4 *Edwardsiella* spp.

*Edwardsiella* cause a septicaemic condition similar to infections with motile aeromonads. Feeding appropriate medicated feeds can control acute outbreaks of septicaemic diseases. Prevention can be aided by avoiding high stocking densities, moving fish during periods of warm weather, maintaining good water quality and generally good husbandry practice.

### 4.2.5 *Proteus rettgeri*

An epizootic associated with *Proteus rettgeri* has been described in silver carp raised in earth ponds in Israel. The clinical signs were deep red ulcerative lesions on the abdomen, base of pectoral fins or around the head. There were secondary infections with *Saprolegnia* sp. and histopathology revealed an acute septicaemic condition. It was suggested that the disease was the result of handling stress, and the organism originated from poultry since ponds in Israel are frequently fertilised with poultry manure.

### 4.2.6 Emerging bacterial pathogens

It is important to be aware of emerging bacterial pathogens in all fish species. Fortunately, few significant new pathogens have been reported in cyprinids in recent years, but there have been increasing reports of new Gram-positive and Gram-negative bacteria associated with dis-

ease in both freshwater and marine fish. The reports have mostly come from the USA and Spain. However, this may reflect the significant amount of fish health investigations being undertaken by these countries rather than implying that Spain and the USA are geographical foci for emerging bacterial problems in fish.

## 4.3 FUNGAL PATHOGENS AND DISEASES

Fungi are a group of organisms that are nonmotile, nonphotosynthetic and chiefly multicellular that absorb nutrients from dead or living organisms. Fungal spores are ubiquitous in all freshwater systems. Several of the aquatic Oomycetes fungi are known to affect freshwater fish, however, members of the genus *Saprolegnia* are most frequently linked with fungal infections, *Saprolegnia parasitica* and *S. diclina* being the most common. All fish species appear susceptible to *Saprolegnia*. Another genus of fungus, which is more specific for cyprinids, is *Branchiomyces*.

### 4.3.1 Clinical signs and diagnosis

*Saprolegnia* spp. are found on all forms of dead organic material in the aquatic environment and may establish on fish tissues that have already been damaged or infected, e.g. open skin lesions. The fungus produces clumps of aseptate hyphal strands that project outwards from the infection site and these resemble white 'cotton wool-like' tufts (Plate 4.10). These cotton wool growths initially appear white-grey, but can in time become discoloured, e.g. brown, or green as the hyphae entrap particulate matter and algae. Although *Saprolgenia* is a secondary pathogen, it may in some cases be the direct cause of death.

*Branchiomyces* sp. is found on gill tissue of carp and other cyprinids. It can be identified macroscopically by blotchiness of the gills, gill necrosis and white fluffy masses covering the gills (Plate 4.11). The latter sign can be confused for infections with *Saprolegnia.* However, subclinical infections of *Branchiomyces* sp. can be detected histologically by the presence of hyphae in the blood capillaries of affected fish (Plate 4.12).

### 4.3.2 Prevention and control

For ponds and aquaria, maintaining good hygiene, increasing water flows in times of hot weather, reducing handling and keeping stocking densities down can prevent fungal infections. In the natural environment, it is more difficult to control. Ponds and lakes used for sport fishing are often prebaited with vast amounts of ground bait and feed to attract fish to the area to be fished. Much of this food material is not eaten and decomposes in the bottom sediments and, as it does so, it attracts growths of fungi and other micro-organisms. This situation is ideal for increasing the amount of fungal organisms in the water. Chemical

treatments involve bath immersion in malachite green (where permitted) or salt. The use of salt (sodium chloride; 2–5 g/L) is effective for those species of fish that are salt-tolerant as fortunately most cyprinids are.

## 4.4 ZOONOSES

A 'zoonosis' is defined by WHO (World Health Organization 1979) as a disease or infection naturally transmitted between a vertebrate animal and humans. There is a paucity of information regarding viral, bacterial and fungal zoonoses associated with cyprinids. The following information is gathered from the literature on general fish/human disease relationships.

### 4.4.1 Bacterial zoonoses

The most well-known zoonosis caused by bacteria is 'tropical fish-tank granuloma' (or fish-keeper's granuloma), which is a condition caused by acid-fast bacteria – *Mycobacterium fortuitum*, *M. marinum* and possibly other *Mycobacterium* spp. It is more likely that 'tropical fish-tank granuloma' will become more common because of increased medical awareness of the disease and the expansion in the hobby of keeping aquarium fish. Infection is via contact of broken skin with infected fish or their water. In man, this disease manifests itself as persistent granulomas and abscesses occurring on the fingers and backs of the hands (Plate 4.13). If aquarists suspect they have this disease, they should seek medical advice from their general practitioner, who should refer them to a dermatologist. The disease in humans can be treated with a prolonged course of antibiotics.

Some of the common genera of aquatic bacteria such as *Aeromonas*, *Pseudomonas* and *Vibrio* include species pathogenic to humans. The fish pathogens in this group of organisms are very close to the human pathogens and it is possible that infection could be instigated from handling fish that have come into contact with what are really common organisms in the aquatic environment. The people most at risk are the young or old, but more likely those that are immuno-compromised. There is a potential threat of antibiotic resistant infections in humans resulting from the overuse of antibiotics in the koi industry. Of note, there are an increasing number of reports of 'disease resistance' strains of aeromonads cultured from imported fish, especially into the UK.

There are some bacteria that are not technically zoonoses but are included in this section since they are considered as pathogens of importance to individuals working with fish. These bacteria include *Campylobacter jejuni* (gastroenteritis), *Erysipelothrix rhusiopathiae* (skin rash), *Leptospira interrogans* (Weil's disease), *Clostridium botulinum* (botulism), *Edwardsiella tarda* (diarrhoea), *Escherichia coli* (diarrhoea) and *Salmonella* spp. (food poisoning). These organisms are contaminants

in the water body that might be found on the surface of the fish, inside their intestines or in fish products. They are particularly prevalent in ponds that contain dead and decomposing fish or other animals, or in fish products that are eaten uncooked or prepared for consumption in unhygienic conditions. Of particular note is *Leptospira interrogans*, the causative agent of leptospirosis or Weil's disease. This spiral-shaped bacterium is carried primarily by rats and passed in their urine into the watercourse. Leptospirosis is an acute infection in rats, humans, dogs and farm animals. In humans the early symptoms may be vague and can be misdiagnosed as influenza. Due to the potentially life-threatening nature of this disease, it is imperative that persons who regularly work on outdoor ponds should carry a Leptospirosis warning card (Fig. 4.4). The disease should be treated as soon as possible with antibiotics. Over the past decade, a number of fishery workers in the UK have contracted leptospirosis and of those there have been at least two fatalities.

People in contact with fish can prevent infections from these contaminant bacteria by:

- avoiding broken skin contact with fish or water in tanks, ponds and other aquatic environments (all cuts and abrasions should be washed and covered by clean waterproof dressings);

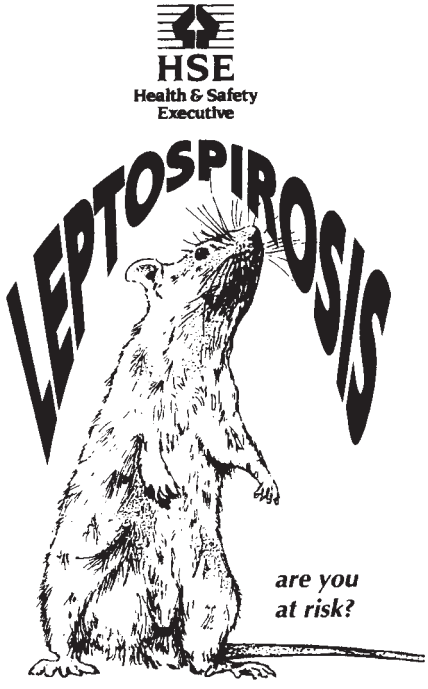


**Fig. 4.4** Leptospirosis warning card produced by the Health and Safety Executive in the UK and issued to all persons who come in contact with natural water sources.

- keeping fish-farm premises and ancillary watercourses free of dead fish;
- thoroughly washing hands and paying attention to personal hygiene and disinfection when conducting post mortem examinations and after handling, transporting and carrying fish;
- wearing rubber gloves and other protective clothing for these tasks;
- disinfecting all ponds and raceways when not in use;
- excluding rats and other vermin from farm sites and ponds;
- disposing of water used to transport fish in authorised sewage systems.

### 4.4.2 Viral and fungal zoonoses

There is no evidence in the literature of viral and fungal diseases transmitted from fish to man. There is concern, however, that pathogenic viruses are transmitted from shellfish and that fish may serve as reservoirs for human pathogens.

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*Diseases of Carp and Other Cyprinid Fishes*
D. Hoole, D. Bucke, P. Burgess, I. Wellby


# Chapter 5
# Infectious Diseases – Parasites

## 5.1 PROTOZOAN PARASITES AND DISEASES

It was in the nineteenth and the early part of the twentieth century that biologists published detailed descriptions of unicellular organisms, in particular, the protozoa. This interest was facilitated by the development of the optical microscope as an important tool in biological and medical science. Biologists' interest in fish parasites was most likely stimulated because fish have always been readily available and are relatively inexpensive animals for research purposes. Of more importance however, they are naturally infested with a variety of parasites.

The emergence of carp farming in eastern and mid-European countries was almost certainly an incentive to investigate diseases associated with the protozoan infections that occurred in the intensive culture systems. The fish health officers of the time soon realised that because carp were originally warm water fish, their introduction into environments that incurred extremes of temperatures led to diseases because the fish existed at the limits of their tolerance. It has been well documented that high protozoan infestations followed by clinical disease and subsequent mortalities in carp were most noticeable in the spring in fish that had over-wintered at low water temperatures. Today, protozoan diseases are still regarded as extremely problematic, especially when they occur in culture systems, over-stocked fisheries, ornamental ponds and aquaria.

Protozoans are single-celled organisms that range from being microscopic to being just visible with the naked eye. The protozoa as a group exhibit a wide range of life histories, some species being transmitted directly between fish whilst others have indirect life cycles that involve several aquatic organisms. In addition, some protozoa are saprophytic, free-living organisms, but can become pests when they settle on fish in large numbers, although they may not actually feed on the host, whereas others are obligate fish parasites. Furthermore, some of the well-known fish parasitic protozoa, such as *Ichthyophthirius* and *Piscinoodinium*, have parasitic and free-living phases in their life cycle.

Most wild cyprinids, regardless of geography, harbour a diversity of protozoan species and in some cases several protozoan phyla may be represented on a single fish. Even seemingly healthy fish sustain small numbers of protozoa, and in the case of most protozoan species, e.g.

*Ichthyophthirius*, the host's acquired immunity plays a role in keeping parasite numbers at low levels. In many situations it is only when the fish becomes immuno-suppressed or otherwise debilitated, perhaps due to adverse environmental conditions, that the protozoans become pathogenic to their host and cause clinical disease. Hence a deterioration in the environment or other stressor often precedes a protozoal disease epizootic within the wild or captive cyprinid stock. Epizootics can also arise if a fish encounters a protozoan to which it is immunologically naïve. This may happen when a parasite is unwittingly introduced to a new locality, e.g. via imported or translocated fish, such that the exposed stock are completely susceptible. Similarly, protozoal epizootics can be a potentially serious problem in rearing facilities because the young cyprinids may not have developed acquired immunity in the relatively sterile conditions of the hatchery.

As will be seen in the following text, some protozoa, especially some of the Ciliophora, are not true parasites, but by their huge numbers and swarming actions they can irritate a fish so much that it ceases to feed. This is important to the survival of fry and fingerlings particularly in aquaria and hatchery conditions. Alternatively, their presence on the skin surface can provoke the epidermal cells to proliferate, resulting in cell sloughing, increased mucus production and scale loss. Or, if the organisms are present in any numbers on the gills, there can be epithelial hyperplasia, cell loss and increased mucus secretion. All of these situations lend themselves to secondary infections by opportunistic organisms.

The taxonomic positions of the protozoa vary slightly according to which authority is consulted. The attention of the reader is drawn to the further reading list at the end of this chapter. This book will deal with a simplistic classification of Phylum, Class, Order, Family and Genus. Thus, the kingdom Protozoa comprises the phyla: Mastigophora, Rhizopoda, Ciliophora, Apicomplexa, Myxozoa and Microspora.

Only the more important protozoa will be described here and the reader should consult Lom and Dykova (1992) for a more comprehensive list, for the key morphological features of each species that may be required for a definitive diagnosis and for the detailed taxonomic status of the protozoans.

### 5.1.1 Mastigophora (flagellates)

These protozoa typically possess whip-like flagella as a means of locomotion. Several of the flagellated protozoa are important ecto- and endoparasites of cyprinid fish. Some of the ectoparasitic species contain chloroplasts and are capable of photosynthesis. The more commonly encountered flagellates in wild cyprinids include *Hexamita* spp., *Trypanosoma* spp. and *Ichthyobodo necatrix*.

***Piscinoodinium pillulare***

This ectoparasitic protozoan attaches to the fish's skin, gills or fins,

causing a disease known as 'velvet', 'rust disease' or 'gold-dust disease' – these various names describing the appearance of the skin of heavily affected fish. *Piscinoodinium* is quite common on barbs and other cyprinids and is reputed to be a potentially serious problem in laboratory stocks of zebra danio. The parasite is generally only a problem in aquaria, hatcheries or other intensive situations. The parasitic trophont stage is between 30 and 100 µm in length and anchors itself to the host via it branched rhizoids. It is thought that the trophont may obtain some of its nutrients from the host, via the rhizoids, however it also possesses chloroplasts and is capable of photosynthesis. The life cycle is basically similar to that of *Ichthyophthirius*, and includes parasitic and free-living stages. After a period of about 3–6 days, the actual time depending on water temperature, the trophont exits its host and forms a reproductive cyst. Within the cyst, the parasite undergoes repeated divisions eventually yielding 256 infective stages known as dinospores that leave the cyst and swim freely in the water. Each dinospore, equipped with a pair of flagella, must locate and infect a fish within about 24–48 hours otherwise it will die.

Affected fish may sustain many thousands of trophonts. When viewed under bright light, large numbers of trophonts may cause the fish's skin to appear as if peppered with yellow-green spots. A host reaction involves localised inflammation, haemorrhaging and, in the worst cases, necrosis and tissue degeneration, causing the skin to slough away. Clinical signs include flashing, clamped fins, and respiratory distress and death is likely in heavily infected fish.

Studies on the closely related *Amyloodinium*, which is the marine counterpart of *Piscinoodinium*, indicate that the host develops acquired immunity to the protozoa; hence the same probably applies to *Piscinoodinium* and its host. Several chemical remedies delivered by bath immersion are suitable for eradicating this protozoan, e.g. copper compounds and sodium chloride. The provision of dark conditions during an outbreak is thought to augment chemical treatments by preventing the parasitic trophonts from photosynthesising.

***Ichthyobodo necatrix* (Costia)**

This ectoparasite has been reported from numerous temperate and tropical cyprinids. The disease, sometimes referred to as costiosis, is familiar to those involved in aquaculture and with ornamental fish. It is an oval or kidney-shaped organism (10 × 20 µm in size) with a pointed end and possesses two flagella. This protozoan affects all species of freshwater fish, and can be particularly problematic for fry and juveniles. However, adult cyprinids, perhaps weakened by the effects of poor water conditions, can be heavily infected with these parasites. The parasite exists in two forms: a feeding, parasitic form, which is attached to the fish's epithelial cells (Plate 5.1), and a nonfeeding, swimming form that exists off the fish. Clinical signs of costiosis include excess mucus production that forms a blue-grey film over the body surface and gills. Heavily infected fish do not feed, may swim in rapid, erratic movements and may also exhibit flashing. Verification of the

organism can easily be made from fresh skin scrapings and gill squashes under the light microscope. It is possible to treat the disease with baths containing sodium chloride or formalin.

***Trypanoplasma* spp.**

These blood-dwelling flagellates, which are common in most fish, and probably affect all cyprinids, are transmitted by fish leeches. The leech acquires these protozoans during a blood meal on an infected fish. The protozoans undergo morphological changes within the leech vector, eventually forming the infective ('metacyclic') stage that can invade the blood of a fish when the leech next feeds. Within the fish's blood, the parasites may exist in different size forms, according to the nature of the infection, acute or chronic. For example, one *Trypanoplasma* species occurs in carp as a slender 20 × 2.5 µm acute form, and as a larger 25 × 5 µm chronic form.

Several *Trypanoplasma* species have been recorded from cyprinids, although it should be mentioned that there is some dispute as to the true number of species within this genus. These include *T. borreli* ( = *T. cyprini*) and *T. carassii* from goldfish and several other cyprinids. Pathogenicity varies according to the *Trypanoplasma* species, with *T. carassii* being linked with mortalities in European cyprinids. Clinical symptoms vary according to the degree of infection, the parasite species and host. Affected fish may exhibit only lethargy, but in some cases there may be kidney damage with exophthalmia and dropsy. Heavy infections can be lethal to the host.

Diagnosis may be possible by examining a drop of blood for the presence of haemoflagellates (Plate 5.2). *Trypanoplasma* species may be distinguished from the related *Trypanosoma* spp. by the presence of two flagella. Examination of blood is not always a reliable means of diagnosis as the parasite may not be observed in low-level infections and in some cases the parasites may leave the blood and invade the extravascular tissues. There are no chemical treatments available to eradicate these blood-dwelling protozoans. The only control method, where feasible, is to exclude leech vectors from the culture facility.

***Hexamita* spp. (syn. *Octomitus* spp.)**

These flagellates, which live in the intestines and gall bladders of fish, are oval shaped, with four pairs of flagella situated at their blunt end. One species causes disease problems in salmonid fishes; however, only a few reports refer to pathogenic hexamitid infections in cyprinids. Catarrhal trails exuding from the vent, and the intestinal lumen filled with creamy mucus have been recorded in infections of grass carp. It is probable that hexamitiasis is more common in cyprinids than is presently realised.

**Other species**

Scientists from time to time describe unusual organisms that do not seem to fit the existing protozoan classifications. For example, it was recently noticed, in the course of diagnostic examinations into patho-

logical changes in the gills of crucian carp, that a number of enigmatic organisms were associated with epithelial cell hyperplasia of the secondary lamellae as 'globoid structures'. They were 17 × 7 µm in size and exhibited undulating (amoeboid) movement in wet preparations. This organism has recently been called *Kinetoplastid sinensis*, a flagellate, although some authorities consider it to be an amoeba.

### 5.1.2 Rhizopoda (amoebae)

Amoebiasis is a disease that is rarely reported in fish, yet is probably very common and widespread in cyprinids. However, amoeba or amoeba-like organisms are now being reported as disease agents more often in various fish species and amoebiasis should now be regarded as a serious emerging disease in fish. Amoebas are not easy to differentiate from host cells in fresh preparations and in histological preparations and are not always clear unless special staining methods are applied. There is still controversy regarding the taxonomic position of pathogenic amoeba in fish and it is beyond the scope of this book to pursue this area in detail. It is likely that some of the pathogenic amoeba belong to the genus, *Acanthamoeba*. Affected fish can show a variety of changes including abdominal distension, lethargy and loss of appetite. Of particular note, post mortem examination of fish with amoebiasis reveals that the kidney and viscera are covered with small white nodules. In histological sections, these nodules are seen to be multiples of granulomas and can occur in nearly all organs including the brain (Plate 5.3). By applying special stains to the sections, e.g. periodic acid–Schiff (PAS), numerous small spherical organisms can be observed on the periphery of some of the granuloma formations. For clearer demonstration of amoeba, the affected tissue is better examined with the electron microscope. Amoeba that have been described using electron microscopy from goldfish tissues were ovoid in shape and approximately 3–4 µm in diameter with pseudopodia, food vacuoles, vesicular nuclei and cyst-wall formation. It is important to apply several techniques when granuloma formations are seen in sections. Many cases of amoebiasis probably go undiagnosed or misdiagnosed as mycobacteriosis, which has similar histopathological characteristics, for example multiple granulomata. The preferred diagnostic method for amoebiasis is to culture the organism from infected fish tissue. Such diagnostic methods, however, can only be used if this organism is suspected, as it requires culture techniques not routinely used in most diagnostic laboratories.

### 5.1.3 Ciliophora (ciliates)

Ciliates are typically covered with cilia and are frequently encountered on the surface of skin and gills of most species of cyprinids. Some ciliates are true parasites of fish and include the infamous *Ichthyophthirius multifiliis*, which causes whitespot disease. Some other ciliates are free-living organisms that settle on fish and other organic and

nonorganic aquatic materials. When present in large numbers on fish, these colonisers become problematic because of the host reaction they invoke. The ciliophora may well be the most significant disease-causing agents in cyprinid culture today. They appear to be increasingly common and the number of different species recorded from a single site appears to be increasing. More worryingly there is increasing circumstantial evidence that members of this parasite group are becoming resistant to the commonly used protozoicides such as malachite green. The more common ciliates encountered on cyprinids include: *Chilodonella piscicola*, *Ichthyophthirius multifiliis*, *Trichodina* spp., *Epistylis* spp., *Apiosoma (Glossatella)* spp., and *Capriniana* spp.

***Chilodonella cyprini* (syn. *C. piscicola*)**

*Chilodonella cyprini* (formerly known as *C. piscicola*) is a highly pathogenic parasite that occurs on the skin and gills of fish (Plate 5.4). It is a fairly large pear-shaped protozoan approximately 50 µm in length. The cilia are mostly situated at the pointed end of the organism, although there are short cilia on one side of the parasite. The parasite propagates by binary fission. Chilodonellosis, for example caused by *C. cyprini*, only becomes significant when large numbers of the ciliates settle on the body surface. It can survive low temperatures, even below 5°C. *Chilodonella cyprini* has been recorded in goldfish, crucian carp, tench and common carp and is most likely present on most cyprinids throughout the world. Heavy infestations can be lethal. The parasite is thought to feed on the epithelial cells of its host, causing a host reaction that appears as a grey film comprising large amounts of mucus, epithelial cell necrosis and ulceration. The fish's skin may become tattered looking and is vulnerable to secondary invasion by bacteria. Outbreaks in fish may be triggered by stress, for example arising from water pollution, or in situations where the stock are held at the lower end of their temperature tolerance range. Diagnosis is based on the examination of a wet mount skin scrape. Distinguishing features of *Chilodonella* are its gliding motion and flattened shape.

***Ichthyophthirius multifiliis* (ichthyophthiriosis, Ich, whitespot)**

The widespread occurrence of this disease, along with the clearly visible and characteristic symptoms it causes, makes whitespot one of the best known of all the protozoal diseases of fish. Whitespot is caused by a ciliated protozoan, *Ichthyophthirius multifiliis*, and is sometimes referred to simply as 'Ich'. This potentially lethal disease is thought capable of affecting most, if not all, species of freshwater teleost fish and therefore poses a threat to all cyprinids. Each characteristic white spot (Plate 5.5), which may reach just over 1 mm in diameter, represents an individual parasite (trophont stage). The parasite resides within the fish's epidermis where it feeds and grows on host skin cells and fluids (Plate 5.6). In heavy infections, some of the parasites may form aggregations. The life cycle of *I. multifiliis* is direct and involves both parasitic and free-living stages (Fig. 5.1). After a certain period of time, the mature trophont leaves its host and drops to the substratum where it

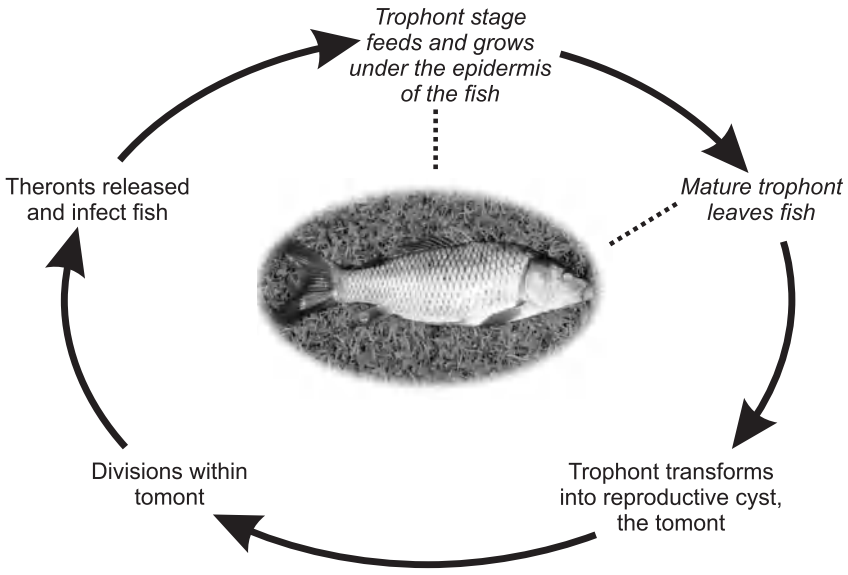



**Fig. 5.1** Life cycle of *Ichthyophthirius multifiliis* (whitespot). Dotted lines and italics indicate stages in which the fish is involved.

transforms into a round reproductive cyst (tomont). This encapsulated cyst undergoes repeated divisions, ultimately yielding between about 100 and 2000 infective stages, known as theronts. Upon release, the microscopic theronts swim actively in the water in search of a fish host. The theront is a nonfeeding stage, which has only a limited time (less than 48 hours) to locate and penetrate a fish host; those theronts that are unsuccessful will die from having exhausted their limited supply of food reserves. Upon contact with a suitable fish, the theront burrows into the epidermis where it transforms into the feeding, growing trophont, thereby completing the life cycle. The duration of this life cycle is temperature dependent, being faster in warmer water. At 20°C, for example, one full cycle requires about 8 days, most of which is spent as the parasitic stage on the fish. Under very cold conditions, the parasite may over-winter on the fish. However, very high water temperatures, above 28°C, are lethal to the parasite. In low level infections, an infected fish may twitch its fins and repeatedly rub its flanks against hard objects such as submerged rocks, presumably in an attempt to rid itself of the parasites residing in its skin. When viewed from above, such as from the shore, this side-on rubbing action causes the fish's silvery flanks to reflect the sunlight – and hence this 'scratching' behaviour is sometimes known as 'flashing'. However, diagnostic care must be taken since fish may flash in response to other skin irritations, not just to whitespot. In moderate infections there may be obvious skin and fin damage, resulting in the detachment of areas of epidermis and mucus, and this is caused by a combination of invading theronts and exiting trophonts, which form holes into the skin epithelium. Secondary skin infections, such as caused by various bacteria and fungi, may follow.

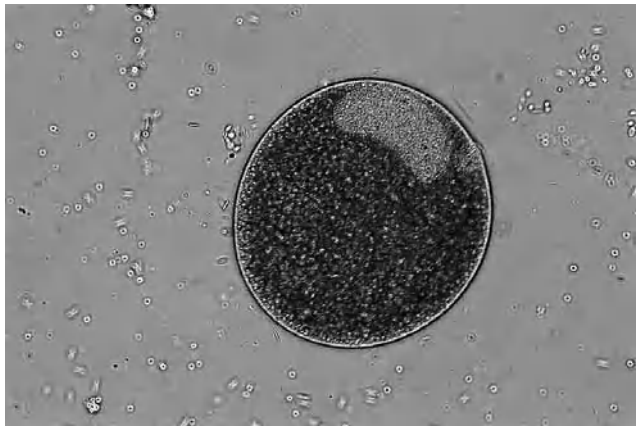


**Fig. 5.2** Mature unstained *Ichthyophthirius multifiliis* in a skin scrape showing the horseshoe shaped nucleus. Magnification ×80 (courtesy of the Environment Agency, UK).

The gills are also sites of attack, such that heavy infections can result in severe gill damage, resulting in respiratory stress. Affected fish exhibit increased gill beat rates and may gulp ('pipe') at the water surface where the oxygen level is richer. In severe cases the fish may experience osmoregulatory stress resulting from the many puncture wounds that breach the skin's water-tight integument. In heavy outbreaks 100% mortality can occur. The low host-specificity of this parasite means that it can also cause total mortalities in facilities housing several cyprinid species, such as in aquaria.

The presence of one or more discrete white spots on the fish's skin and/or fins is a characteristic diagnostic feature. Infection can be confirmed by microscopic examination of an extracted parasite, which will reveal the characteristic 'C' shaped (so-called 'horseshoe') macronucleus of the trophont (Fig. 5.2), and the virtually uniform covering of cilia over the body surface. These cilia cause the organism to spin. Those fish that survive an outbreak of whitespot may go on to develop a specific acquired immunity to this disease. Both the degree and duration of protection varies (there are many complex underlying reasons for this) such that an 'immune' fish may sustain only a few or sometimes no parasites upon a subsequent outbreak of this disease. The fact that fish have been shown to develop immunity has led researchers to try and develop a vaccine to protect fish against whitespot. Preliminary trials are encouraging, such that a commercial anti-Ich vaccine may become available in the not too distant future. Recent studies, primarily carried out in the USA, have led to the possibility of a DNA vaccine based on antigens occurring on the cilia or the use of genetically altered free-living protozoa.

***Trichodina* spp. (trichodinosis)**

Trichodinosis is another ectoparasitic disease that is very familiar

to workers involved with fish. As a result of their interesting visual characteristics, trichodinids rank among the most frequently photographed parasites. The causative organisms are members of the genus *Trichodina* and related genera such as *Paratrichodina*. There are many species that are known to infect cyprinids but only rarely are these parasites identified beyond genus level.

Trichodinids have a complex morphology. The dorsal view shows it to be rounded (Plate 5.7) whilst from the lateral aspect it appears as a saucer or bell-shaped organism (Fig. 5.3). Looking at it from its rounded view, it has rows of cilia both top and bottom. The bottom surface is invaginated and has a row of horny denticles. Internally, it has a horseshoe-shaped macronucleus, micronucleus and a number of vacuoles that contain food, e.g. epithelial cells. These ciliates can affect all species of cyprinids, and the heaviest infections appear on weakened fish. Most of the pathogenic trichodinids inhabit the skin and gills of their host. Clinical signs are seen as white patches on the skin surface and excess mucus exudate on the gills. Usually, these protozoans cause either no visible symptoms, or just a mild disease, but stress factors may predispose the fish to heavy infestations, which may result in impairment of respiratory functions. Heavy infestations can therefore prove life threatening, resulting in chronic low-level mortalities in the stock. The host response involves increased mucus secretion, which may itself attract other pathogens. Furthermore, skin damage caused by large numbers of trichodinids may render the fish susceptible to secondary infections. In otherwise healthy stock *Trichodina* could, therefore, be considered more of as a nuisance organism than a serious pathogen but these ciliates should, if possible, be excluded from hatcheries as the incidence of mortalities from the disease is generally higher in juvenile fish. Verification of trichodinosis can be made by microscopic examination of skin scrapings, particularly the fins, and gill squashes. Trichodinids may also be encountered in the urinary tracts, kidney

**Fig. 5.3** Unstained heavy infection of *Trichodina* sp. in a skin scrape. Magnification × 400 (courtesy of the Environment Agency, UK).

tubules, oviducts and nasal cavities of cyprinids; however, these endoparasitic species are less commonly associated with clinical disease.

***Epistylis* spp.**

These colonial ciliates, which are associated with high levels of suspended organic material, are sometimes found in large numbers on the skin and gills of carp and goldfish and doubtless on other pond cyprinids. *Epistylis* spp. are relatively large sessile protozoans, which have a thin cylindrical or bell shape. They attach to the fish host or other substrate by a long, noncontractile stalk, which may exceed 1 mm in length in some species. Once attached, a single parasite will divide and spread to form large colonies. At the top or anterior end of the cylinder are situated the cilia that assist the protozoa in obtaining food material. This means that the parasite does not feed on the fish, but uses the fish merely as an attachment site. When present in large colonies the attachment sites of *Epistylis* causes a lesion that becomes inflamed and necrotic and eventually ulcerated. The lesion is vulnerable to secondary invasion by pathogens.

***Apiosoma* spp. (syn. *Glosatella* spp.)**

These are close relatives of *Epistylis* spp. and are also found associated with organic material and occur on the skin and gills of fish, but they differ in being solitary organisms, rather than colony formers. *Apiosoma* is a goblet-shaped organism, 30 × 50 µm, which has a flat attachment base and cilia at the anterior end (Fig. 5.4). In common with other ciliates, it divides by binary fission and when present in large numbers *Apiosoma* spp. may cause problems although they are often regarded as relatively harmless ectocommensals. High numbers of parasites attached to the fish's skin and gills may cause inflammation, necrosis and ultimately ulceration of the skin and degeneration of gill tissues,

**Fig. 5.4** Unstained *Apiosoma* sp. in a skin scrape. Note disc (arrowed) by which protozoa attaches to the skin of the fish. Magnification × 400 (courtesy of the Environment Agency, UK).

allowing infection with other pathogens. Species found on cyprinids include *A. siewingi* and *A. filiformis*, both recorded from common minnows.

***Capriniana* spp. (syn. *Trichophyra* spp.)**
*Capriniana* spp. are members of the Suctoria family of ciliates. *Capriniana piscium* may be found attached to the gill lamella of a wide range of freshwater fishes, including cyprinids (Plate 5.8). It is considered to live on the fish as an ectocommensal since it has not been associated with host pathology or host tissue response. The attached form bears no cilia, but possesses several retractile tentacles that are involved in prey capture, which include other ciliates, and food digestion. The body shape and size is relatively plastic, but is approximately oval and may slightly exceed 100 µm in length. Microscopic examination reveals a large meganucleus. These protozoans reproduce by budding, to yield a ciliated migratory stage. Another species, also considered nonpathogenic, is *C. variformis*, which is recorded from grass carp.

### 5.1.4 Apicomplexa

These are parasitic protozoans of which the class Sporozoa is represented in fishes.

***Goussia* spp. (coccidiosis)**
Numerous species of coccidian sporozoans are parasites of fish, however those which probably attract most attention in terms of cyprinid fish are members of the genus *Goussia*, which are found in the fish's intestines. The coccidian life cycle is complex, involving several stages.

In carp, coccidiosis is often associated with debilitation. *G. sinensis* is particularly problematic to the fry of cultured herbivorous carp (silver carp and bighead carp) in Hungary, *G. aurata* is pathogenic to goldfish and *G. subepithelialis* has been described in tench and common carp. These organisms occur in the intestines, and clinical signs of the disease include oedema of the abdominal serous membranes and of the intestinal wall, absence of fat tissue and swollen intestinal mucosa. In severe infections, nodular growths in the intestinal tissues, especially in the connective tissue of the submucosa, may be present (Plate 5.9). The intestinal wall is dark and there is yellowish mucus exudate. Verification of the disease is by microscopic examination of squash preparations (Fig. 5.5). In such samples, the macrogametes (sexual stage) and sporulating oocysts (spore-forming stage) will be identified in the lamina propria and submucosal areas of the intestinal tract (Plate 5.10). Of the many other sporozoan parasites of cyprinids, examples are *Eimeria rutili* recorded from the kidneys of roach, and *Octosporella notropis* found in the intestinal epithelium, swimbladder and spleen of common shiners.

**Fig. 5.5** Unstained squash preparation of spores of *Goussia subepithelialis* from the intestine of a common carp. Magnification × 800 (courtesy of Dr Stephen Feist).

### 5.1.5 Myxozoa (Myxosporea)

It is currently being considered that the Myxozoa should be moved from the Protozoa. This is because myxosporeans are not now technically regarded as single-celled animals since they are multicellular for almost all of their life cycle. As the taxonomic position has not yet been decided for this phylum, for the purposes of this book, this group of parasites is included here rather than with the metazoa.

The phylum Myxozoa contains multicellular spore-forming protozoans that infect invertebrates and ectothermic vertebrates, mostly fish. Within the phylum is the class Myxosporea, which is the largest group of protozoans affecting fish. There are some 1400 species, with new ones continually being discovered. Despite their abundance and, in many cases, significance as fish pathogens, it is only recently that the life cycle of one of the most important fish myxosporean parasites, *Myxobolus cerebralis*, the causative agent of whirling disease in salmonids, has been elucidated. In cyprinids, there are numerous reports of myxosporeans associated with pathological changes in the skin, fins, gills and internal organs (Plate 5.11). The reader is advised to consult the literature at the end of this chapter for more details. Myxosporeans are obligate endoparasites of fish; examples include *Sphaerospora* spp., *Thelohanellus cyprini*, *Myxobolus dermatobius*, *Myxobolus ellipsoides*, affecting the gills, kidneys, fins, skin and spinal column. There are many genera and species of Myxosporea and the classification is continually being revised as more information about morphology and life cycles becomes available. As a generalisation, myxosporidians are typically oval or pear-shaped organisms. Their small size, approximately 10 × 5 µm, varies according to genus and species (Plate 5.12). Internally, there are two spores enclosed in individual shells, which contain usually two polar capsules. Inside these are coiled filaments and a number of vacuoles (Fig. 5.6). The spore stage can remain viable for a long time in the aquatic environment, over a year in some cases, and hence is

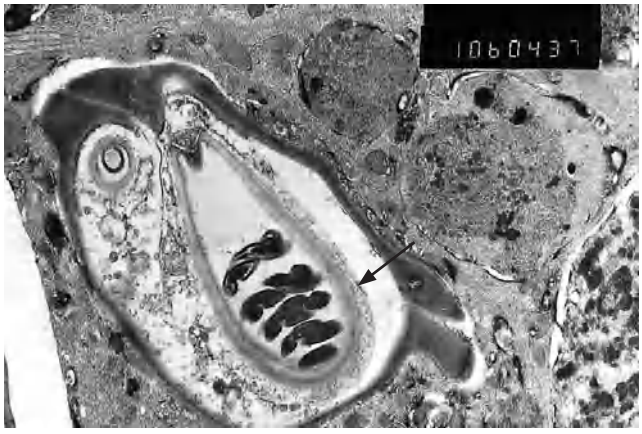


**Fig. 5.6** Picture taken on a transmission electron microscope of a myxosporean showing a polar capsule (arrow) containing a coiled filament. Magnification ×10 000.

of significance in terms of disease control strategies. The differences in the morphology of the myxosporea are important for identification and specialist literature should be consulted for this purpose. There are no treatments for myxosporeosis. For this book, examples of the common diseases associated with the myxosporea are presented.

***Hoferrelus* spp. (kidney enlargement disease)**

Kidney enlargement disease is one of the more common diseases caused by myxosporeans, *Hoferrelus carassii* in goldfish and crucian carp, and *H. cyprini* in common carp. External signs of the disease include swollen lateral flanks (Plates 5.13, 5.14), scale erection, loss of balance and general listlessness. The swelling is the result of kidney enlargement, which is caused by the presence of various stages of the protozoan in the uriniferous tubules. Enlargement and other pathological changes occur through the action of the immature trophozoites for, when the parasites reach the spore stage, they are inert. The parasite causes the tubules to dilate and their epithelia become hyperplastic, infiltrated by inflammatory cells (Fig. 5.7). These changes contribute to kidney failure, necrosis, degeneration and eventual mortality. The worst forms of the disease occur in over-stocked fisheries, including ornamental goldfish farms and poorly managed aquaria. In the case of *H. carassi* it is thought that an oligochaete worm may serve as intermediate host. Identification and exclusion, where possible, of the intermediate hosts serves as a control strategy in the absence of effective chemical treatments.

***Sphaerospora renicola***

Sphaerosporosis caused by *S. renicola* is reported as the most important myxosporeosis of cultured carp. *Sphaerospora* spp. are parasitic to several species of cyprinids. *S. renicola* causes renal degeneration due to

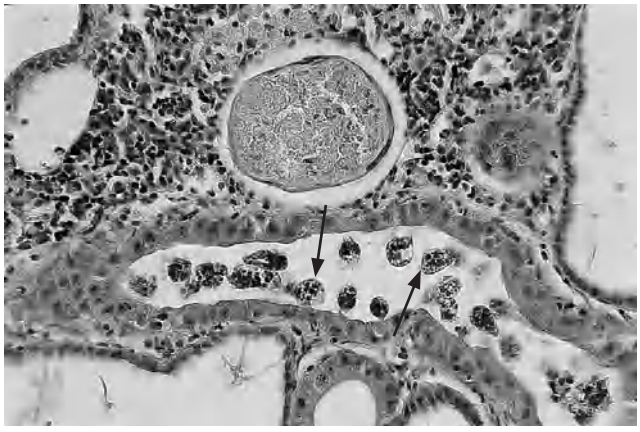


**Fig. 5.7** 'Kidney enlargement disease' caused by the myxosporean, *Hoferellus carassii* (arrowed). Stained with haematoxylin and eosin. Magnification × 400 (courtesy of Dr Marian McLoughlin).

the development of sporogenic pseudoplasmodia in the lumens of the renal tubules (Fig. 5.8). Extrasporogenic developmental stages are found in the blood and the swim bladder. In the latter, infection can lead to epithelial hyperplasia and inflammation that is a particular problem in carp fry. The pathological symptoms in older fish occur in the kidney tubules with hypertrophy of the epithelium.

***Sphaerospora molnari***

*Sphaerospora molnari* occurs in the gills of carp, crucian carp and many other cyprinids. It causes significant pathological changes in the gill tissues. It has been reported in the UK, Czech Republic and Slovenia, where serious losses occur in intensive rearing ponds, with high

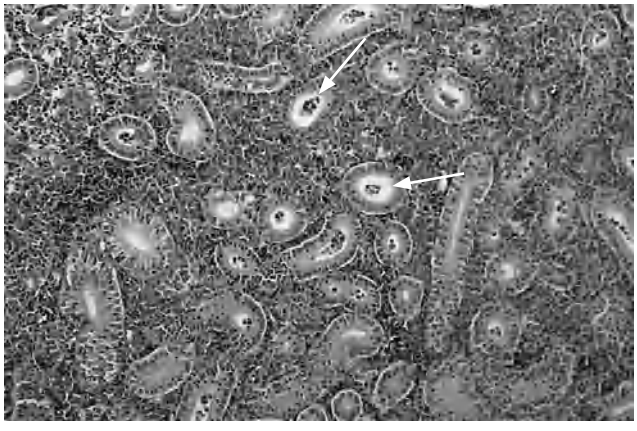


**Fig. 5.8** *Sphaerospora renicola* (arrowed) in the lumens of kidney tubules of a common carp. Stained with Giemsa. Magnification × 200 (courtesy of Dr Stephen Feist).

mortalities in the immuno-compromised fry with prevalences from 30–100%. The typical histopathological changes include epithelial hyperplasia and necrosis. When the infection is heavy, the epithelial hyperplasia of the secondary lamellae becomes extensive, with fusion and in some cases, lamellae disintegration. It is then that respiratory function is affected leading to mortality.

***Myxobolus koi* (gill myxosporeosis)**
Classic cases of gill diseases associated with myxosporea usually involve *Myxobolus koi*, which occurs in common carp and its varieties. It is a potentially lethal disease that is most pathogenic to young fish and is more prevalent in the summer and autumn. The external signs of the disease include flared opercula and masses of 1–2 mm white nodules on the gills. These nodules are cysts containing many parasites at various stages of development.

***Myxobolus ellipsoides* (spinal deformities)**
Spinal deformities have been described in juvenile chub and other *Leuciscus* spp. in rivers in the UK. The cause was identified as a member of the Myxosporea, *Myxobolus ellipsoides*. Histological examination of chub fry showing spinal deformities revealed masses of longitudinal spores within the periosteal tissues of the vertebrae extending anteriorly into the cervical region (Plate 5.15). The spores, which are encysted within cartilaginous and ossified tissues, induce a considerable tissue reaction involving necrosis and cartilage regeneration. It appears that the myxosporeans destroyed the periosteal cartilage, causing the vertebrae to grow abnormally. Further surveys in UK rivers have revealed other *Myxobolus* spp. to cause muscle necrosis in cyprinid fry and juveniles.

### 5.1.6 Microspora (Microsporidia)

Whereas there are many myxosporeans that infect cyprinids, there are relatively few documented microsporidians infecting these fish, and of those studied, few appear to be significantly pathogenic to their host. The Microsporidia are sporulating protozoa and are intracellular parasites. They typically cause tissue damage to their hosts and hence are potentially serious pathogens. Morphologically, they are usually oval shaped, 5 µm in length, and often slightly curved (Fig. 5.9). One end of the spore is darker and this includes the nucleus and polar filament and the clearer area includes the sporoplasm and polar cap. In some genera propagation is by schizogony. Several genera are parasites of fishes. One species, *Pleistophora mirandella*, occurs in several cyprinid (and non-cyprinid) fishes, for example in the ovaries and testes of roach from rivers in the UK and Finland (Fig. 5.10). *Pleistophora ovariae*, which has been described in ovaries from golden shiners in the USA, is considered a pathogenic problem on bait-fish farms. Evidently, the spores develop in the fish's oocytes and destroy them causing sterility of the female stock. There are no treatments for microsporidiasis.

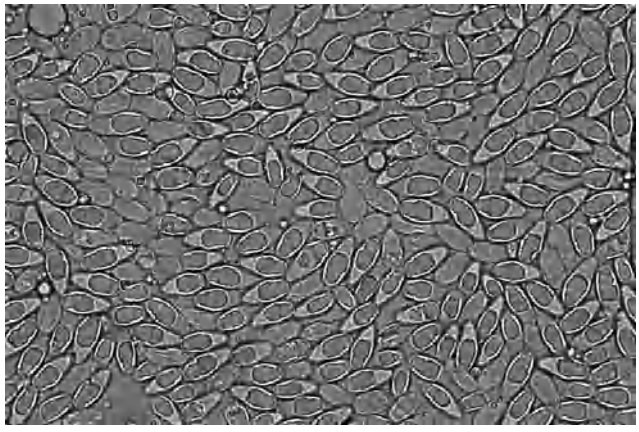


**Fig. 5.9** Unstained squash preparation of microsporideans from a cyst. Magnification × 1500 (courtesy of the Environment Agency, UK).

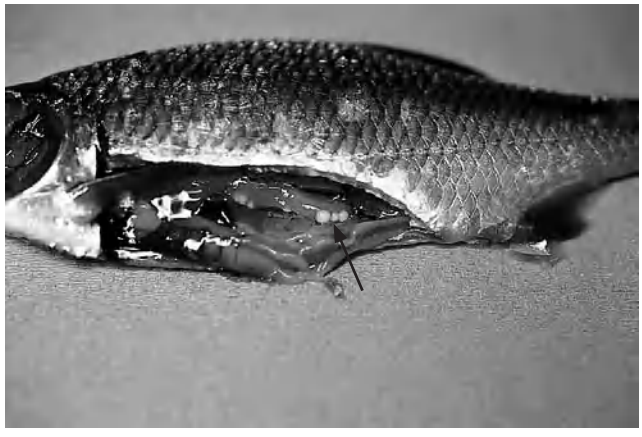


**Fig. 5.10** A rudd with the body cavity opened to reveal microsporidean cysts (arrowed) on the gonads (courtesy of the Environment Agency, UK).

### 5.1.7 Protozoan parasites of uncertain taxonomy

***Dermocystidium* spp.**

This is an enigmatic genus that has caused taxonomists many problems. It was described approximately 50 years ago as a fungal pathogen affecting marine molluscs; however, about 20 years ago these organisms were reclassified as protozoans, but even today experts are still undecided as to their true taxonomic position. *Dermocystidium* is of interest to fish pathologists because several species are known to affect the gills and skin of some fish species. In the past few years, there have been increasing numbers of reports of 'Dermo' disease in common carp and koi. The spores are round in shape, approximately 6–14 µm in diameter and have a signet ring appearance. Cysts containing spores

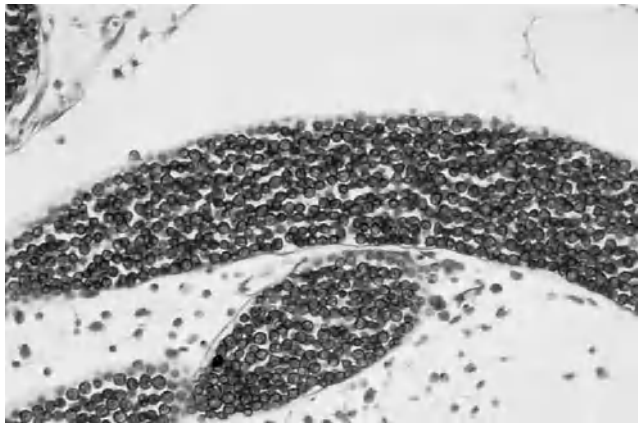


**Fig. 5.11** Sporulating bodies of *Dermocystidium* sp. in the dermal tissue of a common carp. Stained with Ziehl–Neelsen. Magnification × 400 (courtesy of the Environment Agency, UK).

are recognised with the naked eye as large white lesions, especially on the gill filaments and fins (Plate 5.16, Fig. 5.11). Although little is known about the pathogenesis of these organisms, it is likely that their presence inhibits respiratory function when situated on the gills. There is no known method of control.

## 5.2 MONOGENEAN FLUKES

Monogeneans are a class of parasitic flatworms, which are mainly ectoparasitic on ectothermic/poikilothermic (cold blooded) vertebrates such as amphibians and fish, although the group does have members that infect Crustacea, cephalopods, and mammals but they are rare. In fish, monogeneans are found infecting the skin, gills, buccal cavity and pharynx and only very few are truly endoparasitic. They attach to their host by means of a posterior organ that is usually in the form of two large central hooks surrounded by a corona of 12–16 marginal hooks. This attachment organ is modified considerably in some species, e.g. *Diplozoon* sp., and is often useful in identifying different species. Once attached to the host, monogenea feed directly on skin and gill tissue. Of the five major groups there are three that are of concern to cyprinid fish. Two well-known monogenean families have gained justifiable notoriety, the gyrodactylids and the dactylogyrids. The third group is an order known as the Polyopisthocotylida that has several representatives living on cyprinid fish.

The gyrodactylids and the dactylogyrids have confusingly similar names describing two confusingly similar families of parasites. They are usually only differentiated by the simple expedient of being *Gyrodactylus* spp. if they are on the skin and *Dactylogyrus* spp. if they are on the gills. Whilst this is a useful general diagnostic feature, detailed

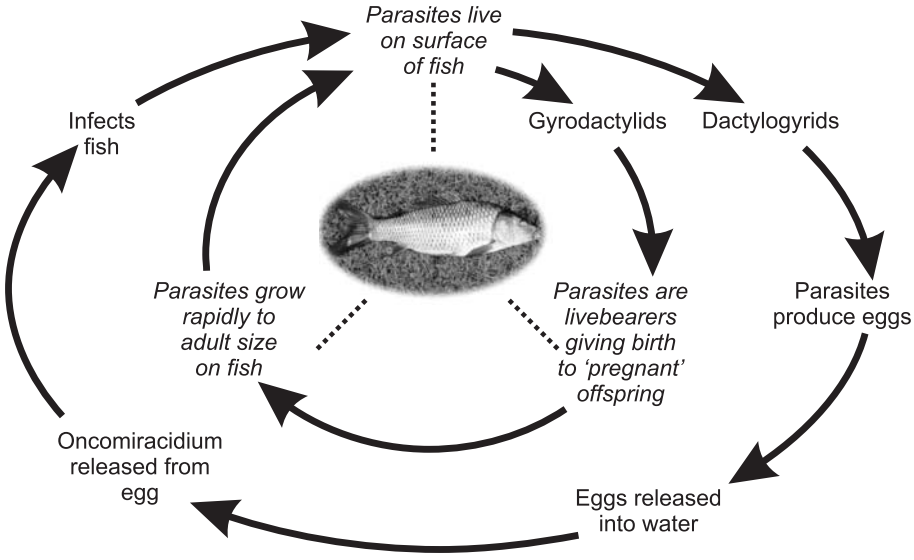



**Fig. 5.12** Typical life cycle of monogenean flukes. Dotted lines and italics indicate stages in which the fish is involved.

investigation is always necessary to identify the exact species present. Both *Gyrodactylus* and *Dactylogyrus* genera conform to the same basic monogenean body shape but they have a substantial and important difference in their mode of reproduction although like all monogeneans they have a simple life cycle involving only one host. The dactylogyrids, as with all the others in this group, lay eggs that hatch to release ciliated larvae that then actively seek a new host. The gyrodactylids, however, give birth to live young, which are fully formed at birth and attach directly to the host (Fig. 5.12). As discussed later the Polyopisthocotylida can have highly unusual body shapes but, like dactylogyrids, they are egg bearers. This reproductive difference has implications for control regimes, which are more problematic in dactylogyrids due to the resistant egg stage.

### 5.2.1 Dactylogyridae

These gill parasites, which have one or two pairs of eye spots, have a complex attachment organ, termed a haptor, consisting of 16 small marginal hooks and two large central hooks.

Once the eggs are laid they tend to be borne away from the fish host and hatch into ciliated oncomiracidia, which only have a very short life, e.g. 10–20 hours in which to find a new host. Research studies on some members of the monogenea suggest that they have adaptations that maximise opportunities for successful invasion of a new host. In particular the hatching process can be controlled by factors such as diurnal rhythms and changes in light intensity. Hatching at particular times of the day or in response to shadow may coincide with the pres-

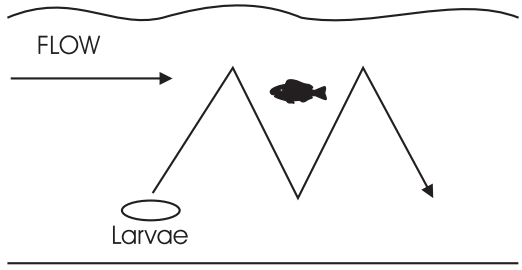



**Fig. 5.13** Movement of larvae of *Entobdella solea* is generally photopositive but exhibit periods of photonegativity. This means they perform a series of ascents and descents through a horizontal current – this amounts to a simple but effective search strategy (redrawn from Kearn 1980, in Williams & Jones, 1994).

ence of the host fish so substantially increasing the possibility of attachment to the host. Some eggs are very sensitive to host mucus, for example *Acanthocotyle lobianchi* will hatch in 2–4 seconds in the presence of mucus from its marine fish host. Once hatched the ciliated oncomiracidium can also adapt to improve its chances of locating a host. Most oncomiracidia are phototaxic and chemosensitive and use variable phototropic and phototaxic responses to initiate an effective search strategy to locate the host (Fig. 5.13). This will give the best possibility of detecting their host with chemosensitivity.

Although these strategies have only been elucidated for a few species of monogenea it seems reasonable to assume that most egg-laying monogeneans have developed some sort of adaptation for correlating hatching rhythms and host behaviour. These sorts of behavioural adaptations can be exploited for controlling the parasite in intensive aquaculture situations. In the example of *Entobdella solea* a light source placed near the abstraction point along with some shading over the fish pool could draw the larvae into the outlet.

There are many different species of *Dactylogyrus* (Plate 5.17) and many of them have the potential to infect cyprinid fish. Two examples, *Dactylogyrus vastator* and *D. extensus*, are given below.

***Dactylogyrus vastator***

This parasite, which is endemic to central Asia, was introduced into Europe, North America and Israel where it infects the gills of carp and goldfish. Infection causes hyperplasia of the gill epithelium and deformation of the gill lamellae. In young fish this damage can be particularly problematic and results in respiratory failure. It has been suggested that this parasite species competitively excludes *D. extensus*, perhaps by producing an unsuitable gill environment.

***Dactylogyrus extensus***

This parasite is very similar to the former and is found in the same fish species and within the same countries as *D. vastator*. The fluke occurs usually between the secondary lamellae and feeds on the epithelial

cells. Adult flukes also cause damage at their attachment sites where necrotic foci form, thus increasing the risk of secondary infections.

Control of dactylogyrids usually entails the application of external treatments, e.g. Praziquantel™, Trichlorfon™ or draining and drying infected ponds.

### 5.2.2 Gyrodactylidae

This family is unique amongst the monogeneans in the fact that they are viviparous. Amazingly, the offspring are sexually mature before they are born and can themselves have a fully formed offspring in their uterus prior to birth. Incredibly, this fully formed second embryo can have a third embryo inside and then a fourth embryo inside the third. This means that there can be five generations present in one worm, giving rise to one of its common names, 'the generation worm' (Fig. 5.14). Not only is this ability quite extraordinary, it is also gives the family a very high reproductive potential.

Although terminology and speciation within the gyrodactylids (Fig. 5.15) is confusing it has been suggested that two species may occur on cyprinids, i.e. *Gyrodactylus kathariner*i and *G. crysoleucas.* The former species occurs on carp throughout Europe, Asia and North America and also has been called *G. cyprini* or *G. mizellei*. Infection in young common carp can cause problems. Fish turn dark blue in colour, become emaciated and eventually die. Some authorities have suggested that mirror carp may be more resistant than the common carp. *Gyrodactylus crysoleucas* primarily parasitises golden shiner, usually increasing in prevalence and intensity in late spring and summer. Both gyrodactylids can damage skin at their point of attachment and can form foci for entrance of secondary infections. Treatment is usually by bath immersion application of formalin or potassium permanganate.

### 5.2.3 Polyopisthocotylida

This third major group has some members that are worthy of note.

**Fig. 5.14** Unstained *Gyrodactylus* giving birth to a live offspring. Another fully developed offspring can be seen within the uterus of the first. Magnification × 60 (courtesy of the Environment Agency, UK).

**Fig. 5.15** Unstained *Gyrodactylus* sp. in a skin scrape. Note the visible haptor of the unborn young (arrow). Magnification × 80 (courtesy of the Environment Agency, UK).

***Diplozoon***

This is another extraordinary fluke, which is very similar to the rest of the monogeneans in that the eggs hatch to give ciliated oncomiracidia that contact the host and then develop into the adult. As they reach maturity however they seek out a mate and enter into a copulatory embrace that sees them fuse together and live out the rest of their lives as a remarkable creature called a twin worm. There are several species of these twin worms that infect cyprinid fish including the most famous *Diplozoon paradoxum*, which is found on bream and other cyprinid fish throughout Europe.

The authors have personally seen many infections from wild fish populations that are never more than 2–3 parasites per gill arch (Plates 5.18, 5.19). There appears to be no significant pathology associated either with the attachment or feeding behaviour of the flukes.

## 5.3 DIGENEAN FLUKES (TREMATODES)

A class of parasitic flatworms infecting a wide range of different animals. They have a complex life cycle involving at least two hosts. In 1946 an eminent European parasitologist, Ben Dawes, wrote a book entitled *The Trematodes*. At the time this group comprised both the monogenetic and digenetic flukes and although they have subsequently been split into two classes the latter group had 45 families, 43% of which occurred in European fishes. Subsequently in 1971 Yamaguti recognised 70 families of fish digeneans many of which occurred exclusively in fish. This perhaps highlights the ubiquitous nature of this class of parasites. As with the cestodes (tapeworms), the life cycle of the parasites comprises at least two hosts and in some cases three hosts. The fish can serve both as the definitive host in which the adult fluke occurs, or as the intermediate host, which usually harbours an encysted life stage. In

life cycles that involve fish, an aquatic snail usually serves as the intermediate host, sometimes in addition to a subsequent second intermediate host. A two-host life cycle is typified by the blood fluke, *Sanguinicola inermis*, that is found in carp. Parasite eggs are either liberated into the water or hatch at an external surface, e.g. in the case of *Sanguinicola* the gills. A free-living ciliated larval stage, the miracidium, is released from the egg. This short-lived stage penetrates the snail intermediate host in which a multiplication phase occurs via the sporocyst and/or redial stage. Eventually a free-living stage, the cercaria, is shed from the snail and penetrates the fish host where it develops into the adult worm. The multiplication phase in the snail is an essential component of the digenean life cycle as the invasion of one miracidium can produce thousands of cercariae. In some species of digenean, direct penetration of the final host does not take place and the parasite becomes encysted on or in another host, a second intermediate host, the final host becoming infected by eating this infected second intermediate host. Fish can also act as second intermediate hosts and contain encysted digeneans. These types of digeneans usually have a snail as a first intermediate host and a bird or mammal as the definitive host. In some cases, e.g. the eye fluke *Diplostomum*, the second intermediate stage does not encyst although a three-host life cycle occurs. Under enclosed culture conditions, such as aquaria, the life cycle of these digeneans can be broken by excluding the aquatic invertebrate hosts. Obviously, those digenean species whose life cycle involves a terrestrial stage, e.g. a bird, cannot complete their life cycle within aquaria. Aquarium fish can, however, harbour digeneans, e.g. eye flukes, *Diplostomum*, black spot, *Posthodiplostomum*, *Apophallus*, indicating that the fish were either once wild or farmed in open outdoor systems prior to being housed in aquaria.

Adult digeneans have a characteristic basic body plan, which comprises a dorso-ventrally-flattened body and external suckers used for attachment and feeding. Internally the fluke's body comprises digestive, reproductive, excretory and nervous systems together with an extensive muscle development. Traditionally the digenea are split into two major orders, the Gasterstomata, which contains a mouth in the middle of the body that leads to a blind ending gut, and the Prosostomata, which has a terminal mouth.

The Prosostomata usually contain two suckers: a ventral sucker used to attach the parasite to the host and a oral sucker located at the anterior end of the fluke and containing a central mouth. The mouth leads to a bifurcating blind ending gut. These parasites are usually hermaphrodite and contain both male and female reproductive organs, i.e. testes and ovary, uterus and the yolk producing vitellaria. Features of the suckers and the gut, reproductive and excretory systems are used to classify the flukes. In recent years, however, with the increased importance of digeneans in fisheries, the need to determine species accurately has led to the use of DNA technology in identification. Identification of digeneans in the second intermediate host is extremely difficult.

### 5.3.1 Digenean flukes with fish as the definitive host

Adult flukes can occur in a variety of organs within cyprinid fish. For example, *Allocreadium* spp. and *Sphaerostoma* spp. infect the intestines of roach, minnow and carp (Plate 5.20), whereas *Phyllodistomum* spp. occur in the ureter of several fish species, and *Sanguinicola* spp. have been found in the blood system of several cyprinids including roach and carp. It is within the latter group that one finds the problematic adult fluke, *Sanguinicola inermis.*

**Sanguinicoliasis and *Sanguinicola inermis***

The Sanguinicolidae contains over 60 species of flukes that occur in the blood system of both marine and freshwater fish. It is those parasite species that infect freshwater fish culture, however, that can cause the most serious economic loss. This loss is not only restricted to cyprinids, as *S. klamathensis* and *S. davisi* occur in salmonid farming in North America. It is however within the cyprinids, given the extent to which they are cultured or manipulated, that sanguinicoliasis has the most serious impact. In China *Sanguinicola lungensis* causes high mortalities in cyprinid fish, but on a worldwide basis *Sanguinicola inermis* is the most problematic. The parasite has been found in a range of cyprinid species from several countries, for example in bream, silver bream, rudd and roach in Lithuania, in France roach, and in nase in Hungary. The parasite is of particular importance however when it infects members of the carp group, i.e. common carp, crucian carp and goldfish in Poland, Hungary, Germany, Bulgaria, Czech Republic, Bosnia-Herzegovina and China. Sanguinicoliasis also occurs in the UK. *Sanguinicola volgensis* was the first sanguinicolid recorded in UK cyprinids, i.e. roach and dace in Lincolnshire in 1964 and later found in chub. *Sanguinicola inermis* was first recorded in the UK causing large mortalities in a carp farm in the south-west of England in 1977. Over the period 1977–93 it was reported in over 170 sites in England including hatcheries, ponds, lakes, rivers and even a palace moat. It has also been found in koi imported from Japan. More recently, in 1982, a third species, *S. armata*, was found in tench in Beverley and Barmstone Drain in Hull, England.

Adult flukes (Fig. 5.16), which reside primarily in the heart, are hermaphrodite, i.e. contain both male and female reproductive systems. The adults live for approximately 60 days dependent on temperature, during which time they produce immature eggs, which are released into the fish's blood system (Fig. 5.17). These triangular-shaped eggs, containing the developing larval stage, the miracidium, accumulate in the capillaries within several organs, e.g. gills, kidney, liver and spleen, and form the site of an intense inflammatory response. Although this response may damage the egg and the enclosed parasite the accompanying degradation of host tissue can be excessive and can result in severe pathological and physiological consequences. Some of the shed eggs become lodged in the capillaries of the gill and are identified in

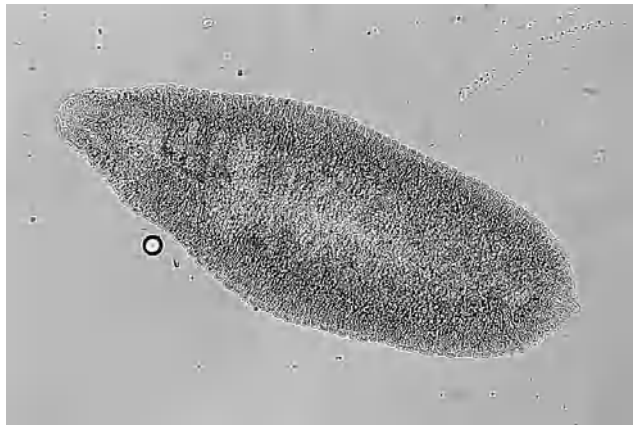


**Fig. 5.16** Unstained adult stage of *Sanguinicola inermis*. Magnification × 400 (courtesy of the Environment Agency, UK).

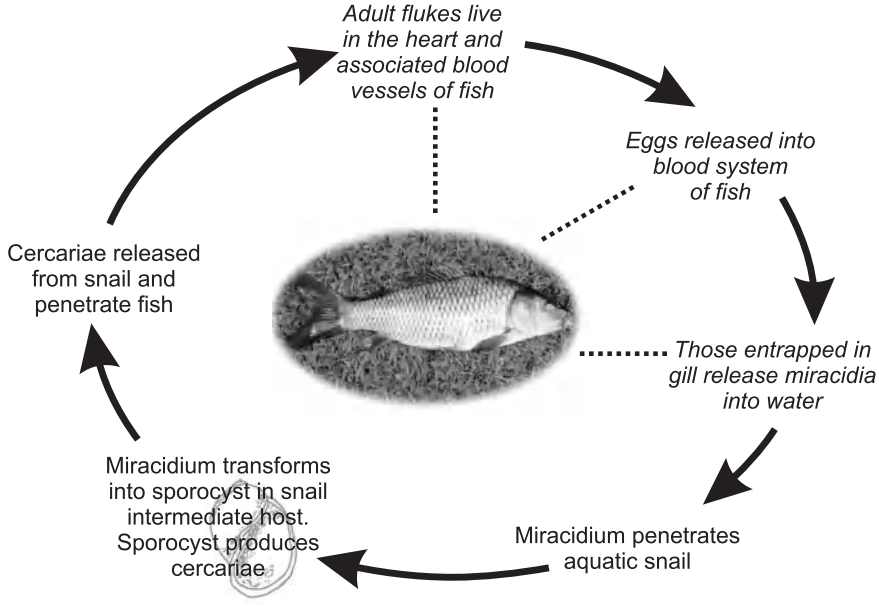



**Fig. 5.17** Life cycle of *Sanguinicola inermis*. Dotted lines and italics indicate stages in which the fish is involved.

the gill tissue by the presence of the triangular-shaped inclusion and the dark pigmented eyespot of the miracidium. In this location the miracidia mature in 7 days at 20°C and are released from the egg, whereupon they migrate through the gill tissue into the water. Unlike the majority of digenean flukes therefore, eggs of *Sanguinicola* are not released from the host. The actual release of the hatched miracidium may cause considerable damage to the delicate gill tissue. Released miracidia pen-

etrate the intermediate host, an aquatic snail. Throughout Europe the pond snails, *Lymnaea peregra*, *L. auricularia* and *L. stagnalis* (Plate 5.21), serve as intermediate hosts; however, curiously the latter species does not serve as a host for *S. inermis* in the UK. Within the snail's digestive gland the miracidium undergoes a multiplication phase, through two further specialised stages of the parasite, termed the mother and daughter sporocyst. The latter releases numerous cercariae (Figs 5.18, 5.19), which are shed from the snail in temperate climates during late afternoon and early evening and penetrate the surface of the fish. If there are a large numbers of cercariae penetrating a single fish then an

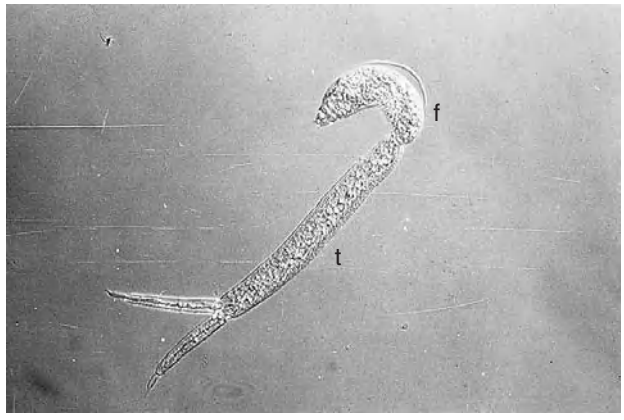



**Fig. 5.18** Unstained cercaria of *Sanguinicola inermis* showing immature fluke (f) and tail (t) used for locomotion by the parasite. Magnification × 200.


**Fig. 5.19** Picture taken on a scanning electron microscope showing immature fluke component of the cercarial stage of *Sanguinicola inermis*. Fluke comprises a fin (f) and tail (t) which is lost when the parasite penetrates a fish. Magnification × 800.

intense pathological response may be evoked and it has been speculated that it may even cause death in young fish. The cercaria on entering the fish tissue loses its tail and the transformed parasite migrates to the heart and develops into the adult fluke.

Although research has established that the fish host is capable of producing an immune response to the parasite this can be ineffectual, allowing the parasite to develop unhindered and produce the next generation of flukes. Recent work however has established that some protection is produced in fish by the initial infection, which reduces the establishment of flukes in subsequent infections. The basis of a vaccine has thus been established. External signs of initial infection with sanguinicoliasis include lethargy and reluctance to feed (Plate 5.22). Once infection has become established and parasite burden increases infected fish begin to swim in spiral movements, become emaciated, dark in colour, develop exophthalmia and bulging opercula. Damage to the gills may cause the fish respiratory distress and to spend considerable time at the surface of the water where the oxygen level is greatest. It should be noted, however, that not all infected fish exhibit these symptoms. The penetrating cercariae and/or the entrapped eggs may cause pathology, and possibly death. The latter causes rupture of capillaries, epithelial hyperplasia and haemorrhage in the gills.

Diagnosis of the disease is by light microscope examination of the dissected fish. The relatively short-lived adult stage can be located in the heart and, with 400–1000 × magnification, triangular eggs can be observed in the gills and several other organs, e.g. liver. Dissemination of the parasite can theoretically occur by movement of water, snails or fish. In practice it is usually the movement of fish that have not been checked for disease that causes the greatest problem and thus the only really effective control is by obtaining fish from disease-free stock. Throughout Europe, however, application of anthelmintics (e.g. Praziquantel™) or molluscicides (e.g. copper sulphate) have been used with some success.

### 5.3.2 Digenean flukes with fish as an intermediate host

Fish can also serve as an intermediate host within the digenean life cycle, usually being infected by the cercarial stage, which has been shed from a molluscan host. Infection can occur from the fish eating the infected mollusc or the cercaria can actively penetrate the surface of the fish. Once within the host the cercaria transforms into a metacercaria (which sometimes is given a specific name appropriate to the parasite species) and can remain either free in some species of parasites or, as is usually the case, becomes encysted in host tissues. Perhaps the most notable example of a group of digenean parasites that remain unencysted as larval stages in the fish are the eye flukes (*Diplostomum* sp. and *Tylodelphys*).

**Unencysted larval flukes: *Diplostomum* sp. and *Tylodelphys* (eye flukes)**
*Diplostomum* and the related eye flukes are ubiquitous parasites occur-

ring in a range of fish species. The disease that they cause, particularly in the case of *Diplostomum*, has been termed diplostomiasis, diplostomastosis, parasitic cataract or eye fluke disease (Plates 5.23, 5.24, 5.25). Over many years there has been much controversy regarding the speciation of *Diplostomum* and whether one species can infect one or several species of fish or whether several parasite species are involved. Regardless of this it would appear that most authorities recognise at least 27 species of *Diplostomum* although the use of enzyme and DNA analysis may clarify the situation in the future. The use of the anatomical location of the parasite as a diagnostic tool is very unreliable as *Diplostomum* sp. have been found in the eye, brain, spinal cord and nasal spaces, unlike *Tylodelphys* spp., which appears to be restricted to the vitreous humor of the eye. *Tylodelphys* spp., the causative organism of the disease tylodelphosis, has been found the eyes of more than 65 species of fish, especially cyprinids. The real difficulties involved in classifying the intermediate stages of digeneans have led to uncertainties about the exact species involved in these infections.

The parasite on which most of the research studies have been carried out is *Diplostomum spathaceum.* This, like all the eye flukes, has a three-host life cycle (Fig. 5.20), two of which are aquatic. The adult flukes inhabit the small intestine of the definitive host, piscivorous birds usually members of the gull family (Laridae), although in some areas of Europe ducks have also been implicated. When the bird consumes an infected fish the metacercaria is released from the fish tissue, becomes activated

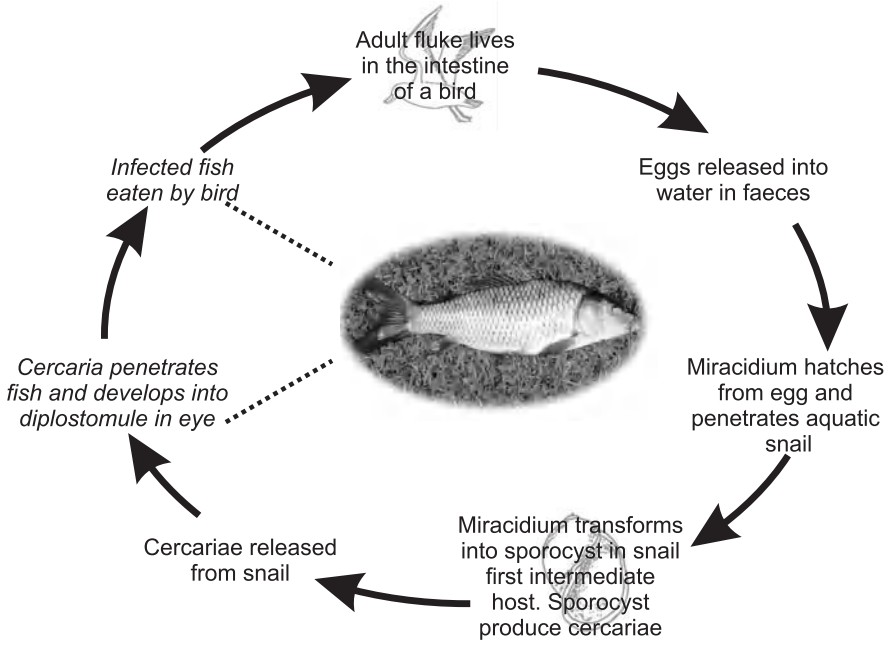



**Fig. 5.20** Life cycle of *Diplostomum* sp. Dotted lines and italics indicate stages in which the fish is involved.

by the bile salts and establishes in the bird's intestine. Sexual development can be completed in three days and eggs are produced for 3–5 months. These eggs are passed out in the bird's faeces into water and release the free-living, short-lived miracidia, which locate and penetrate the snail, the first intermediate host. The most common hosts are *Lymnaea stagnalis* and *L. peregra*, although *Radix ovata* and *Galba* sp. have also known to be infected. Within the snail the mother and daughter sporocysts are formed that produce cercariae, which are shed into the water. These locate and penetrate the fish possibly via the gills, buccal mucosa or eyes and are transformed into the 'diplostomule'. This migrates by an unknown route to the lens of the eye where it develops for 8 weeks before it is infective. Several species of fish including many cyprinids can serve as a second intermediate host, e.g. roach, common minnow, carp, goldfish, bream, rudd, gudgeon, bleak and asp. *Diplostomum* thus represents one of the most common parasites in freshwater fish and can become well established in a fish population within two years of introduction. Individual fish can harbour large numbers, e.g. 550 have been recorded, which has been suggested to reflect the accumulation of parasites from one year to another without significant loss. Prevalence and intensity of infection of fish increases in summer and autumn and active transmission to the fish usually requires a minimum of 10°C, optimum temperature being 17.5°C and cercarial age of less than 5 hours old. Successful host location and invasion by a swimming cercaria usually means that infection is more likely in the still waters of a pond rather than a stream or river environment.

Pathology is associated with several stages of the infection. In carp fry an infection rate of over 3 cercaria/fish has been shown to be lethal. Parasites in the eye cause blindness by cataracts and lens dislocation or induce an exophthalmic response. Blindness reduces food intake and increases the chance of predation and therefore progression into the definitive host. A word of caution, however: nutritional deficiencies and UV radiation can also induce a similar pathological eye response. Within the eye, as indeed in the whole nervous system, the parasite is residing in a somewhat unusual site. The lack of encystment of the fluke may be due to the fact that the eye is an immunologically privileged site where an immune response is not evoked. There has however, been extensive work on the immune response of fish, particularly in rainbow trout, against the cercarial stage and the transformed parasite. Studies both *in vivo* and *in vitro* have revealed that both cellular and humoral immune responses are evoked with the production of activated macrophages, lymphocytes and antibodies. Several workers have also demonstrated that immunisation of rainbow trout with killed cercariae or metacercariae can confer 70–80% protection against infection. The immunisation-induced immune response probably acts against the invading larvae destroying them before they can complete their journey to the eye lens. Although the basis of a vaccination has thus been established it is very unlikely that this will be forthcoming in the near future.

Preventing establishment of the parasite from infected stock therefore usually represents the best control measure, although this cannot

of course prevent contamination of water bodies with infected bird faeces. The sporadic and unpredictable nature of diplostomiasis means that control is usually adopted rather than prevention. Infection is usually greatest after warm dry winters followed by dry springs. These can lead to an increase in snail numbers and reduce water levels, which possibly increases chances of infection and causes physiological stress on the fish. Infection can be reduced by creating turbulent water, which reduces the chances of the cercaria finding a fish, or by treating the pond with molluscicides or introducing molluscivorous fish such as tench or bream. Infected fish can be treated with Praziquantel (Droncit Bayer™). Palmieri and coworkers in 1976 also suggested a novel strategy of biological control, that of employing hyperparasitism where the microsporidean parasite, *Nosema strigeoidae*, infects the fluke.

**Encysted larval flukes**

It is perhaps somewhat unusual that metacercariae of *Diplostomum* are not encysted, as one would expect that the invasion of a fish by a foreign organism would stimulate a pathological and immunological response. This reaction would serve to isolate the parasite from the host tissue and perhaps eventually destroy it. It is therefore not surprising that the metacercariae of a range of digenean flukes do become encysted in fish, either close to the surface of the fish or within the internal organs.

Blackspot disease

This is a common term given to any larval digenean that on infecting a fish becomes encapsulated in the surface layers of the host and evokes a host response resulting in an accumulation of melanin. This pathological melanisation results in the formation of a black spot, hence the name of the disease (Plate 5.26). This pathological response can be stimulated by several species of fluke, e.g. *Posthodiplostomum cuticola* (syn. *Neasus cuticola*), *Apophallus* sp. and can occur in many cyprinid species, including wild or farm-reared aquarium cyprinids.

*Posthodiplostomum cuticola* is widely distributed and in Europe over 50 fish species are thought to be potential hosts. These include cyprinids such as common carp, bream, rudd, roach and ide. Cercariae are liberated from a snail first intermediate host, *Planorbis* spp. and locate and invade the skin of the fish. The invading larvae encyst in the host and the black spots develop within 33 days of infection at a temperature of 22°C (Plate 5.27). Herons usually serve as the definitive host. The cysts that form can be quite pathogenic and affect the normal tissue functioning particularly if they are located in sensitive areas, e.g. the gills. Dark cysts have a diameter of 0.69–0.99 mm and contain a metacercaria that lacks suckers. The other blackspot-inducing parasite, *Apophallus* sp., has, in comparison, smaller cysts, e.g. 0.14–0.28 mm. In some instances infection can be so great that an uninfected fish is the exception, e.g. Spree-Havel Lake in Germany. *Apophallus muehlingi* has been found in a variety of cyprinids, e.g. rudd, roach, bream, asp and tench and uses *Lithoglyphus naticoides* as the first intermediate host and

gulls and mammals as definitive hosts. When the fish is eaten by the definitive host, a piscivorous bird, the digestive process releases the encysted metacercaria, which then attaches to the host's gut wall. Predation by the definitive host is probably dramatically increased by the greater visibility of a fish covered in black spots.

Diagnosis of blackspot is usually by the presence of the dark areas on the surface of the fish. Examination of the enclosed metacercaria is required to establish the causative organism and control measures usually entail obtaining disease-free stock of fish or elimination of snail intermediate hosts.

Visceral and muscle cysts

Trematode metacercariae can also encyst in a range of internal organs or within the muscle of fish. In cases where this occurs in high intensities then organ function and fish health status can be compromised.

Members of the family Strigeidae cause a disease that is usually referred to as 'tetracotylosis'. *Apharyngostrigea cornu* has been reported to occur in roach, bream, rudd, crucian carp, common carp and grass carp where it occurs in the body cavity preferentially encysted on the peritoneum and mesenteries. In severe infections the cysts, which are 680–920 × 390–675 µm in size, form large lumps that can cause numerous haemorrhages and fish death, e.g. grass carp mortalities in Europe. The first intermediate host is a planorbid snail, e.g. *Bathyomphalus contortus*, *Segmentina nitida*, *Anisus vortex* and *Galba palutris*, and fish-eating birds such as herons are the definitive host.

Within this group are the encysted larval flukes that can infect humans. The majority of these infections arise from the consumption of infected under-cooked or raw fish and usually occur in the intestine and associated organs.

## 5.4 CESTODES (THE TAPEWORMS)

To the nonscientist there is perhaps no other parasite that, as a single organism, induces such revulsion as the tapeworm. The usual large size and hence conspicuous nature of the parasites in fish makes anglers, fish farmers and aquarists immediately fear the worst scenario of decrease in fish condition or death with subsequent loss of stock and profit. That this fear is not unfounded has been highlighted on two occasions in which massive fish deaths caused by cestodes, e.g. *Bothriocephalus* and *Khawia* are recorded. Even if fish mortality does not occur, the presence of cestodes, such as *Otobothrium penetrans*, *Nybelinia surmenicola*, *Pyramicocephalus phocarum* in the flesh of fish may reduce the market value of the fish affected. For example, in New Zealand fisheries the high incidence of flesh parasites reduced demand and hence the catch of barracuda. Surprisingly, however, it is only recently that significant advances have been made in our understanding of the interactions between tapeworms and cyprinid fish. This interest has primarily resulted from the increase in transportation, e.g. imports of common

carp has resulted in the introduction of *Bothriocephalus acheilognathi* and *Khawia sinensis* in to the fish stocks of several countries.

The Cestoda or tapeworms, of which there are approximately 3400 species described, 800 occurring in fish, constitute the Platyhelminthes (flatworms), which lead an exclusively endoparasitic life. Although there is much controversy regarding the taxonomy within the tapeworms it is generally accepted that there are 12 orders of these flatworms that occur in fish. Several of these orders, e.g. Tetraphyllidea, Lecanicephalidea and Trypanorhyncha occur in elasmobranchs (sharks and rays) and will therefore not be considered further. However, other orders, e.g. Pseudophyllidea, Proteocephalidea and Caryophyllidea occur in freshwater fish species. The division of the tapeworms into these different orders is based on several variables including body form. The basic body of the adult cestode comprises a scolex (head), which is adapted for adherence of the parasite in the host, usually the gut (Fig. 5.21). The scolex consists of several adhesive structures including hooks, suckers, tentacles or specialised adherence areas, e.g. bothria. The rest of the body, the tape, comprises a series of segments (proglottids) each containing a complete and sometimes doubled set of male and female reproductive organs. Some cestodes, e.g. caryophyllideans that occur in cyprinid fish, lack any segmentation and comprise a uniform, unsegmented monozoic body. Tapeworms lack an intestine and nutritional requirements are met by uptake via the external surface. The life cycle of cestodes involves two or three hosts: a definitive or final host in which the adult parasite occurs and one or two intermediate hosts that contain juvenile stages of the worms, the so-called metacestodes. Fish can serve as both the definitive or intermediate host, and can on some occasions harbour a parasite that does not undergo development. In the latter instance the fish is termed a paratenic host.

The pathology induced by tapeworm infections can be affected by numerous physiological and environmental factors. In general, however, the most intense reaction usually occurs when the fish serves as an intermediate host where the metacestode may become encapsulated both within the body cavity and within various organs. In both types of host (definitive and intermediate) several physiological (e.g. alterations in enzyme and hormonal levels, growth, nutrition, and reproduction) and population effects have been noted.

### 5.4.1 Cyprinids as definitive hosts

***Bothriocephalus acheilognathi* (Asian carp tapeworm)**

*Bothriocephalus acheilognathi* is indigenous to Japan and China where it occurs in the southern area and the Amur River. Classification and names used to describe the parasite have led to much confusion as to its current status in world fisheries. The worm that has been referred to as *B. opsariichthydis*, *B. gowkongensis*, *B. phoxini* has rapidly colonised many countries due to the increase in commercial exploitation and movement of fish, particularly farmed and ornamental carp (e.g. common

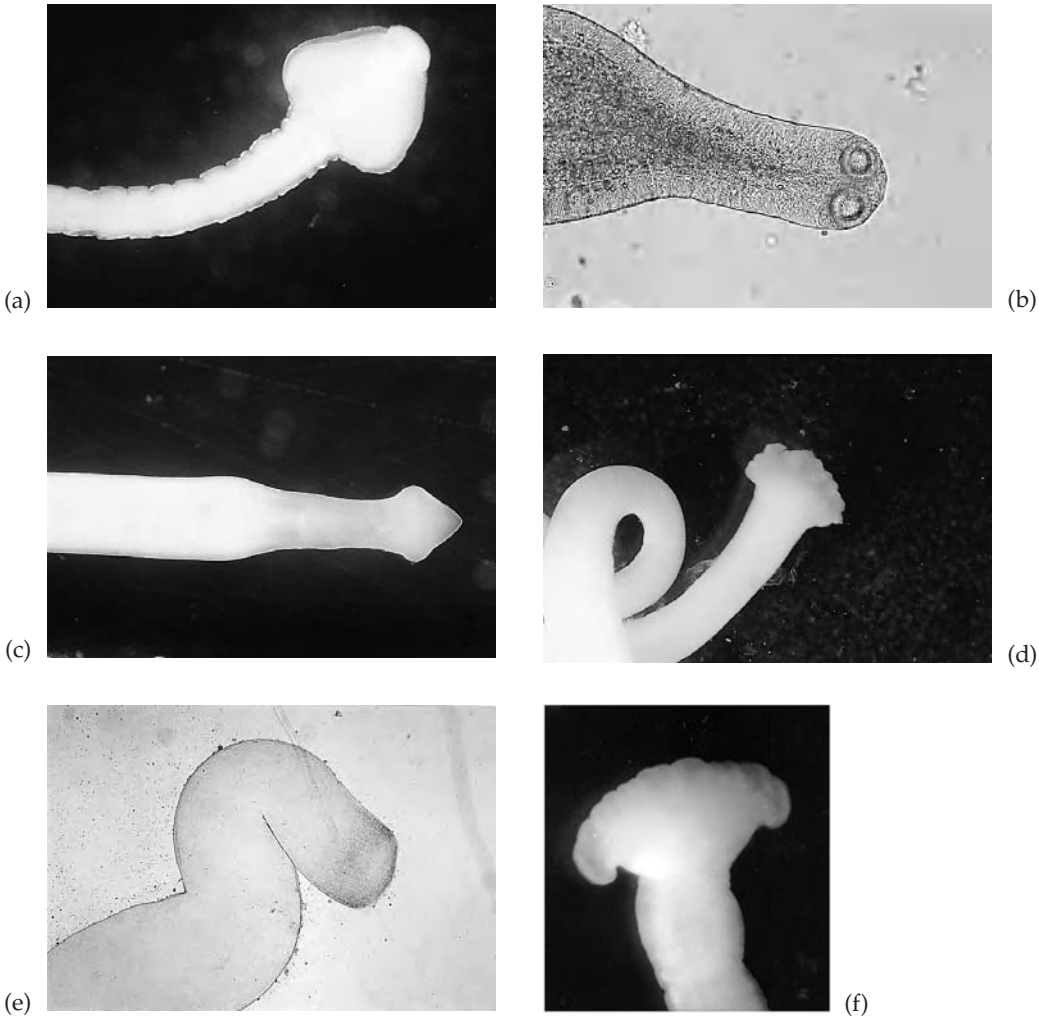



**Fig. 5.21** Scoleces of various cyprinid tapeworms (a) *Bothriocephalus acheilognathi*, (b) *Protocephalus* sp. (c) *Atractolytocestus huronensis*, (d) *Caryophyllaeus laticeps*, (e) *Monobothrium wageneri*, (f) *Khawia sinensis*. Magnification approx. × 60. All specimens are unstained (parts (a)–(e) courtesy of the Environment Agency, UK; part (f) courtesy of Neil Morley).

carp, grass carp). In addition, it has expanded its host range and now occurs in over 40 additional species of cyprinids. It also occurs in representatives of other orders of fish including Cyprinidontiformes (e.g. mosquito fish, blackfin goodeid, plains killifish), Perciformes (e.g. pumpkinseed, *Awos guamensis*, *Hypseleotris* sp.), Atheriniformes (e.g. silverfish *Chirostoma* sp.), Osmeriformes (Australian smelt), Siluriformes (e.g. African walking catfish), Acipenseriformes (*Pseudoscapirhynchus kaumanni*) and even an amphibian (axolotol). It thus represents a threat to stocks of wild, farmed and ornamental fish and has been recorded in endangered fish species, e.g. woundfin minnow, roundtail chub and speckled dace in the United States.

The life cycle consists of two hosts (Fig. 5.22). The adult parasite, which grows up to 320 mm in length and 4 mm in width and comprises a characteristic 'viper-like' scolex with two long attachment grooves, bothria, occurs in the intestine of the fish (Fig. 5.21a). The vitelline, or yolk, glands are evenly distributed in the cortical layer of the segments. Eggs, 0.048 × 0.034 mm in size, are light to dark amber in colour with an operculum and are shed into the faeces of the definitive host. Eggs are sensitive to desiccation and low temperature and die when frozen for 2 hours or within 20 hours when exposed to 20°C. In temperate regions therefore worms are thought to over-winter in the intestine of their definitive host and in young fish two populations of parasite may occur: smaller worms obtained from recent infection and larger individuals acquired the previous spring/summer. Development of the larva, the coracidium, enclosed in the egg is dependent on water temperature and can take as little as 2 days at 25°C to 4 days at 16°C (Fig. 5.23). The intermediate host, several species of copepod, e.g. *Cyclops abyssorum*, *Mesocyclops leuckarti*, *Ectocyclops phaleratus*, *Thermocyclops taihokuensis*, ingests the liberated larval stage in which the procercoid stage of the parasite develops. The fish becomes infected by eating the infected intermediate host.

Several studies have been carried out on the epidemiology and seasonal occurrence of the parasite and it would appear that both transmission and intensity are affected by environmental variables such as water quality and in particular, temperature. For example in Kwangtung Province, China, warm conditions lead to parasite egg production

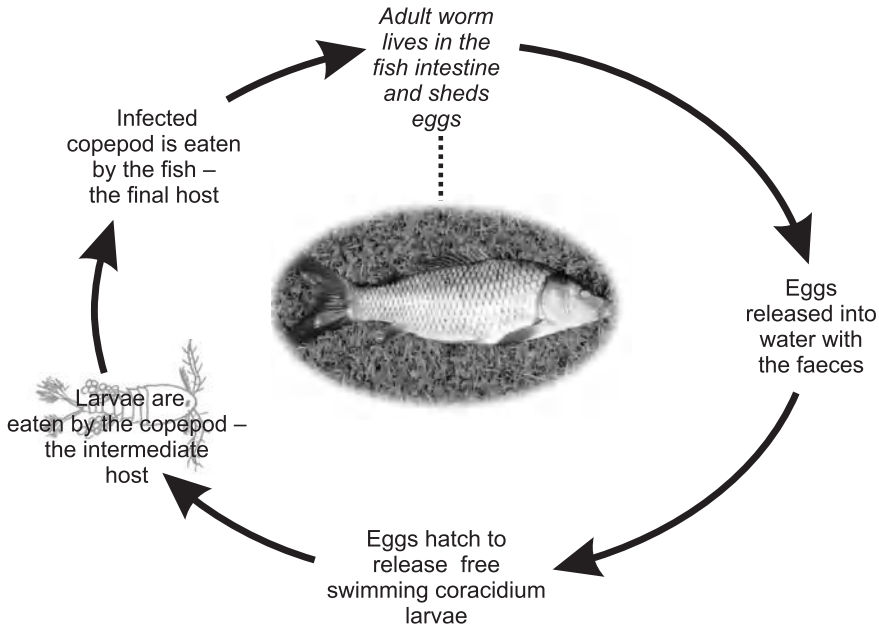



**Fig. 5.22** Life cycle of *Bothriocephalus acheilognathi*. Dotted line and italics indicates stage in which the fish is involved.

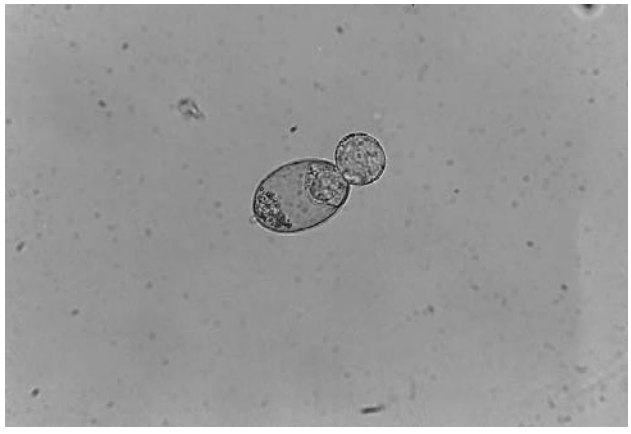


**Fig. 5.23** Hatching coracidium from the egg of *Bothriocephalus acheilognathi*. Unstained. Magnification × 400 (courtesy of Dr Mark Williams).

throughout the year, whilst in the former USSR the intensity and incidence in 0+ carp increases in midsummer and decreases in autumn. In addition, it has also been suggested that the genotype of the fish, which may lead to different expression of the nonspecific immune factor, transferrin, affects susceptibility. Some individuals are therefore more resistant to infection, so-called high responders, than other individuals, so-called low responders.

Symptoms associated with bothriocephalosis include sluggish movements, emaciation and swimming close to the surface of the water. Parasites, which usually accumulate at the anterior part of the intestine, posterior to the common bile duct opening, can cause intestinal blockage, enlargement of the abdomen and on some occasions rupture the intestinal wall (Plate 5.28). At the point of attachment of the parasite the bothria engulf the intestinal folds and induce a local inflammatory response in which separation of epithelial cells or complete loss of the gut epithelium occurs (Plate 5.29). Leucocytes accumulate at the site of infection and an immune response is evoked as indicated by the worm stimulating leucocyte migration, lymphocyte activation and antibody production.

*Bothriocephalus* has been associated with a number of physical and biochemical alterations in infected fish. A reduction in body and organ weight has been recorded together with effects on intestinal enzymes, e.g. trypsin, amylases, and possibly nutrition. In addition, although respiratory enzymes may be affected there would appear to be no alteration in oxygen consumption in infected fish.

Diagnosis of diseased fish entails identifying the tapeworm in the intestine and the preparation of whole specimen mounts for microscopical examination.

Control of the parasite can be directed at either the copepod or fish stage of the life cycle, although it is governed by economic considerations. Ponds can be drained dry and disinfected with lime. Exposure to

the rigours of winter increases the success rate of this treatment. In addition, fish can be given oral administration of a variety of anthelmintics although care must be taken that such substances are ovicidal, i.e. kill parasite eggs, to avoid the discharge of large numbers of infective eggs into the environment when the worm is evacuated from the fish.

***Khawia sinensis* (khawiosis)**

Members of the genus *Khawia* are intestinal tapeworms of cyprinids belonging to the cestode order Caryophyllidea. They were first described in 1935 when Hsü described *Khawia sinensis* as the type-species in common carp at Peiping in the Amur river basin, China. Although there is much controversy regarding classification within the genus *Khawia*, it is generally accepted that *Khawia sinensis* is the most important species and is considered as an economic pathogen throughout Asia, the Far East and Europe. It may however have a wider distribution as indicated in the case of the occurrence of the parasite in the UK. *Khawia sinensis* was first recorded in the UK in 1986 and records in the Natural History Museum in London reveal the presence of the parasite in Great Britain in material collected in the same year. Its introduction may have occurred before this time since *K. sinensis* resembles species of *Caryophyllaeus.*

The adult unsegmented tapeworm occurs in the intestine of a variety of fish species. *Khawia sinensis* primarily occurs in common carp, but it may occur in atypical hosts, e.g. tench and goldfish. In addition, *K. armeniaca* has been found in Lake Sevan, in the former USSR, where it resides in an unusual benthophagus salmonid, *Salmo ischchan*. The scolex of *Khawia* species is typically clover-shaped and is not separated from the rest of the body by a well-defined neck (Fig. 5.21f). Classification of the parasite is however controversial as indeed is the systematics within the Caryophyllidea. Several anatomical structures, e.g. scolex and reproductive organs, have been used to identify *K. sinensis*. The now generally accepted body plan of the parasite is shown in Fig. 5.24. The vitellaria are cortical whilst the testes are located in the medulla region.

The life cycle of *K. sinensis*, shown in Fig. 5.25, comprises two hosts: a definitive host, in the intestine of which occurs the adult tapeworm; and an annelid worm intermediate host in which the procercoid stage of the parasite resides. Eggs (0.04 × 0.03 mm), each with an operculum at one end and a minute knob at the other, are shed into the gut lumen of the fish and are thence passed out in the faeces. In contrast to *K. iowensis*, eggs of *K. sinensis* lack a spine. Embryonic development occurs in water or perhaps within the intermediate host. The rate of development is influenced by temperature, e.g. at 23–24°C development takes 16 days whilst at 10–12°C it takes as long as 57 days. When the egg with the fully developed oncosphere is ingested by an annelid, hatching occurs and the larval stage penetrates into the body cavity and develops into a procercoid. Those species of annelids that are suitable as intermediate hosts include *Tubifex tubifex*, *Limnodrilus udekemianus*, *L. hoffmeiisteri*, *Ilodrilus hammoniensis*, *I. templetoni* and *Euilyodrilus hammoniensis.* Experts

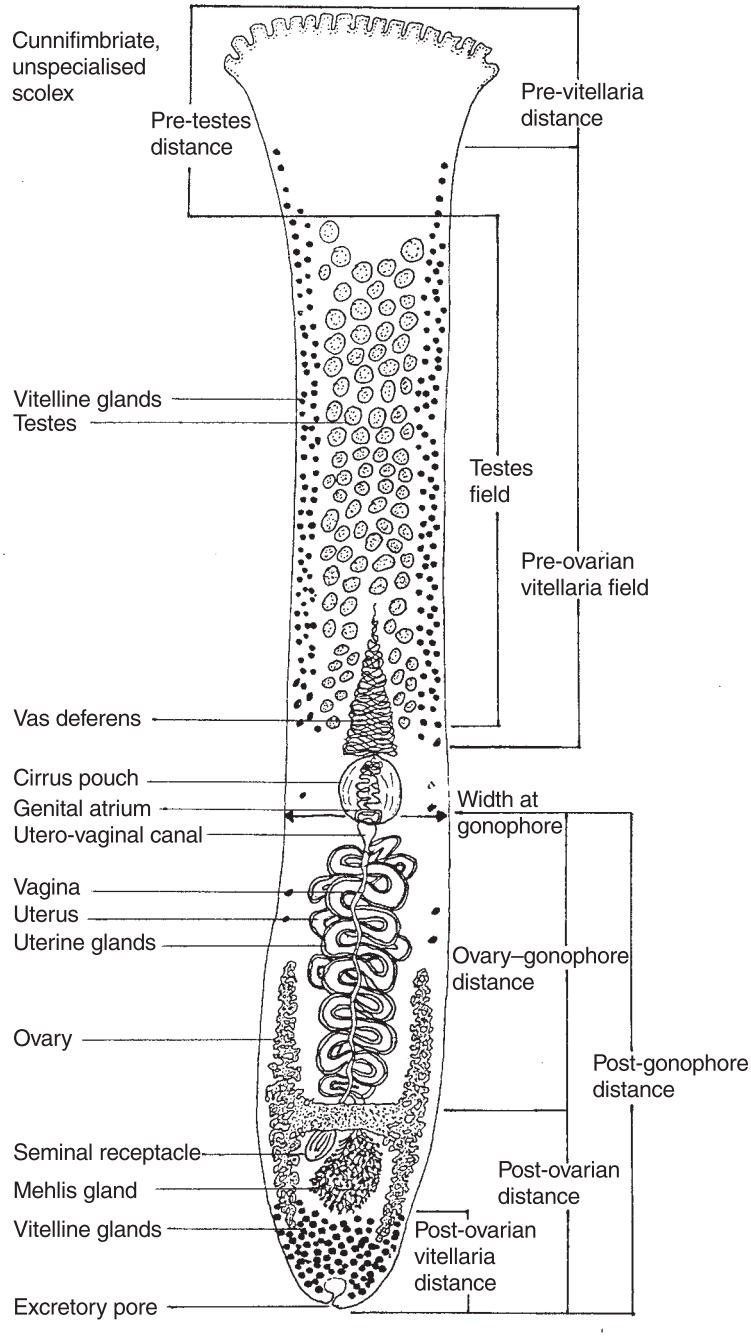



**Fig. 5.24** Diagram of the body plan of *Khawia sinensis* (courtesy of Dr Neil Morley.)

disagree on the length of time the procercoid takes to develop; the range appears to vary between 52 days and 3 months. In addition, the maximum number of procercoids found in the intermediate host differs between natural and experimental infections. In the latter, infected in-

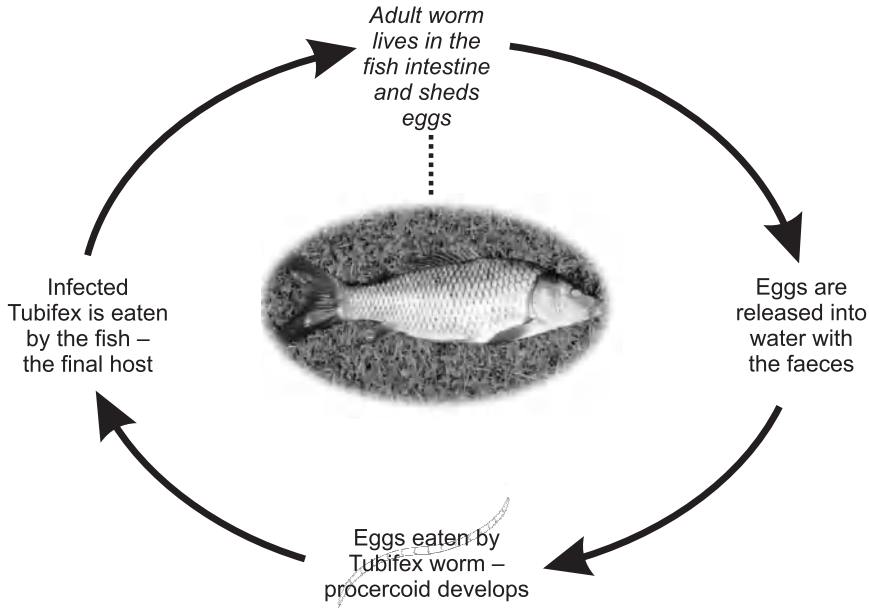



**Fig. 5.25** Life cycle of *Khawia sinensis*. Dotted line and italics indicates stage in which the fish is involved.

dividuals can harbour up to 28–30 parasites, which is substantially greater than the maximum of 7 found in naturally infected hosts.

Transmission to the definitive host, the fish, occurs when the infected annelid is eaten. Development of the tapeworm can take 2–3 weeks at approximately 20°C with an average daily growth of 5mm! Eggs are produced after 7 weeks, but take slightly longer, e.g. up to 11 weeks, at lower temperatures. Survival of the parasite within the fish varies again depending on temperature and host factors. In summer parasites can survive for 40 days but worms can over-winter for 7–8 months. The prevalence of infected fish in a population can range from 4.5–100% with each fish containing 1–455 worms. The colonising ability of *K. sinensis* is illustrated in the Ukraine where the parasite successfully competed with the indigenous tapeworm, *Caryophyllaeus fimbriceps*, replacing it in common carp in several regions.

There is a seasonal occurrence of the parasite in both annelid and fish. In the western Ukraine there is a one-year life cycle where oviposition occurs in April/May from over-wintering adult parasites. The procercoids develop in the intermediate host in spring and summer perhaps, one peak being found in July in carp breeding ponds and two peaks in May and September/October in the growing on and fattening ponds. There is controversy regarding the seasonal dynamics of *K. sinensis* in the fish. In Europe the maximum peak of infection has been noted in 0+ fish in October, whilst in older individuals peak infection has been recorded to occur in May/June. In Japan peaks occur in November with an absence of parasites over-wintering. Recent studies have, however, suggested that in some countries two peaks may

occur with a high incidence in May, followed by a decline to June/July, with subsequent reinfection occurring in July reaching a second peak in September. The variation in the epidemiology of the disease probably reflects various ecological conditions.

Within the fish the parasite is found throughout the intestine primarily being located at the anterior end of the gut (Plate 5.30). The clinical signs of infection include sluggish movements, loss of appetite, emaciation, growth retardation and an anaemic appearance to the skin and gills. In heavy infections the worms can protrude from the anus of the fish and death of the host can occur by the parasites completely obstructing the intestinal lumen. Mortalities have been recorded with infection intensities of 35–45 worms although in carp fry death has been recorded with 3–5 parasites. Localised pathology in the gut has been described and includes lesions, blood loss, inflammation, proliferation of the gut mucosa and increase in mucus production. In contrast, some studies could find no evidence of pathogenicity associated with khawiosis and low mortality of fish infected with *K. sinensis* has been recorded, e.g. in the former GDR. There have been numerous studies on the more general effects of *K. sinensis* on its definitive host. Morphological alterations have been noted in spleen, liver and kidneys and physiological effects on serum proteins and enzyme activity in the intestine, pancreas and liver have been described. Indeed, parasite induced changes in the metabolism of protein in the liver in carp during winter have been suggested to cause a decrease in vitality, and death. In addition, alterations in the erythrocyte and leucocyte component of the blood have also been noted and recent evidence has suggested that infected carp may produce an immune response against the worm.

General control of the parasite usually aims to prevent the formation of new centres of disease and includes the removal of worms from fish before transference. Segregation of fry from adults, control of water supply, surveillance of head ponds and prevention of overcrowding have also been suggested to reduce the effects of the parasite. Destruction of the annelids has also been proposed. Ponds are drained until a soil moisture content of 12% is achieved, the bottom of the pond allowed to freeze and chlorinated lime added. There have been a variety of investigations on the effects of drugs (anthelmintics) on the parasite.

***Caryophyllaeus* spp. (cloverworm)**

This genus of parasites, which gives its name to the order in which it occurs, i.e. the Caryophyllidea, occurs primarily in carp and bream but is also known to occur in other cyprinid species. In some areas of the world it has been designated 'the cloverworm' based on the appearance of the scolex (Fig. 5.21d). However, others members of this group, e.g. *Khawia*, have a similar scolex and therefore the title cloverworm disease is somewhat ambiguous. Three species of *Caryophyllaeus* occur, which differ from *Khawia* in that the former have vitellaria and testes located in the medulla of the segments.

*Caryophyllaeus fimbriceps*, which is usually up to 32 mm in length and 2.3 mm wide, commonly occurs in carp, tench, dace and barbel and occasionally in bream.

*Caryophyllaeus laticeps* is up to 30 mm long and 1.5 mm wide and is principally found in bream and roach, and has an H-shaped ovary.

*Caryophyllaeus fennica* is widespread in cyprinids, particularly roach, and has an inverted A-shaped ovary.

The life cycle of all these species is similar to that of *Khawia* in that the adult tapeworm occurs in the intestine of the fish and the procercoid stage is present in an annelid, usually a species of tubificid. Fish acquire the parasite by eating the infected intermediate host. There has been much work on the seasonality of infection that can be affected by availability of infected tubificids, environmental conditions and host physiological status. In the UK infected intermediate hosts occur throughout the year with the exception of August. Fish only acquire infection between December and March, the parasite matures in April and May and disappears in July. It has been speculated that such a cycle may be affected by fish hormone levels, a temperature dependent immune response and the presence of other parasitic species, e.g. *Ligula intestinalis*. Mathematical models based on the association between *Caryophyllaeus laticeps* and bream have shown that the seasonal variation in the parasite numbers relates to a temperature dependent mortality rate, feeding activity of the host and recruitment rate of the larvae. The increase in intensity and prevalence of infection in fish in spring and early summer has been noted in other countries, e.g. Poland, and has been associated with fish mortality.

Large losses due to infection with *Caryophyllaeus* sp. are rare and usually occur at high intensities, e.g. 300 worms, where the intestine of the fish may be obstructed and exhibit pathological changes, similar to those seen in *Khawia* infections.

***Archigetes* spp.**

In addition to the above, species of the genus *Archigetes* also have an annelid, usually a tubificid, as an intermediate host and represent the only example of a cestode in which sexual maturity is attained in an intermediate host. Several species exist however, where this reduction in the number of hosts has not evolved to extreme and in which the adult tapeworm still resides in a fish gut. In the UK, *A. sieboldi* has been recorded from roach, with the parasite having a fan-like scolex without bothridia and becoming gravid at usually 5 mm long. In North America common carp can serve as a host for *A. iowensis*. Eggs from the parasite are eaten by an annelid host, e.g. *Limnodrilus hoffmeisteri*, in which the procercoid develops, usually within 60 days. The fish becomes infected by eating the infected annelid.

***Monobothrium wageneri***

*Monobothrium wageneri* is a small, white, unsegmented caryophyllidean tapeworm, which can grow to 30 mm in length and 1 mm in width. It appears very similar to *Khawia sinensis*; however, in *M. wageneri*

the scolex is bluntly truncated and reversible in the form of a funnel (Fig. 5.21e). *Monobothrium wageneri* has only been recorded in tench where it is reported to produce a raised plaque-like formation on the gut wall surrounding the firmly anchored scolex. This problem is exacerbated by the tendency of the parasites to attach in one region of the gut (Plate 5.31). Fish become infected by eating infected annelid worms.

***Atractolytocestus huronensis***
This tapeworm, which is synonymous with *Markevichia saggitata*, has only been recorded from carp. It is a small, 25 × 3–7 mm, white unsegmented worm belonging to the Lytocestidae family. Its scolex is bulb-like with a pointed anterior end and a characteristic neck (Fig. 5.21c). The scolex attaches to the gut wall causing displacement of the mucosa, hyperplasia of the submucosal layer and haemorrhaging. The parasite does not appear to be site specific within the gut and can be found throughout the intestine. The parasite has only recently been introduced into the UK, first recorded in 1993, but is indigenous to certain areas of the USSR.

***Proteocephalus* spp.**
Members of this genus have a scolex that comprises four suckers, often with an anterior apical glandular area (Fig. 5.21b). Copepods serve as an intermediate host and several species of *Proteocephalus* have been found to occur in a range of fish species, e.g. eel and stone-loach as well as cyprinids. For example, *Proteocephalus torulsus* has been located in chub, dace and roach. There is a lack of detailed knowledge on the interaction of *Proteocephalus* sp. and their cyprinid hosts. However, studies on *P. exiguus* in northern whitefish (peled) in the USA have revealed infections of up to 1800 worms per fish. Infection can result in a variety of physiological effects, e.g. anaemia and decrease in fat content.

### 5.4.2 Cyprinids as intermediate hosts

***Ligula intestinalis***
This parasite represents probably the most important larval stage of a tapeworm that infects cyprinids. The family Ligulidae occur in both catostomids and cyprinids in both artificial and natural environments and infection can cause significant economic loss and threat to natural fish populations throughout the world and the aquaculture industry, particularly in China and the former USSR. There has been much controversy regarding the taxonomy of the Ligulidae and whilst some countries, e.g. China, propose that several species exist in their fisheries others, such as the UK, consider that only two ligulid worms occur, *Schistocephalus solidus*, which occurs in the three-spined stickleback, and *Ligula intestinalis*, which infests a range of cyprinid hosts. It is possible that in Britain different strains of the parasite occur in roach (Plate 5.32) and gudgeon (Plate 5.33) but, as yet, physiological analysis

has proven inconclusive. Neither has it been ascertained whether gudgeon-*Ligula* can infect roach or vice versa. This is particularly important as the parasite can occur in a diverse range of cyprinid species, for example in the UK, roach, gudgeon, bream, dace and rudd.

*Ligula intestinalis* has a typical pseudophyllidean life cycle (Fig. 5.26). The adult stage of the parasite lives in the intestine of a piscivorous bird. Although this stage of the parasite is relatively short-lived (2–5 days), vast amounts of eggs are produced within this time period, which enter the water in the bird faeces. Eggs hatch, usually within 5–8 days, and release a free-living larval stage, the coracidium. This short-lived stage is ingested by the first intermediate host, a copepod (e.g. *Acanthocyclops bicuspidatus*, *Eudiaptomus gracilis*, *E. graciloides*, *Diaptomus fragilis*, *Mesacyclops leuckarti*) and develops into the procercoid stage in the host body cavity (haemocoel). When the infected copepod is eaten by a fish the parasite burrows through the gut wall and develops into a plerocercoid stage in the body cavity of the fish (Fig. 5.27). This stage has been recorded in numerous species of fish and can exist in the host for more than 425 days. In Europe the parasite has been found in a wide range of cyprinid and goby species. In the former USSR over 49 species of fish are thought to serve as hosts for the family Ligulidae, whilst in the North American continent both the cyprinids (spottail shiners) and various noncyprinids (white suckers, yellow perch, quillback) are successful hosts. In Australasia the parasites have been isolated from noncyprinids (i.e. common bully, the spotted galaxius and the rainbow trout). In China the tapeworm is a problem in the culture of fish

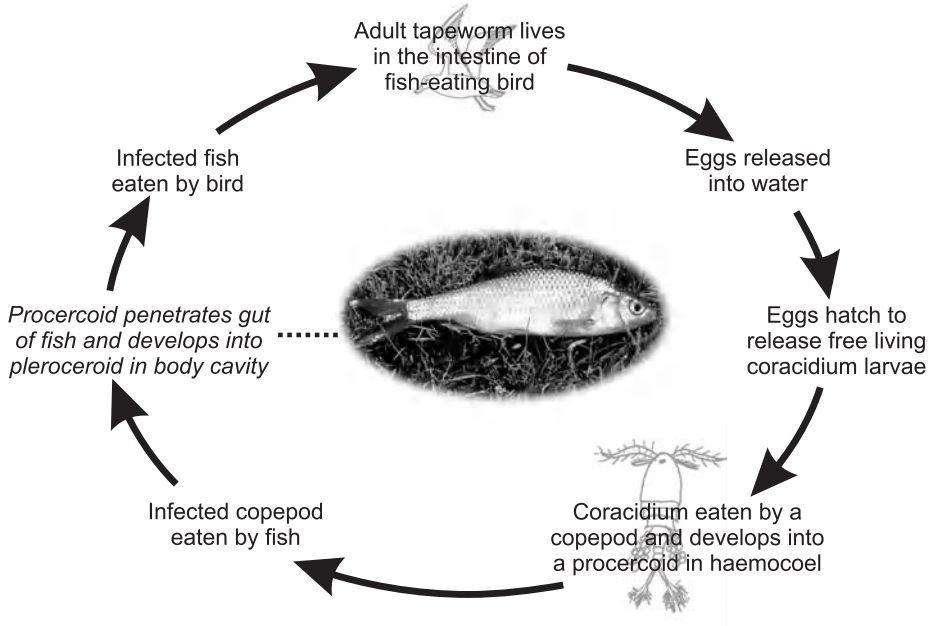



**Fig. 5.26** Life cycle of *Ligula intestinalis*. Dotted line and italics indicates stage in which the fish is involved.

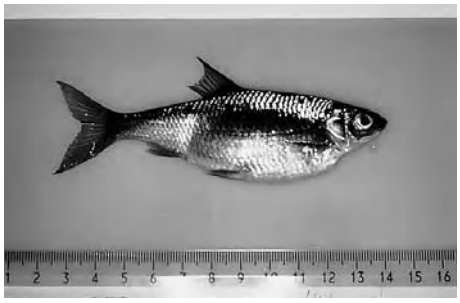
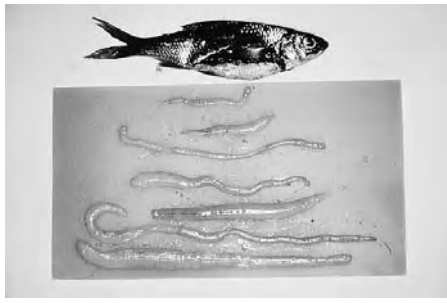
(a)   (b)

**Fig. 5.27** Roach infected with *Ligula intestinalis*. (a) Undissected fish showing gross distension of ventral body and (b) same fish dissected and seven parasites removed (courtesy of the Environment Agency, UK).

belonging to the cyprinid Schizothraciniae. The bird definitive host becomes infected by eating the parasitised fish.

The infected fish can contain a very high parasite burden; the weight of tapeworm can occasionally exceed that of the weight of the host. This is an important consideration to anglers when catching large cyprinids, e.g. roach, as they may actually be catching mainly parasite and not fish! With this amount of parasite material in the fish it is hardly surprising that there have been numerous pathological changes described. These include gross effects on the body such as distension of the body wall, impairment of muscle development and general lowering of condition. The former alteration is very conspicuous and infected fish usually have a very distended body. This probably affects the streamlining of the fish and therefore increases the chances of it being predated by a bird. This increase in the probability of being eaten is further increased by the fact that ligulosed fish usually reside in shallow water adjacent to the bank. The parasite also affects several blood parameters, e.g. cellular composition, serum proteins, haemoglobin content and the physiological status of the fish, e.g. enzyme activities, oxygen consumption, amino acid content, fat and glycogen reserves. Although there is evidence of an immune response to the parasite with the production of aggressive leucocytes and antibodies, these are ineffectual and the parasite is able to have its most serious pathological effect on the host, namely with a decrease in gonad weight and reproductive capacity.

Control of infection is extremely difficult and although the acquisition of disease-free stock can reduce the possibility of infection it will not prevent the contamination of waters by infected bird faeces. Although several anthelmintics have been used, e.g. Praziquantel, their high cost and technical problems associated with their administration usually do not lend them to be considered a viable option in most infected waters.

## 5.5 NEMATODES

Nematodes are known commonly as roundworms and belong to the phylum Nematoda. The adult stage is typically elongated and cylindrical in shape, tapering at each end, and lacks body segmentation. These outward features differentiate nematodes from the tapeworms that, when adult, have flattened and distinctly segmented bodies. In the case of nematodes the sexes are separate, and most species are opaque to white in colour.

The life cycles of nematodes are fairly complex, involving various developmental stages, and require sequential transmission through two or three different host species. In the case of fish parasitic nematodes, the life cycle may involve transmission, for example, through a freshwater invertebrate, then through two fish, the latter typically being a piscivorous species which is the final (definitive) host.

Many of the detailed studies on fish parasitic nematodes relate to those infecting marine fish, as some of these nematodes are capable of infecting man and hence are of medical importance. However, several nematodes are recorded from cyprinids and other freshwater fishes.

Nematodes are rarely life threatening to their cyprinid hosts. They may however damage the parasitised organs and cause general poor health such as emaciation, reduced growth and reduced fecundity. The range and degree of these detrimental effects varies depending upon the nematode species involved, the intensity of infection, and whether the host is final or intermediate.

Cyprinids may be either final or intermediate hosts, depending on the nematode species. For example, bream, dace and orfe serve as second intermediate hosts to *Camallanus lacustris*, the fish acquiring this nematode through eating infected cyclops, which are the first intermediate host. The final hosts to *C. lacustris* are piscivorous fish, notably the pike as well as the freshwater burbot, which contract the parasite by eating an infected cyprinid. In the case of *Philometra abdominalis* (Plate 5.34), this nematode requires only two hosts to complete its life cycle, involving cyprinids as final hosts and cyclops as the intermediate host. Yet another host combination is shown by *Eustrongylides mergorum* in which a cyprinid, e.g. common carp, is the second intermediate host and a piscivorous bird is the final host.

Other nematode species recorded from cyprinids include *Contracaecum microcephalum*, which has been found from the heart and body cavity of fathead minnows and other cyprinids, and *Raphidascaris acus* from the liver, gut and gonads of bream. This latter species is also found in certain species of the Salmonidae, Gadidae (burbot), and Percidae highlighting that some nematodes are not highly host specific. In bream, *R. acus* induces lesions on infected organs, emaciation, exophthalmia and loss of blood into the body cavity.

## 5.6 ACANTHOCEPHALANS (SPINY HEADED WORMS)

Acanthocephalans are endoparasitic worms that occur in the gut of their fish definitive host. They are large parasites, usually between 2 and 3 cm but can attain sizes of over 6 cm. They attach to the gut wall by means of a retractable proboscis, which is covered in chitinoid spines. They have no mouth or gut and so all nutrients are obtained directly through the body wall. Acanthocephalans have a complex, indirect life cycle involving at least one intermediate host, usually microcrustaceans, e.g. amphipods, copepods, isopods or ostracods. The adults show sexual differentiation, the male and female mate in the gut of the definitive host, and eggs that develop in the female are released directly into water in the hosts' faeces. These eggs are eaten by an intermediate host, hatch within its gut and the first stage, the acanthella, burrows into the haemocoel where it develops into a cystacanth. When an infected intermediate host is eaten by a fish host the cystacanth is released from the intermediate host by the digestive process and immediately attaches to the wall of the gut, by inverting its spiny proboscis. Disease problems in cyprinids are rare; however, if the acanthocephalans are numerous, the armed probosces can cause serious damage to the gut wall. In fish an additional problem has been occasionally recorded where the acanthocephalans have migrated through the gut wall and are found in the body cavity or other organs of the host. The most likely explanation for this phenomenon is that the fish may not be the normal definitive host, and although the cystacanth is released from its intermediate host, cues for development and attachment are inappropriate. The parasite may accidentally breach the gut wall in these circumstances, particularly if the gut wall is less robust than that of the normal definitive host.

There are a wide range of acanthocephalans recorded in cyprinid fish but few have been recorded as causing significant long-term problems. As they have relatively simple life cycles it is possible that outbreaks could occur in extensive aquaculture situations. Since both the size and colour of the worms make them obvious, they may reduce the value of any infected fish.

### 5.6.1 *Pomphorhynchus laevis*

There are three biologically distinct strains of *Pomphorhynchus laevis*: a marine strain occurs in the Baltic and North Seas in flounders and plaice, an Irish strain infects rainbow and brown trout and an English strain occurs in barbel and chub. There is no evidence that these isolated 'strains' can interbreed and produce viable offspring. *Pomphorhynchus laevis* attaches to the intestinal wall by a proboscis that has 13–20 longitudinal rows of hooks, with 8–13 hooks in each row (Fig. 5.28). A characteristic feature of this parasite is a bulb at the base of the proboscis that is used to aid attachment. When the proboscis is pushed through the gut wall of the host the bulb is inflated, securing

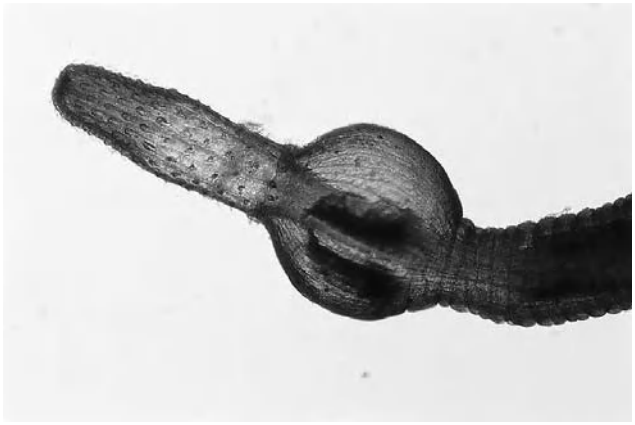


**Fig. 5.28** The anterior end of the acanthocephalan *Pomphorhynchus laevis* from chub showing spiny proboscis and inflated bulb. Magnification × 20 (courtesy of the Environment Agency, UK).

the parasite in position. Adults are orange/yellow in colour and can range from 4 to 25 mm in length. This colour gives rise to the common English name for this parasite, 'the yellow peril' (Plate 5.35).

The definitive hosts of the English strain of *P. laevis* are primarily chub and barbel, but the parasite has also been recorded in substantial infections in grayling, brown trout and rainbow trout (Fig. 5.29). The parasite has also been found in many other fish species where it fails

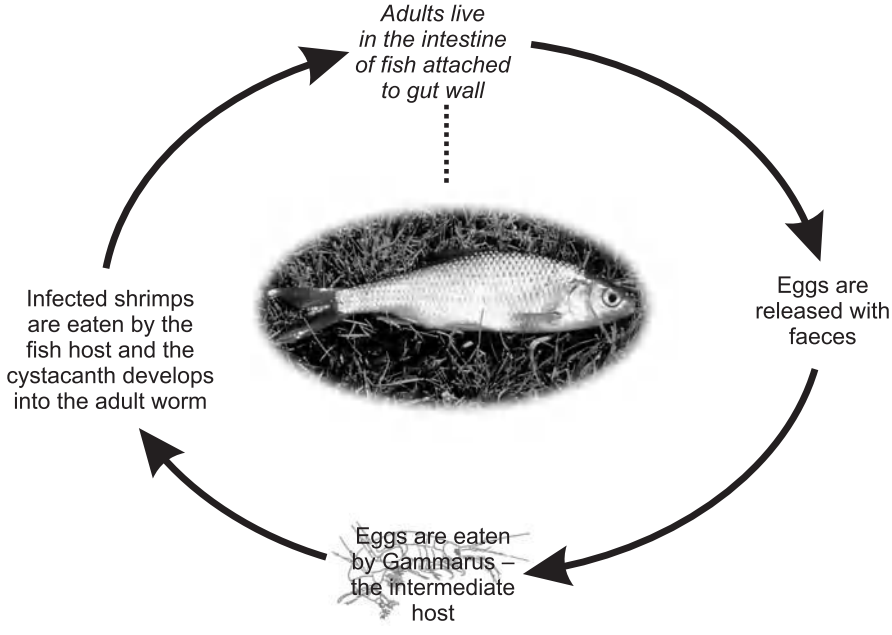



**Fig. 5.29** Life cycle of *Pomphorhynchus laevis.* Dotted line and italics indicates stage in which the fish is involved.

to mature. The eggs when released from the fish, are eaten by the intermediate host, the freshwater shrimp (*Gammarus pulex*), and the larvae hatch in the intestine. The cystacanth stage, which occurs in the body cavity, haemocoel, of the crustacean is bright orange and is clearly visible through the body wall of the shrimp (Plate 5.36). This, in addition to shrimp's behavioural changes, i.e. predisposition to remain in the open water during daylight hours, may increase the likelihood of infected shrimps being predated upon by the fish definitive host in which the adult worm develops attached to the gut wall. Penetration of the proboscis and formation of the bulb cause mechanical damage and inflammation of the intestine wall. Heavy infections can completely destroy the absorptive layers of the gut wall and cause blockages of the intestine. The action of the parasites burrowing through the gut wall into the body cavity may lead to peritonitis. In the UK in the 1970s *P. laevis* was translocated into new water bodies where emaciation and mortality of hosts, particularly barbel, was recorded. Since this time, however, the parasite populations appear to have stabilised and growth and survival of fish does not seem adversely affected.

## 5.7 MOLLUSCA (GLOCHIDIA)

Certain species of freshwater bivalve mollusc utilise fish for the dissemination of the shelled larval stage. Mature freshwater mussels (e.g. *Anodonta*, *Unio*) release their larvae, known as glochidia, into the water. The onset of release may coincide with an increase in water temperature during the spring and summer months, however some species are winter spawners. Evidence suggests that the release of glochidia is heightened when the mollusc is in the presence of a fish, suggesting possible tactile or chemosensory cues. Several cyprinid species serve as hosts to glochidia, however, the fish host range of each mollusc species varies, and most utilise noncyprinids. Glochidia range in size from about 0.05–0.45 mm shell length, depending on the species. The glochidia must locate a fish host and attach onto the skin, fin, or gill epithelia (Fig. 5.30). Studies on the European *Unio* spp. indicate that glochidia must find a suitable fish host within about 3 days otherwise they die. Once attached, they remain parasitic for up to several months and in the case of *Unio crassus*, the glochidia require 27 days on the cyprinid hosts (e.g. rudd, chub) to develop into young mussels. The fish are able to sustain low levels of glochidia without apparent harm, but heavy infestations, especially on the gills of young fish, can cause injury and even deaths.

## 5.8 ANNELID WORMS (LEECHES)

Leeches are parasitic annelid worms belonging to the class Hirudinea, which comprises both terrestrial and aquatic species. Leeches are blood-feeders that are equipped with suckers at each end of the body

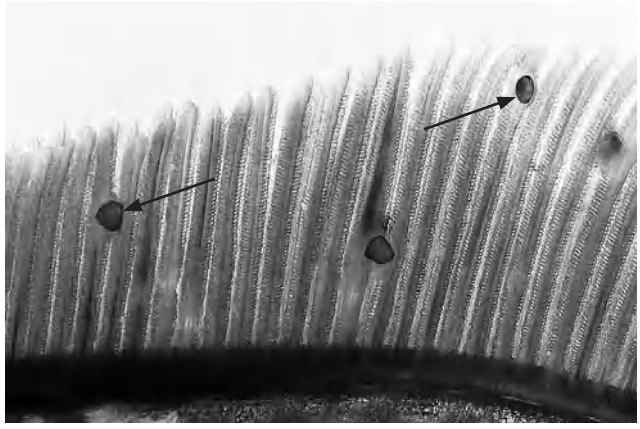


**Fig. 5.30** The glochidia stage (arrows) of the freshwater mussel on a fish gill. Magnification × 10 (courtesy of the Environment Agency, UK).

(oral and posterior suckers) enabling them to maintain a grip on their host (Plate 5.37). Leeches move over surfaces with a characteristic stretching–looping action, and the aquatic forms can also swim in an undulating motion. Several species of leech feed on the blood of cyprinids and other fish, although host-specificity varies according to the leech species. One well-known family of freshwater fish leeches is the Piscicolidae, which includes *Piscicola geometra*, known simply as the fish leech. This species grows to 30 mm in length, but some specimens may reach 50 mm. It lives for less than one year.

Most fish leeches are temporary parasites, detaching from their host after having taken one or more blood meals. When replete, the leech will swim in the water, settling on the substrate where it hides among rocks or plants to digest its meal. The life cycle is direct, with hermaphroditic adults mating and laying eggs in cocoons that are attached to aquatic plants or hard surfaces such as the undersides of rocks. Leeches are more commonly encountered on wild and pond-cultured cyprinids but are rare in aquarium maintained stocks. Leeches themselves are not generally considered to pose serious health threats to cyprinids, being rarely fatal under normal circumstances. In heavy infestations, however, where the amount of blood removed from the host can be significant relative to the size of fish, the host may suffer from anaemia and listlessness. Perhaps of more concern are the leech's bite wounds, which are prone to secondary infections for example by fungi. A more significant health threat however relates to leeches as vectors of fish diseases. Leeches are known to be capable of transmitting certain viruses to fish, such as the spring viraemia of carp virus (SVCv). It is possible that leeches may also be natural vectors of some bacterial fish pathogens, although evidence for this remains scant. In contrast, their role in transmitting various species of blood protozoa, the so-called haematozoic protozoa, is well documented. For example, the leeches *Piscicola geometra* and *Hemiclepsis marginata* are both capable

of transmitting the haemoflagellate *Trypanoplasma carassii* to goldfish, carp and other cyprinids.

As most leeches spend only a proportion of their time attached to the fish, often the only evidence of a leech problem is the presence of circular red or white bite marks on the fish's skin. Distinguishing between the various species of leech is not always easy, and often necessitates expert examination of the worm's external and internal features. It should be borne in mind that not all aquatic leeches feed on fish.

## 5.9 CRUSTACEA

As a taxonomic group the subphylum Crustacea (within the vast phylum Arthropoda) is both large and diverse, consisting of over 38 000 species. Crustacea are primarily aquatic and several species have developed a parasitic lifestyle or close association with fish. A number of these parasites are responsible for diseases of cyprinids, some having provoked considerable attention from ecologists, aquarists and farmers of cyprinids as a result of epizootics in both wild and culture situations. There are few carp farmers worldwide that would not recognise the gregarious behaviour of the 'fish louse' *Argulus* sp. or characteristic appearance of the 'anchor worm' *Lernaea* sp., most from costly experience. Despite being some of the earliest recorded fish parasites and the focus of continued efforts to reduce their commercial significance, little is still known about many parasitic crustaceans. However, with increasing interest in carp fisheries, rising popularity in ornamental cyprinids and steady growth in carp culture worldwide, work is continuing to narrow these gaps in knowledge.

Most crustacean parasites of significance to cyprinids are representatives of the classes Branchiura and Copepoda, the latter containing the majority of species. Although most of the 4500 species of copepod are free-living, a number of species have adapted to a parasitic lifestyle. Of these, members of the families Ergasilidae and Lernaeidae hold particular relevance to cyprinids, as a result of their impact in aquaculture and wild fisheries. Within the class Branchiura there are two families, Dipteropeltidae and Arguluidae, and four genera: *Argulus*, *Dolops*, *Chonopeltis* and *Dipteropeltis*. The most numerous and commercially important parasitic species with respect to cyprinids belong to the genus *Argulus*.

Crustacean parasites display an incredibly wide morphological diversity, so describing a 'typical' crustacean is a near impossibility. However, a number of common characteristics may be outlined. Perhaps most obvious of all is their large size, the adults of most species being easily seen with the naked eye, and their hard exoskeleton. In many species the antennules, antennae, mandibles and primary and secondary maxillae that constitute the five paired appendages on the head region are adapted to parasitic life and are therefore particularly characteristic. Examples of these distinguishing characteristics in-

clude the suckered maxillae of *Argulus* sp. and clawed antennae of *Ergasilus* sp.

The life cycle of crustacean parasites is direct involving only one host (Fig. 5.31). Reproduction involves separate sexes, the males of most species having modified attachment appendages to aid copulation. The production of eggs by the female parasite is followed by temperature-dependent development, after which larval stages emerge. Successive moults take the parasite through a number of larval, juvenile and pre-adult stages before adulthood, the numbers of each differing between groups and species. The point at which the species adopts its parasitic lifestyle varies between species. Copepods tend to become parasitic at the mated adult female stage and *Argulus* species become parasitic immediately after hatching at the first larval stage. The parasitic stages feed on blood, mucus and epithelia.

The effects of crustacean infections on the fish host are often quite similar irrespective of species, being primarily mechanical in nature as a result of the aggressive feeding and attachment behaviour. As crustacean parasites are exclusively ectoparasitic, most primary pathology is associated with the skin, fins and gills, the severity being directly related to the intensity of infection. Disruption of the host integument to varying degrees leads to loss of condition, reduced growth, mechanical damage and osmoregulatory failure. Lesions and haemorrhaging at sites of attachment also typically develop due to secondary infections from opportunist fungi and bacteria.

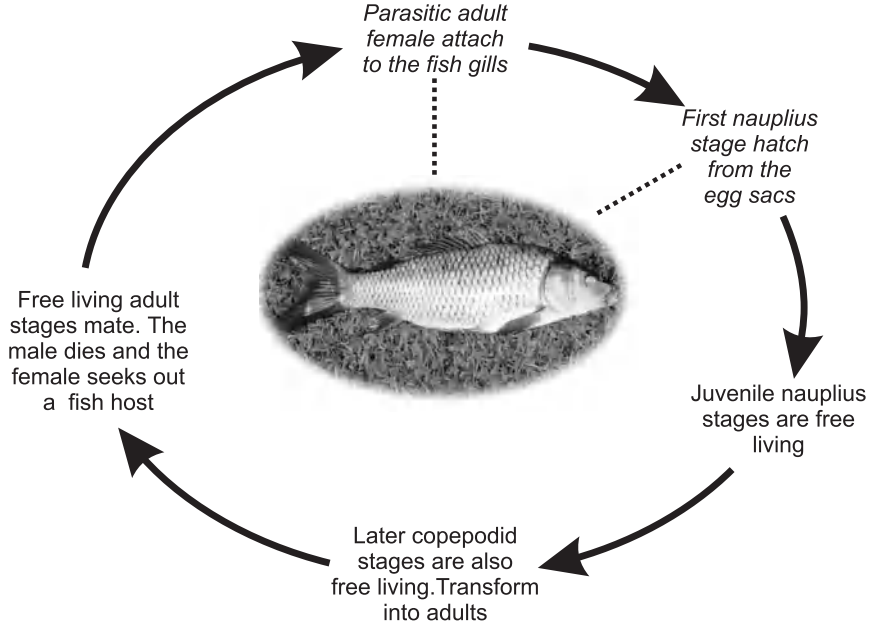



**Fig. 5.31** Life cycle of *Ergasilus sieboldi.* Dotted lines and italics indicate stages in which the fish is involved.

### 5.9.1 *Argulus foliaceus*

Within the Branchiura, the genus *Argulus* represents over 100 of the approximate 150 recorded species. Of these, *Argulus foliaceus* is one of the most common, boasting an extensive distribution, low host specificity and well documented pathogenicity to both wild and farmed cyprinids. Although this low host specificity has led to epizootics in many fish, the occurrence and impact of *A. foliaceus* on cyprinids is such that it has gained the nickname, amongst others, of the 'carp louse'. Native to the British Isles but present in most temperate regions of the world, *A. foliaceus* has made a significant impact on the carp farming industry of east Europe and Asia. The parasite is characterised from other members of the genus by the shape of the abdominal lobes, numbers of setae on the leg segments and morphology of the copulatory structures situated on the swimming legs of males. Speciation, based on female and juvenile morphological features, is generally accepted as being very difficult.

*Argulus foliaceus* has a number of specific characteristics (Fig. 5.32). It has an almost round, dorso-ventrally flattened carapace that engulfs all but the latter appendages and abdominal lobes, which provides a protective and hydrodynamic cover when clamped tightly to the fish. Often green-brown in colour, the slight translucency of the carapace allows the characteristic small black compound eye spots and disc-

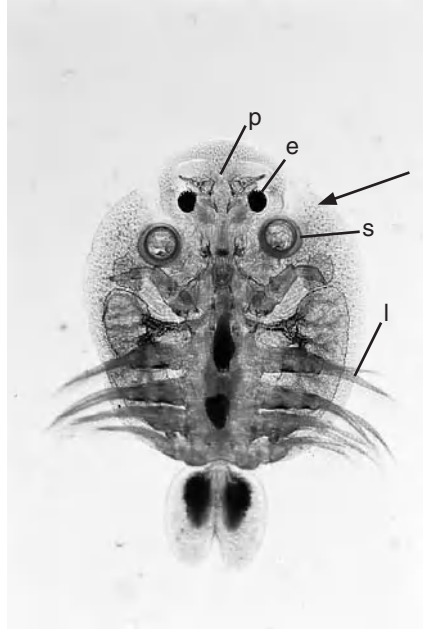



**Fig. 5.32** The crustacean parasite *Argulus foliaceus*. Note carapace (arrowed), black eye spots (e), attachment suckers (s), proboscis (p) and swimming legs (l). Magnification × 12 (courtesy of the Environment Agency, UK).

shaped attachment suckers to be seen *in situ*. These suckered maxillae are characteristic of the genus, allowing firm attachment to the fish as well as being articulated to move like 'legs' and so allow surprising agility. Between the suckers on the ventral surface is the mouth tube or proboscis and pre-oral stylet. Most of the appendages such as the swimming legs, as well as the underside of the carapace, bear numerous tiny spines. These structures, which assist in attachment, also cause host irritation and potential damage.

The feeding mechanism of *Argulus* sp. is very specialised. Once attached to the fish host, nutrition is obtained by a process involving the proboscis and the pre-oral stylet that is inserted into the epidermis. When feeding, this spine is raised from a groove in which it sits, and inserted into the skin. In the past there has been much debate as to the function of this conspicuous structure. Suggestions include that it acts as a blood sucking tube, suction proboscis, tactile sense organ or poison injecting spine. Although still unclear, it has become generally accepted that the stylet has a secretory role, aiding the predigestion of tissue by the release of digestive toxins. In addition, the presence of labial ducts within the mouth tube is thought to further aid tissue breakdown before uptake of the resultant fluids. These secretions are believed to be the primary cause of irritation to the host. However, the repeated insertion of the stylet and rasping of the mouth tube that bears serrated mandibles, are likely to contribute to host discomfort as well as pathological changes.

After copulation, females leave the fish host to deposit eggs in gelatinous 'strings' on any suitable substrate, before returning to the same or new host (Fig. 5.33). This process is unique to the genus, with the eggs of other crustacea undergoing development and hatching whilst still attached to the female. Storing sperm within a collecting duct or 'spermatatheca' a single mating may suffice in all egg depositions a

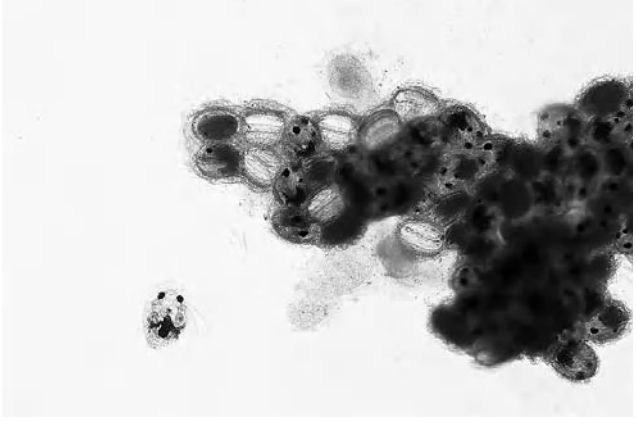


**Fig. 5.33** Egg strip of *Argulus foliaceus.* In bottom left hand corner is a newly hatched parasite. Magnification × 20 (courtesy of the Environment Agency, UK).

female may make in her lifetime. The whitish oval eggs, of which there may be over 500 in a single strip, undergo temperature dependent development, taking as little as 8 days at 26°C, and hatch to release directly parasitic 'metanauplii'. The nauplius, or free-living stage seen in many other crustacean species, occurs within the egg. Measuring little more than 0.6 mm, these metanauplii must find a host quickly to secure survival. It has been documented that chemical, tactile and visual cues play important roles in this process of host location. Attachment of metanauplii to the fish host is primarily achieved by means of hooked maxillae. However, during subsequent moults through nine juvenile stages, these hooks recede to form the paired round suckers, which give the parasite freedom to move about. The whole life cycle may take as little as 40 days under optimal conditions, allowing the completion of four generations in a single season where climates permit.

Seasonal variation of *Argulus* has received considerable attention, and follows a general trend of increasing numbers during warmer summer months, before a decline in infestation with the onset of colder conditions. Below 12°C reproduction ceases, although gravid females can over-winter, laying eggs the following year when temperatures rise.

During adverse conditions, *A. foliaceus* can prove surprisingly resistant, surviving long periods off-host and near drying out. In addition it uses its modified suckers to clamp tightly to the skin, avoiding detachment with considerable tenacity. This resilience may help explain the ease with which the parasite spreads, not only with direct fish movements, but also on nets, plants and in water. In addition, its recorded occurrence on a number of amphibian species allows further potential for dissemination.

Fish suffering argulosis often exhibit behavioural abnormalities during early infestations, including lethargy, irritation and loss of appetite. In heavier infections these changes may be accompanied by excessive mucus, small petechial haemorrhages at the sites of parasite attachment and fin erosion. In severe cases, skin loss may occur, as well as larger lesions, osmoregulatory distress, anaemia and secondary fungal and bacterial infections. Such infestations can severely debilitate individual fish, and quickly decimate heavily infested populations. Although large fish may harbour relatively heavy infestations, juvenile hosts may become moribund with far fewer parasites. Losses of juvenile carp in Asia from intensities as low as five parasites have been recorded and are thought to be a result of osmoregulatory failure. Besides the destructive behaviour, it has been confirmed that *Argulus* sp. acts as a vector for other pathogens, including the viral disease SVC.

Efforts to control argulosis have been considerable in view of the parasites' economic importance. However, despite the introduction of some novel approaches, the control of argulosis in the culture situation still focuses firmly on the use of chemotherapeutics, particularly organophosphate insecticides. In the fishery situation, good management is the only option.

### 5.9.2 *Ergasilus sieboldi*

The family Ergasilidae comprises over 100 species, almost all of which are parasites of fish, and many of which parasitise cyprinids. Two thirds of all ergasilid species are found in freshwater. Each species appears to have a preferred host species and size, and site of attachment, but they will infect other fish. Examples of the ergasilids infesting cyprinids include *Neoergasilus japonicus* on the fins of crucian carp, *Paraergasilus longidigitus* in the nasal cavities of bream (Fig. 5.34) and *Ergasilus briani* between the gill filaments of tench. The ergasilids have been recognised as fish pathogens for a long time. *Ergasilus sieboldi* was first described by Nordmann in 1832 and is commonly called the 'gill maggot' because the large white egg sacs can clearly be seen by the naked eye (Plate 5.38). The adult female is about 1 mm in length. *Ergasilus sieboldi* is widely distributed throughout the world and can be found infecting a very wide variety of species including members of the Salmonidae, Esocidae, Percidae, Siluridae, Cichlidae, and Clupeidae as well as Cyprinidae. Ergasilids exhibit the least adaptation to a parasitic lifestyle of all the parasitic crustaceans; in fact in appearance they are very similar to their free-living copepod relatives, e.g. *Cyclops*. It is the organ of attachment of the parasitic female that shows most adaptation and hence variation and it is the morphology of this that is the usual aid to identification. Males and juveniles are much harder, if not impossible, to speciate. *Ergasilus sieboldi* has been recorded in Britain since 1967 and has disseminated widely, due principally to the movement of fish hosts.

For most of its life cycle *E. sieboldi* (Fig. 5.31) is free-living with six nauplii stages and five copepodid stages leading to free-swimming adult males and females. After mating the male dies while the female, carrying the spermatophores, seeks out a host fish. After attachment

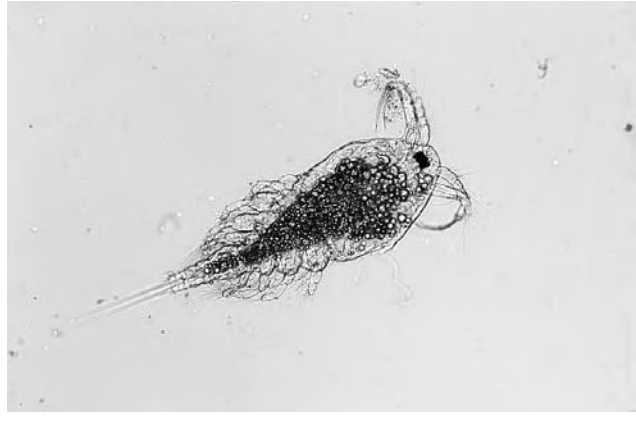


**Fig. 5.34** *Paraergasilus longidigitus* from the nasal cavity of a cyprinid fish. Magnification ×40 (courtesy of the Environment Agency, UK).

the mated adult female is reported to lose the ability to swim although she can move about the gill surface (Plate 5.39). The pair of large antennae have robust claws, which are thrust into each side of a primary lamella. The parasite feeds by damaging the epithelium with rasping mouthparts or scraping legs and then ingesting the debris and mucus. In addition, there may be some extra-buccal digestion using secreted enzymes. Two egg sacs contain 100–300 eggs in which the first nauplius stage develops and hatches out into the water. The life cycle is temperature dependent; in temperate climes there can be three generations a year with egg production occurring from March to November. Females that settle late in the autumn will over-winter before producing eggs the following spring.

*Ergasilus sieboldi* appears to prefer large fish, which tend to carry more parasites. This trend doesn't appear to help the host's survival however as *E. sieboldi* is regarded as the most pathogenic of the ergasilids and intensities of several thousand per fish are often recorded. Heavy infestations lead to serious pathology and even mortality, especially in the summer when water temperatures are highest. Bream, tench and carp are all common hosts, and rainbow trout kept in still waters are particularly vulnerable and often suffer debilitating losses.

Pathology is caused by the aggressive attachment mechanism and feeding action. Both lead to gill hyperplasia, necrosis of tissues and reduced blood flow. Secondary infections of fungus and bacteria are common. Hosts suffer osmoregulatory problems and are very vulnerable to lowered dissolved oxygen levels in the summer months.

The best method of control is to avoid infection by imposing tight restrictions to prevent the import of infected fish into any system. Organophosphate insecticides are used to treat ergasilid infections but these therapeutics are potentially very harmful to the fish, the operator and the environment. The most effective method of eradicating *E. sieboldi* from a lake or fish pond is to remove the water completely, apply lime and leave dry for at least 6 months including one spring period. To limit the impact once these parasites have become established in a water body researchers have suggested reducing the stock level of the more susceptible fish species (e.g. bream and tench). Also increasing the growth of aquatic vegetation can limit the infestation rate as free-living stages of *E. sieboldi* prefer open water. Biological control has been proposed in which flagellate forms of parasitic euglenoids, e.g. *Paradistigma* spp., are used. These invade the eggs of *Ergasilus* sp. and then multiply in the newly hatched first nauplius stage, killing it after 2 or 3 days. This can give a 90% reduction in surviving nauplius stages.

### 5.9.3 *Lernaea cyprinacea*

The family Lernaeidae comprises over 50 species in nine genera. The type species *Lernaea cyprinacea* has been recognised as a problem in carp aquaculture for hundreds of years and is commonly called the 'anchorworm'. *Lernaea cyprinacea* has a worldwide distribution and has

been reported infecting over 40 cyprinid species. It has a very wide host range having been found infecting members of most freshwater fish including cyprinids, salmonids, eels and catfish. It has often been recorded causing heavy losses in a variety of situations. The taxonomy of this group is difficult. The main characteristics used for speciation include the size and shape of the holdfast (Fig. 5.35). However, this structure shows significant morphological differences depending on host species and even the site of attachment. One theory is that *L. cyprinacea* may be specific to goldfish and crucian carp while the very similar *L. elegans* is the species that infects the wide range of hosts.

The life cycle of *L. cyprinacea* begins with three free-living nauplius stages. The following five copepodid stages are all parasitic on the gills of fish but remain mobile. Mating occurs on the fish and mated females either remain on the same host or swim off to find another host. Some lernaeids need two hosts to complete their life cycle but *L. cyprinacea* can complete its whole life cycle on only one host. Attachment can occur anywhere on the fish. The female burrows into the skin of the host and metamorphoses into a long thin worm-like organism with a holdfast buried deep within the host's tissue and its rear end protruding from the fish. The parasite can be up to 12 mm in length and easily seen with the naked eye. Female parasites start producing eggs even before metamorphosis is complete and at water temperatures above 35°C the first nauplii can hatch within two days of initial attachment. The site of attachment may be governed by water flow over the surface of the fish. In lakes attachment occurs all over the body, while in slow flowing water parasites often attach at the bases of the fins.

Although the nauplius stages apparently do not feed, the parasitic copepodid stages feed by grazing the gill tissue. This causes tissue damage and necrosis of the gills and the presence of large numbers, especially on small cyprinids, can cause mortality. The metamorphosing female initially causes a puncture wound as she burrows into the

**Fig. 5.35** Attachment organ (holdfast) of the crustacean *Lernaea cyprinacea.* Magnification × 10 (courtesy of the Environment Agency, UK).

tissue of the host. A scale is often lost and there is more damage to the underlying muscle tissue in which the cephalic horns of the anchor grow. Muscle necrosis, haemorrhaging, inflammation and suppuration are common. Acute pathology is worse if, as often happens, several parasites settle close together. The necrosis of tissues can cause serious lesions that can become secondarily infected by bacteria or fungi. Eventually a connective tissue host response to the presence of the parasite holdfast occurs. Inflammation and then tissue growth around the site of entry encapsulate the parasite's body. Females feed by ingesting tissue debris and blood cells perhaps involving some extra-buccal digestion by secretion of enzymes. Mortality of hosts caused by adult females is generally the result of physical destruction of tissues or secondary bacterial infections.

There is some evidence of acquired host immunity as lower levels of infestation or reduced parasite survival occurs on fish that have been infested before. For example trout, upon reinfestation, suffer most attachment to the eye, an immunologically privileged site and perhaps indicating an activated immune response in the rest of the epidermis.

As with the ergasilids the best method of control is to avoid infestation by preventing the introduction of infected fish into a system. Quarantining fish for at least three weeks at 25°C (where tolerated by the fish) will allow time for any females present on the gills to mature and attach to the skin making them easily recognisable by the naked eye. Organophosphate insecticides can be used, but they are only effective against the copepodid stages so treatment must be repeated every seven days for at least a month at 27°C to kill all the females. *Lernaea cyprinacea* has been reported to develop resistance to insecticides and this therapeutic treatment is not normally an option other than for enclosed systems. Again the most effective method of control of *L. cyprinacea* is to drain the lake or pond and treat with lime. Biological control of larval stages of *L. cyprinacea* by using predatory free-living cyclopoid copepods has been reported as a possibility in Japan.

## 5.10 ZOONOSES

As highlighted in this chapter cyprinid fish can serve both as an intermediate or definitive host in several parasite life cycles. Whilst these cycles may not directly involve humans there are a few parasitic species that can utilise the human body to varying degrees. The relationship between these parasites and the human host may be very transient, as in the case of leeches attaching to the skin, or can be more persistent and thus can induce medically important diseases. Even in this latter instance, the parasite may invade the human body for a limited period and undergo no further development or may actually develop within the human host. Such pathogens are therefore zoonoses. Although in recent years the incidence of these diseases has increased, those arising from fish are relatively few as compared with mammal/human interactions. Fish-borne zoonoses occur mainly in selected

areas of the world where social activities, e.g. eating undercooked or raw fish such as sushi increase the chances of transmission. Cyprinid fish can serve as a source of several medically important zoonoses primarily involving trematodes or nematodes. This section of this chapter is not intended to give an exhaustive account of these parasites nor to give details on the medical condition in humans. For this information the reader's attention is drawn to the further reading at the end of this chapter. In this section it is hoped to highlight the importance of cyprinids in the biology and epidemiology of these medically important diseases.

### 5.10.1 Trematodes

Fish-borne trematode zoonoses occur throughout the world but those originating in cyprinids are of primary importance in the Far East and Southeast Asia. Although the diseases they induce in humans are not usually fatal they can be debilitating and can have social and economic implications, not only to the individual but also the population. Humans usually become infected by ingesting the metacercarial stage present in the muscle or other subcutaneous tissues of fish.

**Clonorchiasis**

This disease is caused by *Clonorchis sinensis*. Adult worms, which usually mature in the bile ducts of rats, cats, dogs and pigs, measure 8–15 mm in length by 4 mm in width. They are characterised by two highly branched testes and a prominent seminal vesicle, although the nomenclature is somewhat controversial. The old name for the fluke, *Opisthorchis*, is still used and in certain areas, e.g. Asia, authorities consider that there are two separate diseases viz. clonorchiasis and opisthorchiasis. Eggs of *C. sinensis* are shed from the fish via the intestine and the enclosed miracidium hatches and infects a snail intermediate host. Several species of snail have been identified as successful hosts of the parasite, e.g. *Parafossarulus manchouricus*, *Bithynia* ( = *Alocima*) *longicornis*, *Bulimus fushsiana*, *Semiisulcospita libertina*, *Thiara granifera*, *Assiminea lutea*, *Melanoides tuberculata*, in which the sporocyst and redial stages develop. The liberated cercariae penetrate the skin of the cyprinid host and develop into the metacercarial stage. Many fish species, particularly the Cyprinidae, have been listed as hosts of *C. sinensis*. Yoshimura in 1965 listed 81 fish species that could serve as hosts for *C. sinensis*; of these the majority, 71 species, were cyprinids. This importance of cyprinids in the transmission of this disease is emphasised when considering areas of the world where the disease has a high prevalence, e.g. in China, Taiwan, and Japan. In addition, cultural practices such as locating latrines over ponds and using human faeces as night soil facilitates the transmission of the parasite. Very little is known regarding the pathological response of the fish to the parasite as most of the research has concentrated on the definitive host, the human, particularly with respect to disease control and diagnosis of the disease.

**Opisthorchiasis**

*Opisthorchis* sp. resembles *C. sinensis* and has a similar life cycle that involves mammals including humans as a definitive host. Two species that are of importance in respect to zoonosis are *O. viverrini* and *O. felineus*. The adults of both species live in the biliary ducts of their definitive hosts (civet; and cats, dogs, foxes and pigs, respectively). The former species differs from *C. sinensis* in that it possesses lobed testes and a different pattern of flame cells (part of the excretory pattern) in both the cercarial and metacercarial stages. The two *Opisthorchis* species differ in that *O. felineus* has less lobate testes, which are situated further from the ovary and also have a different vitellaria distribution. Humans become infected by eating raw or undercooked fish. *Opisthorchis viverrini* predominates in the Far East, e.g. in Thailand a local dish called Koi-pla serves as the source of human infection. In this area of the world important snail hosts include *Bithynia goniomphalus*, *B. funiculata* and *B. leavis* (*B. siamensis*) whilst cyprinid hosts include *Cyclocheilicthys siaja*, *Hampala dispar* and *Puntius orphoides.*

*Opisthorchis felineus* occurs in Siberia and Central Europe where the snail host is *Bithynia leachii* and cyprinid hosts include common carp, bream, barbel, tench, silver bream, rudd and roach.

**Minor trematode zoonoses**

*Echinochasmus perfoliatus* from the family Echinostomidae has been recorded in Leningrad, the Caucasus, Kazakhstan and the Far East and was first recorded in Britain in 1996. The parasite has a typical digenean life cycle. Parasite eggs occur in water bodies and are ingested by a snail intermediate host from which cercariae are liberated. These cercariae swim to find their next host, a fish, and infect the lateral line canal where they become encysted (Fig. 5.36). The next stage relies on infected fish being ingested by a definitive host where the parasite at-

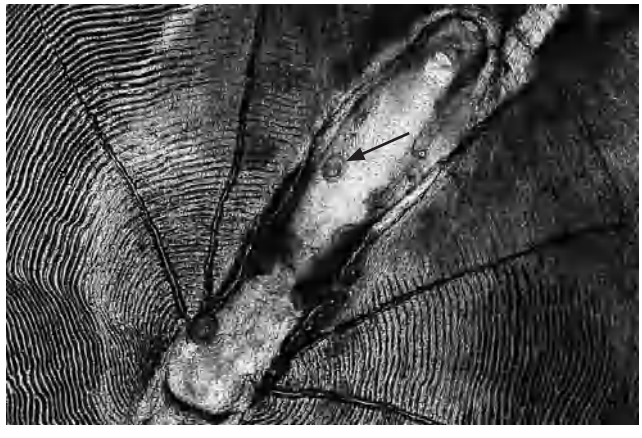


**Fig. 5.36** A scale of a roach showing lateral line canal. Note the intermediate stage of the parasite *Echinochasmus perfoliatus* (arrowed). Magnification ×25 (courtesy of the Environment Agency, UK).

taches to the gut wall and develops into a mature intestinal worm. Definitive hosts include piscivorous mammals, herons and humans. The infected human develops only minor stomach upsets.

### 5.10.2 Nematodes

Nematodiasis, diseases caused by fish-borne nematodes, are usually incidental infections of humans; however, they can cause serious pathogenic responses and even death. Those that infect cyprinids are termed capillariasis and gnathostomiasis; however, it must be pointed out that other fish besides cyprinid fish serve as intermediate hosts.

**Capillariasis**

This is caused by the nematode *Capillaria philippinensis*, which is a trichuroid worm with a narrow and filiform anterior region. The female is usually slightly larger than the male, i.e. 2.5–5.3 mm compared to 1.5–3.9 mm, both sexes being characterised by the presence of a strichosome, a glandular structure located posterior to the oesophagus. Adult worms occur in the intestines of a variety of mammals including humans. After fertilisation, parasite eggs are passed into water where they embryonate and are ingested by a suitable fish intermediate host where the first stage larvae hatch in the intestine. A range of freshwater and brackish fish serve as hosts including cyprinids, e.g. common carp and *Puntius gonionotus.* Capillariasis was originally described in the Philippines but has subsequently been described in the Far East, Asia and the Middle East, e.g. Thailand, Japan, Taiwan, Indonesia, Korea, India, Egypt and Iran.

**Gnathostomiasis**

This disease is caused by members of the nematode genus *Gnathostoma.* Those that occur in humans include *G. hispidium*, *G. spinigerum*, *G. doloesi* and *G. nipponicum* and are characterised by the presence of a headbulb that together with the body cuticle bears rows of spines. The adult worms live in tumour-like nodules in the stomach or oesophagus of a range of mammalian hosts including humans. Parasite eggs pass out of the host via the intestine and, after embryonation in the water, are ingested by an intermediate host, a copepod. Several species are known to serve as host, e.g. *Cyclops strenus*, *C. vicinus*, *Eucyclops serrulatus*, *Mesocyclops leuckarti*, in which a first stage larva develops. When the infected copepod is eaten by a fish the second stage larva enters the muscle of the fish and develops into a third stage larva, which is infective to the definitive host. Cyprinid fish, e.g. common carp and goldfish, can serve as secondary intermediate hosts. Gnathostomiasis occurs throughout China, Japan, Korea, the Indian subcontinent and the Middle East. Prevalence increases where raw fish is part of the diet, e.g. in central Thailand raw fish dishes called Hu-Sae, Som-Fak and Pla-Som are eaten.

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 6
# Noninfectious Diseases

Noninfectious diseases are those that do not involve a pathological agent. A wide range of noninfectious diseases has been documented for both wild and captive cyprinids, and include tumours (neoplasias) and developmental and physiological diseases. In addition, physical injuries and attacks by predators and pests will also be considered here. Environmental factors, notably adverse water conditions, which are a major underlying cause of noninfectious diseases, are also considered.

Noninfectious diseases can affect virtually any part of the fish's body, either external or internal, sometimes both. For example, various tumours have been associated with most organs and tissues in cyprinids. Generally, however, it is only those diseases that manifest as external symptoms that are detected during routine visual monitoring of fish. This is particularly the case with nonlethal developmental diseases, those causing obvious bodily disfigurements, such as distorted backbones or missing opercula, being the most commonly reported. In terms of injuries, such as arising from predator attack, it is typically the external surfaces of the fish that incur the majority of the damage. Sometimes, a noninfectious disease or injury can predispose the fish to an infectious disease, an example being damage to the skin epithelium as a result of rough handling which can lead to invasion by opportunistic pathogens such as Oomycetes (fungi).

## 6.1 SELF-INFLICTED INJURIES

Fish are prone to accidentally injuring themselves, just as any other animal. Physical injury may be sustained during normal swimming behaviour, but is more likely during bouts of heightened activity, such as fleeing from a predator, engaging in spawning chases, or when rubbing its flanks against the substrate in an effort to dislodge irritating parasites. The topology of the aquatic environment can itself damage the fish's skin. For example, in natural waters, human litter such as broken glass and pieces of metal increases the risk of cuts and abrasions, particularly to those cyprinids that forage over the substrate. Under captive conditions, notably in aquaria, injuries to the mouths of goldfish have resulted from the use of sharp-edged substrates such as sharp gravel. Resulting damage to the skin around the mouth predisposes the fish to microbial infections, such as mouth rot. The use of

rounded gravel is therefore recommended for aquaria housing goldfish and other cyprinids that have the habit of 'mouthing the substrate'. Those cyprinids that spend a significant proportion of the time in contact with the substrate, such as *Garra* and *Rhinogobio* spp., are also prone to ventral injuries caused by sharp substrates. Goldfish that are housed in aquaria and other substrate forages are also prone to getting pieces of gravel lodged in their mouths. Affected fish may initially try to dislodge the stone by a choking reflex, manifested by a pronounced opening and closing of the mouth, often with a backward-jerk swimming action. If the stone is sufficiently large then its weight may cause the fish to swim with a slight head-down posture. Removal of the foreign body may require human intervention, involving the fish being held in a net whilst a pair of fine, blunt forceps are gently inserted into its mouth to extract the stone. During this procedure the fish should be anaesthetised in order to reduce the risk of injury. It is not clear whether choking on pieces of substrate commonly occurs in wild cyprinids.

### 6.1.1 Injuries and deaths due to jumping (escape responses)

Certain toxins and other adverse water conditions may cause an avoidance response by fish, which in extreme situations can result in the affected fish attempting to leave the water. This avoidance response to adverse environmental conditions is a common cause of fish deaths arising from jumping onto land. Under pond conditions, particularly agile cyprinids such as orfe may try to escape from an environment with low dissolved oxygen, such as may occur during a summer thunderstorm. Those escapees that do not happen to flip back into the water will eventually die of hypoxia as a result of damage to the gill lamellae. Intensive husbandry practices may subject cyprinids to chronic or acute stressors, which will also increase the likelihood of jumping. For this reason, intensive holding facilities such as aquaria and transportation containers should be adequately covered. Since fish are a wild animal many species do not naturally adjust to confinement and when introduced into a new environment may jump out of the water. It is therefore recommended that tanks, etc., should be covered for a few days after the fish have been introduced

## 6.2 INJURIES CAUSED BY FISH AND OTHER ANIMALS

In the wild, and to a lesser extent under captive conditions, cyprinids are prone to injuries caused by other animals. Often such injuries are the result of a failed attempt by an animal to eat the cyprinid, i.e. predator attack, or they can sometimes be due to aggressive behaviour, usually by other fish.

### 6.2.1 Injuries caused by con-specifics and other cyprinids

Cyprinids are generally peaceful, shoaling fishes, and are far less prone to con-specific or interspecific aggression than are, for example, the cichlids (family Cichlidae) or sunfishes (family Centrachidae). However, in some instances, notably during spawning time, injuries have been sustained by female cyprinids during pursuit by male fish. For example, in the goldfish, which is a chase-spawner involving one or more males herding a ripe female into aquatic vegetation where the eggs are laid and fertilised. In these instances, the pursued female can sustain skin damage due to abrasion from the earthen bank or the woody stems of emergent plants. Shoal spawning fish, e.g. roach, can sustain considerable damage during spawning. This damage, coupled with stress induced by spawning, can lead to a predilection to infection, e.g. *Aeromonas salmonicida*. This can lead to postspawning epidemics of roach ulcer disease causing large mortalities. In addition, barbel spawn in pits they have excavated in gravel, and in doing so they are prone to lesions located on their ventral surface. Aquarium observations reveal that a few cyprinid fishes are quite aggressive, at least in captivity. One well-known example is the tiger barb, a popular ornamental fish from Asia, which has a reputation for biting the fins of other fish, including other cyprinids.

### 6.2.2 Injuries caused by noncyprinid fish

Cyprinids share their habitats with many other groups of fish, some of which exhibit aggressive or predatory behaviour, e.g. pike. Major sites of attack are the eyes, which may be permanently damaged or even dislodged, and the fins, particularly the unpaired fins, which may become torn. These damaged tissues are prone to secondary infections. Loss of one or even both eyes may not necessarily result in incapacitation, as fish can rely on sensory cues other than sight for navigation and collision avoidance. Furthermore, cyprinids in general do not rely heavily on sight for food acquisition. Some fish species such as pike and perch feed by swimming into large shoals of cyprinids, e.g. roach. Such behaviour can cause scale loss and induce stress.

Attacks on cyprinids by parasitic fish known as lampreys deserve special mention due to the unusual behaviour of these primitive jawless fish (Fig. 6.1). Lampreys belonging to the family Petromyzontidae are northern hemisphere fishes and include several European freshwater species. The adult stage of the lamprey is parasitic, although some species such as the brook lamprey, *Lampetra planeri*, are nonparasitic and in fact do not feed at all when adult. The adult lamprey attaches usually to the flank of its victim and uses its highly specialised mouthparts for both attachment and for feeding. The lamprey's suctorial mouth is a disc-shaped organ that is equipped with a complex array of horny teeth for rasping at the victim's skin, body fluids, and underlying musculature. Several lampreys may simultaneously feed on a single fish. Lampreys are largely undiscriminating in their choice

**Fig. 6.1** Lamprey: a primitive jawless fish which is a temporary external parasite on a range of fish species (courtesy of Nick Giles).

of prey species, which can include cyprinids. One study of lamprey feeding behaviour in Loch Lomond, Scotland revealed evidence of lamprey attacks on the local roach population, as well as on two non-cyprinid species (powan and brown trout). Fish that have sustained a lamprey attack exhibit tell-tale scarring to the body. Such attacks may prove fatal as a direct result of physical damage or due to infection of the feeding wound.

### 6.2.3 Cannibalism

The eggs and fry of cyprinids are prone to predation by other fish, including those of their own species. The likelihood of predation is relatively high in cyprinids as compared with fish groups that exhibit parental care and protection of their progeny, such as the cichlids.

It should be mentioned that very few cyprinids are themselves entirely piscivorous, two exceptions being the Colorado squawfish, an endangered species, and the Chinese *Elopichthys bambusa* which may reach 2 m in length.

### 6.2.4 Injuries caused by species other than fish

Throughout their range, cyprinids are exposed to predation by many groups of animals, both aquatic and terrestrial species. These include various piscivorous mammals, birds, reptiles and amphibians as well as a variety of freshwater invertebrates such as certain species of crustaceans and aquatic insects. Apart from causing mortalities, any failed attempt by the predator to secure and consume the cyprinid fish can result in injuries to the prey. Predation attacks are not limited to natural environments. In the case of outdoor aquaculture systems, where cyprinids may be held at high densities, these facilities attract attention from piscivorous animals, notably mammals and birds. Bird attacks

may be intensified during certain routine procedures on the fish farm, for example during pond drainage when the fish become concentrated within shallow water, thereby greatly improving the predator's chances of success. In aquaculture situations where predation reaches a level of economic concern, various deterrents such as traps, poisoned baits, fences and net cover in the case of aerial predators may need to be implemented.

**Mammals**

Piscivorous animals include cats (domestic and wild species), mink, otters, and even certain bats. It is known that both cats and otters may kill cyprinids without eating them, and hence these two mammals are capable of causing far more damage than their size would otherwise suggest.

**Birds**

Many species of bird are piscivorous, well-known examples being gulls, grebes, herons, cormorants and kingfishers (Fig. 6.2). Ornamental pond fish, notably goldfish and koi, are particularly susceptible to bird attack due to their typically bright colours which make them conspicuous from the air. The heron (*Ardea cinerea*), in particular, is renowned for visiting ornamental ponds and taking or injuring ornamental carp. This species is capable of consume cyprinids up to 20 cm in length, but may attack fish much larger causing stab wounds. In Europe, other large avian piscivores for example cormorant (*Phalacrocorax carbo*) are known to cause wounding to cyprinids, and there are documented cases of cormorants inflicting deep skin wounds to fish within a coarse fishery comprising mostly cyprinids.

**Fig. 6.2** Bird attack on a carp. Note white scars (arrowed) on dorsal surface of fish (courtesy of the Environment Agency, UK).

**Fig. 6.3** Male frog in amplexus on the head of an ornamental koi (courtesy of Ron Griggs).

**Reptiles and amphibians**

Certain reptiles and amphibians, for example various newts and the African *Xenopus*, are known to predate on cyprinids. A somewhat bizarre form of injury, though far less common, is attributable to male frogs. During the mating season, male frogs have a tendency to clasp at moving objects in an attempt to secure a female: even a stick placed in front of a lone male may elicit this response. Male frogs may inadvertently seize a fish, mistaking it for a mate. Relatively slow-moving pond fish such as common carp, goldfish and koi appear more vulnerable to such amorous amphibian advances. The frog's amplexus grip may be quite tight and is usually targeted around the fish's head region, such that the frog's hind legs may restrict movement of the fish's opercula, causing respiratory difficulties and possibly death from hypoxia (Fig. 6.3). The frog's forelimbs may also grip around the vicinity of the fish's eyes causing ocular abrasions.

**Aquatic invertebrates**

The predatory aquatic stages of certain insect species are known to prey on small cyprinids and other small fishes. Notable aquatic insect predators are the nymphs of dragonflies (order Odonata) particularly the larger nymphs which can grasp fish of up to 2 cm or more in length. The dragonfly nymph captures its prey by rapidly projecting an elongate pair of fused jaws located ventrally on its head. Their prey, which is grasped by terminal hooks on the jaw apparatus, is then pulled to the insect's mouth. Among those insects which are aquatic when adult, some, such as the water scorpions (family Nepidae), and giant waterbugs (family Belostomatidae) also include small cyprinids and other fish within their diet. In addition, diving beetles, e.g. *Dytiscus* spp., may predate cyprinid fry and therefore must be controlled in fry ponds. Another well-known aquatic invertebrate that may occasionally capture tiny fish is the freshwater coelenterate, *Hydra*. Reaching only a few

millimetres in size, the *Hydra*'s prey is generally limited to *Daphnia* and other plankton, but it can capture fish fry. Large numbers of *Hydra* may sometimes occur in cyprinid rearing facilities (e.g. ponds and aquaria) and can have a significant impact on reducing fry numbers. Piscivory is also known among some arachnids, notably the fishing spiders (family Pisauridae) which enter the water and catch small fish. Among the larger invertebrates, various species of freshwater crayfish and crabs will also capture cyprinids if the opportunity arises.

## 6.3 INJURIES CAUSED BY HUMAN ACTIVITIES

The human exploitation and modification of natural bodies of freshwater has created new hazards for cyprinids and other fish, such as caused by weirs and pumping systems which draw water from rivers. It is known, for example, that the mesh-screened inlet pipes for water-cooled power plants cause numerous deaths and injuries to cyprinids that have the misfortune of venturing too close. Often the fish is macerated as it is sucked through the grid, but some specimens survive with varying degrees of injury. Unfortunately, there is little published data available to give an indication of the extent of cyprinid losses arising from these man-made devices although it has been suggested that these losses are significant but very localised.

### 6.3.1 Aquaculture practices

Cyprinids held in captivity, whether they are kept for food, restocking as in coarse fisheries or ornamental purposes, are typically subjected to various handling procedures which, if improperly carried out, can result in injuries to the fish. In the case of food fish, the stock may frequently be caught and handled out of water, i.e. for grading, moving to larger on-growing water bodies or for the injection of vaccines and antibiotics. For example, grading the fish can be performed by various methods, some quite primitive, such as hand-sorting by eye, to more elaborate devices such as the simple grid box (e.g. the Bachmeyer apparatus) through which small fish pass while larger ones are trapped. Fully mechanised graders, some of which pump the fish from the pond and pass them through an automatic grader, are also available. None of these grading techniques are totally harmless to the fish, and in some cases significant injuries, such as skin damage, are incurred. Broodstock may additionally be handled during the process of 'hand-stripping' in which the eggs and sperm are expelled from the adult fish by massaging the ventral flanks in the direction of the vent (Fig. 6.4). Rough or clumsy handling, and the use of harsh-fabric nets, can inflict skin abrasions or deeper injuries, which can become infected if not properly treated. Transportation of live cyprinids is yet another stage in the production cycle that may cause injuries if not conducted with care. In the case of carp, these have sometimes been transported 'dry' for short periods, the fish being wrapped in wet cloth, or held in

**Fig. 6.4** Hand stripping of a carp (courtesy of Simon Scott).

baskets. Such practices can sometimes result in significant mortalities. Some cyprinid species, e.g. bream, roach, dace and bleak, are particularly sensitive to movement and large mortalities may occur during transportation. In addition, species should not be moved close to spawning and in conditions at the extreme of their biological tolerance, e.g. roach in hot summers.

### 6.3.2 Angling

There is no doubt that sport fishing inflicts countless injuries to fish. The hooking process inevitably results in tissue damage to the mouth region (Plate 3.10), however the extent to which long-term trauma is incurred (e.g. chronic disability, infection, or even death) is difficult to quantify. It will obviously be greater if the hook remains in the mouth or the more times a single fish is caught. Hook gash injuries around the mouth may temporarily impede feeding or in severe cases may cause permanent loss of feeding ability, resulting in starvation. The injuries are also prone to invasion by pathogens such as bacteria and fungi. There is a need for detailed scientific studies on the short- and long-term effects of hook damage, in order to assess the impact of angling on the health and welfare of cyprinid fish stocks. Other aspects of angling, such the use of keep nets, weighing or photographing caught fish, also warrant scrutiny in terms of possible injuries to the fish. Although limited studies suggest that there is no significant harm incurred some countries control the type of keep nets used.

### 6.3.3 Scientific research

A number of cyprinid fishes are used in scientific investigations. Notable laboratory models are the zebra danio, which is used for toxicological testing, and the goldfish, crucian carp or common carp, which are used for a broad range of scientific studies. Some studies on cyprinid fish have provided an insight into wound healing and the complex physiological changes that are triggered by injury and/or the stress it causes. Given the often intense handling of laboratory fish, which may include repeated out-of-water manipulations, the likelihood of injuries resulting from poor handling is very high. In the UK, there is a legal requirement for researchers who are working with fish to prove their competence in basic handling techniques and an understanding of fish husbandry.

## 6.4 DEVELOPMENTAL AND PHYSIOLOGICAL DISEASES

Developmental abnormalities have been recorded in cyprinids as well as other fish groups, and most studies relate to descriptions of visible physical deformities. Some developmental problems are due to genetic factors, whilst others have been linked with adverse environmental conditions, including the presence of pollutants. However in many

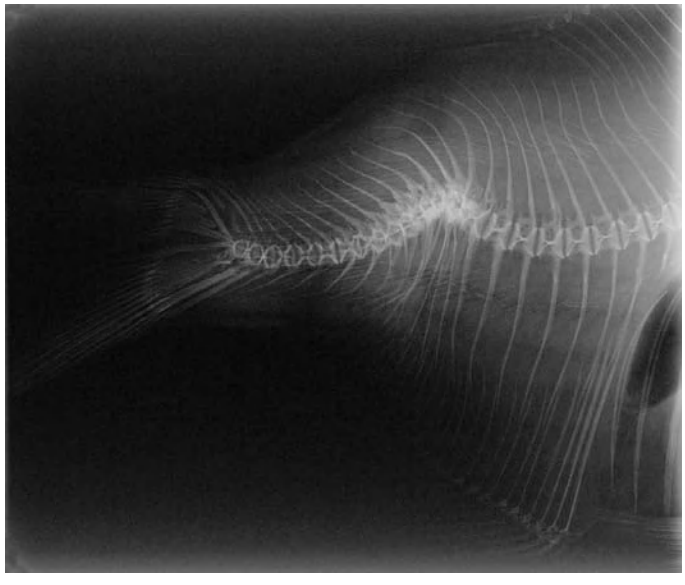

**Fig. 6.5** X-Ray photograph of a common bream with a severe deformity of the posterior section of its backbone.

cases, the underlying cause or causes are unknown although in some instances infectious agents have been implicated.

Deformities of the head, eyes, gills, and in particular the skeletal system are most commonly reported, due to their obvious manifestations (Plate 6.1, 6.2, Fig. 6.5). It appears likely that developmental deformities are responsible for many deaths during the early life history of fish, both in nature and under captive conditions. However, the high incidence of visible deformities among cultured cyprinids probably reflects their increased chances of survival within the relative safety of a captive environment. However, in some cases aquaculture may actually contribute to the incidence of deformities within captive cyprinid stocks, with factors such as unsuitable larval diets, adverse water chemistry, and adverse water temperatures being blamed. For example, research has linked excessively acid pH conditions with the occurrence of spinal deformities in carp embryos. Some of the intensive aquaculture practices which are applied to enhance production have been shown to cause deformities in a range of fish species, and are thus likely to have similar deleterious effects on cyprinids in general. Examples include the use of hormones to induce spawning of broodstock and for promoting larval growth, and the use of manure and other fertilisers in pond culture. As far as wild cyprinids are concerned, it is increasingly recognised that the contamination of freshwaters by various industrial pollutants is causing developmental abnormalities in these stocks.

## 6.5 COMMON DISEASE PROBLEMS

Certain noninfectious disease problems are relatively common, e.g. swimbladder disorders and noninfectious dropsy.

### 6.5.1 Swimbladder disorders

In cyprinids, the swimbladder is typically large and comprises two chambers that connect via a narrow duct. Dysfunction of the swimbladder may cause the fish to float at the surface or sink to the substrate, as a result of over-inflation or collapse of the swimbladder, respectively. Cultivated varieties of goldfish such as 'Orandas' and 'Moors' which have truncated bodies appear more likely to suffer from misshapen swimbladders, predisposing these fish to buoyancy problems (Plate 6.3). Goldfish in general seem prone to swimbladder displacement caused by polycystic kidney disease. Swimbladder inflammation disease has been recorded in several cyprinid species, and appears also to be related to transportation stress. In addition, certain microbial and parasitic diseases can also cause swimbladder damage, and differentiating between infectious and noninfectious causes can be difficult.

### 6.5.2 Noninfectious dropsy

As with swimbladder disorders, dropsy can also arise from one of several causes. Apart from infectious diseases, dropsy may result from physiological disorders of the osmoregulatory system or by tumours or injuries associated with one or more organs of osmoregulation. Senile-induced osmoregulatory dysfunction is a further possible cause of dropsy, which is sometimes seen in captive fish. Fish suffering from dropsy have a distended belly, due to the accumulation of ascites fluid and muscle water content, which causes the scales to raise so as to give the characteristic serrated contour to the body. In some cases, dropsy may coincide with exophthalmia.

## 6.6 STERILITY

Problems with sterility in cyprinids are obviously of great concern to the aquaculturist. Sometimes sterility can be traced to certain infectious diseases of the broodstock whilst in other cases, the inability of sexually mature fish to produce viable eggs and/or sperm may be the result of adverse environmental conditions. In terms of environmental factors, it is known, for example, that the amount of light available to the fish can affect the state of its reproductive organs. This has been clearly demonstrated in the case of goldfish that exhibited degeneration of the gonads after having been experimentally kept in the dark for a prolonged period of time. Other noninfectious causes of sterility include tumours of the reproductive tissues and organs, and senility. In the case of senility, old age is linked with a reduction in egg or sperm counts, and this is a major reason why aquaculture broodstock are used for only a limited period before being replaced.

### 6.6.1 Hybridisation

Another cause of sterility is hybridisation. Interbreeding between different species of cyprinids may give rise to infertile hybrids. Hybridisation may be artificial in that it is occasionally carried out for aquaculture purposes, e.g. hybrids of various Indian carps, or it may be a totally natural phenomenon. Natural hybridisation can occur for example between roach and bream and in various North American minnows which sometimes interbreed when populations of two different shiner species happen to merge at spawning time. Not all hybrids are sterile, however, for example, the North American finescale dace commonly interbreeds with the northern redbelly dace resulting in progeny which are fertile but all female. These monosex hybrids are able to breed with the males of one or both parent species and in some areas the hybrids outnumber the parental species. For aquaculture purposes, sterility may sometimes be advantageous, for example to prevent uncontrolled reproduction. Artificial hybridisation, e.g. as achieved between several species of carps and gynogenesis which yields sterile

triploid fish are two methods which have been applied to cultured cyprinids.

## 6.7 TUMOURS

Tumours have been widely reported in many families of fish, including numerous cyprinid species. The term 'tumour' can be misleading because all kinds of nodules, lumps and swellings have been described in the literature under this category. In the context of this book, the term 'tumour' refers to 'neoplasm'. A neoplasm is defined as an abnormal mass of tissue which can grow independently and uncoordinated from normal tissue. It can be contained within its own capsule, when it may be benign, or it can be invasive and even metastasise when cells migrate to one or more different organs, and is termed malignant.

In cyprinids, tumours are frequently benign and rarely malignant or metastasise. In 1885, Bland-Sutton, a lecturer in comparative anatomy, recorded several fish tumours, including a spindle cell tumour in a goldfish, from specimen collections at the Royal College of Surgeons of England. In recent years, tumours in goldfish and other ornamental cyprinids are frequently reported (Fig. 6.6). This could be because the keeping of ornamental fish is an expanding hobby, and because of the cost of these fish, more attention is paid to their appearance and health. On the other hand, the increase in reports could be because there is some form of environmental trigger that is allowing these tumours to develop.

A common way of classifying tumours is as follows:

- tumours of epithelial tissues (papilloma, adenoma);

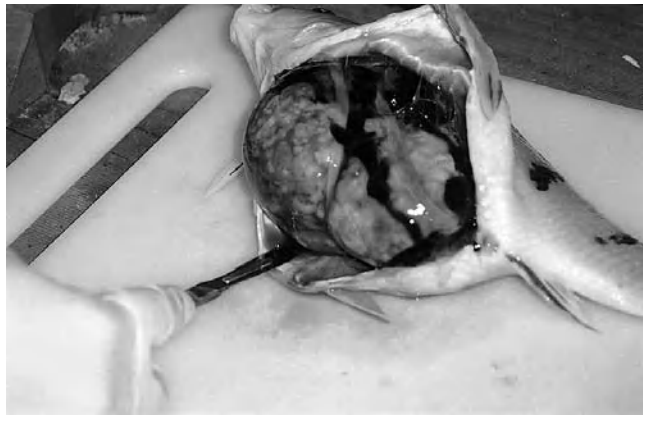

**Fig. 6.6** Tumour mass in the peritoneal cavity of a koi (courtesy of Dr Rohma Newman-Cummings).

- tumours of nonhaematopoietic mesenchymal tissues including muscle, connective skeletal and vascular tissues (fibroma, lipoma, chondroma, osteoma, angioma, leiomyoma, rhabdomyoma, etc.);
- tumours of haematopoietic tissues, that is, lymphatic and other blood forming tissues (lymphoma);
- tumours of neural tissues, including the peripheral nerve system (neurilemmoma and ganglioneuroma);
- special types of tumours, for example from pigment cells and embryos (melanoma, erythrophoroma and teratoma).

A common, but not always accepted rule for plurals of benign tumours is to add 'ata', e.g. 'fibromata'. Similarly, malignant forms of some tumours are termed 'sarcomas' and prefixed by the name of the type of tissue from which they have arisen, e.g. 'fibrosarcoma'. It is not possible to describe all the types of tumours reported in cyprinids, and the reader should consult the further reading section for more information, although some common examples are described in this text. All of the following types of tumours have been reported in cyprinids:

(1) epidermal papilloma, squamous cell carcinoma;
(2) adenoma of the thyroid, kidney, testis, gonad, liver, and gastrointestinal tract and pancreas.

### 6.7.1 Papilloma

Papillomata are wart-like growths on the skin. They can occur anywhere on the body surface, and are most common on the fins and around the oral area. They are considered by some experts to be advanced stages of hyperplasia. However, the relationship is not that simple and remains a controversial subject amongst comparative pathologists. A well-known example of epidermal hyperplasia is the common epidermal condition known as 'fish pox'. This disease is considered to have a herpes-virus aetiology and is known to be transitional in affected fish, and on rare occasions develops into papillomata. It is worth noting the possible differences between hyperplasias and papillomata. There can be a problem when hyperplastic lesions become traumatised through injury, and when inflammatory processes become involved, as these invariably infiltrate into the subdermal tissues. An example of this condition was noted on occasions in an epizootic involving epidermal hyperplasia in a population of barbel in a British river (Fig. 6.7).

Epidermal hyperplasia is therefore a proliferation of simple squamous epithelial cells that extend horizontally with the basement membrane. The cells are uniform in appearance and there are very few mucous cells or other cell types in the lesions. This means that they do not have their own blood supplies or mesenchymal connective tissues. Furthermore, epidermal hyperplasia appears as pale, translucent, slightly raised areas on the body surface, and often at the tips of fins. These lesions can be singular, often circular in shape, or they can

**Fig. 6.7** Epidermal papilloma (arrowed) in a barbel.

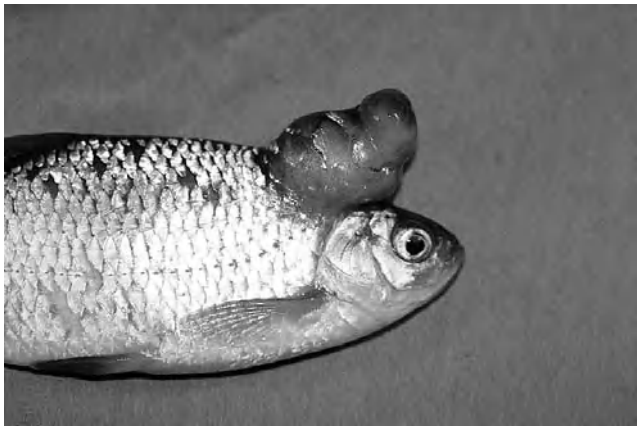

**Fig. 6.8** Epidermal papilloma in a roach (courtesy of the Environment Agency, UK).

be multiple, and in extreme cases, nearly cover the whole body surface of the fish. Epidermal papilloma is usually seen as greyish, translucent, raised growths or nodules (Fig. 6.8). Their main cell-types are again simple squamous epithelial cells, but instead of extending horizontally along the basement membrane, they are sometimes thrown up into folds. Those upward cell growths are supported by mesenchymal connective tissues and blood vascular systems (Fig. 6.9). If these papillomata became damaged through injuries, they can become inflamed and haemorrhagic. The aetiology of both epidermal hyperplasias and papillomata is inconclusive, although adverse environmental conditions, including pollutants, have been suggested. On occasions, inclusion bodies, suggestive of accumulations of virus particles, have been described in both epidermal hyperplasias and papillomata. Based on

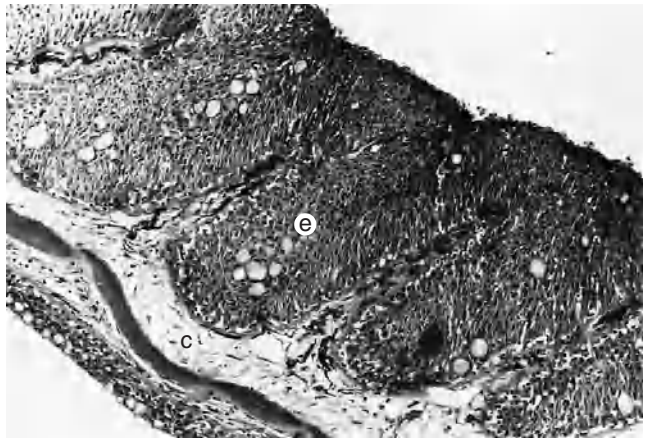


**Fig. 6.9** Section of an epidermal papilloma from a barbel fin. Note the folds of epidermis (e) supported by connective tissue (c). Stained with haematoxylin and eosin. Magnification × 150.

these findings most scientists accept a viral aetiology as a cause of the tumours.

### 6.7.2 Squamous cell carcinoma

Squamous cell carcinomas occur as raised, uncoordinated growths on the body surface, often on the head. Visual examination reveals these lesions to be similar to papilloma (Plate 6.4). However, microscopical examination shows that the squamous epithelial cells are formed into whorls (Fig. 6.10). The lesions are invasive into the hypodermis, and

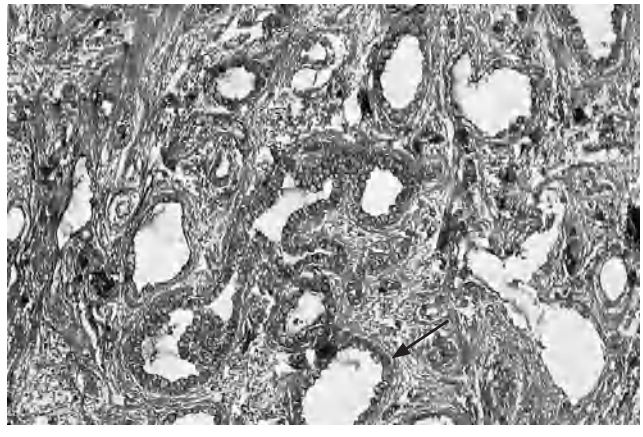

**Fig. 6.10** Section of squamous cell carcinoma in a gudgeon. Note the whorls of epithelial cells (arrowed). Stained with Halmi's Trichrome. Magnification × 150. Slide deposited in the Registry of Tumors in Lower Animals (RTLA) (219) Washington DC, USA.

have good blood supplies and several cell types are included. It is this invasive action and lack of co-ordination of cells that classify this tumour as a malignant lesion. A somewhat similar tumour, also in the epidermis, is a basal cell carcinoma, where the predominant cell-types are basal cells instead of squamous epithelial cells.

### 6.7.3 Adenoma of the thyroid

Tumours involving the thyroid are more controversial than epidermal hyperplasia/papilloma. Probably most of the older reports of neoplasms of the thyroid would not be classified as such if they were reappraised today. This is because they were most likely hyperplastic rather than neoplastic conditions.

Some of this misdiagnosis occurred because scientists did not realise that the thyroid in fish is nonencapsulated and the vesicles, especially in cyprinids, are not only situated primarily between the first and second gill arches, but are also secondarily situated in the kidney, occasionally in the liver and spleen and more rarely in other organs. Thyroid hyperplasia in fish has been attributed to iodine deficiency. Nevertheless, adenoma of the thyroid has been reported in several cyprinids. Although most of the reports are of single fish within a population having a lesion, an epizootic of thyroid tumours has been documented in a population of roach living in a water system which received effluents from a steel processing works (Plate 6.5).

### 6.7.4 Adenoma of the kidney

An epizootic involving swollen kidneys in a population of crucian carp revealed that some of the affected fish had adenomata originating from renal tissue (Fig. 6.11). Interestingly, the majority of the fish had poly-

**Fig. 6.11** Adenocarcinoma in a hybrid cyprinid.

cystic kidneys, a condition fairly common in the closely related goldfish. It is considered that the polycystic condition in fish kidney might be a precursor to an adenoma. However, more frequently in crucian carp and goldfish, the condition is the result of infection with protozoans, e.g. *Sphaerospora* sp.

### 6.7.5 Nonhaemopoietic mesenchymal tumours

Fibroma/fibrosarcoma is the most commonly reported tumour in fish, and has been recorded for well over 100 years in cyprinids. Fibromata, which are mostly recorded in the hypodermis (Plate 6.6), in histological sections, are well defined and consist of whorls of fibroblasts. If they are invasive into surrounding tissue, e.g. malignant, they are termed fibrosarcoma. However, a very similar mesenchymal tumour is the spindle-cell tumour consisting of pleomorphic spindle shaped cells formed into whorls. Such tumours arise from blood vessels and are similar to haemangiopericytomas commonly found in mammals including humans.

A myxoma/myxosarcoma is a variant of a fibroma/fibrosarcoma, and is a tumour that contains mucin. The application of special staining techniques, such as the periodic acid–Schiff method (PAS), will help differentiate such tumours. Osteoma/osteosarcoma is a tumour of bony tissue origin. They are rarely recorded in cyprinids. Rhabdomyoma, a rare type of tumour of skeletal muscle origin, has been reported once in cyprinids, in a tench.

Lipoma is more commonly reported and takes its name from its fat cell origins. The example shown in Plate 6.7 was from a common bream; the tumour measured 8.2 × 9.0 cm, and the fish weighed 4.8 kg. Histological examination of the tumour revealed it to be an encapsulated mass of fat cells, with some interspersed fibrous cell types. There was a well-established blood system within the tumour formation and there was no evidence that it was invasive.

### 6.7.6 Haematopoietic tumours

In fish, these tumours occur primarily in the mesonephros part of the kidney, the spleen, thymus and submucosa of the gut (Fig. 6.12). However, they can occur in any tissue. These tumours are termed lymphoma/lymphosarcoma and in histological section, they appear at first glance as if one is viewing the night sky, and have been described as 'starry sky at night' tumours (Fig. 6.13).

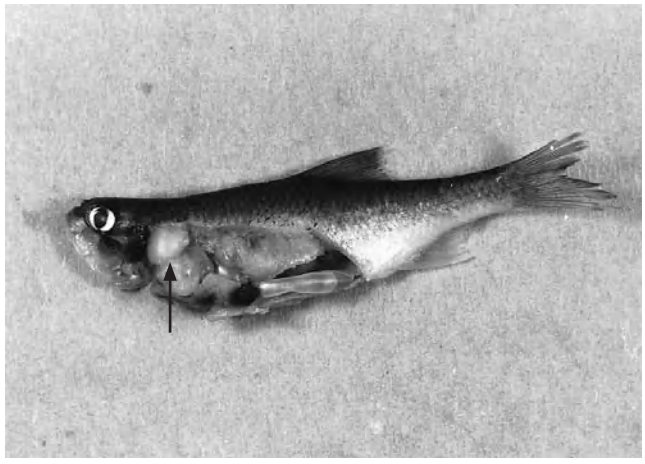

**Fig. 6.12** Tumour mass (arrowed) in the pronephros of roach.

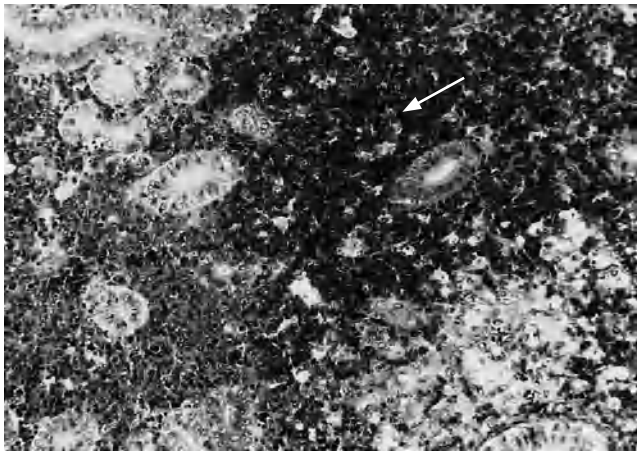

**Fig. 6.13** Section of a lymphosarcoma in roach showing masses of lymphoid cells, likened to a 'starry sky at night' appearance (arrowed). Stained with haematoxylin and eosin. Magnification ×150. Slide deposited in RTLA (1878) Washington DC, USA.

### 6.7.7 Pigment cell tumours

Although melanoma/melanosarcoma tumours are the commonest pigment cell tumours in fish, they are not frequently reported in cyprinids. However, when they are, the orbit of the eye is usually involved, because this is a site of melanisation. It is likely that some form of trauma involving the eye may be the triggering factor for the formation of such tumours.

### 6.7.8 Nervous tissue tumours

Tumours originating from the central nervous system are extremely rare in fish and have not apparently been reported from cyprinids. However, neoplasms originating from the spinal ganglia, which have been reported, and neurilemmoma of goldfish, apparently, are not too uncommon. These tumours which occur on the epidermis and are derived from the nerve endings, are often sited on the dorsal surface of the fish (Plate 6.8). They are usually small, 2–3 mm diameter, greyish in colour, although can have a tinge of pink. They can easily be confused with papillomata.

### 6.7.9 Miscellaneous tumours

Epizootics of gonadal tumours in goldfish/carp hybrids have been reported from the Great Lakes, USA and the cause has been attributed to hormonal imbalances in both males and females. In both sexes, the tumour, which originates from the Sertoli cells, causes sterility in the male and prevents the female spawning, although the oocytes develop to full term stage. Similar tumours apparently occur in goldfish/carp hybrids in the UK. These also occur in nonhybrids, e.g. koi where the cell types are mainly spindle cells found in homogenous sheets of germ cells and other connective tissue cells. Probably more attention is paid to the health and appearance of koi and other ornamental fish and that is why there is an increase in reports of tumours in these fish. However, there could be more insidious reasons, such as genetic imbalances, chemical effects from husbandry procedures or dietary deficiencies. For example in recent years, abdominal masses appear to be frequently reported in koi. The tumour types are difficult to diagnose because the cell-types are always very mixed and there are usually considerable degrees of inflammation and necrosis. Authorities have suggested that the tumours originate from the gonad, kidney, gastro-intestinal tract, pancreas or other visceral organs.

Whether a particular abnormality is inherited (genetic defect) or is the result of adverse environmental conditions during development may not be easy to ascertain. Developmental problems caused by nutritional deficiencies are discussed in Chapter 8.

## 6.8 LONGEVITY AND SENILE-RELATED DISEASES

In fish, as with other animals, old age is accompanied by a gradual deterioration in the efficiency of various organs and tissues and a general reduction in activity. In general, old fish tend to be more sluggish, less fecund, and are more prone to organ failure and infectious diseases.

In the case of wild cyprinids, chronic senile-related diseases are considered to be relatively uncommon, especially among the smaller species. This is because of the increasing likelihood of death as the ageing fish becomes generally weakened and less agile, resulting in a higher

**Table 6.1** Life spans of cyprinids living in the wild.

| Fish | Life span (years) |
|---|---|
| Danionella | <1 |
| Rosefin shiner | 2–3 |
| Bluntnose minnow | 3–4 |
| Northern hogsucker | 11 |
| Tench | 14 |
| Bream | 18 |
| Roach | 18 |
| Barbel | 25 |

risk of predation. As a result, most wild cyprinids are thought to die long before any chronic senility-related disorders manifest. Captive cyprinids, on the other hand, may survive long enough to develop chronic senile diseases, particularly those fish that are held in aquaria or ornamental ponds where food is generally abundant and predators typically absent.

Old age in fishes has been associated with various clinical and behavioural changes, including locomotor dysfunction (e.g. swimming head down; abnormal pitching), paling of skin colour, mild exophthalmia and cataracts. Senile-related disorders of one or more organs associated with osmoregulation can result in an accumulation of fluid within the abdomen, causing the condition known as dropsy. Old fish may suffer from a deterioration in their immune system that predisposes them to infectious diseases. The likelihood of tumours also increases, as shown by the incidence of chromatoblastomas in ageing goldfish. Longevity in cyprinids varies greatly according to species, even when extrinsic factors such as the increased likelihood of predation of smaller species are taken into account. Precise longevity data is usually only possible in the case of captive fish, especially those kept for ornamental purposes. For example, the oldest authenticated record for a goldfish is 43 years, and this relates to a specimen kept as a pet in an aquarium. However, the average life span in captivity for this species is around 25 years for the common goldfish and about 14 years for fancy strains (e.g. Orandas, Moors). Stories of carp living in excess of 200 years are undoubtedly gross exaggerations. Examples of longevity data for cyprinids living in the wild are shown in Table 6.1. The wide range of life spans between these wild species is probably due to a combination of factors, some intrinsic, i.e. innate longevity, others extrinsic, e.g. risk of premature death due to predation.

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 7
# Environmentally Induced Diseases

Most environmentally induced diseases are directly caused by adverse water conditions. Fish do not live in chemically pure water, for all natural water bodies contain numerous other substances which are present in either a dissolved or suspended state. The chemical and physical properties of natural fresh waters vary both spatially (geographical location) and temporally, e.g. diurnally and seasonally.

Fish have adapted to live within a relatively narrow range of water parameters which can be defined in terms of temperature, pH, dissolved oxygen level, hardness, and other physico-chemical properties. There are considerable variations between species in their tolerance range to these various water parameters, to the extent that a given set of water conditions may be optimal for one species but physiologically stressful to another. Temperature tolerance is a prime example: the optimum water temperature for the Indian zebra danio is approximately 24–28°C, whereas this high temperature would not be tolerated by a temperate-dwelling cyprinid such as the gudgeon.

Similarly, there are species differences in terms of tolerance to various pollutants and other stressors. Even for a given cyprinid species, there may be population differences in tolerance levels, and individual fish may even be capable of adapting to water conditions outside of the species' norm. When exposed to extremes of water conditions, or to pollutants, a fish may be able to adapt or it may become stressed with the risk of developing pathological changes. Adverse water conditions may be described as acute, i.e. a rapid change from tolerable to adverse conditions, or chronic, i.e. slow change. Acute, large changes in one or more water parameters are usually the most dangerous to fish, and are often lethal. Sometimes, however, a chronic exposure to mildly toxic conditions can become severe, such as when pollutants accumulate in the fish's tissues, causing an additive toxic effect over time. Clearly, both the duration and magnitude of the change will influence the degree of damage inflicted on the exposed fish.

## 7.1 ENVIRONMENTAL DISEASES CAUSED BY NATURAL EVENTS

Within a given aquatic habitat, the native fishes will under normal circumstances be physiologically adapted to cope with natural chemical

and physical changes to the water. For example, temperate cyprinids are able to tolerate the seasonal changes in water temperature, and in fact such temperature changes can be deemed beneficial to the fish, by serving as cues for spawning activity. Very occasionally, however, natural events cause a rapid change in the water conditions, such that the fish are unable to adapt, and succumb to environmentally induced diseases, or even death (Plate 7.1). For example, there are documented cases of fish having been killed by acute exposure to low pH, as a result of heavy rains flushing acid water from peat bogs into water bodies. Natural floods may also pose health threats to cyprinids. This can arise where the flood waters deposit considerable quantities of suspended matter into the fish's environment, causing clogging to the gills and resultant respiratory difficulties. Floods may also flush large quantities of organic matter such as organically rich topsoil into the water, causing a surge in microbial activity which utilises dissolved oxygen (DO) in order to break down the organic nutrients. In severe cases the DO value may decrease below the minimum physiological requirement of some cyprinids and result in fish death. Influxes of saline water into freshwater because of tidal waves, storms or breached barriers have been reported to have killed large numbers of cyprinids. Even when the salinity decreases in these situations, toxic algae have been identified as the cause of fish deaths. There are also reports of fish being killed by lightning strikes!

## 7.2 ENVIRONMENTAL DISEASES CAUSED BY HUMAN ACTIVITIES

The overwhelming majority of environmentally induced diseases of cyprinids stem either directly or indirectly from human activities. The continuing growth in the world's population has necessitated accompanying increases in intensive agriculture practices, industrialisation, and urbanisation – mostly to the detriment of natural environments, including freshwater habitats. It is probably no exaggeration to say that most bodies of freshwater on the planet have, to a greater or lesser extent, been perturbed by humankind.

The effects of human activities on the freshwater environments inhabited by cyprinids may be physical, e.g. thermal changes caused by warm water discharge from industrial plants, or chemical, for example pollution from agricultural fertilisers, sewage and industrially generated heavy metal wastes. All may cause disease to the cyprinid stocks. Environmentally induced diseases are also well documented in the case of captive cyprinids, such as those held on fish farms or in ornamental ponds and aquaria. Some of the major environmental causes of disease in cyprinid fish will be reviewed in this chapter. Table 7.1 lists some of the water quality standards for cyprinids laid down by the Freshwater Fish Directive of the European Economic Community (EEC, 1978). These are imperative values unless stated otherwise.

**Table 7.1** Water quality parameters for cyprinids, as laid down by the Freshwater Fish Directive of the EEC, 1978. Tolerance values are those at the point of discharge.

| Water parameter | Standard for cyprinids |
|---|---|
| Temperature | Maximum: 28°C (= maximum permitted temperature at the edge of the mixing zone for more than 98% of the time) |
| pH | Range: 6–9 |
| Nitrites | Maximum: 0.03 mg/L $NO_2$ (guideline value) |
| Unionised ammonia | Maximum: 0.005 mg/L $NH_3$ (guideline value) |
| Total ammonia | Maximum: 0.2 mg/L $NH_4^+$ (guideline value) |
| Dissolved oxygen (DO) | Minimum (50% of water samples): 8 mg/L $O_2$<br>Minimum (100% of water samples): 5 mg/L $O_2$ (guideline values) |
| Biological oxygen demand (BOD) | Maximum: 6 mg/L $O_2$ (guideline value) |
| Total residue chlorine | Maximum: 0.005 mg/L HOCl |

Obviously, these standards may not necessarily be appropriate for cyprinid stocks occurring in other geographical localities.

## 7.3 WATER TEMPERATURE

Fish are ectothermic animals, which means that that they do not generate body heat and their body temperature is thus dependent on the surrounding environmental temperature. Water temperature therefore has a profound influence on fish, affecting their rate of metabolism and other biological processes.

As a group, cyprinid fishes span temperate and tropical freshwaters. The temperature tolerance range of cyprinids will therefore vary between species, with many of the temperate cyprinids being capable of surviving near freezing conditions, whereas those inhabiting tropical waters may endure 30°C of more without any deleterious effects. Goldfish have a very broad range, being capable of surviving temperatures ranging from near freezing to the upper thirties degrees Celsius, although at the higher temperature limits respiratory stress may be significant. In some cases temperature tolerance may vary significantly between different geographical populations of a species. One example is the North American longnose dace (*Rhinichthys cataractae*), a normally temperate fish which also exists as a tropical subspecies, *R. c. smithi*, endemic to the hot springs in Alberta's Banff National Park (Fig. 7.1). Sadly, this subspecies is now extinct, probably due to competition from introduced aquarium fish.

Fish will suffer if exposed to water temperatures outside of their physiological tolerance range. An extreme of temperature may not necessarily be lethal, but can have long-term effects on the fish's health status. For example, ornamental koi that have been chronically exposed to

(a)

(b)

**Fig. 7.1** (a) Hot springs, Alberta, Canada. Home to a warmwater population of longnose dace and (b) associated poster board depicting the fish.

extreme temperature changes, as may occur on some koi farms, show signs of thermal stress, manifesting as general listlessness and a thin body, with a concave outline to the dorsal surface. Diseases and abnormal behaviours are associated with exposure to extreme high or low temperatures for the species.

### 7.3.1 High temperatures

Fish that are exposed to high temperatures may suffer respiratory stress and nervous activity. This is because increasing water temperature exerts a dual effect on the fish's oxygen demand/supply ratio. The warmer water carries less dissolved oxygen, yet it increases the fish's requirement for this gas due its increased metabolic rate. High water temperatures can also affect the fish's ability to osmoregulate, by altering the lipids within the fish's gill cells, making the cells leaky and less efficient at regulating salt balance and excretion. Increasing water temperatures also have an indirect effect on the fish's health by increasing the toxicity of certain pollutants, such as heavy metals. High temperature also has a thermal effect on nitrogenous wastes, by increasing the proportion of ammonia that is present as the toxic nonionised form. Anthropogenic activity also increases water temperature, e.g. discharge from industries. However, legislative powers exist in Europe to protect rivers and other natural water bodies against excessive thermal pollution from industrial outflows (see Table 7.1). In the case of captive cyprinid stocks, high temperature problems can occur in outdoor culture systems, for example where shallow water or small volume systems are unprotected from the sun's rays, or in the case of tropical aquaria where the thermostat controlling the heater is faulty.

### 7.3.2 Low temperatures

Fish health problems can occur as a result of low water temperatures. In the case of temperate cyprinids, the fish's immune system may be compromised at cold temperatures, rendering the fish more prone to infections caused by fungi and certain bacteria. Studies on disease problems in over-wintering carps (common, crucian and silver carps) have been traced to pseudomonad infections which may, in part, be caused by the lowered immunity of these farmed stocks. Among the more direct effects of low temperature on fish is respiratory stress and coma. Even though cold water contains more dissolved oxygen than warm water, its suppressive effects on the fish's respiratory rate and heart rate means that fish may become hypoxic at very low temperatures due to insufficient oxygen uptake. Behavioural signs associated with hypothermia include lowered respiratory rate and loss of swimming co-ordination.

## 7.4 DISSOLVED GASES

The three most abundant gases within our atmosphere are nitrogen (78% of all atmospheric gas), oxygen (21%) and argon (1%). Water, however, contains much lower quantities of certain gases and their relative concentrations may be different to those within the atmosphere. The gaseous composition of water will also vary between different water bodies, and can alter on a temporal basis. For example, cyprinids that inhabit fast-moving rivers will be accustomed to higher dissolved oxygen levels than those living in stagnant pools.

### 7.4.1 Oxygen

All fish require oxygen in order to respire, and the vast majority of fish (including cyprinids) obtain this vital gas directly from the water, via their gills. Water is relatively poor in oxygen, containing approximately 20–30 times less than the concentration in air. The oxygen carrying capacity of water decreases with increasing water temperature and also with increasing salinity (Table 7.2).

Oxygen requirements vary greatly between fish species. Carp, for example, are remarkably tolerant of low oxygen conditions, enabling them to survive in organically rich waters. The common carp survives for some time at oxygen levels as low as 0.7 mg/L. This compares with salmonids for which the minimum oxygen requirement is approximately 5.5 mg/L.

The amount of oxygen consumed by a given mass of fish will depend on their metabolic rate, and this in turn is greatly influenced by water temperature. For example, studies on common carp have shown that one kilogram of fish consume 7.2 mg oxygen per hour at 2°C, with oxygen consumption rising dramatically to 300 mg oxygen per hour at 30°C. Given that the oxygen carrying capacity of water decreases

**Table 7.2** Relationship between water temperature and oxygen carrying capacity.

| Water temperature (°C) | Dissolved oxygen (mg/L)* |
|---|---|
| 0 | 14.6 |
| 5 | 12.8 |
| 10 | 11.3 |
| 15 | 10.2 |
| 20 | 9.2 |
| 25 | 8.4 |
| 30 | 7.6 |
| 35 | 7.1 |

*Values are 100% oxygen saturation in freshwater at 760 mmHg barometric pressure.

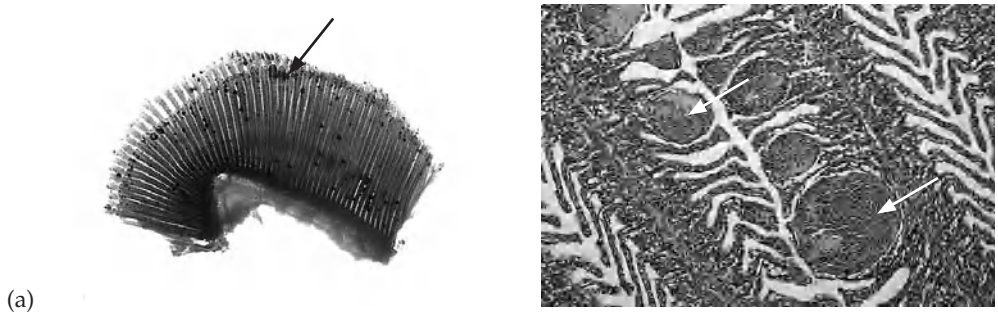
with increasing temperature, it becomes evident that oxygen levels can quickly become critically low at high water temperatures. Several factors can lead to hypoxic conditions such as overcrowding of fish, overfeeding, or high levels of organics, e.g. a sewage spill causing a rise in biological oxygen demand (BOD). In aquaculture, where cyprinids may be held at very high densities, supplementary oxygen may be supplied by bubbling air or oxygen gas through the water.

Fish are however capable of adapting to low oxygen conditions, within certain limits. For example when goldfish are experimentally exposed to low oxygen conditions within a few weeks the fish adapt by increasing their efficiency in utilising oxygen from the blood, by increasing the density of mitochondria within their red muscles.

When oxygen levels fall quickly to critically low levels, the fish can rapidly develop respiratory stress and may die. Respiratory problems manifest as a significantly increased gill beat rate, and in severe cases some cyprinids may gulp ('pipe') at the water surface where the oxygen level is richer. Similarly, affected fish may gather at the relatively oxygen-rich waters of inflow pipes. In extreme cases, mass mortalities due to acutely hypoxic conditions have occurred within a matter of minutes. Chronic hypoxia, on the other hand, is not necessarily lethal to cyprinids but may cause stunted growth, reduced feeding, and increased susceptibility to infections. Fish that have died of oxygen starvation often exhibit gaping mouths and flared and damaged gills (Fig. 7.2). When investigating possible causes of hypoxia an important distinction should be made between environmental hypoxia, i.e. critically low oxygen levels in the water, and physiological hypoxia, i.e. the fish is unable to extract oxygen efficiently from the water, due perhaps to gill damage by parasites or blood poisoning. The major clinical symptoms may be similar in both cases.

### 7.4.2 Gas supersaturation

The solubility of a gas in water is influenced by certain physico-chem-



(a) (b)

**Fig. 7.2** (a) Isolated fish gill arch showing telangiectasis (arrowed). Magnification ×2. (b) Section of a carp gill showing telangiectasis (arrowed) possibly due to oxygen deficiencies. Stained in haematoxylin and eosin. Magnification ×40 (courtesy of the Environment Agency, UK).

ical conditions of the water. Water temperature in particular has a significant effect on gas solubility; the higher the temperature the less gas water can sustain. However, under certain conditions, water may become supersaturated with one or more gases. Fish exposed to this supersaturated water are at risk of sustaining gas emboli due to the excess gas coming out of solution within the fish's blood. This can cause a disease in fish known as 'gas bubble disease' (GBD), and is similar in its effect to the 'bends' (decompression disease) in human divers.

Nitrogen has been widely implicated as the major gas responsible for GBD in fish, although current views suggest that measurement of the total gas pressure is a more reliable indicator of potential gas supersaturation problems. Gas supersaturation can arise in natural waters as well as artificial culture systems, though the actual cause(s) may be different.

Oxygen gas has also been implicated in some circumstances. For example, oxygen supersaturation may develop in ponds on sunny days as a result of intense photosynthetic activity by surface algae. This has been linked to gas bubble disease, especially in ponds in equatorial regions where the sunshine is intense. The level of gas supersaturation necessary to cause GBD varies according to the species and developmental stage of the fish. As a general guide, a level of 110% gas supersaturation is considered dangerous to fish.

Other causes of gas supersaturation are:

- the use of powerful air or oxygen injection systems, e.g. venturi valves on fish ponds and sometimes aquaria;
- the discharge of thermal effluents into rivers and other water bodies causes the localised rise in water temperature and thus reduces its gas carrying capacity, causing gas to come out of solution;
- the use of deep bore-hole water for aquaculture. Depressurisation of the drawn water, which is often nitrogen-saturated, may cause nitrogen gas to come out of solution;

- the sudden heating of gas-saturated cold water, e.g. to increase temperature to a suitable level for the appropriate cyprinid species; for example, the rapid heating of water for intensive food-fish systems and aquaria.

Fish may suffer acute or chronic effects of gas supersaturation, depending on the degree of saturation and the exposure time. In acute cases, the fish may exhibit impaired or unnatural swimming behaviour, e.g. loss of balance; spiralling; bursts of activity interspersed with lethargy; and in severe cases death can occur within a few minutes. Chronic GBD may, however, easily go undetected. In both acute and chronic cases, gas bubbles may be observed within the eyes of exposed fish. Eye damage involving 'pop-eye' (exophthalmia) and eruption of the lens can result. Fish that survive GBD may exhibit varying degrees of impaired vision, even total blindness in both eyes. Gas emboli, appearing as bubbles or raised blisters, may also be visible under the fish's skin and within the fins. Microscopical examination of the gills reveals gas emboli within the gill vasculature. The effects of gas emboli within the blood are considered the major cause of death in GBD and tissues damaged by GBD are susceptible to infections.

Fish affected with GBD are not treatable, however, recovery is possible in chronic situations where the cause(s) of gas supersaturation is removed. Methods to eliminate excess gases from the water, prior to exposure to fish, include vigorous aeration, and 'degassing' systems, the latter including vacuum degassers.

## 7.5 pH

This is a measure of the water's acidity or alkalinity and is based on the relative proportions of hydrogen ($H^+$) and hydroxyl ($OH^-$) ions present, respectively. In fact, pH is defined as the negative $\log_{10}$ value of the hydrogen ion concentration. The pH scale spans from 0 to 14, with the halfway point, pH 7, defined as neutral. As the proportion of $H^+$ in the water increases, the water becomes increasingly acid and its pH value decreases. Conversely, an increasing proportion of $OH^-$ corresponds with increasing pH and alkalinity. Because the scale is logarithmic, a change of one pH unit represents a 10-fold difference in the hydrogen ion concentration.

Most natural bodies of freshwater are partially replenished by precipitation. Rainwater is naturally acid, about pH 5.6, in contrast to pure water which is neutral (pH 7.0). The acidity of rainwater is the result of contact in the atmosphere with carbon dioxide ($CO_2$) and natural sulphur compounds. Sulphur dioxide and other industrial emissions into the atmosphere cause further acidification, resulting in some parts of the world receiving precipitation which is very acid, as low as pH 4.0, so-called 'acid rain'. Run-off water may cause acidification of streams, particularly in areas where the precipitation falls on substrates that have negligible buffering capacity, i.e. those which are poor in alka-

line salts, such as granite. Under closed conditions, such as ponds and aquaria, there is tendency towards acidification, caused by the metabolic activity of various aquatic organisms including fish, which yield acid waste products. The toxicity of various pollutants may also be influenced by pH.

Cyprinids, in common with other fish groups, vary between species in their physiological optimum pH range. In nature, most cyprinids will be found in waters between about pH 6.0 and 8.0. For example, the optimum pH for koi is around 7.5, though some cyprinids, e.g. from parts of Southeast Asia, may be found naturally in water as low as pH 5.5. At an individual level, fish appear capable of acclimating to a change in pH, within limits, such that their pH tolerance range may shift slightly from the species norm. Beyond the tolerance range, a fish will exhibit various degrees of stress, sometimes sudden death, depending on the severity and rapidity of the pH change. Rapid pH change may also stress fish even though the pH shift remains within physiological tolerance limits.

### 7.5.1 Excessively low pH

This can arise from the precipitation of acid rain, or in the case of enclosed systems from the build-up of acidic metabolic waste products from fish and other organisms. Soft waters (i.e. mineral deficient) are more prone to falls in pH due to inadequate buffering capacity. In chronic acidosis, the affected fish exhibit only vague symptoms of disease, such as retarded growth. In acute cases, the fish may become hyperactive and nervous, and there is hyperproduction of gill mucus in response to the acid conditions, causing the fish respiratory problems. Heavy metals are more toxic under acid conditions and at very low pH, below about 5, colloidal iron may be deposited on the gills. Fish suffering from acute acidosis will exhibit significantly faster gill beat rates.

### 7.5.2 Excessively high pH

Extremes of high pH are far less frequently encountered than extremes of low pH. In aquaculture, an unnatural increase in pH can arise when a recently concreted pond has been filled with water before allowing the concrete to cure. The practice of 'liming' an aquaculture pond also raises pH. Lime (calcium hydroxide) may be added to the pond either to counteract acid conditions, or at the end of the production cycle, as a disinfectant. Over-dosing with lime, or insufficient flushing following disinfection with lime, can result in extreme alkaline pH conditions (up to pH 11), which would be rapidly fatal to fish. Contamination of water with sewage or agricultural fertilisers may have an indirect effect on pH by promoting algal blooms that cause the pH to rise. A fish that is exposed to a pH higher than its tolerance range may exhibit clinical disease (alkalosis), which may take the form of a general stress response. In cases of acute alkalosis the fish's skin and gill epithelia will

be damaged and become cloudy in appearance. It should be borne in mind that ammonia toxicity increases with pH.

### 7.5.3 Adjusting pH

Because pH is a logarithmic scale, it must be kept in mind that a small change in pH value will have a large effect on the OH/H ratio. Sudden changes in pH are to be avoided as the fish may suffer acute acidosis or alkalosis, which can be lethal. In general, the pH should be adjusted by not more than 0.5 units per day. There are various ways of adjusting the pH, according to the cyprinid species being kept and the type of environment, e.g. ponds, lakes, or aquaria. Where pH buffering is required this will usually be needed to increase pH. Chemical liquid buffers are widely used for aquaria, and lime is sometimes used to raise the pH in large water bodies.

## 7.6 NITROGENOUS WASTES

The major nitrogenous waste product of fish is ammonia, which is excreted mostly via the gills. In natural water bodies, as well as in biologically filtered culture systems, the toxic ammonia is sequentially broken down into nitrite and then nitrate. These two chemical transformations form part of the nitrogen cycle and are mediated by aerobic bacteria known as nitrifiers. Tolerance to these nitrogenous wastes varies between species and even on an individual level.

### 7.6.1 Ammonia

Ammonia is the most toxic of the nitrogenous wastes and can be acutely lethal to fish under certain circumstances. In water, ammonia exists as two forms: ammonium ions (= ionised ammonia) and free ammonia (= nonionised/unionised), the relative proportions of which differ according to the water conditions. Of the two forms it is the free ammonia which is by far the most toxic. The proportion of ammonia present as the toxic free form increases with increasing water temperature and pH, but decreases with increasing salinity.

Thus:

$$\underset{\text{ammonium ions}}{NH_4^+} \leftrightarrow \underset{\text{free ammonia}}{NH_3}$$

$\rightarrow$ increasing temperature

$\rightarrow$ increasing pH

In addition, ammonia is more toxic to fish at low dissolved oxygen (DO) levels. Aquatic vegetation can also be influential by affecting pH and DO levels. If the water contains a significant biomass of aquatic macrophytes and/or algae then the cycles of photosynthesis (day) and res-

piration (night) will cause rising/falling DO levels and falling/rising $CO_2$ levels, respectively, thus affecting pH.

Ammonia may originate from several sources. In addition to being an excretory product of fish, it is also generated from the decomposition of organic wastes. For this reason, ammonia toxicity problems can occur in natural waters that are contaminated by sewage or agricultural slurries. In closed systems such as aquaria, ammonia toxicity can arise from inadequate biofiltration and specifically an insufficient population of *Nitrosomonas* nitrifying bacteria.

For any given set of water conditions, the toxicity of ammonia to fish will depend on various factors, such as: the species of fish; the exposure level of free ammonia; the period of exposure, and any previous acclimation effects. Scientific studies indicate that fish are capable of developing tolerance to ammonia following chronic exposure to sublethal levels. Fish that are exposed to toxic levels of ammonia may exhibit changes in their blood chemistry, e.g. raised pH, osmoregulatory problems, and respiratory difficulties. Osmoregulatory problems include, notably, an increase in permeability, which the fish must compensate for by increasing their urine excretion. There is evidence to suggest that ammonia harms the gill epithelia, although some experts believe that this damage is caused by other toxic metabolites that tend to co-accumulate with ammonia. At high levels, ammonia is acutely lethal to fish. It destroys the epithelium of the skin and gut, causing haemorrhaging, and affects the fish's central nervous system, causing excitability and other abnormal swimming behaviours. Exposure to chronic sublethal levels can result in growth suppression and increased susceptibility to infections.

Ammonia problems can be easily checked using a test kit, e.g. colorimetric assay. Ammonia test kits measure only total ammonia, i.e. ammonium ions plus free ammonia, thus the pH and temperature of the water must also be measured at the same time in order to calculate the amount of toxic free ammonia present.

In emergency situations ammonia levels can be quickly reduced in closed systems by partial water changes or the addition of zeolite. The prompt transfer of affected fish to ammonia-free waters may also be an option in some situations. As a long-term measure, ammonia problems can be solved by improving biofiltration; reducing stocking density; removing other sources of ammonia, e.g. by reducing the level of organic wastes.

### 7.6.2 Nitrite

Although far less toxic than ammonia, nitrite ($NO_2^-$) is also potentially hazardous to fish. Susceptibility to nitrite varies considerably between fish species, and this may be partly due to species differences in nitrite uptake. Nitrite toxicity is also influenced by environmental factors, e.g. water hardness reduces nitrite toxicity. Nitrite problems may occur when there is inadequate biofiltration, specifically when there are insufficient *Nitrobacter* bacteria present to convert nitrite into

nitrate. Fish that are exposed to nitrite absorb this chemical across their gills and into the blood. Within the blood, nitrite oxidises the respiratory pigment, haemoglobin, into methaemoglobin, which is far less efficient in carrying oxygen to the tissues. This results in hypoxia and respiratory stress. Affected fish may show behavioural symptoms of hypoxia, such as increased gill beat rate and piping at the water surface. Nitrite is also a smooth muscle relaxant and vasodilator, prompting some scientists to suggest that it could cause cardiac arrest in exposed fish. Chronic exposure to sublethal nitrite levels has been linked with increased susceptibility to bacterial infections. Fish that are suffering from nitrite poisoning may have 'brown blood' due to the presence of methaemoglobin and their gills may also appear brownish.

The nitrite level can be reduced in closed systems by partial water changes, and by improving the biofiltration to allow greater colonisation by *Nitrobacter*. For those cyprinids that are salt tolerant, e.g. carp, the addition to their water of chloride, e.g. sodium chloride, at up to 100 mg/L, helps reduce nitrite poisoning, possibly by inhibiting nitrite uptake across the gills. Test kits are available commercially to measure nitrite.

### 7.6.3 Nitrate

Nitrate ($NO_3^-$) is the end-product of bacterial-mediated nitrification under aerobic conditions, and is therefore a natural component of water bodies. In many parts of the world, agricultural practices such as the use of nitrogen-based fertilisers have caused nitrate pollution of rivers and ground waters. When present in high concentrations, however, nitrate has been associated with reduced growth in fish and increased susceptibility to infections, although the mechanism of nitrate toxicity is not well understood.

## 7.7 OTHER POLLUTANTS

Literally thousands of different pollutants have been recorded in water bodies as a result of human activities. In the UK alone approximately 32 000 pollution incidents were reported in 1998. There are numerous studies on the detrimental effects of various pollutants on fish and other aquatic life.

### 7.7.1 Heavy metals

Small quantities of certain heavy metals are essential for fish; zinc, for example, is a component of enzymes. However, when present in high concentrations, many heavy metals become toxic to fish. The four most commonly studied metals are cadmium, copper, mercury and zinc. Heavy metals can harm fish in many ways, both physically and physiologically. For example, cadmium has been linked with vertebral damage in common minnows, and zinc and copper are known to cause

(a) 


(b)

**Fig. 7.3** Photographs of a gill taken with a scanning electron microscope showing (a) chub with normal unfused secondary lamellae and (b) chub with fused tips of the secondary lamellae caused in this case by microbial toxins. Magnification × 100 (courtesy of Professor John Lewis).

respiratory stress by damaging the secondary lamellae of the gills (Fig. 7.3). Water chemistry has an influence on heavy metal toxicity, one notable example being water hardness which reduces the toxicity of copper and to a lesser extent some of the other heavy metals.

### 7.7.2 Pesticides

Many pesticides are harmful to cyprinid fish, both directly and indirectly. Most are nonspecific poisons, which at high concentrations will cause some damage to the fish. For example, organophosphates, a group of common pesticides, inhibit cholinesterase activity in vertebrates, including fish. In affected animals the normal nerve functions are grossly disturbed resulting in death, usually due to asphyxiation. Fortunately most of these chemicals are biologically active for only a few days, so the effects of these modern pesticides are usually observed as acute poisonings or fish kills. Perhaps as importantly, pesticides, even at extremely low concentrations, can severely affect the aquatic ecosystem usually by killing invertebrates. Since many cyprinids rely on invertebrates for much of their diet, this type of pollution is often very damaging as it is not immediately apparent and will result in the slow starvation of the fish, until the invertebrate population can recover.

### 7.7.3 Endocrine disrupting chemicals

Following the discovery in the 1980s of wild fish that had significant egg masses within their male testes, there has been increasing concern over pollutants that can affect the endocrine system of fish and other wildlife. Considerable research effort has shown that there are a number of different compounds responsible for the observed effects. For example, some compounds referred to as environmental oestrogens can mimic oestrogen, the hormone that controls female characteristics and reproduction. Many can eliminate or reduce hormone actions and are called

anti-oestrogen or anti-androgens (the male hormones). All these chemicals lead to feminisation of normal male fish and the production of eggs as well as milt in the testes (Plate 7.2). In England, male fish living near municipal sewage outlets were found with both male and female sex characteristics and their livers produced vitellogenin, a female egg yolk protein not normally found in males. These abnormalities appeared to be reduced in fish that occurred further from the sewage outlet. Several different chemicals, especially the alkylphenols, which are breakdown products of chemicals found in detergents and plastics, are suspected of causing the feminising effects. In a widespread survey, all sampled waters in England were found to contain feminised male fish.

## 7.8 ENVIRONMENTAL STRESSORS AND DISEASE

As indicated throughout this chapter, there is evidence that exposure to environmental stressors such as extremes of water chemistry, temperature, pollutants, etc., can predispose fish to infectious diseases. In cyprinid aquaculture, overcrowding, regular handling, netting, sorting, and other practices may also stress the fish.

A stressed fish will produce high levels of cortisol and other stress hormones which are known to affect immune function in several ways, such as by reducing the level of circulating lymphocytes and the suppression of immunoglobulin synthesis. Another group of stress-mediated hormones, the catecholamines, are thought responsible for the suppression of macrophage activity. Clearly, therefore, methods to minimise fish stress in aquaculture systems will improve the stock's ability to resist infectious diseases. Finally, it should briefly be mentioned that some pollutants are directly immunotoxic, one infamous group of chemicals being the polychlorinated biphenyls (PCBs) which are components of many industrial products, such as hydraulic pump fluids. The effects of PCBs on fish immunity is reviewed by Noguchi (1998).

## 7.9 MEASUREMENT OF SOME ENVIRONMENTAL WATER PARAMETERS

| Parameter | Measuring devices |
|---|---|
| Temperature | Mercury/alcohol thermometers<br>Electronic temperature probe |
| pH | Colorimetric test kit*<br>Electronic pH meter |
| Dissolved oxygen (DO) | Colorimetric test kit<br>Oxygen probe |
| Dissolved nitrogen | Tensionometer (measures total gas pressure rather than specifically nitrogen gas) |
| Ammonia | Colorimetric test kit<br>Electronic meter fitted with specific electrode<br>Note: perform pH and temperature readings to calculate level of toxic free form |
| Nitrite | Colorimetric test kit<br>Titrimetric test |
| Nitrate | Colorimetric test kit<br>Titrimetric test |
| Copper | Colorimetric test kit<br>Atomic absorption spectophotometry<br>Graphite Furness analysis<br>Anodic Stripping Voltometry |
| Iron | Colorimetric test kit<br>Atomic absorption spectophotometry<br>Graphite Furness analysis |
| Chlorine | Colorimetric test kit |
| Biological oxygen demand (BOD) | Oxygen probe<br>Test based on incubation of water sample for 5 days at 20°C and measuring amount of oxygen consumed |

*The aquarium industry produces inexpensive and reasonably accurate colorimetric test kits for measuring various water parameters

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 8
# Nutritionally Induced Diseases

When maintaining cyprinids in captivity, dietary considerations are just as important as providing proper environmental conditions. Fish that are chronically starved of food, or fed nutritionally inadequate diets, have an increased risk of developing health problems.

Nutrition related disorders manifest in various ways, such as poor growth, developmental abnormalities, skin and skeletal problems, failure to spawn, and in severe cases, death. Poor nutrition also reduces the fish's ability to resist infectious diseases. It is not simply the quantity of food that is important, but also its quality. A nutritionally balanced diet is one that provides all of the fish's needs for energy and growth. The major groups of nutrients that together form a balanced diet are the proteins, carbohydrates, lipids, minerals and vitamins. The composition of each of these nutrients is equally important. For example, the protein component of the diet must provide all of the essential amino acids, without which the fish will be unable to synthesise new proteins required for growth and metabolism. Similarly, cyprinids have a dietary requirement for various essential fatty acids, vitamins and minerals. In contrast, dietary carbohydrates are not absolutely essential for fish, although in practice fish-food manufacturers incorporate carbohydrates within the diet.

## 8.1 NATURAL FOODS

The term 'natural' foods is defined here as those food items that are normally encountered in the wild. Taking the Cyprinidae as a whole, natural foods include detritus, plankton, algae, higher plants (including seeds), aquatic invertebrates (e.g. insect larvae, worms, crustaceans and snails). In the case of a few cyprinid species such as the chubs, *Leuciscus* and *Semotilus* spp., live fish may form a significant part of the adult's diet. Furthermore, many cyprinids, including carp, will prey on the eggs and fry of fish, including those of their own species. Only a few cyprinids are highly specialised feeders, whereas most are flexible feeders and can switch from preferred to alternative diets according to food availability.

## 8.2 ARTIFICIAL FOODS

The maintenance of captive cyprinids for food, ornamental, and sport purposes has prompted the commercial production of a wide range of artificially formulated diets. These mostly comprise dry formulations, such as pelleted and flake foods, which are fed either exclusively, or in the case of pond-held cyprinids, as supplements to available natural foods. Commercial dry formulations generally satisfy all of the fish's dietary requirements, however under certain circumstances nutritionally related pathologies can occur, and most relate to nutritional deficiency problems.

## 8.3 NUTRITION RELATED DISEASES

Nutrition related diseases are poorly documented for wild cyprinids, despite the likelihood that human perturbations to the aquatic environment (e.g. chemical or thermal pollution) will have caused the decline of certain types of aquatic food organisms. The relatively wide dietary range exhibited by most cyprinid species helps protect them against any detrimental human impacts on particular food organisms. Starvation, however, can occur in wild stocks, as discussed later in this chapter.

It is under captive conditions where nutritional deficiencies are more likely to occur. Various types of nutritional diseases are discussed below and refer mostly to captive cyprinid stocks.

### 8.3.1 Vitamin deficiency disorders

Vitamin related health problems may occur in situations where the diet is totally or predominantly artificial, as is common in intensive closed aquaculture systems. Approximately 15 different vitamins are known to be important for fish, although not all species require them all. Fish also need particular quantities of each vitamin in their diet, and these quantitative requirements vary according to the species of fish, as well as to intraspecific factors such as stage of development, reproductive status, and environmental conditions notably water temperature and season.

Vitamins can be classified according to their solubility: those which are fat-soluble (e.g. A, D, E, K) and those which are water-soluble (e.g. B group, C, choline, inositol). Deficiencies in water-soluble vitamins are more likely to occur as these vitamins cannot be stored as reserves in the fish's body.

In practice, where vitamin deficiency problems do occur in captive fish stocks, these mostly relate to a deficiency of vitamin C. There are several reasons for this. In contrast to birds and mammals, fish are unable to synthesise vitamin C and hence are totally reliant on dietary sources. Vitamin C is chemically unstable, being prone to decomposi-

tion during food processing and subsequent storage, and inactivated by high temperatures, high humidity, and bright light. Vitamin C losses of up to 95% have been recorded during dry diet manufacture. Vitamin C will also leach from dried foods upon contact with the water. Studies on flake formulations for aquarium fish have shown 65% leaching of vitamin C within 30 minutes of contact with the water. When combining these losses, it can be seen that as little as 2.5% of the original vitamin C composition of a dry diet may actually be ingested by the fish. Fish-food manufacturers compensate for such high losses by enriching diets with vitamin C, or by using stabilised vitamin C analogues, such as ascorbate-2-monophosphate or ascorbate-2-polyphosphate. Proper storage of dry foods in dry, cool conditions helps minimise vitamin degradation. For similar reasons, it is generally recommended that food should be purchased in small quantities at a time, so as not to exceed a storage period of 3 months. In aquaculture practices, problems caused by deficiencies of other vitamins are highly unlikely to arise if the fish are fed with a quality commercial diet that is appropriate for the species.

Vitamin depleted test diets have been formulated in order to assess the specific effects caused by omitting a particular vitamin from the diet. Table 8.1 gives examples of vitamin deficiency disorders in cyprinids, based largely on test diet data. In practice, the clinical symptoms listed are far more likely to arise from nondietary causes such as infectious diseases or adverse water conditions.

**Antibiotics and vitamin deficiencies**

Studies on common carp and other cyprinids have shown that these fish are not totally reliant on their diet to meet all of their vitamin requirements. Commensal bacteria within the fish's gut are capable of synthesising some vitamins such as inositol, B1 and B12, which can be utilised by the fish. Some fish health workers are concerned that antibiotic treatments may destroy the vitamin-synthesising gut bacteria and for this reason a compensatory vitamin B supplement is sometimes administered to fish that are undergoing antibiotic therapy, as a safeguard until the gut bacteria recolonise.

### 8.3.2 Amino acid deficiencies

Dietary amino acid imbalances can arise in cyprinids. Fish require several different amino acids in their diet, without which deficiency problems may arise, hence these protein subunits are referred to as 'essential amino acids'. Most proteins are deficient in one or more amino acids, such that no single protein source is likely to satisfy the entire amino acid requirements of cyprinids. For example, certain oilseeds are deficient in the amino acids lysine and threonine. Lysine deficiency, for example, has been associated with fin erosion disorders, and several other amino acid deficiencies appear to increase the mortality rates in common carp. Care must therefore be taken when formulating

**Table 8.1** Disease symptoms associated with vitamin deficiencies. The studies all relate to common carp, except where otherwise indicated.

| Vitamin | Poor growth | Fin and/or skin haemorrhaging | Blood disorders | Nervous disorders | Exophthalmia |
|---|---|---|---|---|---|
| A (retinol) | X | X | | | X |
| B1 (thiamine) | X | X | | X | |
| B2 (riboflavin) | X | X | | X | |
| B6 (pyridoxine) | X | | | X | |
| B12 (cyanocobalamin) | X (*Labeo rohita*) | | X | | |
| C (ascorbic acid) | X | X | | | |
| D3 (cholecalciferol) | X | | | | |
| E (tocopherol) | | | | | X |

artificial diets to ensure that the various protein constituents together contain all of the essential amino acids in nonlimiting quantities.

### 8.3.3 Mineral deficiencies

Fish require minerals for various metabolic processes, e.g. haemoglobin synthesis, and for enzyme and hormone function. Studies on common carp have shown that dietary deficiencies of certain minerals are linked with various diseases. For example, a deficiency in any one or more of the following minerals may cause reduced growth and cataracts: magnesium, manganese, selenium, and zinc. Other signs of mineral deficiency in carp include dwarfism (manganese deficiency), skeletal deformities (phosphorus) and anaemia (selenium).

It is interesting to note that fish obtain a proportion of their mineral requirement from the surrounding water, via absorption across the gills and/or skin, such that they are not totally reliant on dietary supplies, in contrast to terrestrial vertebrates.

### 8.3.4 Fatty acid imbalances

Dietary fats are a major energy source for fish. Cyprinids and other fish have a dietary need for certain fatty acids, referred to as essential fatty acids (EFAs) and these belong to two families known as the omega 3 series and the omega 6 series. Due to differences in the chemical bonding of fatty acid molecules, some fatty acids will solidify and harden at low temperatures whereas others remain fluid. These physical properties relate in part to the degree of 'saturation' of the fat molecule. The feeding of 'hard' saturated fats, e.g. beef tallow, may cause dietary problems in cyprinids, especially temperate species, because these fats will solidify at low temperatures. The critical temperature therefore depends largely on the degree of saturation of the fat. Hard fats are therefore not easily digestible and can interfere with metabolism. The result of a hard fat diet may be intestinal blockage that results in constipation, and sometimes even death. In some cases, raising the water temperature within the fish's physiological tolerance limits may help unblock the gut by softening the ingested fat.

## 8.4 STARVATION

Chronic starvation will result in weight loss, and manifest as thinning of the body and concave abdomen (Plate 3.2). Growth will effectively cease and reproductive potential may be curtailed. Disease problems relating to a lack of certain essential nutrients may also arise following chronic starvation, notably vitamin deficiency symptoms such as anaemia, eye cataracts, and skin and gill haemorrhaging.

Brief periods of starvation may be normal in some circumstances. Fish are ectothermic ('cold blooded') animals which means that their body temperature and rate of metabolism is greatly influenced by the

temperature of the surrounding water. The feeding response can also be suppressed at low temperatures. In the case of temperate cyprinids, the fish may become torpid and eat very little, or cease feeding altogether during the cold winter months, a time when food supplies may be scarce. Such natural periods of starvation may not necessarily harm the fish provided it was well nourished prior to the onset of winter. However, those individuals that have failed to build up sufficient nutrient reserves may perish, as in the case of the common carp where deaths of over-wintering fish have been recorded in Britain.

Starvation can also occur in captive cyprinids, even in situations where abundant food is being offered. This can arise if the food is inappropriate for the stock, as may occur during larval rearing. For example, cyprinid fry may inadvertently be given pelleted foods that are too large to be swallowed whole (most fish cannot chew their foods!). This mistake is not uncommon amongst novice aquarists when first attempting to raise fish. A similar rearing problem may arise during the time of first feeding when the fry of some cyprinid species may recognise only living, moving foods as being edible. A lack of suitable live prey can therefore lead to heavy mortalities during this critical phase in the fish's early life. Suitable supplies of live foods (e.g. rotifers, brine shrimp nauplii) must therefore be established before spawning programmes are initiated. The delivery method of the food may also be important. For example, floating dry foods are inappropriate for bottom-dwelling cyprinids such as *Rhinogobio*, which are sometimes sold for aquaria, as these fish do not normally rise to the surface to feed. Thus, although the correct food may be offered, it is effectively unavailable to the fish, resulting in starvation. Often, however, captive fish learn to alter their feeding behaviour in order to take foods from other levels in the water column.

Judging the correct quantity of food can be difficult in the case of pond raised cyprinids, especially in large volume, turbid waters where it may be difficult to estimate the fish biomass. Routine monitoring of the stock, in the form of visual inspections of sampled individuals and weight gain estimations, should reveal whether the amount of food is being correctly gauged.

## 8.5 NUTRITIONAL TOXICITY DISORDERS

Experimental diets have revealed disease conditions resulting from the intake of excessively high levels of certain vitamins, e.g. vitamin A. However, cases of hypervitaminosis are unlikely when commercial diets are fed.

In aquaculture, plant-derived proteins are often incorporated into foodstuffs for economic reasons. However, some of these ingredients contain toxins that must be inactivated during food processing. For example, toxins in certain oilseeds including soybean, cottonseed, and rapeseed can reduce the growth rate of fish.

### 8.5.1 Mycotoxins

Dry feeds that are stored at high temperature and/or high humidity can become colonised by various moulds (fungi). Certain species of filamentous fungi notably those belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium* produce substances known as mycotoxins which are highly toxic to fish even at very low concentrations. One type of mycotoxin produced by *Aspergillus* spp. is known as aflatoxin, and is lethal to rainbow trout at only 500–1000 p.p.b. concentration. Although mycotoxin poisoning is uncommon, it is nevertheless prudent for fish culturists to store dry foods under cool, dry conditions so as to minimise the likelihood of mould spoilage.

## 8.6 OVERFEEDING

Feeding excessive amounts of food is the most common cause of death in captive fish, particularly those held at high stocking densities in relatively confined quarters, such as aquaria. Although rarely causing direct harm to the fish, the ingestion of excess food will result in increased ammonia excretion, and any uneaten food will also contribute to the accumulation of this nitrogenous waste. Even if biological filtration is installed, this may not be able to cope with a rapid surge in ammonia production, and hence the fish may be exposed to toxic levels of ammonia. Obesity would rarely be expected in wild fish, and is uncommon in cultured food fish since feed ratios are carefully calculated for economic purposes: overfeeding causes unnecessary production costs.

Obesity is sometimes observed in aquarium fish that have been chronically and frequently overfed. It is also observed in goldfish that have been given the opportunity to feed *ad libitum* on commercial dry diets, using demand-feeders. Several designs of demand-feeders exist, for example one model relies on the fish learning to nudge a submerged rod which triggers the release of a food pellet.

Obesity is also observed in certain coarse fish, notably carp, that are stocked for angling (Plate 8.1). The widespread use of trout pellets and food attractants may result in the carp taking large amounts of nutritionally rich foods. In some coarse fisheries in the UK angling is so intensive that the cyprinid stocks survive predominantly on angler's baits. These baits, some of which are known as 'boilies', are not formulated to provide a nutritionally balanced diet, often being very rich in proteins and fats. Pictures of pitifully obese carp on the front covers of angling magazines highlight this problem. Coarse fish that rely heavily on bait foods may succumb to nutrition related diseases, particularly immunodeficiency problems.

### 8.6.1 Winter bait foods

Anglers who use food attractants during wintertime can cause carp

to suffer from digestion problems. This is because bait foods that are given in mid-winter may encourage carp to feed when the water temperature is very low. Their ability to properly digest foods is poor due to low-temperature suppression of the fish's digestive enzymes below approximately 10°C and this can lead to food rotting in the fish's gut.

## 8.7 DISEASES ASSOCIATED WITH LIVE FOOD ORGANISMS

Several parasites enter the fish host by the oral route, via the food. For example, aquatic annelid worms and copepods may carry the intermediate stages of *Khawia* and *Bothriocephalus* tapeworms, respectively. These parasites gain entry into the fish following its ingestion within the prey organism. Copepods may also harbour the procercoid stage of *Ligula intestinalis*. Freshwater amphipod shrimps, such as *Gammarus*, form part of the natural diet of some cyprinids, e.g. chub and barbel, and these invertebrates are intermediate hosts to certain digenean and acanthocephalan parasites of cyprinid fish, such as *Pomphorynchus laevis*, an acanthocephalan which infects several cyprinid species.

Disease transmission via live foods can also occur under enclosed conditions, such as aquaria, where wild-caught food organisms, such as copepods, are fed to the fish. *Tubifex* worms were once very popular live-foods for aquarium fish but these mud-dwelling annelids are now known to harbour viral, bacterial and protozoan pathogens of fish, such that their popularity has declined over recent years. To obviate disease problems, some live food organisms are specially cultured under controlled (e.g. fish-free) conditions, so as to minimise the risks of disease transmission. Also, several popular food organisms, including *Tubifex*, are nowadays available as frozen-irradiated or freeze-dried preparations, as a means of eliminating disease problems.

Live foods collected from natural sources may also carry chemicals that are toxic to fish. For example, earthworms harvested from gardens are a popular 'treat' for goldfish and other ornamental fish, but can be toxic to the fish if the worms happen to contain ingested soil that has been recently treated with herbicides or insecticides. Some aquatic invertebrates bio-accumulate toxins in their tissues, such that any fish which eats a contaminated prey organism may be exposed to high levels of the pollutant. Certain pesticides, such as dieldrin, may be accumulated this way.

## 8.8 DISEASES ASSOCIATED WITH FEEDING SICK OR DEAD FISH

The feeding of diseased fish carcasses to other fish can also result in disease transmission, and for this reason the practice is extremely un-

wise. Most fish, including cyprinids, will forage on the bodies of dead or dying fish, and thus moribund fish should be removed from the housing facility in order to reduce the spread of disease by this route. Many fish engage in coprophagy, and this too can result in disease transmission via the oral route as in the case of mycobacteria that may be present in the faeces of infected fish.

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# Chapter 9
# Diseases of Eggs and Fry

High losses are incurred during the early development of cyprinids, notably at the egg and larval stages. Factors such as predation, adverse environmental conditions, parasites and pathogens are the major causes of early cyprinid deaths in both wild and cultured stocks.

## 9.1 EGGS

All cyprinid fishes are oviparous: they are egg-layers. Many cyprinids lay their eggs amongst aquatic vegetation where they may be out of sight from major predators such as other fish.

### 9.1.1 Infertile eggs

Sometimes, the fish's eggs may be infertile and hence will not develop or hatch. Infertility can be caused by various conditions. For example, the parent male or female may be producing gametes (eggs or sperm) which are nonviable, perhaps as a result of some physiological dysfunction in the sex organs. Another cause of egg infertility is the absence of sexually mature male fish, a situation that is more likely to arise under captive conditions. External fertilisation in cyprinids means that eggs and milt must be released into the water at the same time. If this does not occur then infertility will result, e.g. infection of the nasal cavities of cyprinids by the copepod *Paraergasilus longidigitus* may reduce the ability of the fish to receive spawning cues. Observations of aquarium cyprinids have also shown that ripe females may sometimes shed their eggs despite no males being present.

### 9.1.2 Hatching failure in fertile eggs

Field observations of the spawning sites of wild European cyprinids suggest very high hatch failure rates among fertile eggs, often well in excess of 50% even under seemingly favourable conditions. Egg production must therefore be very high in order to compensate for such heavy losses. For example, it is known that a female common carp of 85 cm length can produce in excess of 2 million eggs at a single spawning. The ability of some cyprinids to produce several successive batches of eggs within a single season is another strategy to maximise fecundity, as exemplified by the tench.

When compared with the adult fish, the egg stage appears particularly susceptible to rapid changes in environmental conditions and is less tolerant of extremes. Various environmental factors are known to reduce hatching rates. These factors may be natural phenomena, such as excessive water turbulence, which damages developing eggs, and gas supersaturation. The latter is known to occur in tropical lakes and ponds where intensive algal photosynthesis releases large amounts of oxygen into the water. In addition to its harmful effects on fry and adult fish, gas-supersaturated water may cause gas emboli to develop within the eggs, resulting in decreased hatching yields.

Contamination of natural waters with various industrial and agricultural chemicals has also had a deleterious effect on hatchability. Notable examples include the heavy metals, such as zinc, mercury, cadmium, and copper. Zinc, which may occur in the outflow wastes of certain industrial plants, such as steelworks, has been shown experimentally to increase incubation time and reduce the percentage hatch of zebra danio eggs. Various organic pollutants, such as the organochlorine compounds and other pesticides, also exert adverse effects on egg development. It is interesting to note that a significant proportion of the organic contamination of a fish egg is actually derived maternally. In fact, up to 25% of the female fish's organic pollutant burden may be transferred to her eggs during vitellogenesis. In terms of aquaculture, it is recognised that certain chemicals used on the fish farm may also be harmful to eggs, in particular certain disease remedies and prophylactics. One example is malachite green which is sometimes applied as an antifungus treatment. Although considered of value in controlling egg fungus, this dye is toxic to eggs which are close to hatching, and to newly hatched fry. The duration of egg exposure to malachite green must therefore be limited. Certain other disease medications are believed to cause premature hatching, resulting in high mortalities of the under-developed fry. For this reason, some fish health experts recommend the withdrawal of all egg medications 24 hours before hatching.

### 9.1.3 Infectious diseases

Fertile and infertile eggs are prone to a range of infections, mostly caused by viruses, bacteria and fungi.

#### Viral and bacterial infections

A few bacterial, e.g. mycobacteria, and viral fish pathogens are known to be transmitted from parents to progeny via eggs or possibly sperm such that the offspring are infected before hatching. This vertical disease transmission is suspected in the case of a viral infection caused by some rhabdoviruses, e.g. pike fry rhabdovirus, which, despite its name, also affects cyprinids. It is therefore likely that vertical transmission occurs for a number of viruses that infect cyprinids.

#### Fungal infections

Eggs which are infertile or fail to develop are likely to become invaded

**Fig. 9.1** Fungus infection on the egg of a fathead minnow. Of the three eggs shown, only the lower one has been attacked by fungus. The middle egg has just hatched and the upper egg is developing normally. Magnification × 10.

by opportunistic fungi. Most fungal infections of fish eggs are caused by *Saprolegnia* (class Oomycetes), and indeed 'saprolegnia' is the term commonly used to describe egg fungus. However, it is known that fungi belonging to other genera and orders of the Oomycetes may occasionally cause hatch failure. Although the fungus primarily invades nonviable eggs, it may spread to adjacent healthy ones (Fig. 9.1). Eggs that become white are sometimes considered to be 'fungused'. This is not necessarily the case, as the whitening can be caused by the precipitation of protein material within the dead eggs. Fungus, however, visibly manifests as a white to grey cotton wool-like growth smothering the eggs, which microscopically shows as a network of long, branched aseptate hyphae.

### 9.1.4 Parental care as a means of reducing egg infections

In those very few cyprinid species that exhibit parental care of the eggs, the male may periodically clean the eggs, such behaviour serving to reduce the chances of microbial attack. One well-known example is the North American fathead minnow (Plate 9.1) which is used as a laboratory model for toxicology studies. Spawning in the fathead is similar to that of many cichlids, with the adhesive eggs being deposited as a single layered clutch on solid surfaces, usually on the underside of caves or overhangs. After spawning, the clutch is guarded and cared for by the male. The mature male fathead possesses a thick semitranslucent pad on his head, hence the fish's common name, which he uses to stroke the eggs, thereby helping to remove any particulate matter and pathogens that may have attached to the egg surface.

### 9.1.5 Parental care and increased egg survival

A small number of cyprinid species have evolved various strategies

to reduce the likelihood of egg predation. A few, such as fathead minnows, guard their eggs, while some shiners hide their eggs in crevices, though there is no parental care. Nest building, in which the eggs are typically deposited in a gravel pit and then covered, is exhibited by some other North American cyprinids. A more unusual form of egg protection, known as brood hiding, is practised by the bitterlings and certain other cyprinids (*Sarcocheilichthys* spp.). The eggs of these fish species are inserted, via the female's elongate ovipositor, into a live freshwater bivalve mollusc such as *Anodonta* and *Unio*, where they develop and hatch within the protection of their hard-shelled 'foster mother'.

## 9.2 FRY

Once emerged from the partial protection afforded by the egg, the fry are exposed to a range of microbial pathogens and parasites, as well as the ongoing risk of predation. Many direct life cycle skin parasites e.g. *Ichthyophthirius* are particularly serious if fry develop an infection in the early stages of life.

### 9.2.1 Immunity and infectious diseases

In terms of infectious disease risks, the newly hatched fry are disadvantaged, as compared to older stages, by not having a fully mature immune function. Furthermore, they will not have developed any acquired immunity to specific infectious diseases. The ability of fish to develop acquired immunity to microbial pathogens and certain parasites (e.g. *Ichthyophthirius*) is well documented, however, this type of immunity develops only following an initial exposure to the pathogen. High mortalities among fry are therefore likely since even low infection levels may be lethal to small fish. This has important implications for cyprinid culture where extra precautions are required in order to minimise infectious disease outbreaks within the hatchery unit.

### 9.2.2 Gas bubble disease

Fry are particularly susceptible to gas bubble disease. Studies on Indian carp (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*) have revealed occasional serious damage to fry as a result of oxygen gas production by ingested phytoplankton. Freshly consumed phytoplankton is capable of photosynthesising within the fry's gut as a result of the intense tropical sunlight entering through the fish's semitranslucent gut wall. Affected fry exhibit gas-distended abdomens, impaired digestion, and unusual swimming posture, typically swimming with their ventral surface uppermost. This condition can therefore occur in the absence of gas supersaturation of the surrounding water.

## 9.3 PREDATION OF EGGS AND FRY

As mentioned earlier, predation plays a significant role in reducing egg and fry numbers, especially under natural conditions. The majority of cyprinids do not exhibit parental behaviour and typically scatter their eggs amongst vegetation or over the substratum, or disperse their semi-pelagic eggs in fast-flowing rivers, as occurs in some Asian cyprinids. The eggs and fry of most cyprinid species are at the mercy of various fish predators, including possible cannibalism by the parents and conspecifics. This problem is obviated in aquaculture by the removal of broodfish after spawning. Among the nonpiscine predators are various types of aquatic invertebrates, including some species of aquatic snails that may forage on fish eggs. Field observations on the spawning sites of roach in Poland have revealed that a proportion of eggs that were empty had sustained round punctures to the egg wall, suggesting predation by invertebrates. The aquatic larvae of some insects, such as the dragonflies, are notorious predators that can snatch fry and will even take the adults of small cyprinid species. Another invertebrate predator of fry is the freshwater coelenterate, *Hydra*, which uses its elastic stinging tentacles to capture its prey. Of particular note amongst the vertebrate predators of fry are the amphibians, particularly the newts.

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# Chapter 10
# Management of Fish Health

Effective management of fish health in any culture situation is vitally important as it will result in cheaper, healthier, better quality products or longer lived and happier pets. As discussed throughout this book cyprinid fish are held in captivity for a wide variety of reasons in an even wider assortment of systems, from personal aquaria to high productivity fish farms. Management practices therefore will have to take many forms depending on the type of fish and the holding facilities. In this short chapter it would be impossible to cover all eventualities that may occur in this diversity of cyprinid culture situations with any degree of detail. The number of disease processes, the multitude of factors involved in fish health and the sheer variety of different systems make this impossible. This chapter will, therefore, introduce the important principles of prevention, husbandry and cure and show how knowledge of the fish, its diseases and the culture system will allow the formation of sensible site management plans in all circumstances. Management of fish health will here be directed toward open systems, which due to their complexity are far more problematic. However some of the basic principles are appropriate for all systems including aquaria.

The gathering of available information on the physical, chemical and nutritional requirements of a species is essential to maintain suitable conditions for the fish and is important regardless of the type of site, i.e. from a single aquarium to a large fish farm. Thus establishing a site management plan to help utilise all the various elements of this knowledge is an important component in the management of fish health. The importance of disease management is highlighted by the continued development of national and international controls on the movement of fish used by many governments to protect wild fisheries and fish farming. In addition, there is increasing public awareness and concern regarding animal welfare protocols used to rear animals intensively and the impact such systems have on the environment. For example, the recent European Union decision to phase out battery chicken farming may mean that husbandry techniques used in the rearing of fish may similarly be reviewed on animal welfare grounds. Several independently commissioned reports exist regarding animal health and welfare practices on fish farms and numerous recommendations for improvements have been put forward (e.g. Report of pain and stress in fish, commissioned by the Royal Society for the Prevention of Cruelty to Animals, UK).

## 10.1 INTRODUCTION TO SITE MANAGEMENT PLANS

In cyprinid culture an overall strategy for the prevention of disease problems is more important than in almost any other fish culture system. In terms of coarse and food-fish farms, most cyprinids are managed in extensive systems (Fig. 10.1) where they feed on natural food. There is very limited turnover of water and the handling of fish prior to treatment is contraindicated. In most cases, therefore, it is simply not possible to treat the fish effectively once they are suffering an outbreak of disease.

In terms of intensive, enclosed systems, e.g. those based on recirculating filtered water, as used for example by aquarists, these provide ideal conditions for rapid propagation of many disease-causing organisms. Another problem with filtered recycling systems is that many chemical treatments cannot be easily applied, as they are harmful to the nitrifying bacteria of the biofilter. Examples of such treatments include methylene blue and various antibiotics.

In addition, most countries constantly review the human health or environmental effects of adding chemical treatments to water bodies. The result is that whilst effective chemical treatments may exist, their application is restricted or banned because of their toxicity and effects on the environment. In a recent UN Food and Agriculture Organization review on the state of world aquaculture, disease amongst fish was considered as part of a wider picture. Treatment of disease should therefore go beyond consideration of the pathogen alone as conventional approaches have had only limited success. This holistic system management approach is an idea that is now gathering favour in many sectors of fish health.

**Fig. 10.1** Cyprinid farm in the UK.

A site management plan is therefore an effective tool to limit the spread of disease and can be used in all circumstances where fish are held in captivity. It allows all the various health management issues to be integrated into one scheme and shows how each aspect helps in achieving the final result. In most cases this plan will fall into three sections:

(1) preventing diseases gaining access to the site;
(2) good husbandry to create healthy fish;
(3) curative measures.

### 10.1.1 Prevention

The axiom 'prevention is better than cure' is of overriding importance in fish health management. The majority of fish health problems in any fisheries enterprise are preventable, but once established, the problems are difficult if not impossible to cure. Obviously the best way to prevent a transmissible fish disease from causing problems is to prevent its access to the system. To achieve this all possible sources of infection should be monitored and controlled. Each site will be different and will vary depending on the amount of control that can be placed on potential points of entry.

The very first aspect of the plan should be to review the site and secure the perimeter as much as possible. In most cases the perimeter can be secured leaving three main points of association with the wider environment, the main farm gate, the water inlet (abstraction point) and the water outlet (discharge point).

**Restriction of access**

Once the perimeter is secure the main farm gate is the point of contact for most human farm visitors. These visitors and their vehicles do represent a risk in terms of pathogen transfer. Farming enterprises that involve intensive systems usually restrict visitors' access, and in fact many fish-farmers ban all outside vehicles from the yards and buildings. It is recommended that a policy of completely excluding everyone/thing from close contact with the fish be enforced. In many cyprinid farms and other culture situations this can be problematic as a substantial income can be obtained from selling fish at the farm or in the case of aquaria by having the fish on display as a tourist attraction. These problems can be eradicated or reduced with careful planning. The office buildings, reception and car park should be sited well away from the fish-holding facility. The reception area should also include the feed store so that high-risk vehicles such as feed lorries are not allowed near the fish. A proper enclosed feed store also has other advantages in that the food is more likely to stay dry and deter vermin.

Some visitors will obviously still need to be allowed access to the site itself but risks of disease can be minimised by using disinfectants. Disinfection procedures are usually simply a matter of limiting the

number of access points, usually to one, and then implementing a disinfection routine at the point of access; this is known as a disinfection barrier. Disinfection is usually by means of a foot bath and wheel bath.

Once the disinfection barrier is established, site-access protocols should be implemented to ensure that it is used effectively and is never circumvented. Methods to ensure that the barrier is both used and effective include the construction of foot baths that are wider than easy stepping distance and making sure that the bath is always full of disinfectant. It is perhaps ironic that an effective disinfection policy may lead to complacency and poor implementation of the site management plan once that disease's incidences decline.

The most commonly used disinfectants are iodine (iodophores) which are easy to apply, have minimal toxicity to fish and are nonpersistent. Other products available include peroxyacetic acid, para-acetic acid and sodium hydroxide.

A special section of the site management plan should address the most often ignored visitor to the site, namely the fish farm staff. These personnel often pose one of the main risks for introduction of disease as they will often visit other fish farm sites. Farm staff should be supplied with a full set of clothing that is to be worn only on site. Staff should be informed and trained in all aspects of the site management plan, a copy of which should be prominently displayed in a suitable staff area.

Another source of disease introduction is through unauthorised visitors in the form of poachers and animal predators. The perimeter fencing will usually discourage poachers and random visitors, although cyprinids are not usual targets for poaching, being usually maintained in extensive ponds, which make the fish difficult to extract quickly. Intensive systems however do require good security especially where high-value fish, such as koi, are being cultured. It would seem obvious that any fish-holding facility would attempt to control predators but in some circumstances it may not appear cost effective due to the relatively small quantities of fish consumed. However, many predators, especially fish-eating birds, are either hosts to parasites that infect fish or act as transmitters of pathogens. Access by predators to the site can be restricted by simple procedures, e.g. predator netting.

In addition to predators, wild fish may enter the site from the surrounding environment and this can be a source of extraneous, and sometimes repeated, infection outbreaks. The prevention of this, which is a legal requirement in many countries, usually involves installing and maintaining adequate screening on the intake and discharge points.

The incoming water is also an important source of disease as many transmissible fish diseases are waterborne. Cyprinid culture often has a major advantage in this respect as it has relatively low water turnover particularly in comparison to salmonid culture, which may rely on high flow-through rates. Given the low volume of inflow water that needs to be treated, it is well worth considering the total disinfection of

the water supply. It is theoretically possible to disinfect intake water by treatment with ozone or ultraviolet light.

**Food as a source of infection**

In some systems, particularly in enclosed cyprinid culture and aquaria, live or fresh foods are often used to supplement the dry diet. In many cases this is essential as some of the dry diets available are not especially formulated for feeding to cyprinid species and poor growth or nutritional problems may occur. When feeding certain live and fresh foods the risk of infection can be reduced by obtaining food from reputable suppliers who practise food disinfection or disease-avoidance protocols. Dry diet supplementation is not so important in the case of aquarium cyprinids as many high quality complete dry diets are available for pet fish, thereby obviating or reducing the need for live food supplements.

**Restrictions on the site**

In addition to isolating the whole site from possible sources of infection, certain areas within the facility may need special attention either to protect systems that are vulnerable to disease problem, e.g. hatcheries, or to isolate an outbreak of a disease on the site.

Isolation of the above areas will involve total separation of equipment as far as possible and disinfection barriers being placed between the site in question and all remaining areas. Colour coding all the equipment is a good way of reinforcing this idea. Larger and more expensive equipment is more difficult to replicate and should be thoroughly disinfected between uses. In addition, appropriate working practices can be adopted where the isolated area, which is usually more sensitive to disease, is dealt with at the start of the day's activities.

**Quarantine**

Perhaps the greatest risk of introducing infection onto a site comes from the introduction of new stock. Whilst in an ideal situation the site should be self-sufficient for producing offspring, under normal circumstances this is not the case. Fish are introduced into a new site for a variety of reasons, e.g. to introduce new genetic variability and to reduce inbreeding, or to make up order shortfalls. The risk of introducing a pathogen can be reduced by adhering to several simple procedures. The fish should only be purchased from a reputable supplier, and should be checked for health problems by a suitably qualified fish health biologist. Clear and comprehensive farm records should also be kept. When the fish are brought onto site an appropriate quarantine system should be used. For most situations it is relatively easy to provide for quarantine facilities that meet certain basic requirements:

- They should be large enough to hold the fish for a suitable length of time, usually 3 months.

- They should be spatially isolated from the rest of the stock, e.g. by at least 4 m. The possibility of disease spread via aerosols is often completely ignored.
- Only clearly designated equipment should be used in the quarantine facility.

A technique becoming more widely used in the quarantine process is to introduce some fish from the site to the quarantine tanks after approximately one third through the quarantine period. This use of sentinels has the same effect as introducing the new fish into the system but with considerably less risk. A disease outbreak will often manifest if there are any pathogens present in the quarantined fish.

**Vaccination**

Vaccines provide a prophylactic method of disease control, and have been applied successfully in many areas of captive animal husbandry. However, despite over 50 years of fish vaccine research, only a handful of commercial products are currently available, most directed against salmonid pathogens.

Advances in fish vaccine development are partly hindered by the high research and development costs in relation to expected profits. Individual fish may have a very low market value, and hence the unit cost to vaccinate each fish must be a fraction of this. In addition, acquired immunity in fish is poor when compared with that of higher vertebrates, which means that vaccines might not be capable of stimulating fully protective immunity, or be long lasting. Thus, whilst safe and effective vaccines would be extremely desirable for cyprinid health management, so far only one or two are undergoing development specifically for cyprinids.

**National and international controls**

Many countries, recognising the value of their aquaculture and fishing industry, attempt to protect their stocks from imported disease by controlling imports of live fish. In the European Community this has taken the form of identifying serious disease pathogens and placing border controls on live fish that may be infected. To date there is only one pathogen in cyprinid culture that is recognised as being very serious: SVC. In some countries there is also recognition of the deleterious impacts that introduced pathogens have on the local wild fisheries via the release of non-native fish. These countries have imposed legislation to reduce or eliminate these problems, e.g. in England and Wales the Import of Live Fish Act and Section 30 of the Salmon and Freshwater Fisheries Act.

### 10.1.2 Husbandry

Although a policy of preventing the introduction of pathogens should be carried out in all fisheries, fish farms and aquaria, it can never be completely successful, however well implemented. However, if a dis-

ease is accidentally introduced it may not necessarily lead to massive fish mortalities. Fish have an effective immune system similar to other vertebrates and, given the right conditions, they are able to resist or combat most diseases. The chances of the fish overcoming infections can be increased by the use of good husbandry techniques to reduce stress, good farm hygiene and perhaps in future, the use of vaccines and immune boosters.

**Husbandry and the reduction of stress**

Stress is an important consideration in fish farming, where the stock is typically held at high density in a confined space and is routinely disturbed for various purposes, such as grading and movement between sites. In fish farms where health and water quality monitoring is not properly enforced, the fish may be exposed to continuous chronic stress due to pathogens and/or adverse environmental conditions. The deleterious effects of stress on the immune system in fish are now well documented, and it is not surprising, therefore, that stressed fish are more prone to disease outbreaks. Depending on its nature, stress can have other negative effects on the stock, for example suppression of growth rate, reduction in feed intake, and inhibition of spawning. Conspecific aggression within the stock is another form of chronic stress, but fortunately this is less of a problem with cyprinids in general, as compared, for example, with salmonid farming.

Good husbandry and general farm practice can help to reduce stress in captive cyprinids. High stress levels in fish suppress the immune response, possibly via the production of 'stress hormones', the corticosteroids. All aspects of the keeping of captive fish have some impact on the stress levels in a fish. For instance, carp kept in overcrowded conditions release a substance, probably a pheromone, into the water which causes depression of heart rates and growth rates of fish exposed to it. It would be impossible in the space available to give details on all aspects of fish husbandry that can potentially induce high levels of stress in the stock; however, several potential stressors are mentioned below.

Stocking density

Overcrowding is a major cause of stress. The stocking density will vary depending on the species and the properties of the system in which they are held. It is vital however that the maximum stocking density is established and never exceeded, as greatest mortalities are usually associated with overstocking. Some species, particularly shoaling cyprinids, also have a minimum stock density below which they may become territorial. In addition, many species benefit from sharing their habitat with another species, and this can be exploited by co-culturing two or more species, termed polyculture. In polyculture systems, stocking densities in excess of those attainable using monoculture may be achieved in situations where individual species do not significantly compete for swimming space and other requirements, e.g. silver carp and common carp.

### Handling fish

Keeping fish inevitably means that the animal has to be handled. This is obviously the most stressful action for the fish, although handling stress can be reduced by the adoption of good husbandry practices. For example, ponds should be constructed so that they are easily drained and include a catch pit. In addition, the type of equipment used, e.g. grading machinery, should be carefully chosen to reduce stress. Such machines also save on labour costs and are therefore a good economic investment. The grading of fish also gives an opportunity to check for disease or administer treatments or vaccines.

### Maintenance of water quality and acclimation

All fish are sensitive to adverse water quality. In addition, fish are particularly sensitive to rapid changes in environmental parameters, even where the change is to a condition in which the species would normally be expected to survive. Fish therefore must be allowed to acclimate slowly to the new conditions. This will minimise the physiological stress caused by moving the fish and therefore maximise their survival. This is highlighted by consideration of a common situation that occurs in both aquaria and recirculation systems.

**BOX 10.1: CASE HISTORY – AQUARIUM EXAMPLE**

Some tropical ornamental cyprinids developed vague signs of ill-health and a test of the water revealed a very high level of nitrates. Nitrate accumulation may have arisen due to poor aquarium hygiene, infrequent partial water changes, overcrowding or excess nitrogenous waste excretion due to gross over-feeding. Given the known ichthyotoxicity associated with high nitrates, the perceived solution was to quickly dilute the nitrate by changing 75% of the aquarium water. Initially, the fish's health improved but within a couple of days they started dying. Although the high nitrate level was undesirable, the remedial water change resulted in a dramatic increase in pH, causing physiological stress to the fish ('pH shock').

Fish do have the ability to accommodate or acclimate to changes in water conditions, provided such changes occur gradually and remain within the physiological tolerance limits of the species in question. In this scenario, the acclimation to gradually increasing nitrate levels was in fact less life-threatening than the subsequent exposure to a sudden and large change in pH. It is therefore important to avoid subjecting fish to rapid changes in water conditions (e.g. temperature, pH) such as when transporting them, or when undertaking water changes to their captive environment.

Routine health checks of stock

It is vitally important that the stock are regularly checked by a competent person. This can conveniently be carried out daily when the fish are fed. Any abnormal behaviour or disease symptoms should be reported to a qualified fish disease expert as soon as possible. It is essential to investigate and diagnose the problem promptly. It is also important to keep records of all the farm activities that will not only assist in making sure the fish are routinely checked but also, should a disease occur, aid in tracing the source of infection.

Feeding and nutrition

Cyprinid culture is one of the oldest farming practices in the world yet in many countries farmers and fisheries still feed cyprinid fish on a dry pelleted feed specifically formulated for other fish species. Most commonly this means growth formulated trout pellets being fed to carp. There is perhaps no more damning indictment of the fisheries industry than the feeding of cyprinids with pelleted food designed for carnivorous species. Formulation of feed should therefore be tailored to the species, as well as for the time of year, the climate and the required growth. For example, for common carp in temperate areas winter starvation for 5 to 6 months appears to be a normal part of their life history. The fish will have different feed requirements on entering and recovering from these periods of torpor. In addition, during these periods of quiescence, feeding should be reduced or stopped. Storage of food is also important. Dry pelleted feeds all have recommended storage conditions as well as use-by dates. Food that has become mouldy or contaminated by rats and other vermin should be discarded.

### 10.1.3 Therapeutic measures

If a disease occurs in the fish stock then the final control method is the implementation of a treatment regime. This may involve both chemical and physical control measures.

**Chemical treatments**

In some countries the appropriate use and control of chemicals is a legal requirement in all farming activities. Detailed records therefore need to be kept for any chemical treatments whether they are for fish disease control, weed control or whatever. In addition, most chemotherapeutics have a minimum withdrawal period or a maximum residual level of active ingredient in the flesh of the fish, which are important considerations when the fish are intended for human consumption. Also, the indiscriminate use of chemicals prior to disease investigation can often mask the disease-causing organism, making it impossible to detect the cause of a problem. The use of chemicals to treat disease should therefore be used with caution since indiscriminate application may mean culturists are failing in their duty to care for the animals in their charge. As Noga (2000) so eloquently states 'It is unworthy of

a fish professional to treat fish in this way'. It cannot be emphasised strongly enough that chemical treatment should not be administered without a proper diagnosis. Most chemical treatments are harmful to the fish in some way and if administered incorrectly can actually do more harm than good as in the case of the antibiotic oxytetracycline (OTC), which can act as an immunosuppressant. In addition, no product should be used without proper knowledge of the active ingredients and mode of action. Many drugs that are used to treat both food and pet fish are neither licensed for use on fish nor licensed for the way they are being applied.

It is therefore important in all circumstances where a chemical is to be used to:

(1) Accurately diagnose the disease to be treated
(2) Identify the most appropriate chemotherapeutic
(3) Administer the drug in a safe and effective manner
(4) Conform with all legal requirement for the country/region in question
(5) Keep records of all the actions taken to alleviate the problem.

Factors affecting the choice of chemotherapeutic

Several factors need to be taken into consideration when choosing a treatment. These include the species of fish, the system in which the fish are held and the route of administration. Generally, only fish that can be confined and accurately targeted are suitable for chemical treatment. This means that fish held in extensive systems will have to be captured and treated. Obviously, as with most applications of chemical treatments, the capture and preparation for treatment induces stress in the fish.

There are five major routes by which fish are exposed to chemotherapeutics: waterborne, oral, injection, spray and swab.

*Waterborne*

This is the most common method used in any fish culture system and has the obvious advantage that the handling of the fish is reduced or eliminated. The chemicals can be administered by constant immersion, bath or flush through. Not all chemotherapeutics are water-soluble and hence not all can be administered this way.

*Prolonged immersion.* To avoid any handling of the fish the chemical is added to the system. It is mostly suitable for the treatment of external pathogens such as skin and gill parasites, fungi and skin bacteria. This sort of treatment does, however, have the problem that the drugs are usually toxic to a wide range of organisms. They can therefore be problematic in recirculating systems and filtered aquaria where the biological filter bacteria are likely to be affected, also in open water extensive growing ponds where the live food that is essential for the productivity of these ponds will be affected. Copper treatments, for example, are highly toxic to certain aquatic invertebrates. In extensive systems,

which usually contain large volumes of water, it means realistically that chemical immersion *in situ* is not really suitable.

*Bath.* This is usually the cheapest, most effective and commonly used way of administering a chemical. It allows a precise dosage to be given for a controlled time period with greater control over the situation. For bath treatment the following protocol should be carried out:

(1) Take a suitable size container, fill it with water, half fresh and half from the system and aerate. Ensure the fresh water is chemically compatible for the fish, e.g. pH, temperature, etc.
(2) Add chemical to required dosage.
(3) Carry out a trial test with a few of the worst affected fish. Leave these fish for at least 2 hours to check that they fully recover before continuing to treat the rest of the population. When satisfied, add the remaining fish. An exposure time of between 5 and 10 minutes is usually recommended.
(4) Have a recovery tank containing well-aerated water in readiness to receive the treated fish.
(5) At the end of the treatment place fish back into system.

The major problem with bath treatments is the initial capturing of the fish as many cyprinids are held in systems where netting of the stock is required. Since this method of chemical treatment will obviously have no effect on any pathogens or their stages that occur in the water or in intermediate hosts, then repeat administration may be required.

*Flush.* This technique is particularly relevant where fish are held in systems that have a continuous, high water flow-through. Very simply, a measured amount of the chemical is added at the tank inlet and allowed to flush through the system. This technique is really only effective when the chemical will pass through the culture in a predetermined time and where uniform dispersal occurs. In addition, there is also the problem that active fish may flee to the end of the tank in advance of the chemical front, and hence receive suboptimal exposure. Although this method of chemical administration is not really suitable for most cyprinid systems the technique is routinely used in the treatment of eggs held in Zuger jars.

*Oral*

This may be the ideal way for the administration of some chemotherapeutics. Requirements are: that the chemical must be combined with the diet of the fish in such a way that it retains its activity, the fish will still consume the food, the chemical should not be inactivated by the fish's digestive process, and the active ingredients should be absorbed into the fish's body through the gut wall. Unfortunately, many treatments do not fully meet these prerequisites. Generally, oral treatment should take place after a 24-hour period of starvation and the fish should be fed at 2% body weight. The dosage should be calculated to

give an adequate dose to any fish that eats at least 1% of its body weight per day. To work effectively the correct amount of treated food must be consumed by each fish. This is unlikely in any fish system as infected fish may lose their appetites and dominant individuals may eat more food. In addition, young fish may eat only live foods. However, novel delivery techniques are being developed, for example fish can be fed *Artemia* that has been impregnated with chemotherapeutics, and drugs can nowadays be encapsulated to increase delivery efficiency and reduce degradation in the fish's gut. The problem of oral uptake of food impregnated with chemicals can be overcome by oral intubation where the medicine is injected down the throat of an anaesthetised fish by means of a fine soft tubing, allowing a precise dose to be given. This technique should only be carried out by a trained professional, as it is easy to damage the gut.

*Injection*
This is the best means of administering the correct dose of therapeutic to individual fish. Obviously it requires handling, possibly anaesthetising, and is therefore extremely stressful. This technique should never be performed by inexperienced people and is therefore expensive in training or hiring skilled personnel. The recommended sites for most injectable drugs are either into the peritoneum (intraperitoneal) or the muscle (intramuscular).

*Spray*
Some drugs and vaccines can be sprayed onto the skin or gills of fish and this can be conveniently carried out during routine husbandry procedures such as grading.

*Swab*
Some treatments may be given topically to the affected area. This is particularly useful when treating large, expensive fish exhibiting bacterial skin lesions. Although the fish may respond to a systemic antibiotic, the lesion may take time to heal and in the meantime will have a significant impact on the health of the fish, particularly in relation to secondary infection. A broad spectrum biocide is usually applied to all areas of the lesion and after 30 seconds the area is sealed by the application of a water-repellent substance such as Orabase™ or Vaseline™.

It will be obvious that the selection and dose of any chemical or drug is dependent on a wide range of variables including the method of administration, the disease, fish species, age of fish and environmental parameters. It is therefore impossible to give details on all the permutations available. However, to give an indication, some treatments are listed in Table 10.1. The reader should always seek advice from a recognised disease expert before any application is made. The chemicals have been included here because they are well-known and generally effective. Their inclusion and the exclusion of others does not represent an endorsement.

**Table 10.1** Selected examples of chemical treatments of fish pathogens.

| Chemical | Use/target organism | Route of delivery |
|---|---|---|
| Acetic acid | Disinfectant and parasites | Waterborne |
| Acriflavin | Bacteria | Waterborne |
| Antibiotics | Bacteria | Oral, injection or immersion |
| Chloramine T | Parasite | Waterborne |
| Copper sulphate | Protozoa | Waterborne |
| Difluorobenzuron | Crustacean copepods | Waterborne |
| Dimetronidazole | Protozoa | Waterborne |
| Diquat | Parasites and gill bacteria | Waterborne |
| Fenbendazole | Nematodes | Oral and waterborne |
| Flubendazole | Parasites | Waterborne |
| Formalin | Ectoparasites | Waterborne |
| Formalin/malachite green | Ectoparasites (particularly protozoa) | Waterborne |
| Furanace | Bacteria | Waterborne |
| Gentian violet | Fungi and bacteria | Waterborne |
| Hydrogen peroxide | Disinfectant and bacteria | Waterborne |
| Iodine – Iodophors | Disinfectant and bacteria | Waterborne |
| Ivermectin | Crustacean parasites | Oral and waterborne |
| Levasimole | Nematodes | Oral |
| Malachite green | Fungi and protozoa | Waterborne |
| Mebendazole | Cestodes and monogeneans | Oral |
| Mercurochrome | Bacteria | Topical |
| Methylene blue | Bacteria, fungi and protozoa | Waterborne |
| Metronidazole | Protozoa and some bacteria | Waterborne |
| Niclosamide | Cestodes | Oral |
| Organophosphate pesticide – Dichlorvos and Trichorphon | Parasites (particularly crustacea and monogeneans) | Waterborne |
| Phenoxyethanol | Bacteria and anaesthetic | Waterborne |
| Piperazine | Nematodes | Oral |
| Potassium permanganate | Parasites and some bacteria | Waterborne |
| Praziquantel | Parasites, particularly cestodes | Oral and injection |
| Quinines | Protozoa | Oral |
| Quaternary ammonium compounds – benzalkonium chloride | Protozoa and bacteria | Waterborne |
| Salt (pure salt, NaCl) | Protozoan, bacteria and fungi. Environmental stress relief. Reduces osmotic stress and nitrite poisoning | Waterborne |
| Toltrazuril | Protozoa | Oral |

Antibiotics are extremely effective against bacterial infections and are also used as growth promoters. However, as has already been stated in this book, there are increasing numbers of bacteria species and strains that show extensive resistance to antibiotic treatments. It is therefore imperative that before any antibiotic is administered the

causative bacterium is identified and its antibiotic sensitivity established.

**Environmental/physical treatments**

Environmental and physical control measures can prove very effective, particularly where control can be exerted over the system and the fish. These control measures take many forms depending on the disease. For example, with whitespot disease some control can be achieved in aquaria by daily removing the top layer of gravel, which contains the reproductive tomont stage. As another example, the hatching of *Dactylogyrus* eggs can be reduced by placing a mesh 50 cm from the bottom of the tank. This eliminates some of the cues produced by the fish that stimulate the eggs to hatch. Other parameters that can be altered include:

- Temperature. This can be achieved by adjusting the temperature beyond the tolerance range of the pathogen but within the physiological limits of the fish.
- pH. Many disease agents are susceptible to changes in pH. An example is Costia, which thrives at pH 5–6. Therefore raising the pH will have a deleterious effect on the parasite.
- Filtration. In systems using recirculated water, there is an opportunity to filter out infectious disease agents, for example the use of microfiltration.
- Ultraviolet radiation. Used to kill microorganisms.
- Light. Many infectious life cycle stages of parasites are phototaxic, therefore placing lights around the outlet of a tank may help to attract these stages to the outlet.
- Control of intermediate hosts. Detailed knowledge of the life cycle of the parasite may help in its control.
- Ozone. Although strictly a chemical treatment, ozone is often considered as a distinct strategy in specialised facilities.

## 10.2 TRADE ORGANISATIONS AND OTHER BODIES

Many forms of farming have trade organisations that act to support the interests of the whole industry and are funded by the industry. One extremely successful trade organisation is the Scottish Salmon Growers Association, which supports research and is a point of focus for political lobbying and advice in the UK. Unfortunately, similar organisations do not widely exist for cyprinid culture even though cyprinid fish are of economic importance throughout the world. However, several organisations have been established with interests in the health of fish and fisheries. Such organisations include the European Association of Fish Pathologists (EAFP), the Institute of Fisheries Management, UK (IFM), the Ornamental Aquatic Trade Association (OATA), the British Koi Keepers Society (BKKS), the American Fisheries Society, the Fish

Veterinary Society, UK, and the Food and Agriculture Organization (FAO) of the United Nations.

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 11
# Future Developments

In an ideal world, there should be little need for health management of fish. This is because fish stocks that live in uncrowded and unpolluted conditions are generally able to resist serious disease problems. In fact, disease prevention, by providing good water conditions and adequate quarantine procedures, is the best method of health management for both wild and captive fish. It is, however, difficult to attain this goal and produce sufficient quantities of fish to make a system commercially viable.

In terms of cultured cyprinids, there is a growing dependence on aquaculture as a means of feeding the world's human population. This means that research into more efficient and economic production of fish is required, particularly in the area of health management.

Aquaculture conditions created for fish cultivation units can be an ideal environment for inducing poor health and diseases, which result in production losses, fluctuating markets and possible economic disaster. The problems arise primarily because cultivated fish are held at high stocking densities in less than ideal conditions. These disease problems are exacerbated by the worldwide trade in fish that has led to the introductions of ova and fish stocks from wide ranging geographic areas. Unfortunately, although attempts at controlling the spread of fish diseases have not been very effective, despite international laws governing fish and fish product introductions. The industry, therefore, has had to control diseases with the use of drugs such as antimicrobial compounds, chemicals, insecticides, herbicides, expensive diets, food additives, natural substances (e.g. garlic and onions), and more recently, vaccination. In fact, vaccines are regarded by some as the future saviour for the control of fish diseases. Caution is however required because even in vaccinated stocks some fish remain as 'carriers' and act as reservoirs of disease.

Most of the pioneering and current research in fish disease has been directed towards the rapidly growing salmon farming industry and fewer efforts have been applied to cyprinids. This appears to reflect the areas of the world in which salmonids are cultured rather than the economic importance of cyprinids in the global economy.

At present, developments in the field of fish health appear to relate mainly to:

(1) The rapid diagnosis of disease and the diagnosis of disease in asymptomatic carriers

(2) The vaccination of fish against serious pathogens
(3) The development of novel therapeutic methods

## 11.1 RAPID DIAGNOSTIC METHODS

Rapid disease diagnosis is vital in cases of acute infectious diseases, such as those caused by bacteria and viruses. Conventional diagnostic methods for microbial pathogens are often time consuming, requiring isolation and culture techniques that may take several days to yield a conclusive result.

The advent of rapid serological tests, where disease diagnosis relies on testing a small sample of blood, enables the diagnosis to be obtained in as little as a few hours. In simple terms, the principle of serodiagnosis involves the production of relatively specific proteins (antibodies) that react when in contact with an antigen produced by an organism foreign to that host. The traditional way of preparing these antibodies is by inducing their production in laboratory animals, e.g. a mouse, by exposing the mammal to the antigen. These antibodies can then be integrated into simple agglutination and neutralisation tests, which identify organisms, based on these properties. These tests are relatively easy to perform and have proven successful in identifying certain disease organisms. The principles involved in both agglutination and neutralisation assays have been developed to form the basis of serological tests, which rely on measuring antigen–antibody reactions *in vitro*.

### 11.1.1 Antibody detection assays

The presence of antibodies indicates that the fish has either been exposed to a certain pathogen and thus its antigens, or carries a current infection. By sampling the blood of several fish within the population it is thus possible to gather information on the disease history of the stock, even in fish that are no longer infected or do not show overt disease.

### 11.1.2 Antigen detection assays

These assays use antibodies raised in mammals or within cell cultures of mammalian lymphocytes (e.g. monoclonal antibodies) to test for any specific antigens in the fish's blood. The presence of circulating antigens, indicative of a particular pathogen, indicates a current or very recent infection. These types of assay thus rely on the visualisation of the antibody–antigen reaction. This can be done by labelling the antibody, e.g. monoclonal with an enzyme, as used in the ELISA (enzyme-linked immunosorbent assay) which utilises a colorimetric enzyme–substrate reaction to visualise the test result (Plate 11.1). Alternatively, a fluorescent probe can be attached to the antibody as in the FAT

(fluorescent antibody test). The results are read visually using ultraviolet microscopy.

As well as diagnosing disease outbreaks quickly, serological tests hold great promise for future fish health monitoring, as new antibody and antigen reagents become commercially available, enabling a wide range of fish pathogens to be screened for. These novel tests may reduce the need for more invasive and destructive diagnosis. Current problems relate to the specificity of these serological tests. Research into diagnostic techniques has concentrated on improving their specificity and yet, in some cases, an initial simple and easy to perform test for general fish health status is all that may be required.

Advances in serology will also focus on producing simplified assays that are sufficiently robust to be performed and interpreted on-site by the layperson, such as fish farmer or aquarist, rather than, as is the case at present, tests having to be undertaken by specialised laboratories using expensive equipment. In addition, modified serological assays are under evaluation that can be used to test samples of fish mucus, enabling the diagnosis of pathogens without the need for invasive blood sampling.

### 11.1.3 Genetic screening technology

The identification of a pathogen, particularly in the case of metazoan parasites, relies upon distinguishing subtle differences in the shape and size of several structural features. In genera containing large numbers of species this can be problematic. For example there are over 300 species of gyrodactylids. Some are relatively benign, others have a wide host range causing problems on some host species and not on others whilst others, i.e. *Gyrodactylus salaris*, are extremely pathogenic. The difficulties associated with identification of gyrodactylids have serious consequences in determining the host and geographical range of individual species and establishing successful control strategies. To overcome these problems automated statistical databases are being developed to increase the accuracy and rapidity of diagnosis. In addition, molecular techniques now offer an alternative approach to conventional taxonomy. For example, ribosomal DNA (rDNA) probes and restriction fragment length polymorphism (RFLP) analysis are being developed to differentiate *Gyrodactylus salaris* from other gyrodactylids (Fig. 11.1). *In situ* hybridisation is a new technique for detecting subclinical infections, including covert infections and 'carrier' states in fish populations. Basically, this involves a pathogen-specific single stranded DNA (ssDNA) probe, which is labelled with digoxigenin.

## 11.2 VACCINE DEVELOPMENT

Vaccination is an immunological method of disease prevention. There are various types of vaccine which range from utilising whole organ-

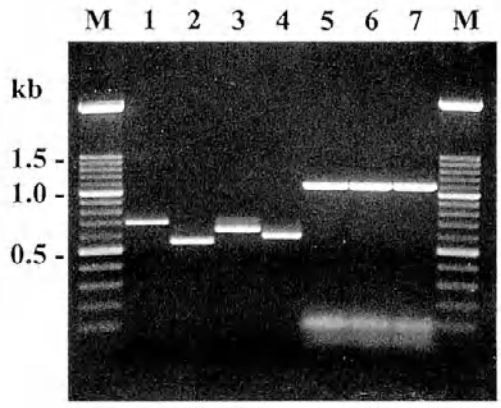


**Fig. 11.1** Gel electrophoresis using polymerase chain reaction (PCR) products from ribosomal DNA (rDNA) from gyrodactylids. M = molecular weight markers; lanes 1–2 using primers (P3 and F3) from *Gyrodactylus salaris* and *G. turnbulli*; lanes 3–4 using primers (R1 and P3) from *Gyrodactylus salaris* and *G. turnbulli*; lanes 5–7 using primers (P3 and P4) from *G. turnbulli*). Note different light bands (PCR products) with different species of parasite (courtesy of P.D. Harris, J. Cable, R.C. Tinsley and C.M. Lazarus in the *Journal of Parasitology*; American Society of Parasitologists).

isms, whether they are alive, dead or attenuated, to individual components of the pathogens, e.g. antigens and, recently, DNA. Basically, a vaccine works by eliciting a protective immune response in the fish but without causing the disease; it therefore simulates an infection.

Vaccines for bacterial diseases of carp were first investigated by Schäperclaus in the 1930s. The first prototype vaccine came to fruition in the 1940s and was developed against a bacterium, *Aeromonas salmonicida*, the causative agent of furunculosis, which had emerged as a serious disease affecting hatchery reared trout. This vaccine was not particularly successful and it took another 20 years for an efficient furunculosis vaccine to become commercially available. Since then, there has been considerable interest in vaccine development for other fish diseases and, over the past 10 years, at least another 30 vaccines have become commercially available. Mostly, this success has been due to the rapid growth of the salmon farming industry and subsequently the need to protect against diseases, reduce the huge cost of chemotherapeutic drugs, and overcome the problems of drug resistant pathogens.

Unfortunately, although there have been considerable advances in the production of vaccines against several species of farmed freshwater and marine fish, the vaccination of cyprinids has not received the same amount of attention, particularly in the case of ornamental cyprinids. The more extensive nature of cyprinid farming and the low value of some fish species has, until recently, discouraged the development of commercial vaccines. The main disease against which farmed cyprinids have been vaccinated is ulcer disease, although a vaccine against SVCv is under development. As far as the vaccination of

ornamental cyprinids is concerned, the main progress in this area relates to the development of effective furunculosis vaccines for salmonids and their widespread uptake and commercial use. A dramatic increase in the incidence of ulcer disease, i.e. goldfish ulcer disease, koi ulcer disease, carp erythrodermatitis (CE), caused by aeromonads, and especially atypical strains of *Aeromonas salmonicida*, and the increase in problems associated with antibiotic resistance has encouraged workers to assess the feasibility of extending the range of the furunculosis vaccine. Therefore, the vaccines that are available for ulcer diseases are simply furunculosis vaccines initially intended for salmonids, but marketed under other brand names. Currently, there are two such vaccines available, Cyprivac (Aquaculture Vaccines Ltd), an immersion vaccine, and Furogen-6 (Aqua Health Vaccines), an injectable vaccine. Traditional methods of vaccination have involved using attenuated virus strains, inactivated virus, protein subunits or peptides. These vaccines are however not in use because of regulatory laws introduced in response to concerns about the safety of the products, and inconsistent efficacy.

There are a number of problems associated with the vaccination of fish, particularly ornamental species. While injectable vaccines may be acceptable for use on farms and possibly at the wholesaler, there are problems with the immunological boosting of fish by the ornamental keeper or fishery owner since it is unreasonable to expect the amateur aquarist to have the necessary skills to vaccinate fish by injection. There can be problems with adjuvanted vaccines in terms of side effects in the fish and possible allergic reactions following accidental self-injection by the operator. Indeed, some of the adjuvants contained in injectable furunculosis vaccines can be quite toxic to cyprinids.

Vaccines applied by immersion may overcome some of the above problems but there are also drawbacks associated with their use. Immersion vaccination usually only provides relatively short-term protection necessitating regular boosters. In addition, these vaccines have been developed for the salmonid industry and have thus been formulated to vaccinate large numbers of fish. It may be economically difficult to scale down the volumes of vaccine to suit the small numbers of fish that are kept for example by the aquarist. Most fish vaccines are only available on veterinary prescriptions and there are logistical problems in providing such vaccines for fish-keepers, aquarists, carp farmers and fishery owners. In addition, there appears to be reluctance by the suppliers of ornamental fish and carp farmers to become involved in pilot vaccination programmes. This is mainly for economic reasons and their apparent unwillingness to abandon the use of established antibiotics and other chemotherapeutics on their extensive farms. Nevertheless, there is current research being undertaken into producing vaccines for the ciliate, *Ichthyophthirius multifiliis* (Ich) and SVC virus. Although there is progress for producing a vaccine for Ich, latest reports suggest that a commercial vaccine for SVC will not be available for some time.

There are new and evolving areas of research into genetic immunisation or DNA-based vaccination methods. These methods involve the direct transfer of plasmid DNA encoding antigenic proteins, which are expressed within cells of an organism. When these probes are introduced, strong responses in both the humoral and cellular arms of immunity occur in the fish. It is now possible to achieve DNA-mediated immunisation against a single protein antigen, and this is said to be enough to provide protection against infection by certain pathogens. For example, DNA-vaccination of rainbow trout by intramuscular injection of the plasmid-encoding gene of the viral G-protein of the VHSv (viral haemorrhagic septicaemia virus) has recently demonstrated that it can effectively protect fish against VHS. This disease is caused by a rhabdovirus. Spring viraemia of carp is also caused by a rhabdovirus and it is possible the same method could be used to control that disease in cyprinids. DNA mediated immunisation to a single protein can also provide protection against infection by incorporating oligodeoxynucleotides and plasmid DNA stimulants into leucocytes to produce supernatants with antiviral activity. Similarly, bacterial DNA and synthetic oligonucleotides have also been used as adjuvants and immunostimulants.

Vaccines rarely comprise just the pathogen or antigen in an inert solution. They usually include additives, i.e. immunostimulants (adjuvants). There has been considerable research into the use of these adjuvants in oral, injection, immersion and spray vaccines. Their inclusion appears to enhance the immune response, increase the duration of protection and at the same time increase general resistance to pathogens. Adjuvants used include a whole range of natural and synthetic compounds, e.g. glucans, lipopolysaccharides (lps), extracts of abalone, natural and synthetic oil-based materials. Those adjuvants that have shown encouraging results in experimental work include: bovine lactoferrin and saponin used orally, levasimole for immersion and glucans for injection.

For some years now there have been attempts to find ways of delivering vaccines more effectively and many of the studies have concentrated on the oral route. However, although the gastro-intestinal tract of most fish can take up whole proteins, many are broken down by the digestive enzymes. To develop this simple, less stressful method of delivering vaccine to a fish it will be necessary to deliver the whole protein vaccine through to the site of absorption without it being broken down and inactivated. Current research into the development and evaluation of biofilms of *Aeromonas hydrophila* for oral vaccination of carp has shown some promise. The presence of a protective glycocalyx coat may confirm resistance of the biofilms to the action of digestive enzymes, antibiotics, antibodies and certain chemicals. Related techniques include the delivery of a vaccine in the body of a food organism, e.g. the brine shrimp, and the use of liposomes or fat droplets to encapsulate the vaccine. The former shows particular promise in small fish.

## 11.3 TRENDS IN CHEMOTHERAPY

Many of the problems relating to the development of more efficient delivery routes for vaccines are also applicable for drugs. The problems are however exacerbated by the varied legislative controls on drug use and how strictly they are adhered to. For example, in the USA in the 1960s, there were about 270 different chemicals and drugs available to the fish farmer, but now only four antimicrobials and one anaesthetic are legally available. The situation is very similar in EU countries. These restrictions are usually applied to the use of chemical compounds for the treatment of fish for human consumption and include oxytetracycline, oxolinic acid, amoxicillin, and co-trimazine (trimethoprim-sulphadiazine). However, even in those countries with rigorous restrictions, a few additional drugs are available through the 'prescription cascade', i.e. certain restricted drugs can be administered to fish only by a veterinarian who is responsible for monitoring the outcome of the treatment. The reason for the strict controls on drugs for fish relates to human health concerns, for example the risk of antibiotic resistance being transferred to human bacterial pathogens. In addition, antibiotic resistance may also be induced in bacterial pathogens of fish, which would make their future control more problematic.

In the case of antimicrobials, they are usually administered as 'in-feed' medications, by surface coating them onto feed pellets or adding them during the food manufacturing process. The doses have to be strictly controlled and, if the fish are being reared for human consumption, a time period following dosing must be observed before harvesting. However, for expensive brood-fish and some ornamentals, e.g. koi, which are not reared for human consumption, some drugs are administered by injection.

## 11.4 FUTURE DISEASE RISKS

Infectious diseases are likely to remain the major health problem facing cyprinid culture, despite expected advances in vaccine technology, rapid disease screening, and the application of novel chemotherapeutics.

The alarming emergence of antibiotic-resistant bacteria remains a health concern for the future. Already, some of the established generation antibiotics for treating fish, such as oxytetracycline, have met with resistance by vibrios, aeromonads and other fish-pathogenic bacteria, limiting the value of these antimicrobial drugs. Even relatively novel antibiotics, such as the fluoroquinolone drug sarafloxacin, are showing evidence of failure against certain strains of bacteria, a worrying situation that is being paralleled in human antibiotic therapy. Worse still, some strains of fish pathogenic bacteria have become multi-antibiotic resistant which means that the aquaculturist may have to resort to novel, and possibly expensive, antibiotics or combination antibiotics.

The indiscriminate use of antibiotics to treat fish diseases serves to accelerate the emergence of drug-resistant bacteria. In many countries, including the USA, the availability without prescription of many antibiotics leads to their over-use or misuse by professional aquaculturists and pet fish-keepers alike. Global restrictions on the use of antibiotics may slow the rate of drug resistance; however, it is difficult to see how such legislation could be enforced and policed internationally. One solution is to ensure that the disease-causing bacteria are isolated and identified so that an appropriate antibiotic may be administered rather than the 'trial and error' approach that is often employed when bacterial diseases occur. The answer probably lies in improving captive fish welfare, perhaps augmented with immunoenhancing vaccines and other drugs that will increase the stock's resistance to bacterial pathogens and hence reduce the need for antibiotic intervention. A new generation of antimicrobials, such as chemicals that inhibit bacterial attachment to host cells, are being evaluated for medical use. Given that many of the modern chemotherapeutics applied to fish were initially developed for the medical and veterinary industries, it is possible that these attachment inhibitors may also find their way into aquaculture.

Towards the end of the 1990s there were great advances in transgenic research, particularly on commercially important animals. There are obvious potential economic benefits of transgenic technology to aquaculture. Selective breeding, particularly in ornamental fish species, has been carried out over numerous years, but the identification, isolation and construction of genes responsible for desirable traits and their transfer into broodstock has important implications in many aspects of fish biology. Several genes that have been isolated, e.g. growth hormone and antifreeze protein (AFP) genes, have great potential value in aquaculture particularly involving salmonids. With the increasing knowledge of the genomes of cyprinid fish, e.g. carp, zebra danio, and the ability to isolate and characterise genes that play an important role in disease resistance, there is a great potential to produce transgenic cyprinids that are resistant to different pathogens.

As mentioned earlier, intensive aquaculture practices demand high fish stocking densities, which provide ideal conditions for disease transmission. Of particular concern is the ongoing risk of introducing new diseases via stock movements and the aquaculture of exotic fish species. It should be borne in mind that noncyprinid fishes may carry diseases that could affect native cyprinid stocks, due to the wide host range of many fish pathogens and parasites. Legislation to control and restrict the import and movement of fish stocks, together with adequate quarantine regimes, will reduce the likelihood of introducing exotic diseases. Such legislation is now enforced in many countries, including the UK. However, the illegal movement of fish (especially ornamentals and exotic coarse fish), which is unlikely to abate, presents a continuing health threat to both wild and cultured cyprinid stocks.

It should be noted that in the case of several metazoan and protozoan parasites, introduction does not necessarily mean establishment. For a parasite to successfully infect a native cyprinid population the

correct environmental conditions and, in some cases, the appropriate intermediate or definitive hosts must be present.

Whilst some diseases have a wide distribution, others have a geographical range which is currently somewhat restricted. The continued expansion of cyprinid fisheries, and thus fish movement, may mean that some of these parasites extend their range to other countries. Given the predominance of cyprinid culture in certain parts of the world, e.g. Eastern Europe and the Far East, there is the potential for parasites within these regions to expand their range. For example, in China *Dactylogyrus vaginulatus* causes problems in the culture of silver carp and *Asymphylodora japonicum* can prove problematic in fry of grass carp, silver carp, bighead carp, common carp and crucian carp. The parasitic crustacean *Sinergasilus undulatus* only occurs in China where it has been noted to cause damage to the gills.

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*Diseases of Carp and Other Cyprinid Fishes*
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# Chapter 12
# Laboratory Procedures in Disease Diagnosis and Control

The diagnosis of morbidity and mortality in any sphere of animal husbandry is a highly skilled role and this is no different for cyprinid fish. The aquaculturist and aquarist, however, do have a role to play and can sometimes diagnose the problem without recourse to a professional. However, it is very important to understand the limitations of what can be done on site and when it is time to call in the veterinary surgeon or suitably qualified fish pathologist. When a disease outbreak occurs that needs expert advice there are two options: either the expert visits the site or samples are sent directly to the fish health laboratory. The fish health inspector must:

(1) Be experienced in a wide range of fish and fishery related aspects apart from his or her knowledge of fish diseases
(2) Always wash and disinfect the work area and protective clothing before leaving the site
(3) Inform the fishery owner/farmer exactly what information is needed and the type of samples that need to be examined
(4) Inform the farmer what has been done, explain any preliminary results, provide information about further tests and advise when results will be available
(5) Present the farmer with a list of samples taken, and inform the farmer's veterinary surgeon of the visit.

## 12.1 EXAMINATION ON SITE

If the sampling and post mortem examination are to be made in the field, some sort of mobile laboratory will be needed, e.g. the back of a car, a shed or building at the sampling site.

There are several advantages of site investigations: the pathologist can discuss the background of the problem with the farmer, husbandry personnel, fishery owner, river-keeper or aquarist and establish if there are any problems with water quality and husbandry that might be associated with disease. Records of fish movements onto and off the site can also be scrutinised. In addition, the samples can immediately be obtained from the diseased fish and where appropriate samples can be dispatched by courier services to specialist laboratories. Examination on site also means that the fish do not suffer further stress from transportation.

There are, however, several disadvantages associated with examination on site. Sites, facilities and weather conditions are not always favourable for post mortem examination and sampling, and the lack of sterile conditions may increase the chances of the samples becoming contaminated. In addition, specialist staff are required for the sampling, which means that it can be labour intensive.

It may be necessary to transport tissue samples to the specialist laboratory. Samples requiring virological examination can be frozen or placed in transport media, whilst samples requiring bacteriological examination must not be frozen, but can be dispatched in transport media. Samples for histological examination must be placed in sealed receptacles containing appropriate fixative.

Selected samples of live fish may also be sent for laboratory examination either using a special fish-transporter fitted with aerated tanks or by placing them in water-filled polythene bags for transportation. For the latter method the fish bags are one-third filled with water. The other two-thirds of the bag are filled with pure oxygen. The bag is then sealed and placed upside-down in another polythene bag. The bag should be placed in a box made of efficient insulating material and, where appropriate, surrounded with ice (Fig. 12.1). Maximum transport time depends on water temperature and the ratio between biomass, water volume and oxygen. As a rule, transport time should be no more than a few hours and the biomass should not exceed one third of the water volume. It is important to note that some countries do not allow live animals to be transported by courier or postal systems. Ad-


**Fig. 12.1** Fish packed in a sealed bag ready for transport (courtesy of the Environment Agency, UK).

ditionally, there are often regulations for transporting biological materials and chemicals, e.g. fixatives. Familiarisation with the regulations is essential.

## 12.2 SUBMITTING A SAMPLE TO THE LABORATORY

When submitting a sample to a laboratory there are several things that can be done to ensure the best possible service. Firstly, the actual samples submitted for post mortem examination should be obtained during the mortality, and be representative of the diseased or moribund fish showing symptoms. It is always preferable that the professional person consulted should view the fish actually on site. However, in some instances this is not possible, in which case live fish samples should be transported to arrive at the laboratory with the least possible delay. Usually five moribund fish is considered a sufficient sample size; however, this may vary depending on the situation, for example on a fish farm five fish from each affected raceway or tank may be more appropriate. It is vitally important to provide the pathologist with as much information regarding the mortality as possible. A proforma sheet showing the range of information is illustrated in Fig. 12.2. It is very important, however, to contact the laboratory and ask what sort of background information they require. It is recommended that a good working relationship should be fostered with both veterinary surgeon and laboratory before any problems arise.

In the case of aquarium fish it may not be possible or desirable to provide more than one specimen for disease diagnosis, and in most cases specimens will be submitted alive, with the aim of seeking a cure. Details of the aquarium or pond water chemistry, e.g. temperature, pH, ammonia, nitrite and nitrate levels may also be useful.

Most laboratories will deal with post mortem examinations using a set of procedures and protocols. After an initial visual examination for gross disease symptoms a thorough inspection for possible parasites and fungal pathogens will be made. Most parasites can be identified to genus level with a reasonable compound microscope and knowledge of the key features for identification.

Samples need to be taken from the fish for examination for possible viral and bacterial agents and to examine for changes in the tissue and organs using histological techniques. Only fish that are alive at the time of arrival at the laboratory will usually undergo full post mortem examination, although fish dead on arrival may be used for partial sampling.

## 12.3 INITIAL EXAMINATION

In situations where on-site examination of the fish is not possible or practicable then analysis will be carried out in the laboratory. Prior to dissection, the condition of the sample and characteristics of the

| | | |
|---|---|---|
| Your name and address | | |
| Contact numbers | | |
| Email | | |
| Origin of sample | | |
| Species in sample | Number | Size |
| Description of system | | |
| Date when mortality started | | |
| Rate of mortality (no. dead per day) | | |
| Number of fish dead | | |
| Species affected | | |
| Any species present but unaffected | | |
| Symptoms, including behavioural abnormalities | | |
| Any treatments given | | |
| Any other relevant information (water quality tests, etc.) | | |

**Fig. 12.2** A proforma sheet showing the range of information that should be included when submitting a sample to a laboratory for analysis.

mortality will be assessed, in order to determine the extent of sampling necessary. If specimens within the sampled population are showing classical signs of bacterial infection, and this is considered to be directly responsible for the mortality, bacteriology samples only will be taken. If initial diagnosis proves inconclusive, a full post mortem examination will be conducted, and a decision made during a second assessment on which tissue/fish samples to process further. This will, it is hoped, prevent unnecessary time and expense being spent processing samples that will be of little help in the diagnosis. Although rapid diagnostic tests are being developed many of the tests currently available are time consuming and results can take several weeks to obtain after sample submission. In the meantime mortalities in the fish stock are likely to continue. The initial assessment prior to euthanasia will also include noting the general condition of the fish and taking a scrape from the gills and skin, the latter from the dorso- and ventro-lateral surfaces, of at least three live fish soon after their arrival at the laboratory. The scrapes are examined using a compound phase contrast microscope at $\times 100$ and $\times 400$ magnification.

## 12.4 EUTHANASIA

There is overwhelming evidence to suggest that fish are capable of experiencing pain and stress, and hence the method of killing must be humane. Overdosing with a suitable immersion anaesthetic is the most favoured method of euthanasia, and enables several fish to be disposed of simultaneously. Several immersion anaesthetics are suitable for fish; the more popular ones are 2-phenoxyethanol, MS222 ( = ethyl-*m*-aminobenzoate) and benzocaine ( = ethyl-*p*-aminobenzoate). The reader is referred to Ross and Ross (1999) for detailed information on fish anaesthetics and their dosages. The dose of anaesthetic required to euthanase a fish will depend on the species, its size, and possibly other factors, e.g. water temperature.

The example below is for benzocaine euthanasia:

Benzocaine is not water-soluble and must be predissolved in absolute alcohol or acetone before being diluted in water to give the working dose. (Note: the chemically related MS222 has the advantage of being water-soluble.) A stock solution comprising 7 g benzocaine to 95 ml alcohol is prepared and a working dilution prepared using 10 ml of this stock per litre of water. This is thoroughly mixed before the fish are added. This dose has proven suitable for the euthanasia of many temperate cyprinid species.

Some fish may exhibit an escape response to the acetone so the container should be covered and some external parasites may become dislodged, hence the need to examine the fish prior to the application of anaesthesia. Fish should be unresponsive in approximately 20 seconds and left a further 5 minutes before removal. Where feasible, death should be confirmed by destroying the fish's brain, e.g. with a scalpel, in order to prevent accidental recovery. On no account should fish be consumed after chemical anaesthesia.

## 12.5 DETAILED EXTERNAL EXAMINATION

Fish should be placed on a dissecting board and the external surfaces thoroughly examined especially the fins and fin bases, noting any lesions, gross abnormalities and visible parasites. A skin scrape is taken from the anterior dorsal surface of the flanks ventral to the pectoral fin. These skin scrapes are examined as described above. Other sample sites should include the posterior side of fin bases and the edges of any lesions/abrasions present. It is also useful, in the case of wild fish populations, to age the fish to give an indication of growth and recent history. This should be done by low power microscopy of at least three scales taken from the anterior dorsal surface. In addition, 10 scales should be removed from the lateral region line and the area examined for parasites at a magnification of approximately × 8.

### 12.5.1 External bacteriology sampling

The presence of any lesions will necessitate taking a bacterial swab. When numerous lesions are found, the smallest one showing typical characteristics is chosen for swabbing. To reduce numbers of environmental background bacteria, any lesion to be swabbed is liberally flooded with 70% industrial methylated spirit (IMS) and dried in air. An incision is then made with a sterile scalpel across the lesion, cutting to the outside of the lesion into healthy flesh. A swab is then drawn across the resultant cut, from the inside of the lesion out into healthy tissue, rolling the head of the swab. The swab is then placed in a sterile container and any bacteria it contains are increased in number by swabbing on petri dishes containing an appropriate culture medium.

## 12.6 BLOOD SAMPLING

Where feasible a blood sample is taken from each anaesthetised or dead fish. The posterior dorsal aorta, which runs ventrally along the backbone, is generally the easiest and most effective site to obtain blood. The fish is held with the ventral side uppermost and the needle is inserted midway between the posterior base of the anal fin and the beginning of the caudal fin. When the needle touches the backbone the aorta will have been penetrated; with a little back pressure on the syringe piston the blood should flow into the syringe. If not, then very slowly withdraw and rotate the needle, stopping as soon as blood flows into the barrel. Before the blood is expressed the needle must be removed from the syringe to prevent damage to the blood cells. In the case of small fish, blood may be only obtainable using the caudal cut technique, which must be performed on dead fish. The caudal peduncle is cleanly cut with a sharp scalpel to reveal the caudal blood vessel. A heparinised microcapillary tube, prepared by flushing the tube with 10–20 units per ml of heparin in saline, is applied directly to the vein and the blood is obtained by capillary action.

One drop of blood is placed onto a clean slide for a blood smear and the rest of the sample should be transferred into an appropriately labelled tube. To produce a blood smear the edge of a cover slip is put into the drop of blood and drawn at an angle across the microscope slide. This is then examined for parasites by viewing at × 100 and × 400 either unstained or preferably after staining using for example Leishman or Giemsa stain.

It is possible to obtain further information from blood. For example, it can be tested for its packed cell volume (an indicator of general health status) by centrifuging it in a micropipette. The packed cell component is expressed as a percentage of the total blood volume. In addition, there are numerous other tests that can be carried out on a blood sample, e.g. red blood cell counts, glucose content, potassium content. These haematological methods should be treated with caution however as 'normal' levels have not been assessed for most cyprinid fish.

Without the knowledge of how the parameters vary depending on the nutritional status, the time of year, spawning condition, age, sex, etc., they are of very limited value. Work is continuing on many of these areas, particularly blood chemistry of carp, and so it may be possible in the not-too-distant future to determine a great deal more about the health status of a fish from a sample of blood.

## 12.7 DETAILED INTERNAL EXAMINATION

The skin and body wall musculature is cut away to reveal the internal organs (Fig. 12.3). The first incision is made parallel to the operculum from just dorsal to the lateral line, to below the pectoral fin-joint and round to the midline of the fish. Holding the pectoral fin with forceps, a second incision is made along the midline of the fish to a point between the opercula. Pulling the pectoral fin up and away from the body exposes the pericardial cavity and the heart.

### 12.7.1 Heart removal and examination

The heart is removed using forceps just in front of the bulbus arteriosus, and pulling the whole heart gently out of the pericardial cavity. On larger fish, it may be necessary to cut away connective tissue from around the heart, to reduce damage and ease removal. The heart is then placed in a Petri dish with phosphate buffered saline (PBS) and examined under a low power dissection microscope. The organ is then cut longitudinally to reveal the interior, this procedure being carried out whilst under observation at ×10 magnification. Any parasites, e.g. *Sanguinicola inermis*, may be active only for a few seconds and are particularly difficult to see when they are inactive. A longitudinal section of the heart, excluding the bulbus arteriosus, should be stored in an appropriate fixative, e.g. 10% buffered formalin for later histological examination. In the case of very small fish, the whole heart may need to be retained.

After removal of the heart a ventro-lateral opening in the body of the fish is made by using blunt-ended scissors from the top of the first incision, along the flank just ventral to the lateral line, curving the cut ventrally to the vent. Care should be taken not to damage internal organs or puncture the swim bladder by cutting too deep into the body cavity. Remove the resulting flap from the fish, making sure that all internal organs remain intact. Some connective tissue may need to be cut away so that the visceral organs can be examined *in situ* before the body wall flap is removed completely.

### 12.7.2 Internal bacteriology sampling

To gain access to the kidneys in cyprinids, the swimbladder is gently removed or pulled to one side. A small cut can then be made through

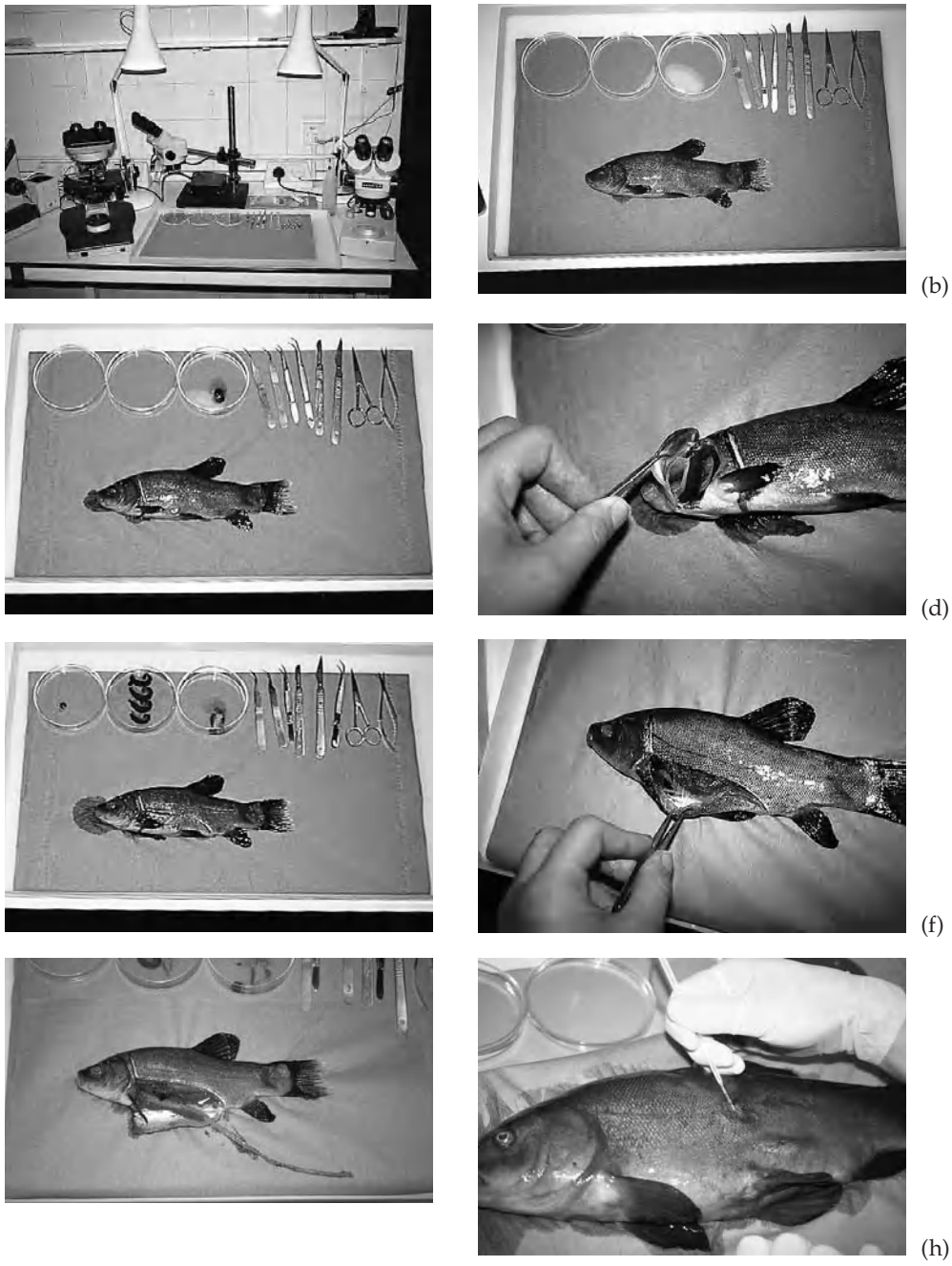


**Fig. 12.3** Post mortem. (a) Workstation set up to perform a routine fish health check; (b) tench laid out ready for examination; (c) the first cut is made to remove the heart which is placed in one of the Petri dishes ready for further examination; (d) the gills are removed and placed in a Petri dish for further examination; (e) the eye is removed and placed in a Petri dish for further examination; (f) the next major cut is from a point adjacent to the pectoral fin to the vent. This allows the lateral body wall of the fish to be pulled ventrally revealing the body cavity; (g) the gut stretched out to allow further dissection; (h) bacterial swab being taken from a small lesion. (Courtesy of the Environment Agency, UK.)

the membrane containing the kidney, with a sterile scalpel, and an appropriately sized swab inserted with a rolling action (Fig. 12.3h). The swab is then labelled and stored in a sterile container in a fridge, prior to plating onto an appropriate culture medium. To swab the kidneys of very small fish the dorsal fin is removed and the dorsal surface of the fish cleaned with 70% IMS, dried in air and an incision is made using a sterile scalpel blade through the vertebral column into the kidney. Care should be taken not to cut into the peritoneal cavity, thus contaminating the sample. Orientate the body ventrally to expose the kidney, and insert an appropriately sized swab, avoiding contact with other areas.

It may also be necessary to swab other internal organs in which case a similar procedure is followed. The exposed surface of the organ is cleaned with 70% IMS and dried before cutting with a sterile scalpel, and a swab is then inserted into the cut.

### 12.7.3 Visceral organs

The spleen, liver and kidney are examined *in situ*, and any discoloration, haemorrhaging, tumours, abnormalities, parasites, etc., noted. Each organ is then sampled as follows:

(1) Small pieces of each organ (approximately 2 mm in size) are taken, placed on a slide with a small amount of saline, squashed using the coverslip and examined under a compound phase contrast microscope at × 100 and × 400 magnification
(2) Larger pieces (approximately 5 mm in size) are removed from each organ (trying to take a clean, undamaged section) and fixed for histological examination
(3) Small pieces (approximately 3 mm) of spleen and kidney are removed and stored at 4°C for virological examination, which should be carried out as soon as possible.

### 12.7.4 Intestine

The gastro-intestinal tract should be carefully removed from the body cavity, noting any discoloration, haemorrhaging, fluid retention, necrosis, tumours, fat deposition, etc. For histology, a small section (approximately 10 mm long) of the whole intestine, approximately one quarter of the length from the anterior end, is obtained and any attached connective tissue/fat gently removed. The remaining intestine is then opened using a longitudinal cut and examined in phosphate buffered saline (PBS) under a low power stereo microscope, noting the contents and any abnormalities and parasites.

### 12.7.5 Gills

Gills are removed intact, by cutting each end of the branchial arches separately, and their general appearance and any abnormalities, e.g. necrosis, clubbing or haemorrhaging, noted. If examining a small fish,

e.g. under 6 cm, a whole gill arch is taken for histology; however, for larger fish a small section of the gill, approximately 10 mm, is taken. Examination of the remaining gills is carried out in phosphate buffered saline (PBS) under a low power dissection microscope, teasing out the connective tissue between the gill filaments and examining for parasites. Squashes of gill tissue are made from a number of filaments, as well as a scrape of the gill tissue/mucus, and examined at a magnification of × 100 and × 400 in phase contrast, for parasites.

### 12.7.6 Eyes and nasal cavity

Following a general external examination of the eye in which any abnormalities, e.g. lens opacity, are noted, the organ is removed by slipping a pair of curved forceps under the eyeball, and cutting the connective tissue below and around it. The lens and humour of the eye are examined in a Petri dish containing PBS under a low power light microscope, taking care not to damage the lens during removal.

Following the removal of the nasal flap, a brief examination of the nasal cavity can be made under low power dissection microscope, and any abnormalities and parasites noted. In very small fish, an incision is made between the two nostrils to expose the tissue within, or the whole nasal region can be removed and examined separately.

### 12.7.7 Brain

A transverse cut is made vertically into the head of the fish, dorsal to the top of the operculum. This can be done with a strong knife, or in the case of larger fish, using a saw with small teeth. The brain, which is located posterio-dorsally to the eyes, can be removed intact and examined for any obvious signs of disease, e.g. tumours, haemorrhaging, and necrosis. Depending on the size of the fish, either half the brain, or an approximately 5 mm longitudinal section, can be processed for histology, and a smaller piece (approximately 3 mm) obtained for virology.

## 12.8 LABORATORY TESTS

### 12.8.1 Parasitology

Identification of parasites can be made using unstained wet preparations observed under appropriate magnification and with the aid of a diagnostic key. However, this is sometimes inadequate and identification should be carried out on stained specimens by a competent parasitologist. There are numerous specific staining and presentation techniques available that will allow the diagnostic features to be visualised. Below is just a representative sample.

**Staining whole mounts of parasites**

Parasites such as flukes, tapeworms, nematodes, etc., can be prepared

either whole or in portions to be viewed using light microscopy. The first step in this procedure is to fix the parasite in an appropriate fixative, e.g. 10% buffered formalin. The large size of some species necessitates them being flattened during the fixation process. This is achieved by placing the parasite between two slides and then submersing them in the fixation fluid. After approximately 24 hours the fixative is removed and the parasite placed into 70% alcohol overnight. A variety of stains can be used to visualise the parasite. For general purposes borax carmine is used and can be purchased from commercial sources. The parasites are placed in the stain for 1–3 days depending upon their size. A few drops of concentrated hydrochloric acid (HCl) is then slowly added until the carmine has precipitated and is brick red in colour. This is left overnight in an equal volume of acid alcohol (3% HCl in 70% alcohol). Acid alcohol destains the tissue and this step should be repeated over several hours until the required amount of stain is retained by the parasite. The parasite tissue is then dehydrated in a graded series of alcohol (90%, 95%, 100%) and then added to xylene. The parasite is then mounted on to a glass slide, covered with a small amount of mounting medium, e.g. DPX™, and a coverslip added. Although the preparation can be viewed immediately using a microscope, it will take several days for the mounting medium to harden.

**Staining blood parasites**

Once a blood smear has been dried in air it should be fixed and stained. This is usually carried out in one process by immersing the slide containing the smear in a specialised stain. These stains are usually based on those established by Romanowsky in 1891 who utilised a stain containing eosin and methylene blue to visualise blood cells and parasites. Now these stains can be obtained already prepared from commercial laboratory suppliers.

Leishman stain

In 1901 Leishman prepared methylene blue combined with eosin and dissolved the resulting precipitate in methyl alcohol. The dried blood smear is covered with the stain for 5–10 minutes and then washed in distilled water to differentiate and turn the smear pink in colour. After the smear has dried it is viewed using a microscope.

Giemsa stain

Giemsa in 1902 introduced a modification of the Romanowsky stain, which was further upgraded by Gurr in the form of 'Gurr's improved Giemsa stain R.66'. With this stain the blood smear has to be initially fixed using methyl or ethyl alcohol for 2 minutes. Ten drops of concentrated stain are diluted in 10 ml of neutral water and the smear immersed in this solution for 10–20 minutes. The stained smear is washed in distilled water, dried and viewed with a microscope.

### 12.8.2 Bacteriology

The swabs taken from the fish should be spread onto a nutrient-rich agar gel as soon as possible. If they are to be retained or sent to a specialist laboratory then special media-filled transport swabs that are commercially available should be used. During transportation the samples should be kept cool on ice but not frozen. On the nutrient agar each individual bacterium will grow and multiply until they each become a visible colony comprising a single species. Some agars provide nutrients that favour certain types of bacterium, but it is usual for a variety of bacteria to grow from a swab, particularly those taken from a lesion. Each bacteria species yields colonies with slightly different characteristics that can aid in identification. A pure culture is required for identification purposes so a single example of each colony type is removed and applied to a new plate and left to grow more colonies.

Identification of each bacteria species is a relatively simple process and is achieved by the use of differential agar. These are agar gels which have ingredients added to favour the development of certain types of bacteria. An example is Coomassie blue agar which contains a blue dye that is utilised only by specific bacteria, such as *Aeromonas salmonicida*, in the development of its cell wall. This gives the colonies of these bacteria a very obvious blue colour. Since each bacterial group has different requirements and abilities to utilise various nutrients it provides the basis of a rapid diagnostic tool. For example the commercial API strip uses a wide series of nutrient tests, the results of which are compared to a database of known bacterial species (Plate 12.1).

### 12.8.3 Serology

Serological tests are used to detect specific antibodies or parasite/pathogen antigens in the fish's blood. Such tests are very useful in situations where alternative diagnostic methods are impracticable or unreliable, for example in the case of many viral and some bacterial diseases. The blood sample obtained from the fish should be left for at least 20 minutes, or more usually 2 hours, at room temperature to clot, before being centrifuged at 3000–5000 *g* for 10 minutes. The serum is removed and dispensed into clean, labelled tubes.

Serological tests are being developed that should enable the rapid diagnosis of all major diseases, particularly those of viral and bacterial origin. The basic principles behind these tests are relatively simple. An infection will introduce foreign substances into the blood of the fish, for example, proteins from the viral coat. These proteins, or antigens, stimulate the fish immune system to produce antibodies, which are specifically designed to recognise these antigens. These two components of the immunological interaction between the pathogen and the fish, i.e. the antigen and the antibody respectively, form the basis of serological tests developed to detect their presence. Such tests rely on

the visualisation of antibody–antigen interactions using markers, e.g. enzymes attached to appropriate probes. These probes are themselves antibodies raised in mammals, e.g. rabbits, against either the antigen or the fish antibody. This is the basis of one commonly used serodiagnostic test, the enzyme-linked immunosorbent assay (ELISA) which can be modified for different uses, e.g. to detect antigens, or antibodies. For example, in an ELISA developed to detect antibodies against a certain antigen, e.g. a protein from *Aeromonas salmonicida*, wells in a plastic plate are coated with either the antigen or a homogenate of the pathogen. This is accomplished by placing the homogenate or a solution containing the antigen into a well and leaving it to bind to the plastic, e.g. at 4°C overnight. After the excess solution is removed and the wells washed with an appropriate buffer, those areas of the plastic that are not covered with the antigen are coated with a nonspecific substance, e.g. bovine serum albumin or a solution of dried milk powder. The well is referred to as being 'blocked'. A serum sample from the infected fish that will contain antibodies to the pathogen is now added to the well. After incubation, e.g. 30°C for one hour, the well is again washed and the probe, e.g. an enzyme-linked antibody raised against a fish antibody, is added. After a further incubation and wash, the substrate of the enzyme is added and a colour develops that can be recorded. What is formed therefore is a sandwich, which comprises antigen, fish antibody, enzyme-linked antibody and enzyme substrate. To develop a sensitive and yet robust assay it is necessary to optimise all components of this sandwich. The detection of antigen entails the formation of a similar ELISA sandwich. In this case however, an antibody is bound to the plate to capture the antigen from the fish's serum. A second labelled antibody is used to confirm both the presence and amount of antigen bound. The technique can be further developed by binding the antigen or antibody not to plastic but to a nitrocellulose membrane. Such an adaptation used to detect protein is termed a Western blot and can be further refined by subjecting the serum containing the antibody, or the pathogen homogenate containing the antigen, to electrophoresis. In this the solutions are loaded into wells in specialised solid media, e.g. polyacrylamide gels, and subjected to an electrical current. The proteins present are separated on the basis of their size and charge and can be electrophoretically transferred to a nitrocellulose membrane and subjected to the staining procedure as with ELISA. This therefore not only allows the antigen to be located but also its molecular weight to be determined.

### 12.8.4 Virology

Examination of tissue for the presence of a virus can be very time consuming and expensive. Detailed virological techniques can be obtained from the references at the end of the chapter, but briefly, the established technique for fish involves isolation of the virus on a tissue culture. In the literature there are also many references to viruses or

virus-like particles observed in ultrathin sections of tissues examined with the electron microscope.

**Virus collection**

Samples of fish tissues selected for virology testing can be held for a short period of time, i.e. up to 2 h before processing at 4°C, or kept on ice. Those needing to be held for longer periods are frozen and stored at –80°C. Otherwise, samples that cannot be dealt with immediately can be transported in 'transport media' and subsequently stored in 'maintenance media'. For transport, a nutrient-rich medium is used. This usually consists of 10% fetal bovine serum with penicillin (5000 Iu/ml), streptomycin (5000 µg/ml), gentamycin (1500 µg/ml) and fungizone (1200 Iu/ml). The maintenance medium is similar but with reduced components, e.g. 2% fetal bovine serum with the following antibiotics added: penicillin (1000 Iu/ml), streptomycin (1000 µg/ml), gentamycin (500 µg/ml), and fungizone (400 Iu/ml). The antibiotics are added to inhibit growth of contaminant bacteria and fungi. Virology samples from small fish are usually pooled in batches of five, but tissues from larger fish are processed individually.

**Tissue culture**

The use of tissue culture to grow viruses is the recommended diagnostic method used in most laboratories. This requires the availability of established fish 'cell lines'. Viruses are fastidious and may require specific live cells to grow in, for example, spring viraemia of carp virus (SVCv) grows best in carp epithelial cells (EPC). These cells are usually grown as monolayers on plastic 24, 48 or 96 well microtitre plates. The virus samples, i.e. tissue extracts or homogenates, are inoculated at predetermined dilutions in maintenance media, usually 1:100, onto the cells in each well. It is important to establish the correct sample dilution in order to prevent false positive reactions due to toxic contamination. The inoculated plates are examined daily and if there is a virus present, the cells will behave abnormally, such as forming syncytias (cells linking together). This is known as the cytopathic effect (CPE) (Fig. 12.4). If there is no CPE after 7 to 10 days, fluids from the inocula are collected from the wells and used to inoculate on a fresh batch of wells and the whole procedure is repeated. This is the first 'blind passage'. If after a further 7 to 10 days there is no sign of a CPE, a further repeat inoculation is made and then if signs of CPE are still absent the sample is considered to be free of virus. The presence of the virus can be confirmed by the addition of antibodies, which have been raised in rabbits against a known viral antigen. If the particular virus is present then there will be a cessation of CPE due to antibody inhibition of the virus.

### 12.8.5 Histology

Histology is one of the most powerful tools in the armoury of a clinician

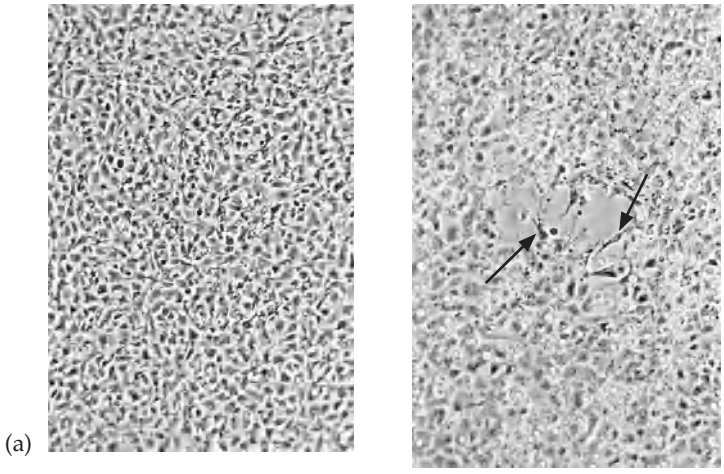


**Fig. 12.4** Fish cells lines used to diagnose viral infections. (a) Normal fathead minnow cells; (b) fathead minnow cell line infected with virus showing destruction of cells (arrowed). The so-called cytopathic effect. Magnification × 200 (courtesy of Keith Way).

for detecting cellular abnormalities. It can, however be time consuming and expensive and results can occasionally be difficult to interpret.

**Rapid methods**

Some protozoa and bacteria in the gill, and protozoans, especially developmental stages of myxosporeans, in the kidneys can be detected rapidly using tissue smears. This involves the following:

(1) a freshly dissected small piece of tissue is taken from the gill or kidneys with forceps and blotted on absorbent paper to remove excess fluid;
(2) the tissue is gently pressed onto a clean microscope slide (several impressions can be made on one slide);
(3) the smear is air-dried and fixed in 5% acetic-methanol for 5–10 minutes; the smear is then stained in May-Grunwald-Giemsa stain.

Protozoa and some bacteria stain pink to blue and, with practice, can be differentiated from the host cells.

**Preparing tissues for histology**

It is usual for routine examination to take samples of gills, intestine, spleen, liver, kidney, heart and brain. The tissues should be handled very carefully to avoid any post mortem handling damage which can mask any cellular changes present, and preserved in a fixative, e.g. 10% neutral buffered formalin. Each sample of tissue should be no more than 5 mm thick to allow complete penetration of the fixative. To achieve this, samples should be placed in at least 20 times their volume of fixative. It will take approximately 48 hours for the tissue to be

preserved enough for further processing. Other less common tissues that can be sampled include sections of skin, muscle and eye. These tissues are best fixed separately, in other fixatives, e.g. 'Bouins' solution for 48 hours, before being washed and stored in 70% alcohol. For large fish, intestine sections may necessitate dripping the fixative through the gut lumen, to allow even penetration and adequate fixation.

Fixed tissues are trimmed to present a large, flat surface area and then further processed. Any tissues comprising a large amount of bone should be decalcified using 5% aqueous nitric acid or 10% formic acid. This is because unless the calcium salts are removed from the bone, the tissue is too hard for microtomy. Whether subsequent processing is carried out manually or using one of the many automated processing machines that are available, the procedure is basically the same. Processing involves first dehydrating the fixed tissue by passing it through a graded series of alcohols, i.e. 90%, 95%, 100% and then placed in a fluid that is miscible with wax. Such a fluid is xylene and because of its refractive index the tissue appears clear and the process is sometimes referred to as 'clearing'. The cleared tissues are now placed into molten wax, sometimes in a vacuum oven, and the wax changed several times. The molten wax penetrates the tissues and is hardened by plunging the molten block into cold water. Thin sections, approximately 5–7 μm thick, are obtained using a microtome and are then attached to a slide. The wax is removed from the tissue slices by immersing them in xylene and then they are rehydrated using a decreasing graded series of alcohols and water mixtures. The tissue can be visualised using a range of stains, some of which react with certain tissue components. For general staining however, haematoxylin and eosin (H&E) is usually used.

## 12.9 DIAGNOSIS

After all these techniques have been completed and the results evaluated the diagnostician is armed with a great deal of information. The art of good diagnosis is to translate this information into usable, practical advice for the fish keeper. It helps if this advice can be given quickly and usually an initial response should be given straight after the post mortem examination, ideally within 24 hours of the sample being submitted. This report should include the results of the parasite and fungal analysis and will also include descriptions of symptoms that may be bacterial or viral in nature. This initial diagnosis should lead to some practical suggestions.

Samples taken for examination take a considerable amount of time for a definite diagnosis, for example:

- Bacterial diagnosis can take about 8–10 days
- Viral results can be about 14 days
- Histology results can be obtained in approximately 7 days.

Therefore, it is important to insist upon an initial diagnosis that can provide immediate practical advice. For example, if high levels of pathogenic parasitic or fungi are evident then these can be dealt with quickly by chemotherapy. However, if the diagnostician considers that the underlying cause may be bacterial in nature, weakening the fish and making them susceptible to ectoparasites, then the administration of an antiparasitic drug may be contraindicated.

## 12.10 POST MORTEM EQUIPMENT

Although diagnosis may require the use of specialised equipment, usually simple instruments are required to carry out the initial post mortem. A dissecting board, scalpels, sharp scissors, forceps and sterilised swabs are needed.

In addition, the most important piece of equipment is the compound light microscope (Fig. 12.5) and to obtain good results it must be set up and used correctly.

To set up a microscope, the following steps should be followed:

(1) Place the microscope squarely on the bench in front of you.
(2) Make sure the condenser, objectives and eyepieces are dust free. They can be cleaned with special lens tissue. Never use tissue paper or any other substitute.
(3) Lower the stage of the microscope.
(4) Swing the ×10 (low power) objective into position and place a microscope slide centrally on the stage.
(5) Use a slide that contains a tissue section or other suitably small object.

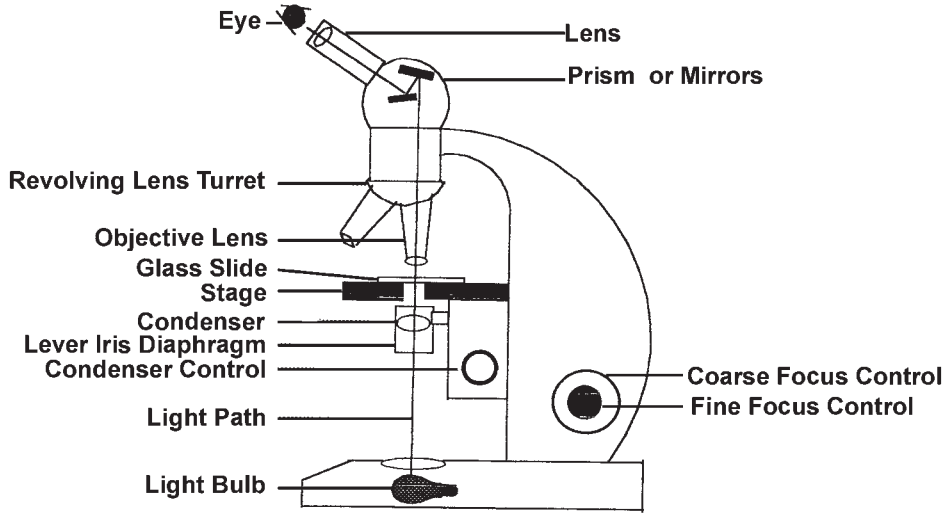


**Fig. 12.5** Diagrammatic representation of a compound microscope.

(6) Raise the condenser until approximately 2 mm below the slide and half open the iris diaphragm.
(7) Move the stage towards the objective lens using the coarse focus control.
(8) Look through the eyepiece lens, which is usually ×10 magnification, and slowly move the stage down, using the coarse control, until the object on the slide comes into view. The image can be sharpened by using the fine control.
(9) Hold a pencil or other sharp object against the light source and focus the condenser by moving it up and down until the image of the sharp object is in view.
(10) Carefully remove the eyepiece and look down the tube. Open and close the iris diaphragm on the condenser and adjust it until its image is between two-thirds and three-quarters of the diameter of the objective lens. Replace the eyepiece.

To increase magnification most microscopes have an additional objective lens, ×40 magnification, and some microscopes may also include a ×100 magnification lens. This latter lens is very specialised and requires oil to be placed between the tissue and the lens. Such a lens is usually used for viewing blood preparations or bacteria. The oil-immersion lens should be used very carefully:

(1) Make sure the object you wish to view is in the middle of the field of view with the ×40 objective.
(2) Move the stage down, rotate the lens turret to locate the ×100 lens in place and add one drop of immersion oil onto the sample.
(3) Watching from the side, slowly lower the lens onto the oil. It is obvious when the lens inserts onto the meniscus of the oil. Viewing the specimen down through the eyepiece, slowly focus the image.
(4) After you have finished the stage should be lowered and the ×100 lens cleaned immediately with lens tissue.

## FURTHER READING

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*Diseases of Carp and Other Cyprinid Fishes*
D. Hoole, D. Bucke, P. Burgess, I. Wellby


# Fish Names Cited in Text

## CYPRINID FISHES

| | |
|---|---|
| Aral sea barbel | *Barbus brachycephalus* |
| Asp | *Aspius aspius* |
| Barbel | *Barbus barbus* |
| Barbs | *Barbus, Puntius* and related genera |
| Bighead carp | *Hypophthalmichthys nobilis* |
| Bitterling | *Rhodeus* sp. |
| Bitterlings | *Rhodeus, Tanakia, Acheilognathus* and related genera |
| Black carp | *Mylopharyngodon piceus* |
| Blageon | *Leuciscus souffia* |
| Bleak | *Alburnus alburnus* |
| Blue bream | *Abramis ballerus* |
| Bluntnose minnow | *Pimephales notatus* |
| Bream | *Abramis* and *Blicca* spp. |
| Carp | *Cyprinus carpio* |
| Cave barb | *Caecobarbus geersti* |
| Chub | *Leuciscus cephalus* |
| Clicker barb | *Pseudorasbora parva* |
| Colorado squawfish | *Ptychocheilus lucius* |
| Common bream | *Abramis brama* |
| Common carp | *Cyprinus carpio* |
| Common minnow | *Phoxinus phoxinus* |
| Common shiner | *Luxilus cornutus* |
| Crucian carp | *Carassius carassius* |
| Dace | *Leuciscus leuciscus* |
| Danubian bleak | *Chalcalburnus chalcoides* |
| Fathead minnow | *Pimephales promelas* |
| Finescale dace | *Phoxinus neogaeus* |
| French nase | *Chondrostoma toxostoma* |
| Golden masheer | *Tor* sp. |
| Golden shiner | *Notemigonus crysoleucas* |
| Goldfish | *Carassius auratus* |
| Grass carp | *Ctenopharyngodon idella* |
| Gudgeon | *Gobio gobio* |
| Ide | *Leuciscus idus* |
| Koi | *Cyprinus carpio* |

| | |
|---|---|
| Longnose dace | *Rhinichthys cataractae* |
| Major Indian carps | *Catla catla, Cirrhina mrigala, Labeo rohita, Labeo calbasu* |
| Mediterranean barbel | *Barbus meridionalis* |
| Mirror carp | *Cyprinus carpio* |
| Nase | *Chondrostoma nasus* |
| Northern hog sucker | *Hypentelium nigricans* |
| Northern redbelly dace | *Phoxinus eos* |
| Orfe | *Leuciscus idus* |
| Roach | *Rutilus rutilus* |
| Rosefin shiner | *Lythrurus ardens* |
| Roundtail chub | *Gila robusta* |
| Rudd | *Scardinius erythrophthalmus* |
| Schneider | *Alburnoides bipunctatus* |
| Silver bream | *Blicca bjoerkna* |
| Silver carp | *Hypophthalmichthys molitrix* |
| Snail-eating carp | *Mylopharyngodon piceus* |
| Silver crucian carp | *Carassius carassius* |
| Speckled dace | *Rhinichthys osculus* |
| Spottail shiner | *Notropis hudsonius* |
| Tench | *Tinca tinca* |
| Tiger barb | *Puntius (Barbus) tetrazona* |
| Toxostome | *Chondrostoma toxostoma* |
| Vimba | *Vimba vimba* |
| White bream | *Blicca bjoerkna* |
| Woundfin minnow | *Plagopterus argentissimus* |
| Zebra danio | *Brachydanio rerio* |

## CYPRINID FISHES (NO COMMON NAMES)

*Catla catla*
*Cirrhinus mrigala*
*Cyclocheilichthys siaja*
*Danionella translucida*
*Elopichthys bambusa*
*Hampala dispar*
*Garra*
*Garra barreimiae*
*Labeo rohita*
*Leuciscus* spp.
*Puntius orphoides*
*Puntius gonionotus*
*Rhinogobio* spp.
*Rutilus frisii*
*Sarcocheilichthys* spp.
*Semotilus* spp.

## NONCYPRINID FISHES

| | |
|---|---|
| African walking catfish | *Clarias* sp. |
| Atlantic salmon | *Salmo salar* |
| Australian smelt | *Osmerus* sp. |
| Barracuda | *Sphyraena* sp. |
| Blackfin goodeid | *Goodea atripinnis* |
| Brook lamprey | *Lamptera planeri* |
| Brown trout | *Salmo trutta* |
| Burbot | *Lota lota* |
| Common bully | *Gobiomorphus cotidianus* |
| Eel | *Anguilla anguilla* |
| Grayling | *Thymallus thymallus* |
| Mosquito fish | *Gambusia* sp. |
| Northern whitefish | *Coregonus peled* |
| Peled | *Coregonus peled* |
| Pike | *Esox lucius* |
| Plains killifish | *Fundulus zebrinus* |
| Powan | *Coregonus lavaretus clupeoides* |
| Pumpkinseed | *Lepomis gibbosus* |
| Quillback | *Carpiodes cyprinus* |
| Rainbow trout | *Oncorhynchus mykiss* |
| Silver fish | *Chirostoma* sp. |
| Spotted galaxius | *Galaxius maculatus* |
| Stone loach | *Barbatula barbatula* |
| Three-spine stickleback | *Gasterosteus aculeatus* |
| Wels catfish | *Silurus glanis* |
| White sucker | *Catostomus commersoni* |
| Yellow perch | *Perca flavescens* |

## NONCYPRINID FISHES (NO COMMON NAMES)

*Awos guamensis*
*Hypseleotris* sp.
*Pseudoscapirhynchus kaumanni*
*Salmo ischchan*

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# Glossary of Terms

**Abrasion** A scraping of the outer layers of the skin or mucous membrane.

**Abscess** A localised cavity containing pus, usually produced by micro-organisms.

**Acclimation** The habituation of an organism to a different climate or environment.

**Acidosis** Condition caused by exposure to a large sudden fall in pH or a pH level which is below the tolerance range of the species affected.

**Acid-fast** Micro-organisms (e.g. *Mycobacterium* spp.) not readily decolourised by acids after staining.

**Acini** Lobules of secretory glands, e.g. the exocrine cell formations in the pancreas.

**Acquired immunity** Specific immune protection acquired following exposure to an antigen presented by immunisation or infection.

**Acute** Having a short and relatively severe course (4 h to several days), e.g. acute toxicity, acute inflammation (inflammation in which progress is rapid and manifestations are immediate and pronounced).

**Acute phase proteins** Those proteins which increase in concentration in the blood of an organism within a few hours, e.g. 24–48 h of being exposed to an insult that induces inflammation, e.g. C reactive protein (CRP).

**Adenocarcinoma** A malignant neoplasm derived from glandular tissue.

**Adenoma** A benign neoplasm composed of glandular tissue.

**Adhesion** Uniting of two surfaces of structures that would normally be apart, e.g. visceral organs attached to the abdominal wall by fibrous tissue, the result of acute or chronic inflammation.

**Adjuvant** Chemical used to assist the action of another chemical, usually associated with a vaccine.

**Aerobic** Micro-organisms which grow only in the presence of oxygen.

**Aetiology** The study of the causation of diseases, or the sum of the factors of a disease; or the cause of a disease.

**Agglutination** Clumping of bacteria, red blood cells or other particles.

**Algae** A heterogeneous group of unicellular (e.g. diatoms), colonial (e.g. *Volvox*) and multicellular (e.g. seaweed) organisms that have a simple structure, usually photosynthetic and traditionally included in the plant kingdom and recently in the kingdom Protista.

**Alkalosis** Condition caused by the exposure to a large sudden rise in pH or a pH which is above the tolerance range of the species affected.

**Alkylphenols** Alkylphenolic chemicals are nonanionic surfactants, used in detergents, paints, herbicides, pesticides and other products. They are thought to mimic oestrogens.

**Amoeboid** Resembling a single-celled organism, an amoeba in shape and having a gliding movement.

**Amino acid** The basic building blocks of protein. Any class of compounds of a general formula $RCH(NH_2)COOH$ where R is a distinct side chain.

**Ammoniotelic** Excreting nitrogen mainly as ammonia.

**Amplexus** Mating embrace in frogs and toads.

**Amylase** An enzyme which breaks down (hydrolyses) starch, glycogen and other glucose polysaccharides.

**Anaemia** A condition characterised by a deficiency of haemoglobin and/or erythrocytes. The more important anaemias in fish are normocytic anaemia caused by acute haemorrhaging, bacterial and viral infections, and metabolic diseases resulting in red cell destruction; microcytic anaemia due to chronic haemorrhaging, e.g. caused by external parasites, iron deficiency and deficiency of certain haematopoietic factors; and macrocytic anaemia (e.g. the absence of juvenile cells; too many mature cells) resulting from an increase in haematopoietic activity in the spleen and kidney.

**Anaerobic** Living in the absence of oxygen, e.g. anaerobic bacteria.

**Androgens** Any male steroid sex hormone concerned with the development of the male reproductive system and production of male characteristics.

**Angioma** A tumour consisting of blood vessels or lymph vessels.

**Annelid** Segmented worm with a distinct body cavity, e.g. earthworms, lugworms, leeches.

**Anoxia** Devoid of oxygen.

**Anthelmintics** Drugs whose action is against helminth parasites, e.g. flukes, tapeworms.

**Antibiotic** A chemical substance originally produced from moulds or bacteria, but now from synthetic substances. Antibiotics are capable of inhibiting the growth of, or killing other micro-organisms.

**Antibody** A specific immunoglobulin molecule produced by vertebrates by an organism in response to an antigen. An important component of the acquired immune response.

**Antigen** A substance which induces the formation of specific immunoglobulins, i.e. antibodies.

**Antimicrobial** Substance which is effective against a micro-organism, usually bacteria.

**Antiserum** A serum containing a specific antibody or antibodies.

**Artefact** An artificially produced fault in a tissue or other structure, e.g. post mortem manipulation.

**Aseptate** Without any septum (internal walls).

**Ascites** Abnormal accumulation of a serous fluid, usually in the peritoneal cavity, e.g. dropsy.

**Asphyxia** A condition of suffocation.

**Asymptomatic carrier** An animal carrying an infectious agent, but showing no overt signs of the disease.

**Ataxia** Failure of muscle co-ordination.

**Atresia** Congenital absence or closure of a normal body opening or tubular structure.

**Atrophy** A wasting away or diminution in size of a cell, organ or part, due to disease, nutritional deficiency, pressure or lack of innervation.

**Atypical** Not corresponding to a normal specimen or condition.

**Autopsy** Post mortem examination and its description.

**B lymphocyte** Leucocyte which upon stimulation with an antigen produces antibodies.

**Bacteriology** The study of bacteria.

**Bacteraemia** Presence of bacteria in the blood.

**Bacteriostatic** Having the ability to inhibit the growth or reproduction of bacteria.

**Basophil** A type of white blood cell that contains granules that stain strongly with basic dyes.

**Basophilia** A condition in which the basophils and polymorphonuclear leucocytes are present in undue numbers, as in lead poisoning, leukaemia, etc.

**Basophilic** Substance which stains with basic dyes.

**Benign** Not endangering life. However, a benign tumour is a neoplasm that is slow growing and noninvasive, but it may eventually grow to a size that may compress or occupy space, thus endangering the life of that organism.

**Benthic** Living on the bottom of a water body.

**Benthophagus** An animal that feeds on or within the substratum i.e. 'bottom-feeder'.

**Bioaccumulate** To accumulate a chemical, e.g. pollutant in the body of an aquatic organism.

**Branchial** Pertaining to the gills.

**Carbohydrate** Compounds of carbon, hydrogen and oxygen of the general formula $C_x(H_2O)_y$, including sugars, starch, cellulose.

**Carcinoma** A malignant tumour derived from epithelial cells.

**Cartilage** Firm elastic skeletal tissue in which cells, chondrocytes, are embedded in a matrix containing collagen fibres.

**Cartilaginous** Gristly, consisting of cartilage.

**Catecholamines** A group of chemicals acting as neurotransmitters or hormones, e.g. adrenaline.

**Cataract** Partial or complete opacity of the crystalline lens or its capsule.

**Caudal** Pertaining to the tail.

**Ceroid** Yellow-brown acid fast pigments representing the end products of peroxidation of unsaturated fatty acids and occurring in many tissues, especially the liver and spleen and within macrophages.

**Cestoda** The taxonomic group of tapeworms.

**Chase spawner** Species in which the males pursue the females during spawning, e.g. goldfish.

**Chemosensitive** Responsive to a chemical.

**Chemotherapy** Treatment of diseases by chemicals.

**Cholangioma** Benign tumour of the biliary ducts in the liver.

**Cholinesterase** Enzyme which breaks down (hydrolyses) acetylcholine.

**Chloroplasts** Organelles found in the cytoplasm of plants which contains the green pigment, chlorophyll and are responsible for photosynthesis.

**Chondroalbumoids** Proteins of cartilage.

**Chondroma** Tumour of the cartilage cells.

**Chondromucoid** Compound of chondriotic acid and mucin, forming the intercellular substance of cartilage.

**Choroid** The vascular layer of the eyeball.

**Chromatin** The basophilic DNA portion of the cell nucleus.

**Chromatoblastoma** A pigmented tumour.

**Chronic** Prolonged. Persists for a long time for example weeks, months or years.

**Clinical** The outward appearance of a disease in a living organism.

**Clinical infection** An infection or disease generating obvious signs of pathology.

**Clubbing (gills)** Swelling of the tips (distal ends) of the gill filaments.

**cm** Centimetre (one hundredth of a metre).

**Coarse fish** Freshwater fish not belonging to the salmonid family (UK term).

**Coarse fishery** A fishery specifically designated for nonsalmonid fish in the UK.

**Coelenterate** Animal phylum. The organism comprises many cells formed into two layers and a simple body plan. Has a single opening (mouth) to the body cavity, e.g. hydra, sea anemones and jellyfish.

**Colloid** A substance of high molecular weight that does not readily diffuse through a semipermeable membrane. A substance composed of two parts (phases), one dispersed in the other.

**Colorimetric kit** Detection kit based on the observation of a change in colour.

**Commensals** Organisms which live together without harm and to the benefit of one or both, e.g. commensal bacteria within the gut of fish.

**Complement** Nonspecific immunological proteins normally present in the blood.

**Condition factor** 'K Factor'. The relationship between weight (W) and length (L) of a fish, expressed usually as: $K = W^3/L$.

**Congestion** Excessive or abnormal accumulation of blood in part of a tissue or organ so that the normal functions are hindered.

**Con-specific** Belonging to the same species.

**Control** The check of an experiment or test in which the experimental substance or condition is omitted (= negative control), or where a known substance or condition is included (= positive control).

**Coprophagy** Ingestion of faeces.

**Corticosteroids** Any group of steroids secreted by the cortex of the adrenal glands or its equivalent, e.g. hormones such as cortisol.

**Cortisol** Steroid hormone usually associated with a stress response which has a marked effect on the organism's metabolism.

**Counterstain** A stain applied to a histological section in order to enhance the effect of an existing primary stain.

**Cues** Stimuli, e.g. temperature may be an environmental cue to spawning in certain fishes.

**Culture** The growing of bacteria or other micro-organisms in artificial media. The propagation of animals (including fish), as in farming.

**Cutaneous** Pertaining to the skin.

**Cytopathic** Destruction of cells infected with a virus.

**Cytopathology** The study of morbid changes in cells.

**Decalcify** To deprive or rid of lime salts (calcium) in tissues, especially in reference to histological technique.

**Deficiency disease** Disease caused by lack of some essential constituents, e.g. in the diet.

**Definitive host** Host organism in which the adult stage of a parasite develops.

**Deformity** Distortion of any part of the body or general malformation of the body.

**Degeneration** Any structural change in an organ, or tissue or cell by which its functional power is impaired. The degenerative change may, or may not, progress to necrosis.

**Deoxygenated** Containing a low concentration of oxygen.

**Desquamation** The shedding of epithelial elements, chiefly of the skin, in sheets.

**Detritivore** An organism that feeds on decaying animal or plant protoplasm so bringing about decay.

**Diabetic** Organism suffering from a condition which is characterised by an abnormally high level of blood glucose, i.e. diabetes.

**Diagnosis** The determination of the nature of a given disease.

**Digestion** The process by which food is broken down in the alimentary canal to aid its assimilation.

**Dipteran** Order of insects which possess two wings in the adult stage, i.e. the flies.

**Disease** A deviation from the state of complete physical or social well-being of an organism involving a well-defined set of signs and aetiology and leading to an impairment of its normal function.

**Distal** Furthest from the centre.

**DNA** Deoxyribonucleic acid. Very large self-replicating molecules found in all living cells, which act as the physical carrier of genetic information.

**Dorsal** Pertaining to the back of the body.

**Dropsy** An accumulation of serous fluid in any cavity of the body.

**Duct** A tubular passage in the body for the conveyance of a secretion or excretion.

**Dysfunction** Abnormal or imperfect functioning of an organ.

**Ectocommensal** Commensal living on the surface of another organism.

**Ectoparasite** A parasite that lives on the outside (e.g. body surface) of its host.

**Ectothermic** An animal whose source of heat is primarily external.

**Emaciated** Extreme weight loss.

**Emboli** Clots of blood that obstruct circulation.

**Embryonate** Egg containing an embryo.

**Encapsulate** To form a covering or surrounding capsule.

**Encysted** Enclosed in a cyst.

**Endemic** Pertaining to a given locality or area, e.g. an endemic disease.

**Endocrine** Glands which liberate their internal secretions into blood or lymph.

**Endogenous** Produced within the organism.

**Endoparasite** A parasite that lives inside its host.

**Enteritis** Inflammation of the intestine.

**Environment** All of the external factors or conditions supporting or influencing the existence of an organism or assemblages of organisms.

**Enzyme** A protein molecule that accelerates (catalyses) chemical changes within an organism.

**Eosinophil** A type of white blood cell which contains granules and stains with the red acidic dye eosin.

**Eosinophilic** A substance in a cell which has an affinity for the eosin dye.

**Epicarditis** Inflammation of the visceral layer of the pericardium.

**Epidemiology** The study of diseases or health factors and their relationships with the population and the environment.

**Epithelium** The thin outer layer of the skin, mucous membranes and the inner layer of internal cavities.

**Epizootic** A disease attacking many organisms in a population at the same time; widely diffused and rapidly spreading.

**Erosion** Superficial loss of the epithelial tissue.

**Erythrocyte** A red blood cell.

**Erythrophoroma** A tumour made up of red pigmented fibrous cells.

**Erythrophores** Erythrophiles, cells which stain readily with red dyes.

**Eutrophic** A water body that is rich in nutrients and hence may have excessive plant growth and may suffer from algal blooms.

**Excretion** The process of eliminating wastes, i.e. undigested food residues and other waste products of metabolism.

**Exocrine** A glandular secretion which is directly eliminated outside via a duct.

**Exophthalmia** Abnormal protrusion of the eye.

**Exoskeleton** Hard supporting structure secreted by and external to the epidermis. An external skeleton, e.g. as in insects.

**Exotic** Foreign plant or animal which has not acclimatised or naturalised.

**Extracellular** Occurring outside of the cell.

**Exudate** A fluid which has entered the tissues from the blood in an active process as a result of inflammation.

**Fatty acid** Long chain organic acid containing carbon atoms with the general formula $CH_3(C_nH_x)COOH$. Constituent of lipid and a fuel molecule in cells.

**Fecundity** Capability of producing offspring.

**Fibroblast** A type of connective tissue cell which is concerned with collagen synthesis and associated with developing or repair tissues.

**Fibroma** A benign tumour composed chiefly of fibrous tissue (plural: fibromata).

**Fibrosarcoma** A malignant tumour of the fibrous connective tissue.

**Filiform** A term used to describe threadlike organisms, e.g. *Flexibacter* sp.

**Fin-rot (Fin erosion)** A progressive erosion and disintegration of the fin.

**Formalin** Solution of approximately 37% by weight of formaldehyde gas in water. In a diluted form it is effective in control of external parasites and fungal infections on fish and eggs. Also commonly used as a fixative for preserving tissues for subsequent histological examination.

**g** gram (one thousandth of a kilogram).

**Gametogenesis** Gamete (sperm/egg) formation.

**Ganglioneuroma** Benign neoplasm composed of nerve fibres and mature ganglion cells.

**Gas supersaturation** Level of a dissolved gas (or gases) in water that is above the saturation point.

**Gastro-enteritis** Inflammation of the mucosa of the stomach and intestines.

**Gender** The sex of an organism, i.e. male or female.

**Gene** The basic unit of inheritance by which hereditary characteristics are passed from parents to offspring. A single length of DNA that exerts its effect on an organism's form and functions.

**Genotype** The genetic constitution of an organism.

**Gland** An aggregate of cells that have secretory or excretory function.

**Glomerulonephritis** Inflammation of the capillary loops in the kidney glomeruli.

**Glucose** A hexose sugar found in all living cells.

**Glycogen** A carbohydrate storage material in liver and other tissues that is converted to glucose when required for energy.

**Glycocalyx** Cell coat.

**Gonad** A testis or ovary.

**Gonadotropin** A hormone that stimulates gonadal function.

**Gram stain** A staining procedure used in identifying bacteria and classifying them as Gram-positive or Gram-negative.

**Granulocyte** Class of white blood cell that characteristically contains numerous vesicles or granules, e.g. eosinophils, basophils and neutrophils.

**Granuloma** A tumour of chronic inflammatory nature (plural: granulomata). Note: it is not a neoplasm.

**Haematin** A pigment formed when unbuffered formalin is used in the fixation of tissues. Also abnormal $Fe^{3+}$ constituent of haemoglobin. A decomposition product of haemoglobin.

**Haemangiopericytoma** A tumour around blood vessels.

**Haematozoic** Living in blood.

**Haemocoel** Blood-filled cavity consisting of spaces between organs.

**Haemoflagellate** Flagellate protozoan found in the blood of its host.

**Haemoglobin** Oxygen-carrying haem protein which occurs in the red blood cells in vertebrates.

**Haemorrhage** An escape of blood from the vessels, either through intact blood vessel walls, or by flow through ruptured walls.

**Haemorrhagic petechiae** Small 'pinprick' haemorrhages occurring where capillaries have ruptured, e.g. throughout musculature or in the cutaneous layers.

**Haemosiderin** A yellow iron-containing substance resulting in the breakdown of the red blood cells.

**Hand stripping** Removal of sperm or eggs from a fish by exerting gentle pressure to the ventral aspects of the animal.

**Haptor** Attachment organ of monogenean flukes.

**Hepatic** Pertaining to the liver.

**Herbicide** Any chemical substance, usually synthetic, that kills or injures plant life.

**Herbivorous** Feeding on plants.

**Hermaphroditic** A state where the animal or plant possesses the organs of both sexes.

**Hermaphroditism** The coexistence in an individual of ovarian and testicular tissue.

**High responder** An animal that produces an intense immune response against an antigen.

**Histology** The science of the microstructure of tissues.

**Histopathology** Pathological histology.

**Horizontal transmission** Transmission of a pathogen from one animal to another usually in the same environment.

**Hormone** A chemical substance secreted in a ductless gland and discharged into the bloodstream which affects the functioning of another target organ.

**Humoral immunity** Specific immunity mediated by antibodies.

**Hyaline** Clear, structureless, homologous, 'glassy' material which occurs pathologically in degeneration of several tissues, and also in normal tissue.

**Hybridisation** Formation of a hybrid usually by the cross-breeding of two species.

**Hyperparasitism** An organism which is a parasite on, or in, another parasite.

**Hyperplasia** Abnormal increase in number of cells in a tissue or organ.

**Hyperthermia** Physiological responses induced by exposure to extreme hot conditions.

**Hypodermis** The layers of tissue immediately under the skin, i.e. subcutaneous.

**Hypothermia** Physiological responses induced by exposure to extreme cold conditions.

**Hypotrophy** Diminution in size.

**Hypoxia** Reduction in oxygen in body tissues below physiological levels.

**Idiopathy** A condition of unknown cause.

**Immune** Possession of immunity.

**Immunity** Resistance to an infectious agent or toxin. Immunity may be natural ( = innate, nonspecific) or acquired ( = specific).

**Immunisation** Process or procedure by which an individual is made resistant to disease, generally through exposure to antigens or immunogens.

**Immuno-diagnosis** The diagnosis of disease, using immunological techniques.

**Immuno-compromised** An animal whose immune response is deficient in its operation.

**Immuno-suppressed** An animal with a suppressed immune response.

**Incidence** The number of new cases of a particular disease occurring within a specified period in a given population.

**Inclusion bodies** Bodies occurring in the nuclei or cytoplasm of cells in cases of viral infection. Can also refer to other intracellular foreign bodies.

**Incubation/incubation time** The period between the time of entry of a pathogen and the onset of symptoms of the disease.

**Infection** The introduction or entry of a pathogen or parasite into a host, resulting in the presence of the pathogen or parasite within the body tissues or cells of the host, whether or not this results in overt disease.

**Infection, latent** An infection which remains dormant for a long period of time.

**Infection, opportunistic** An infection caused by a pathogen that is normally present and innocuous, but arises when conditions are favourable to that pathogen.

**Infection, secondary** Infection by a pathogen or parasite which has gained entry into tissues already damaged by a different infectious agent.

**Infection, sub-clinical** Infection that does not cause outward symptoms.

**Inflammation** In fish, the reaction of the tissues to injury characterised clinically by swelling and redness. Pathologically, by vasodilation, hyperaemia, accumulation of leucocytes, exudation of fluid and deposition of fibrin.

**Inoculation** The introduction of a pathogenic organism into the tissues of a living organism or culture.

**Insecticide** Chemical that kills insects.

**Insulin** Polypeptide hormone produced by the β cells of the islets of Langerhans in the pancreas, which decreases the amount of glucose in the blood by stimulating its uptake by cells.

**Intensity** Number of individuals per given area or unit, e.g. number of parasites per host.

**Interleukins** Proteins produced by various immune cells that act on other cells in the immune system.

**Intermediate host** A host in which a parasite lives for part of its life cycle but in which it does not become sexually mature.

**Interspecific** Between distinct species.

**Intramuscular** Within the muscle tissue.

**Intraperitoneal** Within the peritoneal cavity.

**Invertebrate** General term for all animals without a backbone.

***In vitro*** In reference to tests or experiments conducted in an artificial environment, including cell or tissue cultures.

***In vivo*** In reference to tests or experiments conducted in or on living organisms.

**Iodophore** Disinfectant containing iodine.

**Ionised (ammonia)** A molecule that has had an electron removed or added. For example the ionised ($NH_4^+$) form of ammonia, which is less toxic to fish than the unionised form.

**Iridophores** Sometimes called iridocytes. Guanine-containing granules, bodies or plates of which the reflecting, silvery or iridescent tissue of skin in fish is composed.

**IU** International unit.

**Karyorrhexis** Fragmentation of a nucleus.

**kg** Kilogram.

**Leiomyoma** A benign tumour made up of unstriped or involuntary muscle.

**Lenticular** Relating to the lens; the crystalline lens or the lens nucleus.

**Lepidorthosis** Erection of scales.

**Lesion** Any visible pathological or traumatic alteration in the normal structure of organs or tissues.

**Lethargy** Morbid drowsiness, sluggishness or moving very slowly.

**Leucocyte** A term referring collectively to any nonpigmented blood cell.

**Leucophores** White pigmented cells.

**Lipid** Fat.

**Lipofuscin** Any pigment formed by the breakdown of fat.

**Lipoma** A benign fatty tumour.

**Longitudinal section** A cut taken though the longitudinal plane.

**Lordosis** Dorso-ventral curvature of the spine.

**Low responder** An animal that has a poor immune response against an antigen.

**Lymphocyte** A granular leucocyte of the peripheral blood and haemopoeitic tissue.

**Lymphoma/lymphosarcoma** A tumour of lymphoid cells.

**Lymphohaemopoietic** Generation of both lymphoid and blood cells.

**Macronucleus** The larger of the two types of nuclei found in ciliated protozoa.

**Macrophage** A phagocytic cell belonging to the reticuloendothelial system. It is the major phagocytic cell in fish inflammation.

**Macrophytes** Large aquatic plant.

**Malignant** Pertaining to, or denoting progressive growth of, certain tumours that may spread to distant sites or invade surrounding tissue and terminate in death.

**Medulla** Central part of an organ or tissue.

**Melanin** A tyrosine-derived polymeric pigment responsible for the yellow to black coloration of fishes and quite often associated with parasite infestation.

**Melanocyte/melanophore** Cell in which the pigment melanin is formed. Usually found in the skin and responsible for light/dark colour changes.

**Melanoma** A tumour whose parenchyma is composed of anaplastic melanocytes or any benign tumour of melanocytes.

**Melanomacrophage** Macrophage that contains the pigment melanin. Thought to be involved in the immune response.

**Melanosarcoma** Malignant tumour composed of melanin-pigmented cells.

**Metastasise** To change state, position, form or function, e.g. with reference to a tumour.

**Methaemoglobin** Haemoglobin with the haem iron in the ferric state and unable to bind oxygen.

**mg** Milligram (one thousandth of a gram).

**Micronucleus** The smaller of the two types of nuclei in ciliate protozoans. Involved in sexual reproduction but does not produce RNA.

**Microtome** A machine used in histology for cutting thin sections of tissue.

**Microtomy** The cutting of thin sections of tissues for examination with a microscope.

**Mitochondria** Organelles in the cytoplasm of cells, having a double membrane and responsible for oxidative respiration in the cell.

**mm** Millimetre (one thousandth of a metre).

**$mm^3$** Cubic millimetre.

**Molluscicide** Chemical that kills molluscs, e.g. snails.

**Molluscivorous** An animal that eats molluscs.

**Monoclonal antibody** Antibody that is produced from a single clone of B lymphocytes and thus consists of a population of identical antibody molecules all specific to a single antigenic determinant on an antigen.

**Monoculture** The culture or rearing of one species.

**Monocyte** A large mononuclear leucocyte with a more-or-less deeply indented nucleus, slate grey cytoplasm and eosinophilic granulation. This cell is formed into a macrophage once it migrates into the tissue.

**Monozoic** An unsegmented body; comprising of a single body.

**Morphology** The branch of biology dealing with form and structure.

**Mortality** The proportion of deaths in a specified portion of a population.

**Mortality rate** The number of deaths per unit of population during a specified period.

**Mucin** A protein found in mucus.

**Mucopolysaccharide** Types of carbohydrate containing 1:2 glycol groups such as starch, glycogen, mucins that convert to aldehydes by oxidation becoming coloured when exposed to the periodic acid–Schiff reaction.

**Mucosa** Epithelial cover of internal cavities.

**Mucus** The slimy secretion containing mucin of the mucous glands or cells.

**Multicellular** Many-celled.

**Mycobacteriosis** A chronic systemic bacterial disease of fish caused by *Mycobacterium* spp.

**Myocarditis** Inflammation of the heart muscle.

**Myoid** Resembling or composed of muscle fibres.

**Myomere** One of the vertically marked segments which comprise the lateral muscle mass.

**Myopathy** A wasting condition affecting the muscle tissue.

**Naïve** Without any prior exposure or experience (e.g. immunologically naïve) to a parasite or pathogen.

**Native** A species that is a member of the natural biotic community.

**Necrosis** Dead or dying cells or tissues within the living body.

**Neoplasm** An uncontrolled growth of cells.

**Nephritis** Inflammation of the kidneys.

**Nephrocalcinosis** A condition of renal insufficiency due to the precipitation of calcium salts in the tubules of the kidney.

**Nephron** The individual unit, consisting of a renal corpuscle and a tubule, which makes up the functional excretory component of a kidney.

**Neural** Relating to the nervous system.

**Neurilemmoma** Tumour of a peripheral nerve sheath.

**Neurofibroma** Tumour of peripheral nerves due to abnormal proliferation of Schwann cells.

**Neutralisation** Inactivation of a pathogen, usually a virus complexing with a specific antibody.

**Neutrophil** A leucocyte having no affinity for acid or basophilic dyes, but stainable in neutral dyes.

**Night soil** Human excrement used as fertiliser on the land.

**Nitrifiers** A group of aerobic bacteria that can change (oxidise) ammonia to nitrite.

**Nitrification** The conversion (oxidation) of ammonia to nitrite and then to nitrate, mediated by bacteria.

**Nitrogenous waste** Organic wastes with a high nitrogen content, e.g. animal manure, urine.

**nm** Nanometre (0.000000001 of a metre/one millionth of a millimetre).

**Nodule/node** A small lump, knot or node. This is an embracing term which can include any small tumour whether it be neoplastic or not.

**Nonionised (ammonia)** Also known as unionised. This is the highly toxic $NH_3$ form.

**Noninfectious** Not capable of being transmitted from one organism to another.

**Norm** An accepted standard.

**Notifiable disease** A disease which, when first identified, must be reported to the appropriate authority.

**Nutrition** The sum of the processes in which an animal takes in and utilises food.

**Oedema** Abnormal accumulation of fluid in the tissue.

**Oestrogens** A group of vertebrate steroid hormones produced in the female by the ovary and responsible for growth and function of the female reproductive organs and female characteristics.

**Oesteoma** A tumour of bony (osseous) tissue.

**Olfactory** Relating to the sense of smell.

**Oligodeoxynucleotide** Short piece of DNA, for example a DNA primer.

**Omnivorous** Feeding on a variety of different foods.

**Oogenesis** The process of development of the ovum.

**Operculum** A flap-like protective gill covering, present in cyprinids and other fish.

**Opportunist agent** A pathogen or parasite that infects an abnormal or weakened host.

**Oral** Pertaining to the mouth.

**Organophosphate insecticide** A group of chemical insecticides based on compounds where the carbon atoms are linked directly to phosphorus, such as malathion or dipterex used to eliminate lice and other crustacean parasites on fish.

**Osmoregulatory** The regulation of the osmotic pressure of body fluids by controlling the amount of water and/or salts in the body.

**Osseomucoids** Mucoids of bony tissue.

**Osseous** Bony tissue.

**Ossification** Formation of bone.

**Ossified** Converted to bony tissue.

**Osteosarcoma** Malignant tumour of bony tissue.

**Otoliths** Calcified structures within the inner ear. The concentric growth of rings of the otoliths are counted to estimate the age of fish.

**Outbreak** Occurrence of a disease within a site, region, country during a limited period of time.

**Overt disease** A disease apparent or obvious by gross inspection; a disease state exhibiting clinical signs.

**Ovicidal** Acting against eggs, e.g. killing parasite eggs.

**Oxidise** To combine with oxygen or remove hydrogen.

**Oxygenated** Containing oxygen.

**p.p.b.** Parts per billion.

**Papilloma** A benign tumour involving overgrowth of epithelial tissue.

**Parasite** An organism that lives, at the expense of, in or on another organism (the host) and which depends on the host for its metabolism.

**Parasitology** The study of parasites.

**Parasitism** The mode of life in which one species, the parasite, lives in or on another species, the host. The former gains benefit from the association usually at the expense of the latter.

**Paratenic host** Intermediate host in which a parasite stage resides but does not develop.

**Parenchyma** The functional tissue parts of an organ.

**Pathogen** Any organism which, in living on or within another organism (the host), causes disease to the host.

**Pathogenesis** The origin or development of disease.

**Pathogenic** Producing disease or pathological changes.

**Pathological condition** A deviation from normal condition associated with disease.

**Pathology** The study of disease.

**Pelagic** Living in a water body at the middle or surface levels.

**Peptide** A chain of a small number of amino acids.

**Pericarditis** Inflammation of the pericardium, the sac enclosing the heart.

**Pericardium** The connective tissue surrounding the heart.

**Pericyte** An elongate contractile cell surrounding precapillary arterioles outside the basement membrane.

**Periosteal tissue** Connective tissue surrounding bone.

**Peritonitis** Inflammation of the peritoneum.

**Petechiae** Small red spots formed by the escape of blood.

**Petechial** Pertaining to small red spot(s) caused by the escape of blood from blood vessels.

**pH tolerance** Being able to withstand a change in pH (acid or alkaline) within a certain range.

**Phagocyte** A cell capable of ingesting bacteria, foreign particles and other cells.

**Phagocytosis** The ingestion of foreign or other particles by certain cells of the body.

**Pheromone** A chemical released, usually in small amounts, by one animal that is detected and acts as a signal to another member of the same species.

**Photosynthesis** In green plants the synthesis of carbohydrates in chloroplasts from carbon dioxide and water.

**Phototaxic/phototropic** Movement in response to light.

**Phytoplankton** Plant plankton.

**Piscivorous/piscivory** Fish-eating.

**Planorbid** A type of snail belonging to the genus *Planorbis*.

**Plasma cells** Antibody-secreting cell of the immune system which differentiates from the B lymphocyte after recognition of an antigen.

**Plasmid** Small self-replicating circular DNA independent of the chromosome in bacteria and yeasts.

**Poikilothermic** Animal whose temperature varies with that of the surrounding medium. Known commonly as 'cold blooded'.

**Pollutant** Substance or effect that causes pollution.

**Pollution** The introduction by human activities, directly or indirectly, of substances or energy into the environment resulting in deleterious effects on living organisms.

**Polychlorinated biphenyls (PCBs)** A group of toxic synthetic lipid-soluble hydrocarbons that are used in various industrial processes.

**Polyculture** The culture or rearing of more than one species in a system.

**Prevalence** The total number of disease cases in a population. It can be represented as a percentage of a population.

**Proboscis** A trunk-like extension from the head that in some parasites, e.g. acanthocephalans, comprises teeth and is used for attachment to the host.

**Pronephros** That area of the kidney that develops first in the embryonic or larval life. In fish it loses its excretory function and becomes an important organ involved in the immune response.

**Prophylactic** An agent that prevents disease.

**Protein** One of the chief components of living matter, any one of a group of large organic molecules containing oxygen, carbon, hydrogen, nitrogen and sulphur.

**Protozoal** Belonging to the single-celled animals (protozoa).

**Pycnosis (pyknosis)** In necrotic cells with abundant chromatin, dense masses are formed which break up (karyorrhexis) to form granules inside the nuclear membrane or throughout the cytoplasm.

**rRNA** Ribosomal RNA, a major component of ribosomes and the most abundant form of RNA in cells.

**Regeneration** New growth or repair of structures or tissues lost by disease or injury.

**Reinfection** A second infection by the same type of micro-organism.

**Representative sample** A population subset, chosen according to the same criteria, in such a way that the sample faithfully resembles the population from which it was extracted.

**Resistance** The ability, natural or acquired, of an organism to overcome the process of a disease.

**Reticular** Pertaining to a reticulum; net-like.

**Rhabdomyoma** A tumour originating from striated muscle cells.

**Rhizoids** Filamentous outgrowths.

**RNA** Ribonucleic acid, large linear molecule of varied composition, made up of a single chain of ribonucleotide subunits, containing bases uracil, guanine, cytosine and adenine. Many forms occur in a cell and are formed from transcription of DNA. Can be primary genetic material of some viruses.

**Rodlet cell** Cell that occurs in fish that contains rod-shaped granules. Function unknown. Some authorities consider it to be a white blood cell whilst others suggest it may be a parasite.

**Rotifer** Phylum of microscopic, multicellular animals that live mostly in freshwater. Generally cone-shaped with a crown of cilia at the widest end surrounding the mouth.

**Saline** Solution of sodium chloride (salt) at a physiological concentration.

**Sample** A small representative quantity of a population, making it possible to estimate the characteristics of a population. It can also mean a representative piece of tissue from an organ, or fluid, or bacteria, etc., taken for pathological examination.

**Saprophytic** Obtaining nourishment directly from dead or decaying organic matter.

**Sarcoma** A malignant tumour whose parenchyma is composed of anaplastic cells resembling those supportive tissues in the body.

**Saturation (lipid)** Lipids are composed of a chain of carbon atoms each of which has the potential to join to four other atoms via four single bonds. When all these single bonds occur the lipid is saturated.

**Scoliosis** Lateral curvature of the vertebral column.

**Secretion** The production of a substance that differs chemically and physically from the body producing it.

**Section** To divide by cutting; a slice, e.g. histological section.

**Septicaemia** A clinical syndrome characterised by a severe bacteraemia infection, generally involving the significant invasion of the bloodstream by micro-organisms.

**Serodiagnostic test** A test performed on a serum sample for the detection of disease.

**Serology** The study of immune sera and the use of antisera to characterise pathogens, antigens, cells, etc.

**Serosa** A serous membrane.

**Serous membrane** A membrane that lines a cavity that has no communication with the external environment.

**Serum** The fluid part of the blood after removal of clotted cells.

**Site management plan** A strategy that involves the use of good practice and treatment to reduce the chances of a disease outbreak.

**Slough** A piece of dead (necrosed) tissue that has separated from a wound or inflamed surface.

**Spermatogenesis** The development of spermatozoa.

**Spermatophore** A number of sperm enclosed in a sheath of gelatinous material.

**Sterile** Free from micro-organisms; infertile.

**Squamous** Simple epithelium of nucleated cells.

**Stress** The response of an organism to an adverse stimulus (the stressor).

**Stressor** Any adverse stimulus (e.g. of physical or chemical nature) that leads to stress.

**Subcutaneous** Located under the skin.

**Sublethal** The term applied to a dose which is not fatal.

**Submucosa** The tissue lying beneath the mucous membrane.

**Suppuration** Formation or discharge of pus.

**Swab** A mounted piece of cotton wool used for obtaining specimens of a secretion, etc. for laboratory examination.

**Swelling** The abnormal enlargement of an organ.

**Syndrome** A group of signs which, when considered together, characterise a disease.

**Systemic** Throughout the body, involving the whole body.

**T lymphocyte** A lymphoid cell whose development depends on the presence of the thymus; responsible for cell-mediated immunity.

**Teratoma** Abnormal growth of an oocyte or testes germ cell.

**Therapeutic** Any procedure for the treatment of a disease.

**Therapy** Any type of treatment of a disease, e.g. vaccine therapy, chemotherapy.

**Thrombocyte** Spindle-shaped nucleated cell involved in blood-clotting in fish.

**Titrimetric kit** A kit based on the titration principle where the substance to be measured (a liquid) is quantified by the amount of the reagent required to achieve an end point (= total reaction).

**Torpid** A state of sluggishness.

**Toxicity** A relative measure of the ability of a chemical to be toxic. It usually refers to the ability of a substance to kill or cause adverse effects.

**Transgenic** Animals or plants into which genes from another species have been deliberately introduced.

**Transmission** The transfer of a disease agent from one individual to another.

**Trauma** A wound or other external injury.

**Treatment** The medical management of a disease or disorder as a whole.

**Trypsin** Proteolytic digestive enzyme, involved in food digestion.

**Tubercle** A rounded, solid, raised lump on the skin or surface of an organ.

**Tubificid** A group of annelid worms.

**Tumour** Literally, any swelling, but by common consent the term is not held to include passing swellings caused by inflammation, etc. It should be restricted to neoplasms.

**Ulcer** A break in the skin or mucous membrane with loss of surface tissue, disintegration and necrosis.

**µm** Micrometre (0.000001 of a metre/one thousandth of a millimetre).

**Unsegmented** Lacking segments.

**Ureotelic** Excreting nitrogen as urea.

**Vertebrate** Animal with a backbone.

**Vertical transmission** Transmission of a pathogen, usually a virus, from parent to offspring via the reproductive tract or gametes.

**Virology** The study of viruses.

**Virulence** The relative capacity of a pathogen to produce disease.

**Vitamins** A group of unrelated organic substances that in small amounts are necessary for normal metabolic functioning of the body.

**Vitellaria** Yolk glands.

**Vitellogenin** Protein that is converted into yolk.

**Vitellogenesis** Yolk formation.

**Viviparous** Producing live young rather than laying eggs.

**Western blot** A technique in which proteins/peptides are transferred after electrophoresis from a gel medium to nitrocellulose paper. Aids further analysis of the protein.

**Xanthophores** Yellow chromatophore (pigment-containing cell).

**Zeolite** An ion-exchange resin that is used to remove ammonia from fish culture systems.

**Zoonosis** A disease or infection naturally transmitted between a vertebrate animal and humans.

**Zuger jar** Specialised container used for hatching fish eggs.

*Diseases of Carp and Other Cyprinid Fishes*
D. Hoole, D. Bucke, P. Burgess, I. Wellby


# Index

Due to extensive citation in the text, common carp has not been included in the index