3 Infectious Diseases of Coldwater Fish in Marine and Brackish Water

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Introduction

Salmonids are the primary fishes reared in cold seawater netpens. This component of the industry produces approximately 500,000 t year\(^{-1}\) on a worldwide basis. The principle species reared in netpens are Atlantic salmon (Salmo salar), coho salmon (Oncorhynchus kisutch), chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (Oncorhynchus mykiss). Additional species include minor production of Arctic char (Salvelinus alpinus), Atlantic cod (Gadus morhua), haddock (Melanogrammus aeglefinus), Atlantic halibut (Hippoglossus hippoglossus) and Atlantic wolffish (Anarhichas lupus). The purpose of this chapter is to review the most important infectious diseases affecting fish reared in cold seawater netpens.

The problems in controlling water flow make it difficult, if not impossible, to exclude pathogens in the water column from netpens. Therefore, marine pathogens are among the most important causes of diseases in seawater netpens. As salmonids are reared in fresh water before they are held in seawater, freshwater pathogens may be transferred with them to sea cages. Brown and Bruno (Chapter 4) deal with these freshwater diseases, and our emphasis is on infectious diseases that are contracted after transfer to sea cages.

Viral Diseases

Several viruses are important pathogens of salmonid fishes, particularly during their early development in fresh water (Wolf, 1988). Viral diseases of fishes have historically been of great concern to fish health managers because they can cause high mortality. In addition, the presence of certain viruses in a fish population causes economic hardships to fish farmers due to restrictions on transfer or sale of these fish. At least six viral diseases are of concern for pen-reared salmon: these include infectious haematopoietic necrosis (IHN), infectious pancreatic necrosis (IPN), salmon pancreas disease (SPD), infectious salmon anaemia (ISA), salmonid herpesvirus 2 infections and erythrocytic inclusion body syndrome (EIBS). The erythrocytic necrosis virus has the potential to infect salmon in seawater,
but has yet to be recognized as a serious problem. At least one form of plasmacytoid leukaemia is associated with a retrovirus, but this disease is described under the section on *Nucleospora salmonis*. Cardiac myopathy syndrome (CMS) has recently been associated with a nodavirus, and is thus covered in this section. IHN, IPN and EIBS are also important diseases in fresh water, and are dealt with in more depth in Chapter 4. However, the manifestations of IHN and IPN as they occur in seawater are described. Furthermore, viruses have caused disease in farmed Atlantic halibut and turbot larvae, resulting in considerable losses (Bloch *et al.*, 1991; Grotmol *et al.*, 1995; Wood *et al.*, 1996). However, to date, specific viral diseases of non-salmonids reared in coldwater sea cages have not been identified as serious problems. Vacuolating encephalopathy and retinopathy (VER) is a disease primarily of larval or juvenile marine fishes, but may be carried into cage-cultured flatfish. This nodavirus is dealt with in Chapter 9.

**Infectious haematopoietic necrosis in netpens**

The first confirmed report of IHN in pen-reared Atlantic salmon occurred in 1992 in British Columbia (Armstrong *et al.*, 1993; Traxler *et al.*, 1993). Since this initial finding, IHN has been reported at many netpen farm sites and has become a major disease concern on Atlantic salmon farms in the Quadra Island region of British Columbia. The existence or establishment of marine hosts or reservoirs of IHN virus (IHNV) that may serve as sources of the virus at sea cage sites, and the reports of virus in non-salmonids around netpens during an outbreak is of concern (Traxler and Richard, 1996). In addition, IHNV has been found in Pacific herring (*Clupea herringus pallasi*) collected well away from infected farms (Kent *et al.*, 1998).

Clinical signs of IHN in Atlantic salmon in netpens are often similar to those seen in infected salmonids in fresh water (see Chapter 4). However, because affected fish are much larger than their counterparts in fresh water, IHN in Atlantic salmon reared in seawater is usually chronic. Infected salmon also often exhibit frank haemorrhages in the visceral cavity. As with the freshwater form of the disease, IHN is best managed by avoidance. There is circumstantial evidence to suggest a marine reservoir is the primary source of the infection for outbreaks in seawater netpens. If this were the case, then avoidance of the infection in netpens would be very difficult. Marine-phase chinook salmon may harbour the virus for several months with no signs of the disease, and the virus has been found in healthy chinook reared at netpen farms that have experienced IHN outbreaks in Atlantic salmon (St-Hilaire *et al.*, 2001). Therefore, chinook salmon may act as a subclinical reservoir for the virus when they are reared with Atlantic salmon.

**Infectious pancreatic necrosis**

This is a freshwater viral disease (see Chapter 4) that causes mortality in sea cage culture. The infection is prevalent in pen-reared Atlantic salmon in Norway (Krogsrud *et al.*, 1989). For many years, the virus was extremely widespread in Norwegian sea sites (Melby *et al.*, 1991) without causing clinical disease. However, in recent years clinical IPN has impacted on sea-farmed Atlantic salmon culture and is considered as an emerging problem in Chapter 9. Most outbreaks occur from a few weeks up to a couple of months after transfer to seawater (Jarp *et al.*, 1994), but outbreaks up to 1 year after transfer to seawater may also occur (Smail *et al.*, 1992, 1995). IPNV may be the most important infectious disease of farmed fish in Norway, accounting for losses of approximately Nkr 400 million year⁻¹ (Christie, 1996). In Scotland, significant mortality has been associated with the infection, particularly in combination with SPD.

Clinical signs of IPN in post-smolt Atlantic salmon may be minimal, but some fish stop feeding and show nervous distress. The most significant losses may
sometimes be attributed to the long-term effects of reduced or completely ceased feeding. Fish show hyperaemia and petechial haemorrhage in the visceral fat and in the pyloric caeca.

An epizootiological study of IPN in post-smolts has shown that the risk of clinical disease was related to the mixing of smolts from several suppliers at the same sea site (Jarp et al., 1994). A way to avoid this would therefore be to buy smolts from as few producers as possible. Smolts with no history of IPN in fresh water, but with specific humoral immunity against IPNV prior to smoltification, were protected against clinical IPN for up to 4 months after transfer to seawater (Jarp et al., 1996).

Because IPNV can be vertically transmitted and infected fish can excrete virus for the rest of their life, the only effective control method is avoidance. The use of IPNV-free broodstock, rearing progeny in virus-free water and restricting the movement of fish are measures that can reduce the spread of IPNV. A multivalent vaccine, which includes *Escherichia coli*-expressed IPNV proteins, protects pre-smolt Atlantic salmon against natural exposure to IPN (Christie, 1996). This vaccine is now licensed in Norway, and results from the 1996 season were promising, as mortalities due to IPN were reduced considerably.

**Salmon pancreas disease**

SPD of pen-reared Atlantic salmon is an important disease in Scotland, Ireland and Norway (Munro et al., 1984; Ferguson et al., 1986; McVicar, 1987; Menzies et al., 1996). Histological changes consistent with SPD have been observed in pen-reared Atlantic salmon in Washington State (Kent and Elston, 1987), and rarely in British Columbia.

A toga-like virus has been isolated from fish with SPD (Nelson et al., 1995), and McLoughlin et al. (1996) experimentally reproduced the disease with the virus. Therefore, the evidence is essentially conclusive that the cause of pancreas disease is this virus, referred to as salmon pancreas disease virus (SPDV). SPD is contracted after smolts are transferred to seawater. Although not specifically associated with fresh water (McVicar, 1987), a similar toga-like virus has been isolated from rainbow trout (Boucher et al., 1994).

Fish usually exhibit clinical signs of SPD about 6–12 weeks after introduction to netpens, but fish that have been in pens for as long as 2 years may be affected (McVicar, 1987). Mortality associated with the disease is low. Nevertheless, surviving fish often show poor growth and are more susceptible to other diseases (McVicar and Munro, 1987).

**Clinical and gross pathological changes.** Affected fish are anorexic, dark in colour, lethargic, and usually at the sides of cages and near the surface. Fish with SPD are usually emaciated (Fig. 3.1). Internal signs include haemorrhage in the pancreatic tissue and fat between the pyloric caeca, or the tissue between the pyloric caeca may be severely atrophied.

**Diagnosis.** Haemorrhage in tissues associated with the pyloric caeca in emaciated Atlantic salmon smolts, along with the absence of other infectious agents (e.g. IHN or IPN viruses, *Aeromonas salmonicida* or *Vibrio* spp.), is indicative of SPD. Confirmation of the disease is based on histological changes or by isolation of SPDV from
affected fish. Isolation of the virus can be achieved by co-cultivation of kidney tissues on CHSE-214 cells at 15°C, and cultures are blind passed after 28 days (Nelson et al., 1995) where the cytopathic effect (CPE) may be observed after about 10 days.

Control and treatment. No treatment is known for pancreas disease. Reports from Scotland indicate that reducing stressors (e.g. transport and handling) during the acute phase of the disease may enhance recovery. In addition, some farmers have reported that keeping fish on a smaller pellet size reduces anorexia and the overall mortality associated with SPD. Recovered fish exhibit strong protection against reinfection (Houghton, 1994), indicating that a vaccine could be produced against the virus.

Infectious salmon anaemia

ISA was first observed in southwest Norway in 1984. During the following 10 years, the disease spread to most fish-farming areas along the coast, but only seawater farms and freshwater farms that use some seawater have experienced natural outbreaks (Thorud and Djupvik, 1988). However, ISA can be experimentally transmitted to fish in fresh water and therefore might pose a threat to these stocks. A virus typical of orthomyxoviruses has been shown to be the cause of ISA (Dannevig et al., 1995). The virus is shed from infected carriers before they develop clinical signs of the disease through skin mucus, urine and faeces. Early colonization of the causative virus occurs in the pillar cells of the gills and the endocardium, indicating that the gills are the most likely port of entry (Totland et al., 1996). Natural outbreaks in fish farms are restricted to Atlantic salmon, but other salmonids may harbour the virus and may act as reservoirs (Nylund and Jakobsen, 1995). ISA virus has been considered a Norwegian problem, but recently has been found in Scotland, Canada and the USA. ISA as an emerging disease is reviewed in Chapter 9.

Clinical signs and gross pathology. Most clinical cases occur during rapid temperature increases in the spring, but outbreaks may also occur in the late autumn. Fish are anorexic, lethargic, and tend to stay at the bottom or rest near the edges of cages. Mortality may vary from 15 to 100%. Outbreaks are usually chronic (lasting several months), but are more acute if water temperatures are above 10–12°C. Affected fish show a distended abdomen, exophthalmos, oedema and haemorrhage of the skin. Fish are anaemic, and the gills and heart may be extremely pale. The visceral organs are congested and haemorrhage occurs in the perivisceral fat. In some cases the liver may appear extremely congested and almost black in colour (Evensen et al., 1991b).

Diagnosis. A diagnosis is based upon characteristic gross pathology and light microscopy, anaemia and absence of pathogenic bacteria. Supporting diagnosis of the virus is achieved by culture on the salmon head kidney cell line (SHK-1) (Dannevig et al., 1995), a polymerase chain reaction (PCR) test (Mjaaland et al., 1997) or an enzyme-linked immunosorbent assay (ELISA) test.

Control and treatment. To control ISA, Norway has implemented strategies, including mandatory health control in smolt farms, disinfection of processing water from slaughtering facilities, separation of different year classes, isolation of infected sites from unaffected sites and fallowing of sites after infected stocks are removed. In Canada, commercially developed vaccines to control ISA virus have been deployed. Recently, Jones et al. (1999) demonstrated a reduction in vaccinate mortality ($P < 0.01$) using viral antigen emulsified in mineral oil in Atlantic salmon parr. Although trials and licensing may be incomplete, under Canadian Regulatory Guidelines the release of some vaccine for use in the industry is possible. Under current UK and Norwegian legislation there is a restriction on the use of vaccines for ISA.
Salmonid herpesvirus 2 infections

Several members of the family Herpesviridae are recognized as fish pathogens (Wolf, 1988). In Japan, a herpesvirus type 2 (SH-2) infection has caused up to 30% mortality in pen-reared coho salmon (Kumagai et al., 1994). The disease affects fish from less than 100 g to 1 kg, and epizootics usually last from 30 to 80 days.

Certain strains of salmon herpesvirus 2 (e.g. Oncorhynchus masou virus (OMV) and yamame tumour virus (YTV)), cause liver damage in young fish in fresh water. Fish that survive the infection may later develop epithelial tumours in sea cages (Kimura et al., 1981a,b; Sano et al., 1983; Kimura and Yoshimizu, 1991; Yoshimizu et al., 1995).

Clinical signs and gross pathology. Affected fish are dark in colour, and often have skin ulcers and erosion of the fins. The liver exhibits focal pale areas, and the intestinal tract shows erythema. Surface tumours appear as whitish papillomatous masses around the mouth, eyes, fins or gills. These tumours may also occur in the visceral cavity (Kimura et al., 1981a,b).

Diagnosis. Focal necrosis of the liver in coho salmon reared in Japan is presumptive diagnosis for the disease. Diagnosis is achieved by isolation of the virus from affected livers on CHSE-214 or RTG-2 cell lines. Syncytia formation occurs in the latter (Sano et al., 1983).

Control and treatment. As with other viral diseases, the best method to control the infection is avoidance. The occurrence of the disease is associated with previous infections at freshwater hatcheries, although pen-to-pen transmission in seawater is negligible (Kumagai et al., 1997). Rainbow trout may have subclinical infections and serve as reservoir hosts. Kumagai et al. (1997) recommended the following to control the infection: (i) do not rear other salmonids with coho salmon; (ii) disinfect facilities after out-planting stocks; (iii) avoid smolts from contaminated hatcheries; and (iv) examine fish for virus shortly after introduction to seawater.

Kimura et al. (1983) reported that daily immersion of chum salmon in the antiviral compound acyclovir suppressed the growth of OMV-associated tumours. They also found that oral treatment with another anti-viral drug, IUdR, decreased mortality due to the infection. Surface tumours are often removed manually at harvest from fish before they are sent to market.

Cardiomyopathy syndrome in cage culture

This chronic, progressive disease has been observed since 1984 in farmed Atlantic salmon in Norway and a few cases have been diagnosed in the Faroe Islands (Bruno and Poppe, 1996). The cause(s) has not been determined, but recently Grotmol et al. (1997) reported a nodavirus-like agent in affected heart tissue. Although transmission experiments have been negative, viral particles have been observed using electron microscopy and the lesions and epizootiology are consistent with a viral aetiology. The most serious losses typically occur in the autumn, 12–18 months after transfer to seawater.

Clinical signs and gross pathology. Fish in the terminal stages of the disease are often in good body condition, showing no or few clinical signs before death. They may go off their feed and swim sluggishly around for a few days before they die. Such fish frequently develop skin haemorrhage and oedema, exophthalmia and ascites. Typical findings at necropsy are fibrinous peritonitis, ascitic fluid and blood clots surrounding the heart. The atrium and sinus venosus are usually dilated and may contain blood clots. Sometimes clotted blood may also be found on the dorsocranial surface of the liver (Ferguson et al., 1990).

Diagnosis. The diagnosis is based on the characteristic gross and pathognomonic histopathological lesions. Characteristic
lesions are found in the spongious myocardium of the atrium and ventricle (Amin and Trasti, 1988; Ferguson et al., 1990). These lesions are comprised of muscular degeneration, proliferation of the endocardial cells with macrophage infiltration and lymphocytes subendocardially and in the degenerated muscle. Blood clots are frequently found in the atrium. Focal necrosis in the hepatic parenchyma may also occur. Diseased fish may also be diagnosed by means of ultrasound imaging (Sande and Poppe, 1995). CMS bears little resemblance to other diseases, but haemopericardium may be observed in fish dying from other diseases.

Control and treatment. There are differences in susceptibility to CMS between fish families, and selective breeding may be a possibility for controlling this infection in the future. Fallowing of sites for a year or two before new fish are introduced into problem areas has reduced the problem considerably.

Bacterial Diseases

A number of bacterial diseases cause serious and recurring losses in pen-reared salmon and other coldwater fishes. Some important bacterial diseases, such as bacterial kidney disease (BKD), furunculosis and yersiniosis, primarily occur in fresh water and are dealt with in Chapter 4. Important bacterial infections on sea pens include: typical vibriosis, caused by *Vibrio anguillarum* and *Vibrio ordalii*; coldwater vibriosis or Hitra disease caused by *Vibrio salmonicida*; ‘winters ulcers’ caused by *Moritella viscosa* (*Vibrio viscosus*); myxobacteriosis, caused by *Cytophaga-Flexibacter* spp.; and salmonid rickettsial septicemia or piscirickettsiosis, caused by *Piscirickettsia salmonis*. All salmon species reared in netpens are susceptible to these bacterial diseases, but some diseases are more problematic in certain species and particular areas. For example, chinook, coho and sockeye salmon appear to be more susceptible to BKD than Atlantic salmon, whereas furunculosis and myxobacteriosis represent an increased problem for Atlantic salmon compared with Pacific salmon species. Almost all non-salmonid marine fishes are susceptible to vibriosis and furunculosis. Typical furunculosis is caused by *A. salmonicida* subsp. *salmonicida*. The atypical strain of *A. salmonicida* is usually the aetiological agent of furunculosis in non-salmonid marine fishes.

Vibriosis

Vibriosis is a systemic disease that affects many marine fishes and invertebrates (Anderson and Conroy, 1970; Colwell and Grimes, 1984; Egidius, 1987). Frerichs and Roberts (1989) considered vibriosis to be the most significant disease in wild and cultured marine and brackish water fishes. *V. anguillarum* accounts for most of the outbreaks of vibriosis in farmed salmon worldwide, and also causes disease in Atlantic cod. *V. ordalii* occasionally causes disease in salmonids reared in the Pacific Northwest and in New Zealand (Evlyn, 1971; Harrell et al., 1976; Novotny, 1978; Schieve et al., 1981; Wards et al., 1991). Diseases caused by other *Vibrio* spp. include coldwater vibriosis or Hitra disease, caused by *V. salmonicida*, and winter ulcer disease caused by *M. viscosa*. The latter is considered as an emerging problem and is discussed in Chapter 9.

*V. anguillarum* strains show heterogeneity in both phenotypic (Tajima et al., 1985) and serotypic (Kitao et al., 1984; Tajima et al., 1985; Sorensen and Larsen, 1986) characteristics. The strains of *V. anguillarum* that cause vibriosis in pen-reared salmon worldwide represent only one or two serotypes (based on the ‘O’ antigens present), which simplifies the formulation of anti-vibriosis vaccines for controlling the disease. Vibriosis caused by *V. anguillarum* usually occurs between 15 and 21°C, and most outbreaks occur in smolts during their first summer in seawater. Vibriosis in the Pacific Northwest is mainly a problem of Pacific salmon (e.g. chinook...
and coho), although Atlantic salmon are also susceptible to the infection. Interestingly, all cases of vibriosis due to \textit{V. ordalii} in pen-reared salmon reported to date have involved Pacific salmon in Japan and the Pacific coast of the USA (Schiewe \textit{et al}., 1981).

Coldwater vibriosis is a bacterial septicaemia caused by the psychrophilic bacterium \textit{V. salmonicida}. Since its first occurrence in farmed Atlantic salmon in northern Norway in 1977 (Egidius \textit{et al}., 1981), coldwater vibriosis has been diagnosed in most fish-farming areas, as well as in salmon-producing countries surrounding the North Atlantic (Bruno \textit{et al}., 1986), including eastern Canada and the USA (O’Halloran and Henry, 1993). The condition is also known as ‘Hitra disease’ after severe outbreaks occurred in the Hitra region of Norway in the early 1980s. The disease is usually most severe at low temperatures during the winter months, but may occur throughout the year. Environmental stressors and poor nutrition may predispose fish to coldwater vibriosis. Although the bacterium may cause disease in other fish, such as Atlantic cod (Jutorgensen \textit{et al}., 1989), serious losses occur mainly in Atlantic salmon. Schroder \textit{et al}. (1992) showed in experimental studies that cod are more resistant to the bacterium than salmon.

Clinical signs and gross pathology. In small fish, mortality caused by vibriosis may be high and rapid, and these fish may exhibit no gross pathological changes other than darkening and lethargy. Typical of bacterial septicaemias, fish with vibriosis may exhibit erythema at the base of the fins, petechiae in the skin and haemorrhage on the body surface. Fish may also exhibit bilateral exophthalmia and frayed fins. Haemorrhagic abscesses in the muscle are often seen in Atlantic salmon with vibriosis in Europe. Congestion and petechiae are usually evident in visceral organs, particularly in the gut and liver. Large multiple coalescing haematomas in the liver (peliosis hepatitis) are often seen in vibriosis caused by \textit{V. anguillarum}. Affected fish also exhibit pallor of the gills (due to anaemia) and enlargement of the spleen and kidney.

Clinical signs of coldwater vibriosis may be non-specific, but usually include lethargy and cessation of feeding. Affected fish turn dark, exhibit exophthalmons, a swollen vent and pinpoint haemorrhage along the belly and at the base of the pectoral, pelvic and anal fins. The gills are usually pale. Internally, ascites and petechial haemorrhage in the perivisceral fat, pyloric caeca, peritoneal surfaces, liver and swimbladder are typical findings (Figs 3.2 and 3.3). The latter may be filled with a blood-tinged fluid.
and the liver typically has a yellowish discoloration. In chronic cases, skin ulceration, fin rot, a pseudomembranous peritonitis and epicarditis may also be found. The spleen usually has a colour slightly lighter than normal. In cod, the pathological changes are rather diffuse and non-specific, but keratitis is frequently seen.

Diagnosis. For typical vibriosis, presumptive diagnosis is by macroscopic examination if the characteristic haematomas in the liver are present. The causative Gram-negative bacilli are usually easy to detect in Gram-stained kidney smears. The highly motile bacteria are also detectable in fresh preparations of blood or in wet mounts of the kidney or spleen, or in lesions. The other gross and clinical changes are not specific to vibriosis and are associated with a number of bacterial or viral systemic diseases.

A diagnosis is based on culture and identification of the causative organism from the kidney of infected fish. Both *V. anguillarum* and *V. ordalii* are easily cultured on tryptic soy agar with 1.5% NaCl or on marine agar (Difco) at room temperature. Bacterial colonies are round, raised and off-white in colour. *V. ordalii* and *V. salmonicida* grow more slowly than *V. anguillarum* and form smaller colonies. Optimum growth temperature for *V. salmonicida* is from 12 to 16°C and growth will occur between 0 and 22°C. In contrast, *V. anguillarum* and *V. ordalii* will grow at about 25°C. The bacteria can be distinguished using biochemical tests (Schiwe et al., 1981; Holm et al., 1985; Tajima et al., 1985; Scalati and Kusuda, 1986). API-20E test strips (Analytab Co., Plainview, New York, USA) can be used for rapid identification of marine vibrios from fish (Kent, 1982; Grisez et al., 1991). These bacteria can also be identified serologically using slide agglutination tests.

With *V. salmonicida*, microscopical demonstration of the bacterium in Giemsa-stained smears or paraffin sections, or by immunohistochemistry (Evensen et al., 1991a) is useful for locating the organism in tissues. However, immunofluorescence tests applied directly to tissues infected with *V. anguillarum* are not useful for rapid diagnosis of this bacterium. Apparently salmonid tissues contain substances that block receptor sites on the vibrios that would normally react with the vibrio-specific antibodies in the diagnostic antisera (T.P.T. Evelyn, Pacific Biological Station, Nanaimo, British Columbia, personal communication).

Control and treatment. Vibriosis is best controlled by prevention, and commercial vaccines are available. With salmonids, vaccination is best carried out on fish that have attained immunocompetent size (at least 5–10 g) and before they are introduced...
to netpens. The vaccines are conveniently administered by immersion methods, and if applied properly, they afford excellent protection (Evelyn, 1984, 1988). Some farms revaccinate fish shortly after introduction to netpens, and results with revaccination in seawater have been promising. Revaccination should, however, be conducted with caution because the handling of fish shortly after their introduction to seawater is very stressful. The most recent trend is to administer the vaccine by intraperitoneal injection, usually in combination with other vaccines, e.g. with furunculosis vaccines, which are most effective when injected. Vaccines for controlling vibriosis in cod are also promising for protecting against *V. anguillarum* (Espelid et al., 1991; Groman et al., 1992) and *V. salmonicida* (Schroder et al., 1992).

Antibacterial drugs (e.g. oxytetracycline, potentiated sulphonamides, quinolones and florfenicol) incorporated in feed are available for treating vibriosis. Treatment is usually efficacious if the infection is recognized early when fish are still actively feeding, and if care is taken to select a drug to which the pathogen is sensitive. However, in some countries not all of the drugs have been approved for use in fish intended for human consumption. Thus, control of vibriosis should be conducted primarily through a vaccination programme.

*V. salmonicida* is not considered to be a highly pathogenic bacterium and significant exposure is required to infect fish. As with other diseases, optimization of the environment and reduction of stressors, particularly during the winter months, are important measures to avoid outbreaks. Multivalent vaccines protecting against furunculosis, vibriosis and coldwater vibriosis give excellent protection provided the vaccination programmes are carried out in a proper manner (e.g. fish are vaccinated at an appropriate size and given adequate time to develop immunity before exposure). Nevertheless, outbreaks do occur in properly vaccinated fish, particularly in northern Norway. Although the bacterium occurs commonly in the water and sediments close to cages, its numbers escalate during outbreaks and it is therefore important to isolate diseased fish from healthy fish (Enger et al., 1989) and to remove dead and moribund fish from the cages.

**Marine myxobacteriosis**

*Cytophaga* and *Flexibacter* spp. (commonly referred to as ‘myxobacteria’ or ‘gliding bacteria’) are important bacterial pathogens of cultured fishes and usually cause external lesions in freshwater and marine species (Anderson and Conroy, 1969; Pacha and Ordal, 1970). In marine aquaculture, infections by *Flexibacter maritimus* have been observed in Japanese flounder (*Paralichthys olivaceous*) and seabreams (family Sparidae) in Japan (Masumura and Wakabayashi, 1977; Hikida et al., 1979; Wakabayashi et al., 1984, 1986; Baxa et al., 1986, 1987) and Europe (Bernardet et al., 1990). In Tasmania, Handlinger et al. (1997) identified *F. maritimus* associated with skin and gill lesions in pen-reared Atlantic salmon and rainbow trout. Myxobacteria that have not been precisely identified have been associated with skin lesions in seawater-reared salmonids for many years (Borg, 1960; Rucker et al., 1963; Anderson and Conroy, 1969; Wood, 1974; Sawyer, 1976). These bacteria have at times been identified as *Sporocytophaga* spp. However, the presence of microcysts (an important diagnostic feature of this genus) has not been clearly demonstrated in these isolates.

Proper taxonomic identifications have not been conducted on many *Cytophaga* and *Flexibacter* spp. that have been associated with disease in marine fishes. However, to date, marine *Cytophaga* and *Flexibacter* species have not been transferred to *Flavobacterium* (Bernardet et al., 1996), as was proposed for certain freshwater species. Fish health workers and aquaculturists usually refer to these bacteria as ‘myxobacteria’. This is technically incorrect because these bacteria belong to the order Cytophagales, and not to the order Myxobacteria. It would, therefore, be more appropriate to refer to the marine forms using collective terms such
as ‘cytophaga–flexibacter-like bacteria’ or ‘gliding bacteria’. However, to remain consistent with the common terminology and to avoid confusion, reference is made to these bacteria as myxobacteria in this review.

Two types of myxobacterial infections have been associated with high mortality in pen-reared Atlantic salmon in the Pacific Northwest; one type causes large body ulcers, and the other causes lesions primarily in the mouth. Myxobacteria infections are also seen in pen-reared Pacific salmon, but are not usually associated with severe epizootics. In Pacific salmon, myxobacteria are usually associated with frayed fins and erosion of the tail.

A *Cytophaga* sp. causes large skin lesions in Atlantic salmon smolts (Fig. 3.4) shortly after transfer to seawater (Kent *et al*., 1988). Very similar skin lesions associated with *F. maritimus* infections were observed in pen-reared Atlantic salmon and rainbow trout in Tasmania (Handlinger *et al*., 1997). Lesions and associated mortalities usually peak at about 1–3 weeks after introduction, and based on our observations, the infection subsides after about 3–4 weeks. There appears to be a seasonality, and fish introduced later in the spring and summer usually exhibit fewer body lesions. Fish with large lesions apparently die from osmotic imbalance (Kent *et al*., 1988a).

A particularly lethal form of myxobacteriosis occurs in the mouth of pen-reared Atlantic salmon, referred to as ‘mouth rot’ by fish farmers. Infections of the mouth and snout by myxobacteria are observed in post-smolt Atlantic salmon during their first summer in seawater. The condition has occurred at many netpen sites in the Pacific Northwest, and is often associated with high mortalities (Hicks, 1989; Frelier *et al*., 1994). Pen-reared Arctic char have also been afflicted with the infection. The infection appears to begin around the teeth. It has been suggested that the infection is initiated in periodontal tissue that has been abraded by feeding on spiny crustaceans such as crab larvae and *Caprella* spp. amphipods. Other potential predisposing factors suggested by farmers that may lead to the infection are: (i) feeding on hard pellets; (ii) fish biting net surfaces; and (iii) stress-induced lesions in

![Fig. 3.4. Atlantic salmon with Cytophaga-associated skin lesions. Note severe lesion with exposure of underlying muscle.](image-url)
the mouth. Fish farmers in British Columbia, Canada report that the condition is particularly troublesome in waters with high salinity. Based on preliminary culture analysis, the myxobacterium from mouth lesions appears to be different from the myxobacterium causing skin lesions. A similar myxobacterial stomatitis has been observed in wild Atlantic cod in the North Sea (Hilger et al., 1991).

Clinical signs and gross pathology. Skin lesions are large, white patches on the caudal peduncle and the posterior region of the flanks when the dermis is intact. Fish with more severe lesions have areas of the skin completely destroyed and the underlying muscle exposed (Fig. 3.4). Fish with mouth myxobacteriosis are often lethargic, emaciated and anorexic, and some affected fish may exhibit flashing or head shaking. Early in the infection, examination of the mouth reveals focal, yellow bacterial mats around the palate and teeth, including the vomer. The lesions may be single, but the opposing surface is often affected (Frelier et al., 1994). As the disease progresses, affected fish show multiple ulcers in the mouth with large bacterial mats overlying the lesions. The lesions may extend to the branchial arches and proximal oesophagus, and the lower and upper jaw may be completely eroded in severe cases. Severely affected fish do not feed and hence the stomach is devoid of food.

Diagnosis. Diagnosis of myxobacterial infections can usually be accomplished by observing large numbers of filamentous bacteria in wet-mount preparations from the lesions (Fig. 3.5). Isolation of the bacteria can be accomplished by culture on either Cytophaga medium made with 50% sterile seawater or marine agar (Difco). Isolation of myxobacteria in pure culture may be difficult from skin lesions due to contamination with other faster-growing bacteria (e.g. vibrios). However, the lesions usually contain large numbers of the myxobacteria and serial dilutions of affected tissue in sterile 50% seawater facilitates the isolation of the myxobacteria in pure culture. Reichenbach (1988) has described the general characteristics of Cytophaga and Flexibacter spp.

Treatment and control. External treatments with antibiotics are often used to control myxobacterial infections in fresh water, but such treatments are not usually practical in seawater netpens. These bacterial infections are often initiated in the skin where there are abrasions. Physical trauma during transport of smolts may allow the bacteria to establish an infection. According to fish farmers, improved transport techniques and careful handling of fish greatly reduces the prevalence of the disease. There are unconfirmed reports that treating fish with oxytetracycline can control mouth rot.

Fig. 3.5. Cytophaga bacteria in wet mount of skin lesions showing ‘myxobacteria’. Bar, 5 µm.
Salmonid rickettsial septicaemia

_Piscirickettsia salmonis_ causes a severe septicaemia in pen-reared salmon, particularly in Chile (Branson and Nieto Diaz-Munoz, 1991; Cvitanich _et al._, 1991; García _et al._, 1991; Fryer _et al._, 1992; Almendras and Carmeu Fuentealba, 1997). In British Columbia, the disease was first observed in seawater-reared pink salmon (_Oncorhynchus gorbuscha_), held for experimental purposes, at the Pacific Biological Station on Vancouver Island in 1970. More recently, infections of Atlantic salmon with rickettsia-like organisms have been reported from Norway (Olsen _et al._, 1997), Ireland (Rodger and Drinan, 1993) and Scotland (Grant _et al._, 1996). A PCR method developed by Mauel _et al._ (1996) for detecting and identifying the pathogen showed that the isolates from Norway, Ireland, Canada and Chile were all _P. salmonis_; it was clear that at least two variants of the pathogen occurred in Chile. House _et al._ (1998) showed that the strain from Chile was more pathogenic than common strains from British Columbia and Norway.

In British Columbia, the infection is usually coincidental with other infectious diseases (e.g. bacterial kidney disease) in the population, but may occasionally cause epizootics in which it is the primary cause of mortality. In contrast, piscirickettsiosis is the most important infectious disease of pen-reared salmonids in Chile, where it caused losses of around US$48 million in 1995. In Chile, the disease was first recognized as a serious problem in coho salmon, but it now is also common in both rainbow trout and Atlantic salmon. Several outbreaks of the disease may occur in the same population of fish during their seawater grow-out period, particularly with coho salmon.

Information to date on the epizootiology of the organism suggests that it is normally acquired in seawater from a marine source. However, a marine reservoir has yet to be identified, although certain salmon ectoparasites may be involved in the transmission, perhaps serving as vectors (García _et al._, 1994). Furthermore, Cvitanich _et al._ (1991) found evidence of the organism in crustaceans and molluscs around netpens, based on histology and serology. Nevertheless, _P. salmonis_ survives well in seawater (Almendras, 1996) and is easily transmitted directly from fish to fish.

The disease may also occur in brackish water, and the infection has recently been reported in rainbow trout and coho salmon held in fresh water (Bravo, 1994; Cvitanich _et al._, 1995; Gaggero _et al._, 1995). However, _P. salmonis_ does not survive in fresh water away from the host (Lannan and Fryer, 1994). Bustos _et al._ (1994) conducted field trials that suggested that vertical transmission may occur naturally, and Larena _et al._ (1996) detected the infection in 10% of fertilized ova from infected fish. This may explain its occurrence in fresh water. However, the poor survivability of the organism in fresh water may explain the rarity of the infection before fish are introduced to seawater.

Clinical signs and gross pathology. Clinical and gross pathological changes associated with _P. salmonis_ infections have been outlined by Cvitanich _et al._ (1991), Branson and Nieto Diaz-Munoz (1991) and Brocklebank _et al._ (1992). Affected fish are lethargic, anorexic, exhibit pallor of the gills due to anaemia, are dark in colour and may swim near the surface. There are marked differences in clinical signs between salmonid species. For example, infected rainbow trout often do not accumulate near the surface before they die. In Atlantic and coho salmon the nervous system is often affected, with flashing and side swimming being common in the former. Ulceration often occurs on the skin with coho salmon and rainbow trout, whereas this is rare with Atlantic salmon in Chile. However, in Norway, Atlantic salmon with the disease occasionally show skin lesions, e.g. raised nodules or white spots (Olsen _et al._, 1997).

The liver of affected fish may have large, whitish or yellow, multifocal, crater-like lesions or nodules (Fig. 3.6). These lesions often rupture, resulting in shallow crater-like cavities in the liver. Fish may have ascites, an enlarged spleen and a grey, enlarged kidney. The spleen is extremely enlarged in infected pink salmon. Pallor and
petechiae are observed in the visceral organs and muscle, and a whitish pseudomembrane may cover the heart.

**Diagnosis.** Presumptive diagnosis can be achieved by observing the distinctive crater-like lesions and nodules in the liver, but they may not occur in many infected fish. Definitive diagnosis can be achieved by observing the organism within phagocytic cells in liver or kidney imprints stained with Giemsa, Gram or methylene blue stains (Fig. 3.7A), or in macrophages in tissue sections (Fig. 3.7B) along with the distinctive histological changes described above. Acridine orange-stained tissue smears are also useful for demonstrating the organism (Lannan and Fryer, 1991). Further confirmation includes the isolation of the organism in culture using CHSE-214 cells (Fryer et al., 1990; Cvitanich et al., 1991), an indirect fluorescent-antibody test (Lannan et al., 1991) or using specific primers with PCR (Mauel et al., 1996). A commercial ELISA test for *P. salmonis* was developed by Microtek Ltd-Bayer (Sidney, British Columbia) and used extensively by Chilean farmers in brood stock segregation programmes.

**Control and treatment.** Various antibiotics, such as oxolinic acid, flumequine and oxytetracycline, have been used to treat the infection, often with limited success. In extreme cases some Chilean farmers have

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**Fig. 3.6.** *Piscirickettsiosis in salmon. Multiple, white, crater-like lesions in the liver (courtesy of P. Bustos).*

**Fig. 3.7.** *Piscirickettsia salmonis.* (A) Gram-stained kidney imprint. (B) Organisms in macrophages from kidney section, stained with H&E. Bar, 10 µm.
had success with employing injectable treatments with fluoroquinolones. The microorganism is intracellular, and this probably contributes to the difficulties of treating the disease with antibiotics. Although the role of vertical transmission is unknown, the techniques used for preventing vertical transmission of bacterial kidney disease (e.g. brood stock screening) are being employed in Chile with some success.

Protozoa and Myxozoa

Protozoans and myxosporeans (phylum Myxozoa) are some of the most important pathogens of cage-reared fishes. For example, the amoeba Paramoeba pemaquidensis is an important gill pathogen in cage-reared salmon in Australia, and the flagellate Ichthyobodo (= Costia) spp. (see Chapter 4) and trichodinid ciliates infect gill surfaces of salmon and some marine fishes. Systemic infections by Cryptobia salmositica (see Chapter 9) and a diplomonad flagellate similar to Hexamita salmonis have caused disease in chinook salmon in British Columbia. Another diplomonad (Spironucleus barkhanus) has caused extra-intestinal infections in Atlantic salmon in Norway. Four myxosporeans (Parvicapsula sp., M. aeglefini, Kudoa thyrsites and Chloromyxum truttae) and three microsporidians (Loma salmonae, N. salmonis and Microsporidium cerebralis) infect internal organs or gills. Some protozoan infections are contracted in fresh water (e.g. Cryptobia, Ichthyobodo) and are dealt with in Chapter 4. In the present chapter, the most important marine protozoan and myxosporean parasites affecting coldwater marine net pen culture, i.e. Paramoeba sp., K. thyrsites, extraintestinal diplomonads, L. salmonae and N. salmonis, are discussed.

Paramoeba pemaquidensis gill infections

Paramoeba sp. (Sarcomastigophora: Paramoebidae) has caused devastating losses in pen-reared rainbow trout and Atlantic salmon in Tasmania (Roubal et al., 1989; Munday et al., 1993) and Europe (Rodger and McArdle, 1996). A similar (if not identical) amoeba (identified as P. pemaquidensis) was associated with severe gill disease in coho salmon reared in netpens in Washington State and land-based seawater tanks in California (Kent et al., 1988b). This species is an opportunistic pathogen that is normally free-living in seawater. Intensity and prevalence of the amoeba on fish gills varied from year to year, with infections being most prevalent in the late summer and autumn. The exact environmental conditions or health status of the fish that allow the organism to proliferate on fish gills are unknown. Presumably fish already compromised by other diseases are more susceptible to the infection, and in Washington State many of the infected fish had pre-existing diseases or smoltification problems (Kent et al., 1988b).

Clinical signs and gross pathology. Heavily infected fish are lethargic, accumulate at the surface and have flared opercula. Excessive mucus is often observed on heavily infected gills. Focal, whitish patches may be observed on heavily infected fish.

Diagnosis. Paramoebiasis of salmon is diagnosed by the detection of large numbers of the amoebae on the gills. The organisms are best identified in fresh wet-mount preparations of the gills. Floating and transitional forms of the amoeba on the gills are 20–30 µm in diameter and have several digitiform pseudopodia. In wet mounts, amoebae will attach to the slide after about an hour, resulting in a locomotive form measuring about 20 × 25 µm. Paramoeba spp. possess a unique organelle, called a parasome or Nebenkörper, which is adjacent to the nucleus (Fig. 3.8). The parasome can be observed in wet mounts of locomotive forms, and is readily visible with Feulgen DNA stains. Amoebae can also be identified on gill surfaces in histological preparations, but many detach during processing. The amoebae can also be identified with specific
polyclonal antibodies in tissue sections or imprints (Howard and Carson, 1993a).

Treatment and control. Most compounds typically used as external treatments (e.g. formalin, chelated copper, diquat, malachite green and chloramine T) are not effective against the organism (Munday et al., 1993), but the amoeba is readily eradicated from fish gills with freshwater bath treatments. Kent et al. (1988b) confirmed that the parasite survives poorly in low salinity water. Cameron (1993) reported that reducing seawater concentrations to 4 ppt was needed for effective treatment. Reducing the salinity has been effective for eradicating infections in fish held in land-based tanks, but this treatment is usually difficult to apply and impractical in netpens. Hydrogen peroxide bath treatments at concentrations between 200 and 400 ppm are moderately effective at controlling the infection (Cameron, 1993). Howard and Carson (1993b) reported that 100 ppm hydrogen peroxide for 2 h killed all the amoeba. However, Cameron (1994) found that hydrogen peroxide did control the infection in field situations, even when used at 300 ppm. Hydrogen peroxide treatments should be applied with caution because of potential toxic effects to the fish, particularly at higher temperatures (Cameron, 1993; Johnson et al., 1993; Bruno and Raynard, 1994).

Diplomonad flagellates

Extra-intestinal infections by diplomonad flagellates have cause disease in caged-reared salmon in Norway and British Columbia. One outbreak of a severe systemic infection by a diplomonad flagellate (family Hexamitidae) resembling H. salmonis caused close to 50% mortality in chinook at one netpen site in the Sechelt area, British Columbia (Kent et al., 1992). The fish were introduced to seawater in the spring of 1990 and showed high mortality starting in September 1991. Interestingly, about the same time, extra-intestinal infections by a similar parasite were reported in post-smolt to adult Atlantic salmon reared at netpen farms in northern Norway (Mo et al., 1990; Poppe et al., 1992).

Whereas gut infections in fish by diplomonads are common (Woo and Poynton, 1995), systemic infections by diplomonad parasites in fish are rare. Only one other report of such infections is known, and Ferguson and Moccia (1980) reported a similar disease in Siamese fighting fish (Beta splendens). Although the flagellates

![Fig. 3.8. Paramoeba pemaquidensis from coho salmon gill. The parasome (arrowhead) is adjacent to the nucleus. Bar, 10 µm.](image)
observed in pen-reared salmon are morphologically indistinguishable from the relatively non-pathogenic *H. salmonis* that infects the intestinal tract of salmonids in fresh water, they probably represent new, highly invasive strains or species. Sterud *et al.* (1997) recently named the organism from pen-reared Atlantic salmon and Arctic grayling (*Thymallus arcticus*) from fresh water as *S. barkhanus*.

Water-borne exposure of the fish to infected blood and viscera, or cohabitation with infected fish in either fresh or seawater (Kent *et al.*, 1992) can reproduce the systemic disease in chinook salmon. However, the parasite of Atlantic salmon from Norway could not be transmitted to healthy fish by cohabitation.

**Clinical and gross pathology.** In chinook salmon from British Columbia, infected fish appeared normal except that some fish had a distended abdomen. The gills were pale due to anaemia. The hallmark gross pathological change of the disease is an extremely enlarged liver. In some fish, the liver was also mottled and had petechial haemorrhage and whitish, friable areas. Affected fish consistently had serosanguinous ascites and blood clots in the visceral cavity. The spleen and kidney were moderately enlarged, and petechiae occurred throughout the skeletal muscle (Kent *et al.*, 1992).

In Atlantic salmon, the infection differed in that the parasite caused large, multifocal, white, lesions in the musculature, liver, spleen and kidney (Poppe *et al.*, 1992). Yellow or white cysts filled with the parasite were also in the fins, and infected fish often had exophthalmia (Poppe and Mo, 1993).

**Diagnosis.** The infection is identified by wet-mount preparations or Giemsa-stained imprints of the gut or other visceral organs. Because the parasite is highly motile, it may be easier to identify the parasites in wet-mount examination. Wet-mount preparations of the visceral organs reveal massive numbers of flagellates that are 10 × 5 µm (Fig. 3.9A). The parasites are also readily detected in DifQuick or Giemsa-stained imprints (Fig. 3.9B), where they appear as dark-staining, oval bodies with two clear bands, representing the flagella pocket, running the length of the organism. The two nuclei at the anterior end of the parasite may be visible.

**Control and treatment.** Several drugs, most of which are added to the diet, have been recommended for the control of *H. salmonis* infections in the gut of salmonids (Yasutake *et al.*, 1961; McElwain and Post, 1968; Hoffman and Meyer, 1974; Becker, 1977). However, presently, none of these compounds is approved for treatment of food fish in Canada or the USA.

Although it has been determined that the disease is transmissible in seawater, it is not established whether salmon contracted the infection in seawater, or were subclinically infected when they were transferred to netpens. At present, the best guess is that infections in both Norway and Canada are contracted in seawater. In addition, Poppe and Mo (1993) suggested that fish could become infected by exposure to untreated water from fish processing plants. An understanding of the source of the infection would be helpful for implementing effective control strategies.

**Kudoa thyrsites (Myxozoa)**

Myxosporeans of the genus *Kudoa* and related genera infect the muscle of many marine fishes, and heavy infections can cause unsightly white cysts or soft texture in fillets (Kabata and Whitaker, 1981; Patashnik *et al.*, 1982). These parasites can lower the market value of the infected fish, although they seldom cause morbidity. *K. thyrsites* is a cosmopolitan parasite that infects many species of marine fishes (Whitaker *et al.*, 1994). Infections in pen-reared Atlantic salmon have been reported from the Pacific Northwest (Harrell and Scott, 1985; Whitaker and Kent, 1991), Spain (Barja and Toranzo, 1993) and Ireland (Palmer, 1994). In one
instance, Harrell and Scott (1985) attributed mortalities in Atlantic salmon smolts to this parasite. More importantly, heavy infections are associated with soft flesh in pen-reared Atlantic salmon that are either held on ice for 3–6 days or cold-smoked. *K. thyrsites* infections and associated soft flesh have also been observed in farmed coho salmon (Whitaker and Kent, 1992) and brown trout (Baudin-Laurencin and Bennassar, 1993).

The infection is much more prevalent in Atlantic salmon grilse or reconditioned grilse (i.e. fish that have reabsorbed their gonads and do not exhibit external signs of sexual maturation) than in market-size fish that have not undergone sexual maturation (St-Hilaire et al., 1998). For example, infection in grilse may reach 70%, whereas market-size fish that are not sexually mature usually show infections below 10%. There is a positive correlation between intensity of infection and severity of soft flesh in Atlantic salmon held on ice (St-Hilaire et al., 1997a). Heavily infected fish always have soft flesh, whereas lightly infected fish (i.e.
fewer than 20,000 spores g⁻¹) usually do not have the condition. Soft flesh is not noticed on the processing line, and only becomes apparent after fish are held for about 3–6 days on ice or when fillets are smoked. In an investigation of *K. thyrsites* infections in Pacific hake (*Merluccius productus*), the flesh softening was apparently caused by a proteolytic enzyme produced by the parasite (Tsuyuki et al., 1982). This enzyme remains active below 70°C. Therefore, tissue breakdown will continue through cold-smoking processes, which are normally conducted at about 50°C or less. Seymour et al. (1994) suggested that the flesh degradation is due to cathepsin L from the host inflammatory response to the parasite, instead of a proteolytic enzyme from the parasite.

Very little is known about development and transmission of *K. thyrsites* in fish. It takes about 5–6 months after infection before spores are detected in the flesh and a high prevalence occurs in post-smolts (Moran et al., 1999a). As the infection progresses in Atlantic salmon, pseudocysts in the muscle fibres enlarge and ultimately rupture. A prominent inflammatory response is associated with ruptured pseudocysts, and fish eliminate the detectable infections after about a year in seawater (Moran et al., 1999a). It is not known if the high prevalence of the infection in grilse is due to reinfection, or proliferation of a cryptic infection that originally occurred shortly after fish were transferred to seawater. An infectious stage of the parasite occurs in the blood. Moran *et al.* (1999b) showed that direct *per os* exposure of Atlantic salmon with heavily infected tissue did not cause infections. As with other myxosporeans, an annelid alternative host is probably involved in the life cycle.

Analysis using small subunit ribosomal rDNA (SSU rDNA) suggests that *Kudoa* species are phylogenetically different from other myxosporean genera examined (i.e. *Myxobolus, Henneguya* and *Myxidium*), and that *K. thyrsites* in Atlantic salmon is indistinguishable from that infecting tubesnout (*Aulorhynchus flavidus*) and probably other marine fishes in the Pacific Northwest (Hervio *et al.*, 1997).

**Clinical signs and gross pathology.** Heavily infected fish held on ice for 3–6 days may develop extreme softening of the flesh texture. Occasionally discrete white patches are visible with the naked eye. The soft flesh also will occur following smoking at cool temperatures (below 70°C) where white patches in the muscle are readily seen.

**Diagnosis.** Diagnosis is based on the observation of the characteristic stellate spores of the parasite, which are about 13 µm in diameter (Fig. 3.10). The spores are best detected by microscopic examination of

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Fig. 3.10. Wet mount of *Kudoa thyrsites* spores. Bar, 15 µm.
fluid collected from the freshly cut surface of a fillet or by crushing a small piece of muscle. The parasite shows up well in Giemsa-stained histological sections. Detection of the parasite in whole fish is a problem that is, as yet, unresolved.

Wet-mount examination of the hyoid muscle in the underside of the operculum is a relatively sensitive and specific method for detecting the infection without damaging the body musculature (St-Hilaire et al., 1997b). Although this method may miss a few light infections, this is not a great concern because light infections do not cause soft flesh.

Many copies of the rDNA sequence occur within an individual eukaryotic cell, and thus this sequence is useful for developing very sensitive PCR-based tests. Hervio et al. (1997) developed a sensitive PCR test for K. thyrsites, which will be used to identify the source of infection for salmon.

Control and treatment. There are no commercially available drugs against myxosporeans. Fish become infected in seawater, so it would be very difficult to eliminate exposure to infections. Because sexually mature fish and reconditioned grilse are more prone to the infection, removing such fish from the population before harvest (e.g. by thorough screening for grilse in the winter) will greatly minimize the problem.

**Loma salmonae (Microsporidia)**

*L. salmonae* is an obligate intracellular microsporidian and infects the gills and other vascularized tissues of salmonids reared in fresh water (Putz et al., 1965; Putz and McLaughlin, 1970; Morrison and Sprague, 1981; Hauck, 1984; Poynton, 1986; Markey et al., 1994; Bruno et al., 1995). Severe gill infections have been reported in rainbow trout, steelhead trout and kokanee salmon (Wales and Wolf, 1955), and Hauck (1984) observed high mortality in chinook due to systemic infections by a *Loma* sp. (presumably *L. salmonae*).

Infections can persist after fish are transferred to seawater, and the associated lesions in the gills can become severe in the pen-reared salmon (Kent et al., 1989; Speare et al., 1989). Although the gills are the primary site of infection, parasites and associated lesions can occur in the heart, spleen, kidney and pseudobranch. Infection can originate in fresh water, but there is a high prevalence of *L. salmonae* in chinook salmon from netpens that have been reared solely on ground water during the freshwater phase (Kent et al., 1995). Furthermore, infection has been reported in ocean-caught adult salmon (Kent et al., 1998). Potential vertical transmission of microsporians via the ova has been reported by Vaney and Conte (1901) and Summerfelt and Warner (1970). Vertical transmission of *L. salmonae* from infected females to progeny should also be considered as a possibility. *L. salmonae* occurs in the ovaries, but not in the eggs, of sexually mature salmon (Docktor et al., 1997a). Therefore, the progeny of infected females could become exposed to the parasite through contaminated ovarian fluid. Furthermore, spores of *L. salmonae* can survive iodine treatment at 100 ppm for 15 min (Shaw et al., 1999), a dose typically used for disinfecting salmonid eggs after spawning.

Although other *Loma* species infect non-salmonid fish, it is doubtful whether these fish are reservoirs for *L. salmonae*. Shaw et al. (1997) demonstrated that a *Loma* sp. from shiner perch, *Cynmatogaster aggregata* (a common fish found around sea cages in British Columbia), was a different species from *L. salmonae*. Although the two microsporians were morphologically indistinguishable, Shaw et al. (1997) showed that the parasite from shiner perch could not infect salmon.

*Loma morhua* is a common gill parasite of Atlantic cod (Morrison, 1983), and causes similar gill lesions to those induced by *L. salmonae* in salmon. Therefore, this parasite also has potential to cause disease in farmed cod.
Clinical signs and gross pathology. Fish with heavy gill infections are usually lethargic, and small white cysts may be seen in the gills (Fig. 3.11). A consistent finding in pen-reared salmon is the presence of multiple petechiae in an otherwise pale gill. Infected gills may also appear nodular. Systemic infections in chinook may cause enlargement of the spleen and kidney. In Atlantic cod, obvious white cysts are also apparent in the gills and visceral organs (Morrison, 1983).

Diagnosis. Spores of Loma spp. can be easily detected in wet-mount preparations of moderately to heavily infected gills. The parasite–host cell complexes, xenomas, appear more opaque than the surrounding tissue, and high magnification reveals masses of the spores within the xenoma (Fig. 3.12). Individual xenomas may occlude blood vessels, and rupture results in a marked inflammatory response by the host as spores are released (Kent et al., 1989). The spores are bean-shaped and are about 5×3 µm. Spores in infected tissue are Gram-positive.

A sensitive PCR test for L. salmonae using an rDNA sequence has been described (Docker et al., 1997a). This may be useful for screening fish (i.e. broodstock) for subclinical infections. Furthermore, these
specific primers can differentiate \textit{L. salmonae} from other \textit{Loma} species.

Control and treatment. Currently, there are no licensed pharmacological agents or vaccines (Speare et al., 1998). In laboratory studies, feeding fumagillin at 10 mg kg$^{-1}$ fish day$^{-1}$ for 30 days (Kent and Dawe, 1994) prevented infections in chinook salmon. Our recent experiments demonstrated that infections can be controlled with lower doses of fumagillin, i.e. 2 or 4 mg kg$^{-1}$ fish (Kent and Poppe, 1998).

The synthetic analogue of fumagillin, TNP-470 (Takeda Chemical Industries, Ltd, Japan), can also be effective in reducing \textit{L. salmonae} infections (Higgins et al., 1998). Oral treatment with this compound at 0.1 or 1.0 mg kg$^{-1}$ fish for 4 weeks greatly reduced the intensity of infections, with no apparent clinical toxic side effects. Speare et al. (1998) showed that rainbow trout have strong protection against reinfection, which suggests that \textit{L. salmonae} infections may be prevented by vaccines.

The susceptibility of Pacific salmon strains to \textit{L. salmonae} infection using feeding trails was examined by Shaw et al. (2000). Differences in strain susceptibility were noted and this may assist with future breeding experiments.

\textit{Nucleospora salmonis}

\textit{N. salmonis} is an unusual microsporidium that infects the nuclei of haemoblasts, particularly lymphoblasts or plasmablasts, in salmonid fishes (Chilmonczyk et al., 1991). This microsporidium was first observed in pen-raised chinook in Washington State, where it was associated with anaemia (Elston et al., 1987). The parasite has also been reported in freshwater-reared chinook, kokanee (\textit{Oncorhynchus nerka}) and steelhead trout (\textit{O. mykiss}) (Hedrick et al., 1990, 1991b; Morrison et al., 1990) The infection is common in caged-reared chinook salmon in British Columbia and in Atlantic salmon in Chile (Bravo, 1996).

This microsporidium was originally described as \textit{N. salminis} (cf. Hedrick et al., 1991a), but was described shortly thereafter as \textit{Enterocytozoon salmonis} by Chilmonczyk et al. (1991). Rules of zoological nomenclature, morphological data and rDNA sequence data support the validity of the genus \textit{Nucleospora}, and its placement in the family \textit{Enterocytozooidae} (see Desportes-Livae et al., 1996; Docker et al., 1997b). Similar intranuclear microsporidia were reported in Atlantic lumpfish (\textit{Cyclopterus lumpus}) (Mullins et al., 1994) and Atlantic halibut (Nilsen et al., 1995).

\textit{N. salmonis} infections are usually associated with a concurrent neoplastic condition involving massive lymphoproliferation, known as plasmacytoid leukaemia (PL) in chinook salmon in British Columbia (Kent et al., 1990). However, the actual cause of PL is controversial. Laboratory transmission studies indicated that \textit{N. salmonis} may not be the primary cause of all cases of PL (Kent and Dawe, 1990; Newbound and Kent, 1991), and Eaton and Kent (1992) described a retrovirus associated with the condition.

It is possible that PL actually represents two separate diseases; one caused by the virus and one caused by the microsporidium. Studies with fumagillin and TNP-470 (Hedrick et al., 1991b; Higgins et al., 1998) support the microsporidian hypothesis, i.e. treatment with these anti-microsporidian compounds prevented \textit{N. salmonis} infections and PL. Moreover, in contrast to the late 1980s and early 1990s, \textit{N. salmonis} is consistently observed in the proliferating plasmablasts in essentially all cases that have been investigated in recent years in British Columbia.

\textit{N. salmonis} is transmitted by cohabitation or feeding infected tissues to fish in fresh water (Baxa-Antonio et al., 1992). These findings have been reported in our laboratory, but we were unable to transmit the infection by cohabitation in seawater. Circumstantial evidence (e.g. the occurrence of the parasite in Chile) suggests that the parasite may be transmitted via eggs.
Clinical signs and gross pathology. Heavily infected fish are anaemic. Fish with PL are often dark, lethargic and may swim near the surface. Many of the fish with PL exhibit severe bilateral exophthalmos (Fig. 3.13). The exophthalmos is due to massive accumulation of white or hyperaemic tissue in the orbit of the eye. The spleen and kidney are enlarged when systemic infections occur. Petechiae may occur in the liver, mesenteric fat, pancreas, heart and skeletal muscle. The lower intestinal wall may be markedly thickened. Some fish have ascites consisting of a clear or serosanguinous fluid.

Diagnosis. This microsporidium is small (about 2 µm) and is identified by careful examination of nuclei of haemoblasts in histological sections or in Gram-stained imprints (Fig. 3.14). Following Gram stain, the spores stain Gram-positive. They have a characteristic bean shape, and measure about 2 × 1 µm. Sensitive and specific PCR tests have been developed for the detection of *N. salmonis* based on a rDNA sequence.

Fig. 3.13. Severe exophthalmos in chinook salmon with plasmacytoid leukaemia associated with *Nucleospora salmonis* infection.

Fig. 3.14. Gram-stained kidney imprint of *Nucleospora salmonis* showing spores (arrowhead) in a remnant of a nucleus. Bar, 10 µm.
from the small subunit region (Barlough et al., 1995) or internal transcribed spacer region (Docker et al., 1997b).

Treatment and control. There is no commercially available drug for treating *N. salminis* infections. However, Hedrick et al. (1991b) controlled the infection in experimentally infected chinook by oral treatment with fumagillin at 1.0 mg kg$^{-1}$ fish day$^{-1}$ for 2 weeks. Higgins et al. (1998) found that the fumagillin analogue, TNP-470 (Takeda Chemical Industries Ltd), was very effective at controlling experimental infections when fish received an oral treatment at either 0.1 or 1.0 mg kg$^{-1}$ fish day$^{-1}$ for 4 weeks.

**Crustacean Parasites**

**Sea lice – caligid copepods (family Caligidae)**

Sea lice are the most economically important parasites afflicting salmon in cage culture. ‘Sea lice’ refer to several species of marine ectoparasitic copepods of the genera *Lepeophtheirus* and *Caligus* of the family Caligidae that infect marine fishes, particularly salmonids (Costello, 1993; Johnson, 1998). *Lepeophtheirus salmonis* has a circumpolar distribution and is restricted to salmonids, except as a result of accidental transfer from salmonids (Kabata, 1979). In contrast, *Caligus* species that infect salmon have broad host ranges that include both non-salmonid teleost and elasmobranch hosts.

Heavy infections greatly reduce the market value of the fish and ultimately result in death. Mortality may occur due to the development of secondary diseases (e.g. vibriosis, furunculosis) exacerbated by the high levels of accompanying stress. In severe cases where the epidermis is breached, death may be due to a loss of physiological homeostasis including osmotic stress, anaemia and hypoproteinaemia (Wootten et al., 1982; Tully et al., 1993). Sea lice may also function as vectors of bacteria and viruses such as infectious salmon anaemia virus (Nylund et al., 1994).

Sea lice have ten developmental stages: two free-living planktonic nauplius stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two pre-adult stages and one adult stage (Johnson and Albright, 1991; Schram, 1993). The copepodid, chalimus, pre-adult and adult stages all feed on mucus, skin and blood of fish (Kabata, 1970; Brandal et al., 1976).

**Clinical signs and gross pathology.** Pre-adult and adult parasites actively move on the surface of fish, and lesions caused by these stages may be severe and widespread. In contrast, damage by the non-motile copepodid and chalimus larvae is generally focal (Bron et al., 1991; Johnson and Albright, 1992). Infected salmon commonly have grey patches (extensive areas of skin erosion) and haemorrhaging on the head and back. They often exhibit distinct areas of erosion, dark coloration and subepidermal haemorrhage in the perianal region (Wootten et al., 1982; Urawa and Kato, 1991; Nagasawa and Sakamoto, 1993; Johnson et al., 1996). Severely infected salmon have ulcers in which the epidermis is breached and the underlying tissues exposed. These lesions often occur on the head and behind the dorsal fin (Jónsdóttir et al., 1992).

**Diagnosis.** Copepodids and chalimus larvae of sea lice are small (< 4 mm in length) and can occur on the body surface and fins as well as in the buccal cavity and on the gills. Their small size requires the use of a magnifying glass or dissecting microscope to detect their presence.

Pre-adult and adult sea lice are visible to the naked eye. They are on the body surface, especially on the head, back and in the perianal region. It is these stages that usually cause the most damage to the fish. Pre-adult and adult stages of *Caligus* species can be distinguished from *Lepeophtheirus* species by the presence of lunules on their anterior margin (Fig. 3.15). There is a key to aid in the identification of adult sea lice of the northern hemisphere (Johnson and Margolis, 1994).
Control and treatment. There are excellent reviews on the control and treatment of sea lice (Costello, 1993; Johnson et al., 1993; Roth et al., 1993a; Johnson, 1998). Management strategies are useful for reducing the impact of sea lice in cage farms. Farms should be located in areas with strong water currents to flush away copepodid stages and in areas where wild fish reservoir hosts are not numerous. Fallowing of sites between production cycles and maintaining only single year classes at sites can also significantly reduce the need for treatments for _L. salmonis_ (Bron et al., 1993; Grant and Treasurer, 1993). This approach may be applied to several sites within a common area. In cases where farms belonging to different companies are in close proximity to each other, cooperative agreements between companies with respect to single-year class stocking, periods of fallowing and timing of sea lice treatments have been effective for controlling sea lice outbreaks (Grant and Treasurer, 1993).

Chemotherapy has played a significant role in attempts at controlling sea lice. Bath treatments with dichlorvos, trichlorfon, azamethiphos, cypermethrin, carbaryl, pyrethroids and hydrogen peroxide have been employed (Brandal and Egidius, 1979; Costello, 1993; Johnson et al., 1993; Roth et al., 1993a,b; Thomassen, 1993a,b). These treatments have mainly been developed for treating Atlantic salmon, and caution should be used when applying them to other species, as they often vary in their ability to tolerate sea lice treatments (see Johnson and Margolis, 1993; Johnson et al., 1993).

The organophosphorus insecticides, dichlorvos, marketed as ‘Nuvan 500EC’ or ‘Aquaguard SLT’, or in its related trichlorphon form as ‘Neguvon’, were the first chemicals widely used to control sea lice (Brandal and Egidius, 1977; Grave et al., 1991a,b). Dichlorvos and trichlorphon have been used since the 1960s as a bath treatment for parasites in pond fish culture (reviewed in Schmahl et al., 1989). These treatments effectively remove both the pre-adult and adult stages of sea lice (Brandal and Egidius, 1979). Therefore, successive treatments, usually at 2–4 week intervals, are required to control infections (Wootten et al., 1982). Repeated treatment with dichlorovos may induce resistance in sea lice (Jones et al., 1992).

Another organophosphate, azamethiphos (marketed as Salmosan® and Alfa-cron®), is presently used in Europe and
Canada for sea lice control. It is administered as a bath treatment, and like the other organophosphates shows little efficacy against the attached chalimus stages (Roth et al., 1996). This chemical is efficacious against *L. salmonis*, has a wider therapeutic margin and appears to be more tolerated than the other organophosphates by Atlantic salmon (Hodneland et al., 1993; Roth et al., 1993a, 1996).

A drawback with these organophosphates is that they may be toxic to a wide variety of marine organisms when they are released into the surrounding waters after treatment. However, their impact on non-target species may be minimal due to dilution and the rapid breakdown of these pesticides (Egidius and Moster, 1987; Cusack and Johnson, 1990; Dobson and Tack, 1991).

Pyrethrin and pyrethroid compounds are currently being used for sea lice control (Boxaspen and Holm, 1991a,b; Roth et al., 1993b). The synthetic pyrethroid cypermethrin is believed to be more efficacious than azamethiphos for the control of *L. salmonis* on Atlantic salmon. Clinical field trials are ongoing in Maine, USA, using cypermethrin (under the market name Excis®). Presently this is the most widely used bath treatment against sea lice in Norway.

Bath treatments with hydrogen peroxide may also be another effective method for treating sea lice (Bruno, 1992; Thomassen, 1993a,b; Bruno and Raynard, 1994). Thomassen (1993a,b) reported that bath treatments of hydrogen peroxide at a concentration 1.5 g L\(^{-1}\) for 20 min effectively removes from 85 to 100% of the pre-adult and adult stages of sea lice without being toxic to Atlantic salmon. The market name of hydrogen peroxide for use in fish farming is Salartect 500 FLT®. Twenty minute bath treatments of 1.5 g L\(^{-1}\) hydrogen peroxide at 11°C effectively removed approximately 80% of the pre-adult and adult stages of *L. salmonis*, but had no significant effect on the intensity of infection with the attached chalimus stages. In addition, a high proportion of the pre-adult and adult stages removed from the fish recovered after treatment (Johnson et al., 1993; Bruno and Raynard, 1994). With respect to *L. salmonis*, these stages are less likely to reinfect the treated hosts. Pre-adults and adults of species of *Caligus* are generally more active swimmers and reinfection is possible if they recover. The use of hydrogen peroxide is rather impractical and it has been essentially abandoned in Norway. Furthermore, caution should be used when applying hydrogen peroxide treatments, particularly at higher temperatures (Johnson et al., 1993; Roth et al., 1993a; Bruno and Raynard, 1994). Fortunately, Atlantic salmon are less sensitive to hydrogen peroxide than Pacific salmon species, such as chinook salmon (Johnson et al., 1993).

Application of external treatments in netpens is often expensive and difficult. Therefore, considerable effort has been directed toward development of oral treatments for sea lice. Palmer et al. (1987) reported the results of preliminary studies on the efficacy of oral doses of ivermectin for the control of sea lice on Atlantic salmon. Ivermectin has been demonstrated to be effective in controlling all developmental stages of sea lice (Smith et al., 1993; Johnson and Margolis, 1993). Although this drug was found to be effective in reducing populations of sea lice, it had a relatively narrow margin of safety with salmon. Johnson et al. (1993) showed that ivermectin can be very toxic to Atlantic salmon, and that the level of toxicity varied between salmon species. Atlantic salmon fed 0.05 mg kg\(^{-1}\) on alternate days became anorexic after 20 days, and ivermectin was lethal to fish fed at higher doses (Johnson et al., 1993). Fish suffering from ivermectin toxicity are listless, show ataxia and then die in a few days. Due to long tissue withdrawal times and concerns about the impact of ivermectin residues in the sediments beneath the netpens, this drug may never be licensed or registered for use in aquaculture (Burridge and Haya, 1993; Costello, 1993).

Oral administration of diflubenzuron (a chemical that inhibits chitin synthesis) reduces infections by both adult and larval stages of sea lice (Roth et al., 1993a). Treatments using Lepsidon (containing diflubenzuron) and Ektobann (containing
teflubenzuron) are being conducted on a limited scale in Norway and the Faroe Islands under special permit. These compounds are highly effective against the copepodid, chalimus and pre-adult stages. However, they have no efficacy against the adult stages because they no longer moult. A concern with these drugs is the possible detrimental effects on non-target arthropods that dwell around sea cages. Licensing of these compounds in some areas, such as the USA, may be very difficult due to laws that limit the use of diflubenzuron within 5 km of the coast (Roth et al., 1993a).

Cleaner-fishes (i.e. wrasse species in the family Labridae) are used in Norway, the Shetland Isles, Scotland and Ireland to control sea lice (reviewed in Costello, 1993; Kvenseth, 1993; Treasurer, 1993; Tully et al., 1996). In laboratory and field studies, wrasse remove sea lice from salmonids but not always in a predictable manner. A survey of fish farmers in Scotland who have tried wrasses to control sea lice showed that the majority felt that their use was beneficial, particularly when used in conjunction with dichlorvos treatments (Anon., 1991). Wrasses are also useful for reducing fouling on cages. The disadvantages of using wrasse to control sea lice include the requirement for smaller mesh size in nets to prevent their escape, intimidation by larger salmon and a tendency not to clean them, aggressive behaviour and infliction of scale and eye damage to the salmon (Anon., 1991). In addition, wrasse exhibit high over-winter mortalities, their supply is limited and their cost is usually high. Nevertheless, wrasse are used in over half of the fish farms in Norway.

A vaccine for sea lice would be useful. Although Atlantic salmon can produce antibodies to sea lice extracts in controlled studies (Grayson et al., 1991), salmon naturally infected with L. salmonis and Caligus elongatus fail to produce an antibody response (Grayson et al., 1991; MacKinnon, 1991). Furthermore, there was no difference in the number of copepods carried on control and immunized Atlantic salmon when exposed under laboratory conditions (Grayson et al., 1995).

**Family Pennellidae**

Members of the family Pennellidae may cause problems in netpen aquaculture. *Haemobaphes disphaerocephalus* has been reported in pen-reared Atlantic salmon (Kent et al., 1997). This parasitic copepod normally infects eulachon (*Thaleichthys pacificus*) and this was the first report of a *Haemobaphes* species infecting salmon. The parasite penetrates the branchial vasculature and causes anaemia. Fortunately, the infection has been observed in only a few Atlantic salmon reared in British Columbia. In contrast, *Lernaeocera branchialis* is a common parasite of Atlantic cod (Kabata, 1984). The parasite causes reduced growth and anaemia (Khan, 1988), and Khan et al. (1990) concluded that it is a potential threat to cod farming.

**Clinical disease and gross pathology.** Fish infected with either *Haemobaphes* or *Lernaeocera* are usually anaemic and may be lethargic. Examination of the opercula cavity reveals the coiled egg sacs and blood-engorged body of the parasite penetrating the gill (Fig. 3.16). The long neck and anterior holdfast are internal within the gill arch.

**Diagnosis.** *Haemobaphes* and *Lernaeocera* spp. are characterized by attachment at the gill arch and coiled egg sacs. Specific identification requires examination of the anterior holdfast (see Kabata, 1988), which must be very carefully dissected from tissues. Atlantic lumpfish (*C. lumpus*) is an intermediate host of *L. branchialis*, and thus simultaneous rearing of this fish with cod should be discouraged.

**Control and treatment.** There is no suitable drug available for treating this infection. The infective larvae are free-swimming and it would be difficult to prevent the infection in netpens.
Isopods – *Ceratothoa gaudichaudii*

Four species of isopods, *Ceratothoa gaudichaudii*, *Rocinela maculata*, *Rocinela belliceps pugettensis* and *Gnathia* sp., have been reported from seawater-reared salmonids (Novotny and Mahnken, 1971; Awakura, 1980, 1983; Drinan and Rodger, 1990; Inostroza et al., 1993). The only economically important isopod parasite of marine coldwater netpen-reared fish has been *C. gaudichaudii*. In Chile, *C. gaudichaudii* has been reported from a wide variety of native hosts. This low host specificity has allowed this parasite to successfully infect coho and Atlantic salmon. Disease caused by this parasite has been a serious problem at certain farm sites in Chile (Inostroza et al., 1993).

**Clinical signs and gross pathology.** *C. gaudichaudii* feeds on host blood, attaching to the inner mouth surfaces and less frequently to the gills (Sievers et al., 1995). Disease is caused by their attachment and feeding activities. Damage to the host includes severe erosion of gill lamellae and ulcers on the gill arch and inside the mouth.

**Diagnosis.** *Ceratothoa* is readily identified on the fish by examination of the mouth and gills (Fig. 3.17).

**Control and treatment.** Sievers et al. (1995) evaluated the efficacy of eight commercial insecticides against *C. gaudichaudii* on Atlantic salmon. Sixty-minute bath treatments with the organophosphates, trichlorfon (Neguvon) and dichlorvos (Nuvan 1000) at concentrations of 300 and 3 ppm, respectively, were found to be 100% effective against this parasite without toxicity to the fish.

**Helminth Parasites**

Fish are infected with a wide variety of parasitic worms, collectively referred to as helminth parasites. Although these parasites are very common in wild fish, and occasionally infect cultured species, generally they do not cause severe disease. However, certain helminths can cause damage when infections are heavy or when they infect a critical organ. In addition,
some helminth parasites of fish can infect humans or cause unsightly lesions. The following are helminth parasites of importance in coldwater marine netpen-reared fish.

**Cestodes (tapeworms)**

Two life stages of cestodes are found in fish: adults infect the digestive tract and metacestodes (juveniles) are usually on the internal organs or muscle. The first intermediate hosts of tapeworms that infect fish are usually crustaceans (e.g. copepods). Fish may also be the second intermediate hosts for tapeworms, and a fish-eating mammal or bird, or another fish, are definitive hosts. Therefore, fish usually acquire metacestode infections by eating infected crustaceans. Metacestodes in fish tissues often cause an inflammatory response to the encapsulated or migrating parasite. The only reported significant metacestode disease of salmon reared in marine netpens is caused by *Gilquinia squali*, which infects the eyes of chinook salmon (Kent et al., 1991).

**Adult cestodes – Eubothrium spp.**

*Eubothrium* spp. are common cestode parasites of salmonid fish in both fresh and salt water, in which adults develop in the gut. Infections with one species in pen-reared Atlantic salmon in Norway have been associated with reduced growth and, occasionally, mortality (Bristow and Berland, 1991a,b; Håstein and Lindstad, 1991). The weight of infected market-size farmed Atlantic salmon in Norway is 10–15% less than uninfected salmon (Berland and Bristow, 1994). It has been noted that a similar infection occurs in the cestodes of broodstock from pen-reared chinook salmon in British Columbia.

The fish acquire infections of *Eubothrium* species by ingesting first intermediate hosts (presumably copepods) infected with the procercoid stage, or possibly transport hosts infected with plerocercoids. The life cycle of this tapeworm in marine fishes has not been elucidated, but its freshwater counterpart, *Eubothrium salvelini*, uses copepods (*Cyclops* spp.) as its intermediate host. Procercoids that develop in *Cyclops* are directly infective for juvenile salmon (Boyce, 1974). *E. salvelini* is known to affect survival, growth and stamina, and to have other debilitating effects on juvenile sockeye salmon (Boyce and Behrens-Yamada, 1977; Boyce, 1979; Boyce and Clarke, 1983).

**Clinical signs and gross pathology.** Heavily infected fish are often smaller than average. Dissection of the gut will reveal numerous, white, flat, ‘tape-like’ worms in the intestine and pyloric caeca (Fig. 3.18). Heavy

![Adult Eubothrium cestodes in the gut of Atlantic salmon (courtesy of B. Berland).](image-url)
infections may induce anaemia, and when extremely severe the cestodes may cause death due to blockage of the intestinal tract (Mitchell, 1993).

**Diagnosis.** Adult cestodes are usually long, flat, whitish and segmented. Identification as a cestode is based on a segmented body, a scolex (anterior end), and on the structure and arrangement of the reproductive system within the segments (Schmidt, 1986; Khalil et al., 1994). *Eubothrium* lacks hooks on the scolex, which is elongate with two shallow grooves—one dorsal and the other ventral.

**Control and treatment.** Oral treatment for adult tapeworms with anthelmintic drugs, such as praziquantel, may be effective (Mitchell, 1993). Avoiding cestode infections is difficult because infected intermediate hosts (i.e. crustaceans) move freely throughout netpens.

*Gilquinia squali* metacestodes

Eye infections by metacestodes of *G. squali* (order Trypanorhyncha) have been associated with mortality of young chinook salmon at netpen sites in British Columbia (Kent et al., 1991). Avoiding cestode infections is difficult because infected intermediate hosts (i.e. crustaceans) move freely throughout netpens.

**Diagnosis.** The infection is identified by detecting trypanorhynch metacestodes in the vitreous humour. Trypanorhynch cestodes are identified by the presence of four reversible, spiny tentacles, which emerge from the apex of the scolex (Fig. 3.19).

**Control and treatment.** There is no known treatment for *Gilquinia* infection in fish. Infestation can only be controlled by preventing infection. Fish that are feeding well on commercial diets and thus feed less on natural biota appear to have lower infestations. The complete life cycle of the parasite is unknown, so precise recommendations for avoiding infection are not available. Furthermore, preventing transmission of the parasite from dogfish to salmon via the arthropod first intermediate host would be difficult because of uncontrolled water movement into netpens and unrestricted movement of dogfish around netpens.

**Digenetic trematodes (flukes)**

As with cestodes, fish can be intermediate hosts or definitive hosts for digenetic trematodes. Almost all flukes have either a two-host or three-host life cycle, but there is a wide variety of life cycle patterns
With a few exceptions among the marine fish blood flukes of the family Sanguinicolidae, molluscs (either snails or bivalves) are the first intermediate hosts of digeneans and second intermediate hosts may be an invertebrate or a fish. Except in occasional circumstances where the life cycle has been foreshortened, the definitive hosts are vertebrates. The species of concern in marine farming of salmon use either birds or fish as definitive hosts, with the fish serving as the second intermediate host. Cercariae of these species emerge from molluscs and infect the salmonid host by direct penetration of the skin or gills, subsequently developing into a resting stage known as a metacercaria, which may be encysted or unencysted depending upon the final site of infection in the fish.

Heavy infections by metacercariae are of concern because they can cause morbidity. In addition, metacercarial infections of the skin or muscle can be important because they may reduce the aesthetic quality of the fish. Except for blood flukes and a group of tissue parasites of the family Didymozoidae found mainly in scombroid fishes, most adult flukes of fish infect the alimentary tract and seldom cause significant tissue damage.

The metacercariae of four digenean trematodes have caused problems in seawater pen-reared salmonid fishes: ‘neascus’-type, *Diplostomum* sp., *Cryptocotyle lingua* and *Stephanostomum tenue*.

**Skin diseases caused by digenean trematodes**

Black grub (larval type neascus) is the metacercarial stage of certain species of the family Diplostomatidae, which includes several genera (Gibson, 1996). This parasite infects a wide variety of freshwater fishes, including salmonids. Freshwater snails are the first intermediate hosts, and cercariae released from infected snails penetrate beneath the scales in the dermis of the fish host. Fish-eating birds serve as the definitive hosts. These infections can persist after fish are transferred to seawater. The problem has been recorded in pen-reared coho salmon in British Columbia. A condition similar to black grub in coho salmon is ‘black spot disease’ in Atlantic salmon caused by metacercariae of *C. lingua*. In contrast to the former condition, this parasite has a marine life cycle, which involves a definitive (adult) stage in fish-eating birds and a cercarial stage in snails. A *Cryptocotyle* sp. has also caused similar infections in cage-reared Atlantic cod (Lysne *et al*., 1994).

**Clinical signs and gross pathology.** Infected fish exhibit few to numerous raised black spots up to 1 mm in diameter in the skin,
fins, cornea and gills (Fig. 3.20). Occasionally, encysted metacercariae may be found in internal organs. Fish seldom become clinically affected unless they are heavily infected.

**Diagnosis.** Diagnosis of metacercarial infections in general is relatively easy using wet mounts or histological sections. However, more precise identification to the genus or species level usually requires careful preparations of the metacercariae in stained whole mounts and examination of the internal anatomy (Fig. 3.21). Information on the first occurrence of the infection (i.e. marine vs. freshwater) is useful for differentiating neascus from *Cryptocotyle*.

**Control and treatment.** As the cercarial stage of *Cryptocotyle* is common in the periwinkle (*Littorina littorea*), cages located in shallow water and close to the shore are more prone to the infection. There is no known treatment for this infection. With neascus, fish become infected in fresh water by exposure to surface water containing infected snails. Disinfection of the water supply or using ground water should eliminate or greatly reduce the infection. Based on reports from one fish farm in British Columbia, removing fish from freshwater hatcheries and introducing them to netpens before June or July may reduce the intensity and prevalence of the infection.

**Stephanostomum heart infections**

Heart (pericardial cavity) infections by metacercariae of *S. tenue* caused high
mortalities in pen-reared rainbow trout in Atlantic Canada (McGladdery et al., 1990). Rainbow trout are accidental hosts for this marine fluke, which normally infects mummichog (Fundulus heteroclitus) or silverside (Menidia menidia) as second intermediate hosts. Teleost fish, such as the American eel (Anguilla rostrata), are the definitive host for the parasite in the vicinity of the affected netpen sites. The first intermediate host is the mud dog whelk (Nassarius obsoletus), which is common around the affected netpens.

Clinical signs and gross pathology. The infection was associated with high mortalities in the summer months when water temperatures increase, presumably due to cardiac dysfunction (McGladdery et al., 1990).

Control and treatment. Maintaining netpens in water with over 7 m clearance from the bottom may reduce the intensity of infection (McGladdery et al., 1990).

Nematodes

Nematodes (roundworms) are common parasites of fish, and occasionally infect pen-reared salmon. As with cestodes and digenetic trematodes, fish can be either definitive or intermediate hosts for nematodes. Crustaceans and, less frequently, other invertebrates are the first intermediate hosts for nematodes that infect fish.

Members of the family Anisakidae are the only important (and reported) nematodes of pen-reared salmon. The nematode that has been associated with disease in salmon netpens is Hysterothylicium aduncum, which in its adult stage infects the fish digestive tract.

Large numbers of adult Hysterothylicium (=Thynnascaris) aduncum were found blocking the anterior part of the intestine of pen-reared rainbow trout in Norway some months after the trout were fed fresh wild sprat (Sprattus sprattus) that containing juvenile H. aduncum in their viscera (Berland, 1987). Intestinal infections of seapen-reared coho salmon and rainbow trout with Hysterothylicium spp. have also been observed in Chile (González and Carvajal, 1994).

Clinical signs and gross pathology. Carvajal et al. (1990) suggested that heavily infected fish exhibit poor growth, and Berland and Egidius (1980) have attributed mortalities in pen-reared rainbow trout in Norway to heavy intestinal infections with H. aduncum.

Diagnosis. Diagnosis is based on the identification of the worm. Presumptive diagnosis is based on the observation of nematodes in gut lumen. The worms are whitish and cylindrical, and adults are about 40–80 mm in length (González and Carvajal, 1994). Confirmation of identification requires microscopical examination of cleared or dissected worms for certain pathognomonic anatomical features of the worm’s digestive tract (Möller and Anders, 1986; Berland, 1989). Hysterothylicium and Anisakis spp. can be differentiated from Pseudoterranova and Contracaecum in that they have straight digestive tracts, without a ventricular appendix or intestinal caecum. Hysterothylicium can be separated from Anisakis in that the former has the excretory pore at the level of the nerve ring, whereas it occurs near the anterior tip with Anisakis.

Control and treatment. To prevent infections with Hysterothylicium or Contracaecum spp., or to keep infection low, the use of fresh wild marine fish or fish offal as feed for farmed fish should be avoided (Berland and Egidius, 1980; Vismanis et al., 1984). These fish are intermediate or paratenic (transport) hosts for the nematodes. González and Carvajal (1994) also referred to use of anthelmintics to reduce infections but did not specify the anthelmintics they employed.
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