Chapter 1

Overview of Fish Immune System and Infectious Diseases

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Introduction

Cultured finfish are an important source of animal protein worldwide (Naylor et al. 2009), and the Food and Agriculture Organization (FAO) reported that over half of the world's supply of fish and shellfish is now from aquaculture (FAO 2008). As fish consumption increases and natural fish stocks decrease, aquaculture practices will need to intensify in order to meet global demand. Intensification will likely lead to an increase in disease problems, due to a higher number of animals in a limited and confined environment and the influence of poor environmental conditions (i.e., water quality) on the fish immune system. For example, limited disease-related problems were reported in the channel catfish (*Ictalurus punctatus*) industry prior to 1980 because stocking densities were less than 10,000 fish/ha and maximum feeding allowances were about 50 kg/ha/day with most farms using a single crop system (Hawke and Khoo 2004). Production intensity increased following that time with >12,000 fish/ha stocked, and feeding increased accordingly to 90–112 kg/ha/day. Multi-cropping systems (i.e., various sizes of fish cultured together) that utilized limited water exchange were also employed (Hawke and Khoo 2004). As a result, up to 45% of on-farm losses were reported to be due to infectious disease (USDA/APHIS 1997). The emergence or re-emergence of pathogens will likely be seen in many aquaculture ventures as production intensifies and degrades environmental parameters.

Immunity is the inherited ability to recognize and respond defensively against foreign living and non-living agents. The immune response is a coordinated response of immune cells and molecules and memory in vertebrate animals (including fish) that occurs as a result of recognition of foreign agents. Fish have evolved with both non-specific (innate immunity) and adaptive (acquired) immune mechanisms. The innate immune response is limited in specificity via germline encoded pathogen recognition receptors (PRRs) that respond to pathogen-associated molecular patterns (PAMPs) such as bacterial or fungal glycoproteins and lipopolysaccharides (Kawai and Akira 2010; Boltana et al. 2011). The innate response is an important first line of defense, especially in larval fish. Research suggests that the innate immune response is important in priming and regulating adaptive immunity (Fearon and Locksley 1996). Adaptive immunity allows for specificity and memory (Pilström

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2005; Secombes et al. 2005). This chapter provides an overview of the fish immune system and the infectious diseases of fish (bacterial, viral, parasitic, and fungal).

Immune Organs and Tissues

Thymus

The thymus of fish is composed of lymphoblasts (early immune cells) in a reticular endothelial cell network; it is the first organ to obtain mature lymphocytes during immune maturation (Manning 1994; Rombout et al. 2005). Evidence in fish supports the notion that the thymus is responsible for the development of T-lymphocytes (T-cells), as is the case in other jawed vertebrates. T-cell selection occurs in the thymus, and only T-cells that recognize foreign antigenic peptides in the context of self major histocompatibility complex (MHC) molecules are released (Kuby 1994). T-cells that recognize self antigen and self MHC are killed via programmed cell death or apoptosis. Mature T-cells are then released from the thymus and become distributed in the immunological organs and tissues (Rombout et al. 2005). In adult fish, as in mammals, the thymus decreases significantly in size.

Kidney

The kidney is important in hematopoiesis and contains two segments: the anterior or head kidney and the posterior or trunk kidney. Blood cell differentiation occurs here instead of in bone marrow, as in mammals. Early in development, the entire kidney is involved in production of blood cells and early immune responses. The anterior kidney is considered the primary B-lymphocyte (B-cell) organ and is where the B-cells develop. As the fish matures, the posterior kidney is primarily involved in filtration and/or urinary functions. The kidney also contains the reticuloendothelial system, which is a network of sinusoids lined with phagocytic cells that have roles in antigen presentation. There is usually a concentration of melanomacrophage centers consisting of macrophages, lymphocytes, and plasma cells, and these centers are involved in antigen trapping and immune responses (Agius and Roberts 2003).

Spleen

The spleen is a secondary immune organ in fish and is involved in antigen processing, antibody production, and memory. Most fish spleens are not organized into red and white pulp, as in mammals. Manning (1994) demonstrated in carp (*Cyprinus carpio*) that the proliferative response to antigen was scattered and not organized into thymus-dependent and -independent regions. Melanomacrophage centers are also located in the spleen and are primarily responsible for the breakdown of erythrocytes. However, as discussed above, they may be involved in antigen presentation and immunologic memory. In rainbow trout (*Oncorhynchus mykiss*), Hadidi et al. (2008) demonstrated that the spleen size predicted the resistance to *Flavobacterium psychrophilum*, suggesting a role in innate immunity.

Gut

Gut associated lymphatic tissue (GALT) consists of lymphoid aggregates and follicles in the lamina propria of the intestine (Rombout et al. 1989). Immunoglobulin (Ig) + and Ig – cells (B- and T-cells) are present in the intestinal epithelium, suggesting importance as an immune tissue (Rombout et al. 1993). Antigen-specific antibody is secreted onto mucosal surfaces of the intestine. Fish do not have lymph nodes; most likely, their kidney, spleen, and GALT play an equivalent role to the lymph system in mammals with respect to antigen processing and presentation. Rombout et al. (2011) published an excellent review of the present state of fish intestinal immunology.

Natural Defense Barriers

Skin and Mucus

The mucus and skin/scales of fish act as a natural barrier to foreign substances and pathogens. Mucus consists of glycoproteins (lectins) or mucopolysaccharide proteins produced by goblet cells in the skin epidermis, gills, and mucosa of the gut (Dalmo et al. 1997; Sadovy et al. 2005). The mucus can serve as a non-specific defense mechanism, as it can result in sloughing off the

gills, skin, or gut lining, thereby preventing colonization by fish pathogens. The mucus also contains non-specific humoral molecules and specific antibodies.

Innate Immunity and Disease Resistance

Non-specific Immune Cells

Fish phagocytes (macrophages and neutrophils) express receptors on their surface that recognize invading pathogens and activate an innate immune response. These receptors are termed pathogen recognition receptors (PRRs), and these will sense the presence of pathogens through recognition of pathogen-associated molecular patterns (PAMPs). The interaction between PRRs on phagocytic cells and PAMPs of pathogens leads to the initiation of the innate immune response. Recent reviews provide current status of PRRs in fish (Boltana et al. 2011; Hansen et al. 2011; Palti 2011) and the antimicrobial mechanisms of fish that can be induced through PRR and PAMP interactions (Rieger and Barreda 2011).

Monocytes/macrophages

Monocytes and/or tissue macrophages are probably the single-most important cell in the immune response of fish. Not only are they important in inflammation and the production of cytokines (Clem et al. 1985), but they are also the primary cells involved in phagocytosis and killing of pathogens upon initial recognition and subsequent infection (Shoemaker et al. 1997). Macrophages also have an important role in antigen-presentation, thus linking the non-specific and specific immune responses. Forlenza et al. (2011) recently provided an excellent review of macrophage activation in fish.

Neutrophils

Neutrophils (granulocytes) are the primary cells involved in the initial stages of inflammation (12–24 hours) in fish (Manning 1994); their function includes phagocytosis and production of cytokines to recruit immune cells to the damaged and/or infected area. In channel catfish the neutrophil is phagocytic, but it appears to kill bacteria by extracellular mechanisms rather than via intracellular mechanisms (Ellis 1981; Waterstrat et al. 1991). The role of the neutrophils in immunity likely varies among different species of fish.

Non-specific Cytotoxic Cells

Non-specific cytotoxic cells (NCC) are present in fish (Evans and Jaso-Friedman 1992) and their functions are closely related to those of mammalian natural killer cells. These cells can kill a variety of target cells including tumor cells, virally infected cells, and protozoan parasites. NCCs function by lysis of target cells following receptor binding and signaling of the lytic cycle. These cells are important in parasite (Evans and Gratzek 1989) and viral (Hogan et al. 1996) immunity.

Non-specific Humoral Molecules of Fish

The serum, mucus, and eggs of fish contain a number of non-specific humoral molecules that can act against invading pathogens. Magnadottir (2006) and Whyte (2007) provide good reviews of these, a few of which are discussed in the following.

Lectins

Lectins are glycoproteins that non-specifically bind to sugars located on the surface of bacteria, viruses, and parasites, resulting in precipitation and agglutination reactions. Some lectins can act as opsonins for phagocytosis and can also be involved in activation of the complement system. Known lectins in fish include C-type lectins, mannose-binding lectin, pentraxins (C-reactive protein and serum amyloid protein), and ficolin. Sharon and Lis (1993) suggest that lectins are also involved in cell recognition and binding, and are also important in cellular communication. Xu et al. (2001) demonstrated their potential defensive action against parasites.

Lysozyme

Lytic enzymes, such as lysozyme, have been described in fish. Lysozyme is an antibacterial molecule that cleaves the 1-4-β-linkages between N-acetylmuramic

acid and N-acetylglucosamine in the cell wall of bacteria, resulting in cell lysis. Similar to lectins, lysozyme can also act as an opsonin for phagocytosis and activate the complement system. The molecules are also important in opsonization of target cells and in the attraction and activation of cells that are essential in inflammation.

Complement

The complement system is a group of serum molecules involved in the control of inflammation, opsonization of immune complexes and microorganisms, and lysis of pathogens. The liver is responsible for the production of components of the complement cascade. Fish complement can generally be activated through the classical pathway (i.e., specific immunoglobulin or IgM), alternative pathway (i.e., bacterial cell wall components, viral components, or surface molecules of parasites), and lectin pathway (i.e., interaction of mannose-binding lectin with surface molecules of microbes) (Boshra et al. 2006). Even though all three pathways exist in fish, full characterization of all components and their actions are incomplete (Sakai 1992; Boshra et al. 2006). The most important components of the complement system, namely C1r, C2, C3, C4, and C5–C9 (formation of the membrane attack complex), have been characterized and, for the most part, function demonstrated (Boshra et al. 2006). Each of the components reacts in an enzymatic cascade and generates products that are able to clear antigenic molecules and immune complexes, participate in the inflammatory response, lyse microorganisms, and aid in phagocytosis by macrophages and neutrophils. Nakao et al. (2011) provide a review of the current status of research on the teleost complement system.

Transferrin

Transferrin, which is found in the serum of fish, is an iron-binding glycoprotein that plays an important role in transporting iron. This protein acts as a bacterial defense mechanism by binding iron within the fish in order to make this essential element inaccessible to bacterial pathogens, thus preventing their growth.

Protease Inhibitors

Different fish species have protease inhibitors, such as α-2-macroglobulin. Such enzymes may have important roles in non-specific immunity by neutralizing proteolytic enzymes that are produced by different bacterial pathogens.

Signaling Molecules

A number of different cytokines are produced in fish and function to modulate both innate and adaptive immune responses. These include tumor necrosis factors, interleukins (IL), interferons, and chemokines. Recent reviews have been provided on these cytokines (Goetz et al. 2004; Robertsen 2006; Alejo and Tafalla 2011; Secombes et al. 2011).

Adaptive Immunity

Adaptive immunity is characterized by its specificity and association with immunological memory; however, these responses take time to develop in comparison to the innate response. The adaptive immune system of fish is divided into two branches: cell-mediated and humoral immunity, discussed in the following sections.

Cell-mediated Immunity

Cell-mediated immunity is important for defense against intracellular pathogens (i.e., intracellular bacteria, parasites, and viruses). Cell-mediated immune components consist of thymus-dependent lymphocytes, or T-cells, which express T-cell receptors (TCR) on their surface and provide specificity. T-cells consist of cytotoxic T-cells (Tc) and T-helper cells (Th). Both types recognize foreign antigen presented to them (from antigen-presenting cells) in the context of major histocompatibility complexes (MHC), and the interaction between the TCR and MHC molecule results in activation of the cells. Tc cells are identified by surface glycoprotein CD8, which is a co-receptor for binding to MHC class I molecules. These cells kill intracellular pathogens, viral infected cells, and foreign cells. Th cells are identified by the surface glycoprotein CD4 that binds to MHC class II molecules; these cells are further subdivided to Th1 and Th2 cells. Th1 cells secrete gamma interferon, tumor necrosis

factor beta, and IL-2, which activate antimicrobial activity in macrophages. Th2 cells secrete IL-4, IL-5, and IL-13, which promote strong antibody responses. Similar to humoral immunity, research is advancing the understanding of these processes in fish and the current state of knowledge on fish T-cells was recently reviewed (Laing and Hanson 2011).

Humoral Immunity

Antibody, or immunoglobulin (Ig), is the primary effector molecule of humoral immunity; it provides specificity. Antibody circulates in the serum of fish, and can also be found at mucosal sites and inside of some fish eggs. The primary antibody in fish serum has been described as a tetrameric IgM-like molecule (i.e., structurally similar to mammalian IgM). In teleosts, the antibody molecule comprises eight heavy chains and eight light chains. Hikima et al. (2010) describe the organization of fish Ig genes, the expressed Ig isotypes and their transcriptional control. Additional Ig isotypes were recently identified in fish: IgD in channel catfish (Edholm et al. 2011), and IgT in rainbow trout (Hansen et al. 2005), and IgZ in zebrafish (*Danio rerio*) (Flajnik 2005). Zhang et al. (2010) recently described the protein structure of IgT as monomeric in serum and polymeric in the gut of rainbow trout. The authors also demonstrated B-cells expressing surface IgT and suggested their importance in the GALT of rainbow trout.

The primary function of an antibody is to bind antigen. One effector mechanism of an antibody is to bind to bacterial pathogens, viruses, and toxins, which can result in neutralization. Binding of an antibody to a pathogen can also serve as an opsonin, in which macrophages can recognize the antibody via receptors and phagocytose the pathogen. Fish IgM activates complement and is also efficient at agglutinating bacterial cells that can aid phagocytosis.

Fish possess B-cells (surface Ig positive cells), which are considered similar to mammalian B-cells. However, Li et al. (2006) demonstrated that rainbow trout B-cells were both phagocytic and microbiocidal. The surface immunoglobulin of B-cells serves as the receptor for antigen recognition and has the same specificity of the antibody molecule produced. Peptide, protein, polysaccharide, lipopolysaccharide, and lipoprotein, but not lipids, are potential antigens. In a general sense, B-cells become activated following antigen binding to the immunoglobulin surface, either from circulating antigen or from presentation by antigen presenting cells (i.e., macrophages). Different antigens can invoke different mechanisms of B-cell activation. T-independent antigens can stimulate B-cells to produce antibody following binding without needing to interact with T-helper cells. T-dependent antigens need to be recognized by both B-cells and T-helper cells to elicit an antibody response. The activated B-cells then proliferate, differentiate, and generate a population of antibody secreting cells.

Immunologic memory and affinity maturation has been suggested in fish; however, the mechanisms for these are different than in mammals. An interesting difference between primary and memory humoral responses of fish and mammals is that fish do not switch to class IgG. The memory response of fish is IgM, which is same as the primary response. Ye et al. (2011a) suggest affinity maturation of fish Ig; however, the mechanism may be structural changes rather than germline or class switching (i.e., isotype changes). Fish IgM possesses eight antigen binding sites and, while affinity at each site may be relatively low, the molecule has a greater effective binding strength or avidity due to the presence of these multiple binding sites. Research is advancing the understanding of these processes in fish and different models have been proposed (Ye et al. 2011a, b; Zwollo 2011; Costa et al. 2012).

Bacterial Pathogens of Fish

Bacteria are microscopic prokaryotes that are grouped into two categories – Gram-negative or Gram-positive – based on differential staining using the Gram stain. Bacterial cells are generally grouped into three categories based on shape: coccus (round, oval, circular), bacillus (rod), and spirillium (spiral). Bacterial pathogens are typically identified following isolation in pure culture by growth in or on media, and are characterized using Gram-staining, acid fast-staining, and biochemical (nutrient) tests. New tests include serological or antibody-based techniques (immunofluorescence antibody test or enzyme-linked immunosorbent assay, IFAT or ELISA, respectively), fatty acid profiles, and nucleic acid probes such as polymerase chain reaction (PCR) methods. Fish

pathogens may be grouped into primary and secondary pathogens. Primary pathogens are capable of causing disease in a healthy host without other pathogens or environmental problems (e.g., *Edwardsiella ictaluri*, *Renibacterium salmoninarum*, *Francisella* spp.). Secondary pathogens typically cause disease due to environmental problems and/or co-infection (e.g., *Streptococcus* spp., *Aeromonas* spp., *Flavobacterium* spp., *Vibrio* spp.). To establish the ability of an isolated microorganism to cause disease, Koch's postulates need to be fulfilled as follows: (1) pathogen is present in all diseased animals; (2) pathogen is isolated and grown in pure culture; (3) pathogen from pure culture was placed back into fish and reproduced the same disease; and (4) the pathogen is re-isolated from the diseased fish in pure culture.

External/behavioral signs of bacterial disease may include anorexia or lack of feeding response, lethargy, abnormal swimming, excess mucus production, darkened body coloration, necrotic lesions and/or fin erosion, swollen abdomen (ascites), increased opercular movement, and pale or necrotic gills. Internal clinical signs may include reddening of intestine, swollen organs (enlarged spleen), pale or mottled organs (liver), white nodules (granulomas), and hemorrhagic organs (swim bladder).

Table 1.1 lists the bacterial pathogens that are responsible for major economic losses to all cultured fish species worldwide. Numerous books discuss in detail the etiological agents, disease signs, epizootiology, pathology, diagnosis, and control of bacterial pathogens important to cultured fish (Austin and Austin 2007; Plumb and Hansen 2010; Woo and Bruno 2011). The focus of the next sections of this chapter will be on emerging or re-emerging diseases considered by the authors to currently have a negative impact on aquaculture.

Flavobacterium spp.

Flavobacterium spp. are Gram-negative, rod-shaped, filamentous, and yellow-pigmented bacteria that are believed to be ubiquitous in freshwater environments (Bernardet and Bowman 2006). These bacterial species require specialized low-nutrient media for growth because they do not grow effectively on standard bacteriological media. Numerous formulations have been used in culture; some of the more common types include tryptone-yeast extract salts (TYES), Shieh or modified Shieh, Anacker and Ordal (also referred to as Cytophaga), and Hsu-Shotts (Cain and LaFrentz 2007). Two main bacterial species are responsible for most of the diseases affecting farm-raised fish: *F. columnare* and *F. psychrophilum*. However, new species are being identified and can also have negative impacts on fish and potentially aquaculture (Loch and Faisal 2013).

Flavobacterium psychrophilum is the causative agent of bacterial coldwater disease (CWD) (Borg 1960) or rainbow trout fry syndrome (RTFS), and is probably one of the most significant bacterial disease agents of trout and salmon in freshwater worldwide. *Flavobacterium psychrophilum* has a broad geographic distribution and all salmonid species, as well as some non-salmonid fish species, are believed to be susceptible (Starliper 2011). CWD typically occurs in young fish at low temperatures in the range 4–16 °C. Clinical signs of *F. psychrophilum* infections often depend on the size of fish affected and may vary between epizootics. In general, *F. psychrophilum* causes a septicemic infection that can be isolated from most organs of heavily infected fish. In alevins, the epithelial tissue covering the yolk sac may become eroded and, in some cases, the yolk sac may rupture. Common clinical signs in fry and fingerlings include yellow-pigmented lesions on the caudal peduncle, frayed and eroded fins, and dark coloration. If lesions appear on the caudal peduncle, necrosis may progress deep into the muscle tissue and expose the vertebrae. Although these are the classical clinical signs, fish may not exhibit any external lesions but instead may display general disease signs such as loss of appetite, lethargy, exophthalmia, and hanging at the water surface. Internally, petechia hemorrhaging may be visible on the pyloric caeca, adipose tissue, heart, swim bladder, and the peritoneal lining. The spleen of infected fish is commonly enlarged. Fish surviving an epizootic of CWD may exhibit spiral swimming and spinal compression types of deformities.

Preventative measures for CWD include the use of management strategies to reduce risk factors such as stress, poor water quality, and cutaneous lesions, since these factors tend to increase disease transmission. Removal of mortalities and morbid fish from rearing units is important to reduce the potential for bacterial shedding. Due to the ubiquitous

Table 1.1 Economically important bacterial pathogens of fish.

Disease	Bacteria	Fish affected	Distribution
Vibriosis	*Vibrio anguillarium* *Vibrio* spp.	Freshwater and marine	Worldwide
Coldwater vibriosis	*Vibrio salmonicida*	Salmonids	Worldwide
Wound disease	*Moritella viscosa*	Salmonids	Northern Europe
Furunculosis	*Aeromonas salmonicida salmonicida*	Salmonids	Worldwide
	Aeromonas salmonicida achromogens	Freshwater	
Enteric redmouth disease (ERM)	*Yersinia ruckeri*	Salmonids	Worldwide
Piscirickettsiosis	*Piscirickettsia salmonis*	Salmonids	Worldwide
Columnaris disease	*Flavobacterium columnare*	Freshwater	Worldwide
Coldwater disease/Rainbow trout fry syndrome (RTFS)	*Flavobacterium psychrophilum*	Salmonids	Worldwide
Bacterial kidney disease	*Renibacterium salmoninarum*	Salmonids	Worldwide
Enteric septicemia of catfish	*Edwardsiella ictaluri*	Catfish species	USA/Asia
Edwardsiellosis	*Edwardsiella tarda*	Freshwater and marine	Worldwide
Motile *Aeromonas* septicemia (MAS)	*Aeromonas hydrophila*, *A. caviae*, *A. sobria*	Freshwater	Worldwide
Pasteurellosis	*Photobacterium damsela piscida*	Marine	Worldwide
Streptococcosis	*Streptococcus iniae*, *S. agalactiae*, *S. dysgalactiae*, *S. phocae*	Freshwater and marine	Worldwide
Francisellosis	*Francisella noatunensis*, *F. asiatica*	Freshwater and marine	Worldwide
Tenacibaculosis	*Tenacibaculum maritimum*	Marine species	Worldwide

nature of *F. psychrophilum*, CWD can even occur with management strategies in place. External treatments have been used to reduce mortality associated with the bacteria, including bath administration of salt (sodium chloride) and potassium permanganate. However, due to the systemic nature of *F. psychrophilum* infections, CWD is commonly treated with antibiotics. In the United States, florfenicol (AQUAFLOR®) and oxytetracycline (Terramycin®) are approved for use in freshwater-reared salmonids to control mortality due to CWD. The development of an efficacious vaccine for CWD has been difficult and there are no commercially available vaccines at this time, largely due to the lack of consistent protection in fish immunized with killed whole-cell preparations using mass delivery methods. Recently, LaFrentz et al. (2008) developed an attenuated strain of *F. psychrophilum* that was able to confer protective immune responses to rainbow trout following immersion vaccination, making it a promising candidate for vaccine development. Such a strategy was further supported by Alvarez et al. (2008) and Lorenzen et al. (2010).

Flavobacterium columnare is the causative agent of columnaris disease (Bernardet et al. 1996). The bacterium is a significant pathogen of cultured fish species due to its worldwide distribution and its ability to infect most freshwater fish and cause disease over a wide range of temperatures (>15°C). Columnaris disease is responsible for large economic losses in aquaculture and is one of the leading causes of mortality in the channel catfish industry in the United States. All ages of fish are susceptible to columnaris disease, but the disease is more prevalent in young fish. In general, clinical signs of columnaris disease are easily recognized and include frayed fins, depigmented lesions on the skin, and necrotic gill lesions. Wet mounts of gill tissue or skin lesions from diseased fish will reveal long slender rods with gliding

movement, and the cells will aggregate into columns or 'haystacks' of cells (thus the name columnaris disease). Skin lesions often begin around the dorsal fin and then increase in size, eventually resulting in a gray to white lesion that has the appearance of a saddle. In some cases the margin of the lesion is yellow in appearance due to the proliferation of the yellow-pigmented bacterium. Gill tissue can exhibit severe necrosis and may appear white to brown and also yellowish due to the presence of large quantities of the bacterium. Internal pathology is rarely present. Although these are the classical clinical signs of disease, some diseased fish may exhibit no external lesions but instead will appear dark in color and lethargic.

As with the other *Flavobacterium* spp., preventative measures include the use of good management practices to provide proper environmental conditions for fish and reduce risk factors such as stress, poor water quality, and cutaneous lesions. Treatment for columnaris disease generally includes external bath treatments and/or antibiotics. External treatments that have been used include bath administration of salt, copper sulfate, potassium permanganate, hydrogen peroxide, chloramine-T, and quaternary ammonium compounds (i.e., Roccal®, Hyamine, Diquat). In the United States, hydrogen peroxide (35% PEROX-AID®) is approved for use in freshwater-reared coolwater finfish and channel catfish to control mortality due to external columnaris disease. Additionally, florfenicol (AQUAFLOR®-CA1) and oxytetracycline (Terramycin®) are approved for the control of columnaris disease in channel catfish and freshwater-reared rainbow trout, respectively. Two vaccines are available in the United States for the prevention of columnaris disease. One is a *F. columnare* bacterin (FryVacc1) (Bowker et al. 2012) approved for use in salmonids, and the other is a modified live vaccine (AQUAVAC-COL®) approved for use in channel catfish (Shoemaker et al. 2009, 2011).

Francisella spp.

Francisella spp. have probably caused disease in fish for a number of years; however, difficulty to culture on standard media likely lead to the under-reporting of these bacteria to date (Birkbek et al. 2011). *Francisella* spp. are aerobic, facultative intracellular Gram-negative coccobaccilli (Foley and Nieto 2010). The media required to culture *Francisella* spp. require cysteine and a source of iron from blood (Mikalsen and Colquhoun 2010) and/or supplemental hemoglobin (Soto et al. 2009). The two species commonly associated with disease in fish are *Francisella noatunensis noatunensis* and *F. noatunensis orientalis* (*F. asiactia*). *Francisella noatunensis noatunensis* is associated with disease of Atlantic cod (*Gadus morhua*; Nylund et al. 2006) and Atlantic salmon (*Salmo salar*; Birkbeck et al. 2007). *Francisella noatunensis orientalis* is associated with disease in tilapia (*Oreochromis* spp.), three-lined grunt (*Parapristipoma trilineatum*; Kamaishi et al. 2005; Hsieh et al. 2006; Birkbeck et al. 2007; Mikalsen and Colquhoun 2010), and hybrid striped bass *(Morone chrysops* x *M. saxitalis*; Ostland et al. 2006). Disease signs include lack of appetite and emaciation. The most notable internal signs are white–cream-colored nodules or granulomas present in the spleen, heart, kidney, and liver (Olsen et al. 2006; Mauel et al. 2007). Control strategies for *Francisella* include vaccination and antibiotics. Oral administration of florfenicol at the early stages of *Francisella* infection was effective at treating francisellosis in tilapia (Soto et al. 2010). Soto et al. (2011) produced an attenuated *F. asiatica* iglC (gene of the intracellular growth pathogenicity island that aids intracellular survival of *Francisella* sp. in macrophages) mutant that showed vaccine potential in laboratory trials. Due to the nature of *Francisella* spp. as intracellular pathogens, modified live vaccines will probably be needed to induce adequate protection in the field.

Aeromonas spp.

Motile *Aeromonas* septicemia (MAS) is usually an infectious disease of warmwater fish (channel catfish, cyprinids, eels, centrarchids, striped bass); however, trout and salmon can also be affected. The most common species isolated and characterized from fish are *A. hydrophila*, *A. sobria*, and *A. caviae* (Austin and Austin 2007). The bacteria are motile, cytochrome oxidase-positive Gram-negative rods that have the ability to ferment glucose and are resistant to vibriostat (0/129). Definitive identification requires a battery of tests (Plumb and Hansen 2010). Rimler–Shotts

selective media may aid in the identification by yielding orange-yellow colonies when incubated at 35°C (Shotts and Rimler 1973). Clinical signs include poor feeding response, lethargy, pale gills, exophthalmia, and hemorrhagic eyes. In scaleless fish, fins are often frayed and necrotic lesions develop. Internally, organs are friable and hyperemic, the liver may be mottled, ascites may be present, and intestine is generally void of food. Since 2009, *A. hydrophila* has emerged as a significant pathogen in the channel catfish industry in the United States. Between June and October of 2009, an estimated loss of more than 1.36 million kg of food-size channel catfish was reported in West Alabama alone (Hossain et al. 2013). Infections are often associated with stress including temperature shock, low dissolved oxygen (DO), high ammonia, handling or hauling, and co-infection. Control of MAS infections may rely on husbandry practices (e.g., maintain high DO levels and reduce parasite load), feeding antibiotics, and/or vaccination. At present, there are no commercially licensed vaccines for MAS in fish.

Streptococcus spp.

Streptococcal disease is caused by three main species of facultative anaerobic encapsulated Gram-positive streptococci. *Streptococcus iniae*, *S. agalactiae*, and *S. dysgalactiae* are responsible for disease in more than 30 species of freshwater, estuarine, and marine fish worldwide. The bacteria are typically grown on sheep blood agar and may or may not be beta-hemolytic. Other standard microbiological media – such as tryptic soy broth, brain heart infusion broth, or agar – may be employed to routinely culture the bacteria. The growth temperature is 28–30°C with a range of 10–45°C. Lancefield grouping is commonly employed to differentiate *Streptococcus* spp. (Lancefield 1933). *Streptococcus iniae* is not groupable, *S. agalactiae* is Group B (Vandamme et al. 1997), and *S. dysgalactiae* is group C (Nomoto et al. 2008). Disease signs typically include loss of appetite, dark skin pigmentation, erratic or C-shaped swimming, eye opacity, and exophthalmia. Eye opacity or cloudiness appears to be associated with chronic disease. Other disease agents also cause similar clinical signs; definitive diagnosis should therefore rely on culture and identification of the bacteria using biochemical (Facklam et al. 2005; Shoemaker et al. 2006) or molecular techniques such as PCR (Zlotkin et al. 1998; Berridge et al. 2001; Kawata et al. 2004; Mata et al. 2004). The significance of *S. iniae* as a fish pathogen was reviewed by Agnew and Barnes (2007); all three bacteria may be potential zoonotic agents and may therefore possess the ability to infect humans (Weinstein et al. 1997; Lau et al. 2006). Shoemaker et al. (2001) suggest the greatest zoonotic risk appears to be associated with older or immunocompromised people who incur a puncture wound while handling or preparing fresh, whole fish for cooking.

Control of streptococcal disease is via culture management, antimicrobial treatments, and vaccination strategies. Shoemaker et al. (2000) demonstrated that a reduction in fish density resulted in decreased mortality, and suggested this was a result of less fish-to-fish transmission. Feeding of medicated diets is practiced and reduces overt disease signs (Gaunt et al. 2010). However, disease often reoccurs upon discontinuing of feeding. Various vaccination strategies using killed and attenuated vaccine candidates have been practiced (Eldar et al. 1997; Klesius et al. 2000; Evans et al. 2004; Buchanan et al. 2005; Locke et al. 2010; Shoemaker et al. 2010). The strategies have demonstrated effectiveness in the laboratory, and success has also been documented in the field. Work in Israel and Australia has demonstrated the emergence of new serotypes after continued use of autogenous killed vaccines in the field (Bachrach et al. 2001; Agnew and Barnes 2007). Martins et al. (2011) demonstrated that coinfection with parasites led to decreased vaccine performance in tilapia, thereby verifying in part the reported ineffectiveness of vaccines used on commercial farms in the United States.

Viral Pathogens of Fish

Viruses are submicroscopic particles (i.e., 18–300 nm by electron microscopy) that require a host cell to replicate. Most individual virus particles (virion) consist of a single type of nucleic acid, either DNA or RNA (not both), contained within a protein shell or coat (capsid) that may or may not be enveloped (Smail and Munro 1989). Viral infections are typically confirmed following isolation of the virus in tissue culture (Plumb and Hanson 2010). Cytopathic effect

(CPE) or cell injury is often unique to each virus type and may be indicative of the type or group of virus. Following isolation in cell culture, viruses can be characterized by electron microscopy and/or serum neutralization with antiserum specific to the virus. New technologies for virus identification include serological tests (IFAT and ELISA) and nucleic acid probes (e.g., PCR and RT-PCR).

Disease signs in fish infected with viruses may range from exophthalmia, hemorrhagic lesions, distended fluid-filled abdomens, pigment changes, and lethargy to no signs and/or rapid death. Viral pathogens may also result in tumor-like growths both internally and externally. An important consideration for viral pathogens is the limited treatment options available due to the requirement for a living cell for replication. Effective biosecurity measures are prudent to prevent pathogen introduction and spread (Lee and O'Bryen 2003). Effective vaccines have been developed and used for some fish viral agents, and one of the first DNA vaccines approved and commercially available in Canada was developed for use against infectious hematopoietic necrosis virus (Salonius et al. 2007). Another equally effective DNA vaccine has been developed against viral hemorrhagic septicemia virus, but it is not yet commercially available (Lorenzen et al. 2001).

Viral pathogens that are of economic importance to cultured fish are listed in Table 1.2. Numerous available books discuss in detail the viral agents, disease signs, epizootiology, pathology, diagnosis, and control of the viruses that cause loss in cultured fish (Smail and Munro 1989; Plumb and Hansen 2010; Woo and Bruno 2011). The main focus of this section is emerging or re-emerging viral diseases affecting fish that are significant or potentially significant for aquaculture species.

Infectious Salmon Anemia Virus

Infectious salmon anemia (ISA) is a relatively new viral disease caused by infectious salmon anemia virus, which has a virion around 100 nm in size and is of the orthomyxoviridae family. The viral genome is composed of eight single-strand RNA segments (Cottet et al. 2011). The disease was first seen in Norway in the late 1980s and has caused devastating losses in Atlantic salmon (*Salmo salar*) culture in Scotland, Chile, North America, and the Faroe Islands (Miller and Cipriano 2003). Clinical signs of ISA usually appear about 2 weeks post-infection and include severe anemia, swelling and hemorrhaging in the kidney and other organs, pale gills, protruding eyes, darkening of the posterior gut, fluid in the body cavity, and lethargy. Hematocrits can be reduced from around 35–48% to 12–25% (Dannevig et al. 1993), indicative of anemia. The spread of ISA virus may occur as a result of the purchase of infected smolts that did not exhibit clinical disease, farm-to-farm transfer, or from fish processing plants or industries where organic material (especially blood and processing water) from ISAV-infected fish is discharged without treatment (Bruno et al. 1995). Both wild and cultured Atlantic salmon are susceptible to infection. ISA virus also infects brown trout in the marine environment but apparently does not cause disease; instead, it may serve as a reservoir for infection (Nylund and Jakobsen 1995). Extreme strategies including eradication have been employed to control this viral disease; however, there is some evidence that other fish species may carry the virus and can transmit it to susceptible fish. ISA is a World Organization for Animal Health Office International des Épizooties (OIE) reportable disease (OIE 2010a).

Spring Viremia of Carp Virus

Spring viremia of carp virus (SVCV) is caused by *Rhabdovirus carpio*, a bullet-shaped RNA virus around 70 × 180 nm in the *Lyssavirus* genus of the family Rhabdoviridae. SVCV infects a broad range of fish species and causes high mortality in susceptible hosts in cold water (12–17°C; Ahne et al. 2002). Infections have occurred in common and koi carp (*Cyprinus carpio*); grass carp (*Crenopharyngodon idella*); silver carp (*Hypophthalmichthys molitrix*); bighead carp (*Aristichthys nobilis*); cruian carp (*Carassius carassius*); goldfish (*C. auratus*); roach (*Rutilus rutilus*); ide (*Leuciscus idus*); tench (*Tinca tinca*); and sheatfish (*Silurus glanis*) (Plumb and Hansen 2010). Long indigenous to Europe, the Middle East, and Asia, the disease was reported recently in North and South America. In the spring of 2002, SVCV was isolated from koi carp farmed in North Carolina (Goodwin 2002). In the same year the virus was also reported from carp in lakes and rivers in

Table 1.2 Economically important viral diseases of fish. World Organization for Animal Health, Office International des Épizooties (OIE) reportable viruses are marked with an asterisk (*).

Disease	Virus (type)	Fish affected	Distribution
Infectious hematopoietic necrosis*	Infectious hematopoietic necrosis virus (rhabdovirus)	Salmonids	Worldwide
Infectious pancreatic necrosis	Infectious pancreatic necrosis virus (birnavirus)	Salmonids, marine species	Worldwide
Infectious salmon anemia*	Infectious salmon anemia virus (orthomyxovirus)	Atlantic salmon	Worldwide
Pancreas disease	Pancreas disease virus (togavirus)	Salmonids	Europe
Viral hemorrhagic septicemia*	Viral hemorrhagic septicemia virus (rhabdovirus)	Freshwater and marine	Worldwide
Spring viremia of carp*	Spring viremia of carp virus (rhabdovirus)	Carps	Worldwide
Koi herpes*	Koi herpes virus (herpesvirus)	Carps	Worldwide
Channel catfish virus disease	Channel catfish virus (herpesvirus)	Channel catfish	USA, Asia
Viral nervous necrosis	Viral nervous necrosis virus (betanodavirus)	Marine species	Worldwide
Iridovirus disease*	Red sea bream (iridovirus)	Marine species	Asia
Epizootic hematopoietic necrosis*	Epizootic hematopoietic necrosis virus (ranavirus)	Redfin perch, rainbow trout	Australia

Wisconsin and the Mississippi River. SVCV causes impairment of the salt–water balance in fish, resulting in edema and hemorrhages. Liver, kidney, spleen, gill, and brain are the primary organs containing the virus during infection. Horizontal transmission most likely occurs when waterborne virus enters via the gills, whereas vertical transmission may be possible as adult carp shed virus during spawning (Bekesi and Csontos 1985). Reservoirs of SVCV are infected fish and carriers from either cultured, feral, or wild fish populations (Goodwin et al. 2004). This virus may remain infective for long periods of time in water or mud. Once the virus is established in a pond or farm, it may be difficult to eradicate without destruction of all fish at the farm. SVC is an OIE (OIE 2010b) reportable disease. Recommendations for preventing the disease and its spread include the use of a water source free of virus, disinfection of eggs and equipment, and proper disposal of dead fish (Schlotfeldt and Alderman 1995). The OIE (2010) Manual for Aquatic Animal Disease has specifications for surveillance programs to achieve and maintain the biosecure status of aquaculture facilities.

Viral Hemorrhagic Septicemia Virus

On a worldwide scale, viral hemorrhagic septicemia (VHS) is probably the most significant disease. VHS is caused by an enveloped bullet-shaped rhabdovirus in the *Novirhabdovirus* genus of the family Rhabdoviridae that is about 65 nm in diameter and 180 nm long, called viral hemorrhagic septicemia virus (VHSV; Zwillenberg et al. 1965). The genome of VHSV is single-stranded RNA. VHSV has been genetically grouped into four genotypes (I, II, III, and IV) based on the N and G genes (Einer-Jensen et al. 2006; Nishizawa et al. 2006). Genotype I is typically associated with freshwater farmed trout in Europe (Al-Hussinee et al. 2011). Genotype II and III are associated with marine fish from the Baltic Sea and North Atlantic Ocean (Nishizawa et al. 2006). Genotype IV was reported from marine fish in the Pacific Northwest, Japan, and Korea (Hedrick et al. 2003). In 2005, VHSV was isolated and characterized from freshwater fish in the Great Lakes Basin of North America (Elsayed et al. 2006). Interestingly, the viral isolate was genetically related to VHSV genotype IV, but distinct enough to be considered genotype IVb

with the other viral isolate from the marine fish designated IVa (Al-Hussinee et al. 2011). Clinical signs of VHS include severe hemorrhaging in the musculature and internal organs of the fish; however, definitive diagnosis relies on isolation of the virus in tissue culture and subsequent viral neutralization and/or molecular confirmation via RT-PCR (OIE 2010c). VHS has historically been responsible for severe fish losses. The recent identification of VHSV in freshwater in the United States is significant because at least 28 fish species are reported to be susceptible. Due to VHSV being a reportable virus, the national and international trade of baitfish and cultured fish could be severely restricted. USDA-APHIS has outlined a number of regulatory requirements that must be followed if VHSV is suspected (Bowser 2009).

Koi Herpes Virus

Koi herpes virus (KHV) disease is an important viral disease of koi and cultured carps worldwide. The causative virus is koi herpes virus or cyprinid herpes virus 3 (Hedrick et al. 2000). The virus is an enveloped icosahedron about 200 nm in size with a DNA genome (Hedrick et al. 2000; Miyazaki et al. 2008). Hedrick et al. (2000) first reported the disease in Israel and the Unites States in both carp and koi (ornamental carp). Since the first description it has been detected in many countries, most likely as a result of the live koi trade (Haenen and Hedrick 2006). Mortality events can be significant with 80–100% death rate occurring (Haenen et al. 2004). Clinical signs include loss of equilibrium and erratic swimming, gill discoloration, and necrosis (Sano et al. 2011). Other signs include anorexia, exophthalmia, fin erosion, hemorrhage on skin and base of fins, and patchy-appearing skin related to mucus production. The disease is an OIE reportable disease (OIE 2011d). Diagnostic methods rely on tissue culture (i.e., virus isolation), viral antigen detection (IFAT), and PCR methods (Bercovier et al. 2005; Yuasa et al. 2005). The PCR assay to detect KHV genomic DNA followed by sequencing the PCR product is considered to be the best method for virus detection currently available (OIE 2010d). A newly licensed vaccine (Cavoy™-Norvartis Animal Health) against KHV (Cyprinid herpes virus-3) is now available through veterinarians for prophylactic management of KHV disease.

Iridovirus Diseases

Epizootic hematopoietic necrosis disease (EHND) and red sea bream iridoviral disease (RSIVD) are caused by viruses in the Iridoviridae family (Whittington et al. 2010; Sano et al. 2011). Iridoviruses possess icosahedral virions about 120–200 nm in size with a single linear double-stranded DNA genome (Chinchar et al. 2005). Epizootic hematopoietic necrosis virus is in the genus *Ranavirus* and affects mainly wild redfin perch (*Perca fluviatilis*) and cultured rainbow trout in Australia (Langdon et al. 1986; Whittington et al. 1999). Red sea bream (*Pagrus major*) iridovirus is in genus *Megalocytivirus* and mainly affects high-value marine aquacultured fish in Asia. EHND and RSIVD are both OIE reportable diseases and have been shown to infect a number of economically important freshwater (EHND) and marine fish (RSIVD) experimentally. Diagnostic methods for detection of EHND rely on tissue culture and subsequent immunological analysis or PCR with DNA sequencing (Marsh et al. 2002; OIE 2010e;). Detection of RSIV is also based on immunological analysis and PCR methods from fish tissue, as viral isolation in tissue culture is difficult (OIE 2010f).

Parasitic Diseases

Parasites may be microscopic or macroscopic in size and need a fish host for survival and/or to complete their life cycle. Parasites in general gain some benefit (e.g., nutrients) from the fish host. Most fish parasites may cause mechanical injuries to the gill, skin, or internal organs. Some parasites may inhibit the function of vital organs in fish, such as digenetic trematodes and tapeworms. Parasites may secrete harmful substances and cause toxic effects (Sindermann 1990; Zhang et al. 1999). The mechanical injury caused by the parasite provides a portal of entry for pathogenic microorganism, and may enhance fish susceptibility to bacterial diseases (Sindermann 1990; Xu et al. 2007; Martins et al. 2011).

Protozoan Diseases

Diseases caused by protozoans are among the most significant of all parasitic diseases. Cultured fish losses due to parasites are mainly caused by protozoan

parasites (Rogers 1985). In freshwater fish, white spot disease caused by *Ichthyophthirius multifiliis* (Ich), whirling disease, and proliferative gill disease caused by Myxozoan protozoan are some of the severe parasitic diseases that often lead to significant losses of cultured fish. In marine fish, marine white spot disease (caused by *Cryptocaryon irritans*) and marine velvet (caused by the dinoflagellate, *Amyloodinium ocellatum*) both lead to serious mortalities.

Ciliate Protozoans

The protozoan parasite *Ichthyophthirius multifiliis* (Ich) infects virtually all freshwater fish and causes damage to their gills and skin. Epizootics have been reported worldwide and result in severe economic losses for aquaculture producers. The life cycle of the parasite includes three stages: an infective theront, a parasitic trophont, and a reproductive tomont. The parasitic trophont lives completely within the host and feeds on damaged cells and body fluids of fish. The movement of theront and trophont (penetration, rotation, and relocation) cause severe tissue damage (Xu et al. 2000). The parasite feeds on host cells until it is mature; the mature trophont then drops off the host, attaches to substrates, and undergoes multiple divisions to produce 512–1024 infective theronts in less than 24 hours. At optimum temperature (22–24°C), Ich can reproduce rapidly and cause high fish mortality within a short period of time (Matthews 2005; Dickerson 2006). *Cryptocaryon irritans* is a ciliate protozoan that parasitizes marine fish and is one of the most common causes of disease in marine aquaria. The symptoms and life cycle are generally similar to those of *Ichthyophthirius* in freshwater fish, including white spots, so the disease is also known as marine white spot disease. *Cryptocaryon* requires a much longer time for tomont division than in *Ichthyophthirius*. *Cryptocaryon* tomonts take 8 days at 25°C and longer at lower temperatures to divide and produce infective theronts (Dickerson 2006).

Other commonly reported ciliated protozoans are listed in Table 1.3. Trichodinid species that infect freshwater and marine fish include *Trichodina*, *Trichodinella*, and *Tripartiella* species. Most of the clinically important species infect fish skin and/or gills (Shoemaker et al. 2006). Parasitism of the gills is more detrimental than of the body. Signs of disease include respiratory distress, loss of appetite, depigmentation, or loss of scales. *Chilodonella* is a leaf or heart-shaped ciliate that has distinct parallel rows of cilia along the body margin. *Chilodonella* infects the skin, fins, and gills of freshwater fish. A heavy infection can cause detached scales, necrosis of branchial epithelium, and mass mortalities. *Apiosoma* and *Ambiphyra* are similar ciliates; both have a barrel-shaped body with a ring of cilia. The major difference is the shape of macro-nucleus, with the rounded nucleus in *Apiosoma* and the ribbon-shaped nucleus in *Ambiphyra*. These protozoans have a direct life cycle and reproduce by binary fission on the skin and gills. *Epistylis* attaches to fish with its stalk and commonly forms branched colonies. A severe infection causes erosion of skin, scales, and spines and bloody lesions, thus the common name is "red sore disease." Most of these parasites may be treated with salt, formalin, or other approved parasiticides; however, it may take multiple treatments to effectively control these ciliates.

Flagellated Protozoans

Marine velvet is caused by the dinoflagellate *Amyloodinium ocellatum* and leads to serious mortality in brackish and marine warmwater fish at aquaculture facilities worldwide (Noga and Levy 2006). Outbreaks occur rapidly and may result in 100% mortality within a few days. The total life cycle for this parasite approximates 3 weeks and is very similar to *Cryptocaryon irritans*. It has a free-swimming infective dinospore stage, a parasitic trophont stage that feeds on fish skin and gill epithelium, and an encysted tomont stage that divides to produce infective dinospores (Noga and Levy 2006). Dinoflagellate *Piscinoodinium* is similar to *Amyloodinium* in morphology and causes freshwater velvet in tropical freshwater fish (Noga and Levy 2006). *Ichthyobodo necatrix* (*Costia*) is an important flagellated parasite of freshwater fish. Transmission of *Ichthyobodo* is by direct fish-to-fish contact. Common signs indicating the presence of this parasite are respiratory distress and flashing. *Ichthyobodo* can cause severe losses in a short time if not treated. *Hexamita* is flagellate protozoan found in the gastrointestinal tracks of many freshwater and marine fish in the world. Among them, *Hexamita salmonis* may be present wherever trout or salmon are reared. The disease is commonly found in fingerlings and outbreaks are usually sporadic in aquaculture facilities (Woo 2006). Effective treatments for *Ichthyobodo*

Table 1.3 Economically important parasitic and fungal diseases of fish. World Organization for Animal Health, Office International des Épizooties (OIE) reportable parasitic and fungal diseases are marked with an asterisk (*).

Disease	Parasite	Fish affected	Distribution
"White spot" disease	Ciliated protozoan: *Ichthyophthirius multifiliis*	Freshwater fish	Worldwide
Marine white spot disease	Ciliated protozoan: *Cryptocaryon irritans*	Marine fish	Worldwide
Trichodinosis	Ciliated protozoan: *Trichodina* spp., *Trichodonella* spp., *Tripartiella* spp.	Freshwater fish	Worldwide
Trichophrya infestation	Ciliated protozoan: *Trichophrya* spp.	Freshwater fish	Worldwide
Ambiphyra and Apiosoma infestation	Ciliated protozoan: Ambiphyra spp., Apiosoma spp.	Freshwater fish	Worldwide
Chilodonella infestation	Ciliated protozoan: *Chilodonella* spp.	Freshwater fish	Worldwide
Epistylis infestation	Ciliated protozoan: *Epistylis* spp.	Freshwater fish	Worldwide
Dinoflagellate infestation	Flagellated protozoan: *Amyloodinium* spp., *Piscinoodinium* spp., *Crepidoodinium* spp., *Ichthyodinium* spp.	Marine, brackish, and freshwater fish	Worldwide
Hexamitosis and Sprironucleosis	Flagellated protozoan: *Hexamita* spp., *Spironucleus* spp.	Marine and freshwater fish	Worldwide
Ichthyobodosis	Flagellated protozoan: *Ichthyobodo* spp. (*Costia* spp.)	Freshwater fish	Worldwide
Whirling disease	Myxozoan protozoan: *Myxobolus cerebralis*	Salmonids	USA, Europe Asia
Proliferative gill disease	Myxozoan protozoan: *Aurantiactinomyxon ictaluri*	Channel catfish	USA
Proliferative kidney disease	Myxozoan protozoan: *Tetracapsula renicola* n. sp	Salmonids	Europe, N. America
Henneguyiasis	Myxozoan protozoan: *Henneguya* spp.	Freshwater fish	Worldwide
Gill flukes	Mongenetic trematodes: *Dactylogyrus* spp.	Freshwater and marine fish	Worldwide
Gyrodactylus	Mongenetic trematodes: *Gyrodactylus* sp., *Gyrodactylus salaris**	Freshwater and marine fish	Worldwide
Trematode *Bolbophorus*	Digenetic trematodes: *Bolbophorus* spp.	Freshwater fish	USA
White grub and Yellow grub	Digenetic trematodes: *Posthodiplostomum* spp., *Clinostomum* spp.	Freshwater fish	Worldwide
Asian tapeworm infestation	*Bothriocephalus acheilognathi*	Freshwater fish	Worldwide
Thorny-headed worm infection	*Acanthocephalus* spp.	Freshwater fish	Worldwide
Red worm	Nematode: *Camallanus* spp.	Freshwater fish	Worldwide
Fish louse	Crustacean parasite: *Argulus* spp.	Freshwater fish	Worldwide

Table 1.3 (*Continued*)

Disease	Parasite	Fish affected	Distribution
Copepod ectoparasite	Copepod ectoparasite: *Ergasilus* spp.	Freshwater fish	Worldwide
Anchor parasite	Crustacean parasite: *Lernaea* and *Lernaeocera* spp.	Freshwater and marine fish	Worldwide
Sea lice	*Lepeophtheirus salmonis* and *Caligus elongatus*	Marine fish	Worldwide
Water molds	Fungi: *Saprolegnia* spp., *Achlya* spp.	Fresh, brackish water fish	Worldwide
Epizootic ulcerative syndrome*	Fungi: *Aphanomyces invadans* or *A. piscicida*	Fresh, brackish water fish	Asia–Pacific region
Branchiomycosis or "gill rot"	Fungi: Branchiomyces spp.	Freshwater fish	Europe, Asia

include copper sulfate and formalin. Management practices, such as quarantine to avoid transmission to un-infected fish, should be practiced.

Myxozoan Protozoan

Whirling disease is a severe parasitic disease that was first described in rainbow trout in Germany in 1893 (Bartholomew and Reno 2002). Since then, whirling disease caused by *Myobolus cerebralis* has been reported in 26 different countries, including some African and European countries (i.e., Russia), the United States, and other countries. Whirling disease in the United States was first reported in Pennsylvania in 1956 (Hoffman 1990; Bartholomew and Reno 2002). Presently, whirling disease has occurred in 22 states located in the eastern and western United States. The spread of the whirling disease parasite has been attributed to transfer of live fish (Hoffman 1990; Bartholomew and Reno 2002).

Myxobolus cerebralis has a two-host life cycle that involves fish and an alternate host: a common bottom-dwelling tubifex worm. *Myxobolus cerebralis* mainly infects farmed salmonid fish, but is also found in wild fish populations. When an infected fish dies, large numbers of spores are released into the water and become myxospores. The myxospores are then ingested by the tubifex worm and develop into infective triactinomyxon (TAM). Fish can be infected by TAM attaching to fish bodies and/or by fish eating infected tubifex worms. Whirling disease affects juvenile fish and causes fish skeletal deformation and neurological damage. The parasitized fish show "whirling" swimming behavior instead of normal swimming, have difficulty feeding, and are more vulnerable to predators. The mortality rate for fingerlings can reach 90% or higher in the infected populations (Hoffman 1990; Bartholomew and Reno 2002).

Management strategies to prevent and control whirling disease include control of the worm host and its habitats, disinfection of water containing triactinomyxons spores, stocking larger fish into infected waters, eliminating infected fish, enforcement of disease regulation, and stocking less-susceptible species or strains of fish (Wagner 2002).

Proliferative kidney disease (PKD) is an economically important disease of salmonids in Europe and North America. PKD of salmonid fishes is caused by *Tetracapsuloides bryosalmonae*, a myxozoan parasite of salmonid fishes. *T. bryosalmonae* uses a bryozoan as an alternate host, rather than an oligochaete or polychaete worm. Five bryozoan species belonging to the genera *Fredericella* and *Plumatella* have been found to develop infection with *T. bryosalmonae*, resulting in the development of spherical sacs that release spores (Anderson et al. 1999). These spores are released into the surrounding water where they can infect salmonid fish. PKD primarily occurs during summer (April–June), and mortalities may range from 10% to 95% in infected populations (Noga 1996). *T. bryosalmonae* affects mainly the kidney and spleen but can become systemic in most susceptible fish hosts. Other diseases caused by myxozoan parasites include ceratomyxosis, which is a disease of trout and salmon caused by *Ceratomyxa shasta*. Ceratomyxosis has caused disease and deaths in both juvenile and adult hatchery and wild salmonids. Aside from

disinfection and quarantine, there are no good control methods for myxozoan infections. Myxozoan spores are long-lived with some surviving for well over 1 year, so disinfection is mandatory for eradication (Noga 1996).

Monogenetic Trematodes

Among monogenetic trematodes in fish, *Gyrodactylus* spp. and *Dactylogyrus* spp. are the most common. *Dactylogyrus* sp. is often found on the gills, whereas *Gyrodactylus* is found either on the gill or skin of freshwater fish. Monogenetic trematodes use haptors at the posterior end to attach to fish. They have large centrally located anchors and hooks around the margin of the haptor. Anchors and hooks of monogenetic trematodes penetrate into the surface layer of skin, fins, and gills, causing tissue damage. These worms move on the body surface and feed on dermal and gill debris (Post 1983). Monogenetic trematodes are hermaphroditic and contain both male and female reproductive organs. The main difference between the two is that *Gyrodactylus* is viviparous (produce live offspring) and *Dactylogyrus* is oviparous (produce eggs). One of the species that is of concern to salmon fisheries and fish farms is *Gyrodactylus salaris*, which has been responsible for heavy losses of Atlantic salmon in European countries (Buchmann and Bresciani 2006). *Gyrodactylus salaris* is the only parasitic disease at present that is OIE reportable (OIE 2010g). Other monogenetic trematodes are usually not a severe problem unless they increase to high numbers. In heavy gill infections, fish become lethargic, swim near the surface while experiencing partial suffocation, and do not feed (Post 1987). With large numbers of monogenetic trematodes on the skin, fish show excessive mucus, rubbing against sides of holding tanks and occasionally jumping out of water. Formalin, potassium permanganate, and copper sulfate may be used as prolonged treatments for monogenetic trematodes. Recent work has demonstrated the importance of these pathogens in increasing the susceptibility of infected fish to bacterial infections (Xu et al. 2007).

Digenetic Trematodes

There are many digenetic trematodes that infect both freshwater and marine fish. Digenetic trematodes require more than one host to complete their life cycle. Fish may serve as final hosts or intermediate hosts in their life cycle. Very few adult-stage digeneans can cause major damage to the fish host. Metacercarial (larval) infection is the main source of mechanic damage in fish, which leads to economic loss (Paperna and Dzikowski 2006). In recent years, a freshwater digenetic trematode, *Bolbophorus damnificus* or *confusus*, has caused problems in commercially raised channel catfish in Louisiana, Mississippi, and Arkansas (Terhune et al. 2003; Flowers et al. 2005). The trematode is vectored by pelicans (*Pelecanus* spp.), the final host, and snails (*Planorbella trivolvus*), the first intermediate host. *Bolbophorus* infections have caused high mortality and decreased production in channel catfish (Terhune et al. 2003). Other digenetic trematodes, such as white grubs (*Posthodiplostomum* spp.) and yellow grubs (*Clinostomum* spp.), occur in wide-ranging fish populations. These digenetic trematodes usually cause minimal effects on growth, reproduction, and survival of fish (Post 1987) unless fish are heavily infected. Methods employed to keep pelicans (aquatic birds) away from aquaculture facilities and reduce snail populations (Hoffman 1999) are helpful in controlling digenetic trematodes.

Tapeworm, Acanthocephalons, and Nematodes Infection

Asian tapeworm is one of the most serious cestodes that affects fish. The body of a tapeworm is ribbon-shaped and divided into short segments. Asian tapeworm has an unusually wide range of hosts including various carp species, minnows, golden shiner, catfish, and many other aquarium fish. It can cause up to 90% mortality in grass carp and juvenile common carp. The adult tapeworms live in the intestines of many species of fish. Eggs of this tapeworm pass out of the fish with its feces and into water. After hatching, the larvae are eaten by crustaceans (copepods); fish are then infected when they eat the copepods.

Acanthocephalan comprises worms with an anterior proboscis covered with many hooks (Thorny-headed worm). These acanthocephalans are widely distributed in various fish species and almost all are endoparasites in the digestive tract of fish. Fish can serve as final hosts for those acanthocephalans, which become

sexually mature while in host fish. Fish can also serve as intermediate hosts for acanthocephalans, which spend their adulthood in marine mammals (Post 1987). Acanthocephalan epizootics are rare in cultured fish and these parasites are not usually regarded as economically important pathogens of fish.

There are many parasitic nematodes in fishes. These nematodes infect freshwater, marine, and brackish water fish species. Fish nematodes usually have a life cycle involving one or two intermediated hosts (Yanong 2002). Most nematodes infect fish as adults, but some occur as larval stages (Molnár et al. 2006). Adult nematodes usually occur in the intestine while larval stages can be found in almost any part of the fish, including the body cavity, internal organs, external muscle layers, and deeper layers of the skin or fins (Yanong 2002). Disease related to nematodes is surprisingly rare among fish. In fact, a fish may live a relatively normal life with hundreds of nematodes in various organs and body tissues (Post 1987). However, fish may show mortality when they are heavily infected, especially juveniles. Juvenile fish infected by nematodes are more likely to show signs of illness and also have reduced growth rates (Yanong 2002).

Cleaning and sterilizing ponds is an effective way of reducing the numbers of the intermediate hosts of some helminthes (Noga 1996; Yanong 2002). Control strategies for helminthes are generally aimed at disrupting the life cycle and eradicating the helminthe intermediate hosts (Noga 1996). Quarantine and restricting the movement of infected fish will prevent the spread of helminthes and reduce infection loads. Live foods, such as oligochaete worms (e.g., tubifex worms), may act as carriers, and fish should not be fed live feeds if possible (Noga 1996; Yanong 2002).

Crustacean Parasites

There are many crustacean parasites that can infect freshwater and marine fish. Crustacean parasites possess attachment organs that are deeply embedded in the host's tissue. Some species that move freely on the surface of the fish can rupture the protective skin, destroy the mucus cover, and open wounds for subsequent bacterial infections. Anchor worms (*Lernaea* spp.), fish louse (*Argulus* spp.), and copepod ectoparasite (*Ergasilus* spp.) are some commonly seen crustacean parasites in freshwater fish. A more severe crustacean parasite affecting marine cultured fish is sea lice, which has caused heavy losses in salmon culture farms in European countries. *Lepeophtheirus salmonis* and *Caligus elongatus* are the most important species affecting farmed fish (Lester and Harward 2006). Other species of parasitic copepods are also becoming a problem as finfish aquaculture expands worldwide and new species of fish are being cultured.

Fungal Diseases

Saprolegniasis

Saprolegniasis, commonly called "water mold," is a fungal disease of fish and fish eggs. Water molds are caused by aquatic fungi, primarily *Saprolegnia* spp., *Achlya* spp., and *Aphanomyces* spp. These fungi are common in fresh or brackish water and affect all species and ages of freshwater and estuarine fish. The likelihood of infection by fungi is increased when fish are injured physically or infected by parasites. Saprolegniasis also commonly occurs when the water temperature drops below 15°C, and often after a cold front rapidly reduces the water temperature (Noga 1996). Infected fish show a gray or whitish growth in and on the skin and/or fins. Eventually, these growths look like cotton. Once fungus is established on fish, it may grow and spread to healthy tissue and eventually kill its host. Epizootic ulcerative syndrome (EUS) is one of the most destructive diseases of fresh- and brackish-water farmed and wild fish in the Asia–Pacific region (an OIE reportable disease). The EUS is caused by the oomycete pathogen, *Aphanomyces invadans* (also called *A. piscicida*), a non-septate, broad, sparsely branching type of fungus (Vishwanath et al. 1998). The invading fungus causes significant necrotic changes in the skin and muscle tissue, produces granulomas, and ultimately results in the formation of dermal ulcers. If EUS outbreaks occur in small or closed water bodies, liming of water, improvement of water quality, and removal of infected fish are helpful in reducing fish mortality (OIE 2010h).

Branchiomycosis

Branchiomycosis or "Gill rot" is caused by the fungi *Branchiomyces sanguinis* and *Branchiomyces demigrans* in freshwater fish in Europe and Asia (Noga

1996). Infections are located primarily in the blood vessels (intravascular) of the gill, and are confined to the gill arches and the base of the primary lamellae. The pathogen destroys the branchiate membranes and gills (Post 1987). Diseased fish refuse food, congregate near the edge of rearing units, and rise to the surface of the water. The disease usually occurs in the summer and results in high mortality of fish (Post 1987). Prevention of all fungal infections can be accomplished by good management such as maintaining good water quality, removing dead fish, and preventing the accumulation of decomposing organic matter (Post 1987).

Conclusion

This chapter provided a brief overview of the fish immune system and of the emerging or re-emerging bacterial, viral, parasitic, and fungal diseases considered by the authors to negatively impact aquaculture. Maintaining good water quality, minimizing stress, and providing adequate nutrition are major factors impacting the immune system of fish, and ultimately the innate and acquired immune responses to pathogens. The knowledge presented in this chapter provides basic information to enable the understanding of specific examples that are presented throughout the remainder of this book.

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