9 Sporadic, Emerging Diseases and Disorders

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Introduction

Emerging diseases can be divided into two general categories. First, those that are sporadic and generally have a local geographical effect (e.g. Diphyllobothrium infestation, Vibrio viscosus (Moritella viscosa)) and second, those diseases where prevalence has progressively increased in susceptible populations. In both cases frequent or seasonal losses may occur. Sporadic outbreaks of disease may remain insignificant, or build up in a population with increasing mortality, partly because transmission of the pathogen is poorly understood. During the 1950s, the movement of rainbow trout eggs into Japan is believed to represent the source of infectious pancreatic necrosis virus (IPNV) (Yamazaki, 1974). These movements occurred before it was known that fish eggs might be a source of infection. Later, infectious haematopoietic necrosis virus (IHNV) was introduced into Japan, also with salmon eggs (Kimura and Awakura, 1977). The first occurrence of viral haemorrhagic necrosis virus (VHNV) in Canada was attributed to the import of infected fish. However, in this case, improved diagnosis established this virus as endemic and infection in farmed fish was attributed to a wild fish origin (Bernard et al., 1992).

Emerging fish problems continue to be highlighted in many areas of the world and discussion on transfer and interaction with naive populations remains a contentious issue. Disease transfer between farmed and wild salmon stock has been questioned, although some pathogens will have been initially described in wild fish (Kent, 1992; Reno, 1998). The exposure of feral fish to pathogens of farmed origin will depend upon several factors, including the number of infected fish, concentration of pathogen, environmental survival time, presence of susceptible wild hosts, rate of shedding, routes of entry and the role of carrier and escaped fish. The environmental impact of salmon cage culture is poorly understood and currently there is no clear evidence of adverse effects of disease on wild fish stocks (Phillips et al., 1985; NASCO, 1993). Hästein and Linstad (1991) considered that the inability to control, prevent or treat diseases in wild fish suggested that this group presented a greater risk to farmed stocks than vice versa.

Consequently, the role diseases play in regulating wild fish are largely unknown (Möller and Anders, 1986). The factors contributing to or altering the known status
of a particular infection include environmental changes, withdrawal of aquaculture treatments, and emergence of new strains and antibiotic-resistant bacteria. Legislation and health checks may prevent fish movements between some countries. However, in some regions of the world where cage culture is developing there is a lack of enforcement or legislation. Although assessing the consequences of all disease outbreaks is prudent, this again may fail through lack of knowledge or understanding of fish health, as the determining factors are typically multifactorial and complex (Smith, 1997).

The aquaculture industry is generally faced with two types of disease, namely those that are already endemic and those perceived or recognized as emerging problems, although neither is mutually exclusive. Historically, fish diseases, from both wild and farmed salmon, have occurred in one country or region and later emerged elsewhere, possibly with a similar severity or increased impact. Classic examples include bacterial kidney disease (BKD), furunculosis, piscirickettsiosis and, recently, infectious salmon anaemia virus (ISAV). Health management programmes should include appropriate inspections and disease reporting requirements, policies on slaughter, quarantine and adequate disposal of dead animals. The development of such programmes and workshops for improved diagnostic methods will also contribute to our understanding of fish disease. This chapter will consider the status of emerging infectious and non-infectious diseases of cultured finfish. Risk to susceptible stock and their local or global impact, clinical signs, diagnostic techniques and means of prevention will be discussed.

**Infectious Pancreatic Necrosis Virus in Marine-reared Atlantic Salmon**

Infectious pancreatic necrosis (IPN) is a significant disease of salmonid fish primarily occurring in fry and young fish in fresh water. However since the late 1980s there has been an increasing number of reports of clinical IPN and mortality in Atlantic salmon post-smolts from Norway and Scotland (Smail et al., 1992, 1995; Jarp et al., 1994). In addition, farmed turbot and halibut are susceptible (Novoa et al., 1995; Wood et al., 1996). The reason for this trend, particularly in salmon smolts, is not fully understood. Infectious pancreatic necrosis virus (IPNV) is an infectious agent of freshwater fry and is discussed in greater detail in Chapter 4.

**Impact**

Currently there is a low incidence of IPNV in freshwater salmon farms and this has been achieved in part though a successful programme of broodstock testing, the destruction of infected eggs, surface disinfection of ova and other statutory measures. However, the emergence of IPN in the marine environment is having an increasing impact on the industries of Scotland and Norway. The source of the virus is speculative, and could include the transfer of infected fish from freshwater, strain differences emerging in certain areas, failure of fish to adapt to seawater and expression of low level virus or a wild reservoir. With respect to the latter, IPNV has been isolated from wild sea trout. IPNV is stable for long periods in sea and brackish waters and this could also have an impact on seawater outbreaks. At present, one serotype (Sp) appears to be responsible for the outbreaks in Scottish waters, but pathogenic and non-pathogenic forms are indistinguishable using currently available methodology. Therefore, it is difficult to determine the origin of the marine isolates. Stress may contribute to acute IPNV in marine fish, and its emergence in post-smolts would support this theory. The losses from cage sites around the Shetland Isles and in Norway in the Hitra/Frøya region are higher than the other regions where salmon farming is also concentrated. The Shetland Isles and certain regions along the Norwegian coast have a lower water temperature at smolt transfer, which is possibly a factor in the emergence...
of IPN. In Norway, 90% of the losses in 1998 were attributed to IPNV, amounting to 8.3 million individual fish (T.T. Poppe, 1999, personal communication). This situation may be compounded where there is mixing of smolts of different origins, and this has been shown to increase the risk of fish developing IPN. In the UK direct losses in 1998 were estimated at £1 million, although in terms of economic loss to the industry this is higher and around £5–10 million.

Clinical signs

Evidence of clinical IPN begins around 6–8 weeks post-transfer in the UK, with mortalities around 3–4 weeks later. A focal necrosis of the pancreatic acinar tissue with acute enteritis, necrosis and sloughing of the intestinal epithelium is recorded. Eventually the whole pancreas may be destroyed (Fig. 9.1). These lesions may extend into the gut mucosa and renal haematopoietic elements. Infection with IPNV affects feed intake and growth in Atlantic salmon (Damsgård et al., 1998). However, Smail et al. (1995) suggested the digestive functions of the pancreas were not impaired, although the condition factor was reduced. In general, titres to IPNV in older experimentally infected fish decline with age and a small proportion of older fish will remain carriers (D.A. Small, 2000, personal communication).

Diagnosis and control

The diagnosis of IPNV from fish in the marine environment is the same as described for freshwater fish and therefore based on clinical signs, the examination of stained histological sections and the isolation of the virus by cell culture from the kidney and other tissues (Chapter 4). In 1995, the first recombinant IPN vaccine was introduced in Norway and in 1999 an inactivated vaccine became available; both have contributed to the control of IPN.

Fig. 9.1. Infectious pancreatic necrosis virus (IPNV) pathology showing a focal necrosis of the pancreatic acinar tissue with acute enteritis, necrosis and sloughing of the intestinal epithelium.
Future studies

The emergence of IPN in farmed marine fish is an increasing problem for the salmon industry in Scotland and Norway. The examination of the virus genome using IPN strains from these fish will help to establish whether a genetic basis or virus virulence exists. Additional information from the host, environment and husbandry factors will also have to be considered, as well as selecting for genetic IPNV resistance.

Infectious Salmon Anaemia Virus

Infectious salmon anaemia virus (ISAV) is a significant viral disease of Atlantic salmon. The disease is discussed in Chapter 3 but is considered here as an emerging disease for salmon. High losses occurring within the Atlantic salmon farming industries of Norway, Canada, Scotland and Maine, USA, have been documented (Thorud and Djupvik, 1988; Mullins et al., 1998; Stagg et al., 1999; Bouchard et al., 2001). Clinically infected fish frequently show anaemia, often with a dark-coloured liver, ascites and petechiae on the caeca. Culture of the virus in a head kidney cell line and arrangement of the genome has demonstrated that ISAV is an enveloped RNA virus and typical of Orthomyxoviridae (Mjaaland et al., 1997).

ISAV was first recorded in farmed Atlantic salmon in November 1984 along the Southwest coast of Norway and designated ‘Bremmes syndrome’ after the region in Norway where it was first recorded; consequently ISAV was made a notifiable disease in 1988. The 1986/87 outbreaks occurred in smolts and adult salmon in Norway over a wider area. Further outbreaks spread to adjacent areas, particularly those near processing plants and receiving infected fish. Currently, the number of infected farms has been reduced in Norway, but eradication has not been achieved. All outbreaks have been confined to seawater sites.

Towards the end of the summer of 1996 in eastern Canada (New Brunswick, Bay of Funday), rapidly rising mortalities in farmed Atlantic salmon resulted in the description of a condition named ‘haemorrhagic kidney syndrome’ (HKS) (Byrne et al., 1998; Mullins et al., 1998). On the basis of histopathological lesions including necrosis of kidney tubules and associated interstitial haemorrhage, the lesions were considered pathognomonic. Later, ISAV was determined to be the cause of HKS in Canada (Bouchard et al., 1999; Lovely et al., 1999).

In the UK, primary legislation regarding fish diseases is under the Diseases of Fish Acts 1937 and 1983, and Council Directives 91/67/EEC and 93/53/EEC, and are transposed under regulations made under this legislation. ISA is a List I of Annex A to Directive 91/67/EEC and notifiable in the UK since 1990. In May 1998, ISA was diagnosed in salmon farms on the west coast of Scotland at Loch Nevis and Loch Snizort (Bricknell et al., 1998; Rodger et al., 1998). The outbreak represented the first report of ISA within the European Community. During August 1998 the first case of ISAV was confirmed in the Shetland Isles, Scotland, and further outbreaks confirmed on mainland sites. At present, 11 outbreaks have been confirmed in Scotland, with no new cases since November 1999.

ISA was reported in Atlantic salmon reared in the Faroe Islands, Denmark (Anon., 2000) and these fish stocks were destroyed. Other outbreaks were reported in 2001 with variable mortality. The first documented appearance of ISA at an Atlantic salmon farm in Maine, USA has also been documented (Bouchard et al., 2001).

Recently, a report of ISAV from farmed coho salmon in Chile causing high mortality was published (Kibenge et al., 2001). This work highlighted similarities to the Canadian isolates and demonstrated that coho salmon were susceptible to ISAV.

Characterization

Filtered liver homogenate used to challenge experimental fish established the cause of ISA as an infectious virus. Instability of
the homogenate and infected plasma when exposed to diethyl ether and chloroform suggested the presence of enveloped and pleomorphic spherical virions (Christie et al., 1993; Dannevig et al., 1995). Electron microscopy studies on the Norwegian isolates revealed the virus as a single-stranded RNA virus, which was enveloped, with a typical diameter of 100–140 nm (Hovland et al., 1994; Dannevig et al., 1995; Falk et al., 1997). The multi-segment genome consists of eight segments of RNA ranging from 1.0 to 2.3 kb with a total molecular size of approximately 14.5 kb (Mjaaland et al., 1997). ISAV contains four major structural polypeptides with estimated molecular sizes of 71, 53, 43 and 24 kDa (Falk et al., 1997).

Impact

During the spring of 1985, increasing mortality in Norwegian hatchery salmon resulted in 80% losses over several months (Thorud and Djupvik, 1988). Other reports suggest mortality may range between 15 and 100% (Thorud, 1991). During 1997, in the Bay of Fundy, Canada, a loss of $20 million in reduced growth and fish losses was reported. Mortality increased from 3 to 33% per week, despite low temperatures (S.M. Jones, 1998, personal communication).

The Loch Nevis site on the west coast of Scotland was stocked with approximately 1.8 million photoperiod-manipulated S2 smolts during October and November 1997 and mortality changed from a low level to extreme losses of 25% of the total stock within one week (Turnbull, 1999). This apparent variable mortality may be related to a long incubation period, variation in the nature and quantity of the virus, age of the fish, water temperature, patchy expression of disease among fish in sea cages and the sudden rise in mortality without obvious morbidity.

Blood tests confirm the severe anaemia is due to a reduction in haematocrit, haemoglobin and erythrocyte diameter. Circulating erythrocytes show fragmentation, vacuolation and nuclear fragmentation, particularly in the acute phase of the disease. Falk et al. (1997) found ISAV agglutinated salmon and trout erythrocytes, concurrent with an increased osmotic fragility of the red cells with nuclear disintegration and vacuolation of the cytoplasm. Infected salmon show an increase in plasma glutathione (GSH), although levels in the liver are significantly lower (Hjeltnes et al., 1992). A decrease in hepatic GSH may affect transformation and excretion of xenobiotics whereas the raised levels in the plasma may result from leakage of lysed cells (Hjeltnes et al., 1992).

Transmission

Horizontal transmission is considered the main route of infection in farmed fish. The gill lamella and pillar cells are the probable portals of entry for ISAV. Endothelial cells of blood vessels are initial sites of infection following experimental intraperitoneal challenge and it has also been established that urine, faeces and skin mucus of injected salmon smolts contained sufficient virus to establish further infection, suggesting that virus was adsorbed with high affinity to mucus (Totland et al., 1996). Similarly, brown trout have been shown to transmit virus by cohabitation with salmon (Rolland and Nylund, 1998) demonstrating that shedding of ISAV occurs. Recently, it was also demonstrated that Arctic char (Salvelinus alpinus) showed experimental resistance to ISA disease (Snow et al., 2001). Survival of ISAV in seawater was examined by Nylund et al. (1994), who showed that infectivity in sonicated blood occurred for at least 20 h at 6°C and for 4 days in tissue samples. ISAV binds to blood cells and replication of the virus occurs in less than 7 days (Dannevig et al., 1994). Vertical transmission within the contents or surface of the gametes has not been demonstrated (Melville and Griffiths, 1999).

High mortality among Atlantic salmon could indicate that this virus is a newly introduced pathogen to this species (Nylund...
et al., 1997), and salmonids in other regions of the world must be considered at risk. It is noteworthy that the origin of ISA has not been identified (Nylund et al., 1995; Fisheries Research Services, 1999).

Clinical signs and gross pathological changes

Affected fish stop feeding, are lethargic and have problems maintaining a horizontal position in the netpen. They eventually sink to the bottom of the cage. Occasionally, some fish with hyperactive behaviour and presumably nervous movements have been observed. Externally affected fish may show gill pallor, ocular haemorrhage, exophthalmos (chronic phase), slight abdominal distension with occasional scale oedema and haemorrhage. A transient drop in haematocrit is recorded (Snow et al., 2001) correlated with a drop in total plasma protein (Simko et al., 2001).

The presence of ascites and an often dark-coloured enlarged liver, ranging from deep red to black or ‘nutmeg’ in appearance, is noted and attributed to haemorrhage in the parenchyma (Fig. 9.2). The dark liver is usually accompanied by extreme anaemia with a haematocrit of 1–5%. Typically the gills and heart are pale, with punctate haemorrhage noted in the perivisceral fat (Evensen et al., 1991). Splenomegaly is noted and the organ is darker than normal. Lesions in this organ and the kidney are characterized by congestion. Within the foregut, congestion can be evident (Evensen et al., 1991). The content of the alimentary tract is sparse, often with an increase in mucus, possibly corresponding to the absence of food. In tank experiments, sloughed haemorrhagic faecal casts have been noted.

Histopathology

A typical multifocal, haemorrhagic hepatic necrosis is evident (Evensen et al., 1991). Early changes include moderate congestion and a tendency towards dilatation of the sinusoids, followed by marked congestion (Evensen et al., 1991) and rupture of the sinusoidal endothelium with increased erythrophagocytosis. Zonal degeneration of hepatocytes results in a multifocal confluent haemorrhagic necrosis. An anastomosing, ‘bridging’ necrosis typically leaves the parenchyma around the veins intact (Fig. 9.3). Hepatocytes may become swollen with pyknotic nuclei, degeneration and necrosis. The dominant cells in these liver lesions are erythrocytes and necrotic hepatocytes. There is little evidence of

Fig. 9.2. Atlantic salmon infected with ISAV. Clear ascites, dark enlarged liver and splenomegaly are shown.
an increase in inflammatory cells. In the kidney, interstitial haemorrhage, necrosis, tubular degeneration and trapping of erythrocytes have been observed, contributing to a circulatory disturbance. Frank haemorrhage and desquamation of the endothelial cells may follow a marked congestion and bleeding in the lamina propria of the foregut. In experimental studies, a sudden depletion of liver glycogen has been recorded between days 14 and 18 post-infection (Speilberg et al., 1995).

Ultrastructural lesions

Electron microscopy studies show that this virus may be found in all tissues, but the primary target is the vascular endothelium (Hovland et al., 1994; Totland et al., 1996). The presence of intact virus particles within polymorphonuclear leucocytes suggests these are also target cells (Nylund et al., 1995). A protective immune response occurs in Atlantic salmon surviving an infection. Fish that received ISA-convalescent antisera were protected against ISA, suggesting that humoral factors may be important in a successful immune response (Falk and Dannevig, 1995). In the early stage of infection, viral particles are detected exclusively in the pillar cells of the gills and heart endocardial cells (Nylund et al., 1996; Totland et al., 1996; Koren and Nylund, 1997). Particles form clusters at the surface of endocardial cells with assembly of the virions occurring at the site of budding. Later stages of the budding process are as particles connected to the plasma membrane through a short stalk (Koren and Nylund, 1997). In experimentally infected Atlantic salmon post-smolts, Speilberg et al. (1995) noted large membrane-bound vacuoles accumulated in the cytoplasm of perisinusoidal macrophages (PSMs), causing an increase in cell size and blocking of the sinusoidal lumen.

Diagnostic techniques

The first isolation of ISAV was made in 1995 in a new Atlantic salmon head kidney (SHK-1) cell line (Dannevig et al., 1995).
The authors indicated that cell-associated ISAV infectivity could be demonstrated after 3 days following infection with a positively infected tissue homogenate. After 7 days, cell and medium infectivity was demonstrated. Sommer and Mennen (1996) showed that macrophages from SHK-1 cell lines released infectious ISAV after 18 days in culture. Optimal replication of ISAV occurs at 15°C, with replication significantly reduced at 20°C and not observed at 25°C (Falk et al., 1997). The screening of kidney cell cultures and tissue sections has been used to locate ISAV (Falk and Dannevig, 1995) with a monoclonal antibody (mAb, 3H6F8) (Dannevig et al., 1995; Falk et al., 1998). Titration of the mAb showed strong reactivity of infected cell cultures at 1:1000 without loss of fluorescence (Falk et al., 1998). However, the mAb failed to react with viral polypeptides in Western blots under reducing and non-reducing conditions, suggesting the antigenic determinant recognized by the mAb is a conformational-dependent epitope. A new cell line designated TO, derived from head kidney leucocytes, in contrast to SHK-1, provides a high yield of virus and has the potential for use in diagnostic investigations as well as for antigen production (Wergeland and Jakobsen, 2001).

In Scotland, an immunofluorescent kidney imprint test for ISAV has been used with a strong fluorescence in infected haematopoietic cells, tubules and glomeruli from kidney tissue (Stagg et al., 1999). Imprints that are positive for ISAV consist of cells with red nuclei with fluorescent green staining of the cytoplasm (Falk et al., 1997). The staining of the cytoplasm is described as granular; however, epinuclear staining may also be seen as spider-web staining in which the cytoplasmic membrane fluoresces especially where it touches adjacent cells. Negative slides have red nuclei but do not have any specific fluorescence (Stagg et al., 1999).

Diagnosis using reverse transcriptase–polymerase chain reaction (RT–PCR) provides additional confirmatory data on ISAV. The virus contains eight RNA segments, and RNA extracted from kidney can be reverse-transcribed into complementary DNA (cDNA) and the cDNA used as a template for PCR (Mjaaland et al., 1997). The presence of ISAV can be shown by amplification of a 155 base pair fragment corresponding to part of segment 8 as described by Mjaaland et al. (1997). PCR products can be sequenced directly or from clones, and nucleotide sequences from Scottish isolates of ISAV have been compared with those from Norway to confirm that the PCR product was indeed from ISAV and was not non-specific amplification of fish RNA. The complete sequences of segments 2 and 8 of Scottish ISAV have been submitted to the EMBL nucleotide database under accession numbers AJ242808 and AJ242016, respectively (Cunningham and Snow, 2000).

**Risk factors**

Seawater is the major route of transmission of ISAV to salmonid net pen sites in Norway. Furthermore, the proximity of stock to slaughterhouses, processing plants and ISA-positive sites has been highlighted as critical for the spread of infection. Vågsholm et al. (1994) noted that the location of sea sites in relation to other Norwegian ISA-positive sites was a significant risk factor; however, the actual distances between the farms were not recorded. In Scotland, outbreaks of ISA have been linked with movements of fish prior to confirmed outbreaks and tentatively also with farm practices involving the transport of fish via well-boats (Fisheries Research Services, 1999). There is circumstantial evidence that the increase in cage size and hence large farm capacity, which is now common practice in the industry, might offer this virus the potential to multiply such that clinical outbreaks occur. In Canada, the rapid spread of ISA among east coast operations could be linked to the natural strong tidal movements in the area (S.M. Jones, personal communication). Horizontal transmission has been demonstrated in cohabitation experiments (Thord and Djupvik, 1988), supporting the evidence
that waterborne transmission is important for the spread of ISA.

In Scotland, control zones are imposed on suspect/confirmed sites. These are based upon tidal excursion data. Jarp and Karlsen (1997) performed a matched case–control study of ISA risk factors in Norwegian salmonid sea sites. A location within 5 km of a salmonid slaughterhouse gave an ISA ratio of 13:0 compared with a location further away. The risk of infection increased to 8:0 if the site was situated closer than 5 km to another ISA-positive site as compared with the risk when the site was more than 5 km away. The disinfection of waste water from slaughtering and processing plants seemed to prevent transmission of ISA. Outbreaks of ISA were associated with the number of hatcheries delivering smolts to the sea sites, and the risk increased if the hatcheries were located outside the site’s home county. ISA is mainly transmitted from infected salmonid sources to clean sites through seawater, which strongly contributed to the outbreaks of ISA in Scottish waters.

The occurrence of ISA in Canada resulted in a review of Atlantic salmon-producing countries (Stewart, 1998). It was concluded that fallowing and year class separation when coupled with single bay or single area management agreements were effective additions to health care. However, the benefits from these measures were only fully realized when combined with a basic set of sound farming practices. Stagg et al. (2001) recently produced an epizootiological report into the outbreak of ISA in Scotland. The first evidence of increased survival following experimental ISAV infection in vaccinated freshwater-reared salmon was demonstrated by Jones et al. (1999).

**Nodaviruses and Nodavirus-like Viruses**

Nodaviruses and nodavirus-like viruses have been recognized as significant pathogens of marine finfish worldwide. This group is responsible for an infectious neuropathogenic condition described as vacuolating viral encephalopathy and retinopathy, viral nervous necrosis (VNN), encephalopathy and retinopathy (VER), fish encephalitis (FEW), seabass viral encephalitis (SVE), piscine neuropathy nodavirus (PNN) and striped jack nervous necrosis virus (SJNNV). Significant losses are reported in juvenile and adult fish and for many fish species, and the Nodaviridae are considered to cause the most economically important emerging viral diseases. The range of susceptible cage-cultured marine fish species has increased rapidly with the progression of nodavirus infections (Comps et al., 1994; Muroga, 1995; Nakai et al., 1995; Castric, 1997). Nodaviruses and nodavirus-like viruses are spreading into areas including the Indo-Pacific region, the Mediterranean, France and Scandinavia (reviewed by Munday and Nakai, 1997). Despite a broad range of susceptible fish hosts, the consistent neuropathology associated with nodavirus infection has resulted in the proposed generic term, piscine neuropathy nodavirus (PNN) (Frerichs et al., 1996). The Office International des Epizootes (OIE, 1997) refers to the term viral encephalopathy (VER).

**Characterization**

The family Nodaviridae contains two genera, and the Betanodavirus genera infect fish (van Regenmortel et al., 2000). PNN and related viruses are the cause of viral nervous necrosis, and they belong to the family Nodaviridae based on biochemical characterization of the viral nucleic acid (Mori et al., 1992; Munday et al., 1994; Frerichs et al., 1996). Electron microscopy studies show an isometric, non-enveloped virus measuring 25–34 nm in diameter. The virus is primarily found within inclusions or in the cytoplasm of cells in the brain, spinal cord and retina of infected fish (Mori et al., 1991; Frerichs et al., 1996; Jung et al., 1996; Munday and Nakai, 1997). The virions form crystalline arrays and are packed in membranous structures (Fig. 9.4). Two pieces of single-stranded RNA make up the genome with molecular weights of
$1.056 \times 10^6$ and $0.495 \times 10^6$ Da, respectively (Breuil et al., 1991; Mori et al., 1992). Present evidence suggests that there is more than one viral agent or strain, as Nguyen et al. (1994) found differences in the antigens in the coat protein gene (RNA2) (Arimoto et al., 1992, 1993; Delsert et al. 1997). SJNNV and other fish nodavirus genotypes share a significant number of common antigenic determinants, although SJNNV is clearly distinguishable (Nishizawa et al., 1999). The occurrence of two distinct isolates of nodavirus infecting seabass, *Dicentrarchus labrax*, in the Atlantic and the Mediterranean coasts of France (Thiéry et al., 1999) that have a distinct genotype may suggest the absence of a species barrier. The SJNNV coat protein has been sequenced and compared with four known insect nodavirus and other fish nodaviruses causing VNN (Nishizawa et al., 1995a). Their results indicated that the fish nodaviruses that cause VNN are related, but are clearly distinct from the insect nodaviruses. However, the nodavirus isolated from barramundi, *Lates calcarifer*, larvae of striped jack (*Pseudocaranx dentex*) and VNN from Japanese flounder (*Paralichthys olivaceus*) and striped jack were suggested as antigenetically related (Munday et al., 1994; Nguyen et al., 1994). A new genus, *Piscinodavirus*, has been proposed to incorporate fish nodavirus (Nishizawa et al., 1995a; Delsert et al., 1997).

**Impact**

Throughout the 1990s, a number of significant or mass mortalities attributed to PNN affected larval, juvenile and sometimes adult farmed marine fish (Boonyaratpalin et al., 1996; Bovo et al., 1996; Le Breton et al., 1997). The fish species susceptible to PNN or picornavirus-like
infections are listed in Table 9.1. In hatchery-reared Japanese flounder, Nguyen *et al.* (1994) reported total loss at two sites. Similarly, Yoshikoshi and Inoue (1990) reported similar losses in the Japanese parrotfish, *Oplegnathus fasciatus*. In one outbreak in Japan, the mortality was 80% in juvenile redspotted grouper, *Epinephelus akaara* (Mori *et al.*, 1991). Furthermore, in 1990 no juvenile stocks of striped jack were reared, with losses of 400 million hatched larvae due to repeated outbreaks of VNN at two aquaculture facilities (Arimoto *et al.*, 1993). Mortality among farmed seabass at different locations in Greece has been reported to vary between 11 and 60% (Le Breton *et al.*, 1997). In Norway, mortality close to 100% has occurred in juvenile farmed Atlantic halibut, *Hippoglossus hippoglossus*, during the summer months (Grotmol *et al.*, 1995, 1997a). Recently, Aspehaug *et al.* (1999) reported VNN in adult and mature halibut.

The absence of specific histopathological changes or positive fluorescent signal in the gills of infected striped jack larvae suggest this site was not a portal for entry or an initial site for viral multiplication (Nguyen *et al.*, 1996). The authors proposed a specific neurotropism by the virus that might indicate entry to the host via sensory or motor nerve cells linked to the epithelium. However, Grotmol *et al.* (1997a) observed virus-like particles in the pillar cells of halibut indicating the gill epithelium could be a site of entry, although it is possible that virus particles were transported via the capillary network. Recently, Skliris and Richards (1999) demonstrated that marine-reared tilapia, *Oreochromis mossambicus*, could be experimentally infected with a naturally recovered nodavirus isolate and

**Table 9.1.** Fish species susceptible to piscine neuropathy nodavirus (PNN) or picornavirus-like infections.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Species</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic halibut</td>
<td><em>Hippoglossus hippoglossus</em></td>
<td>Norway</td>
<td>Grotmol <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>Atlantic salmon</td>
<td><em>Salmo salar</em></td>
<td>Norway</td>
<td>Castric (1997); Grotmol <em>et al.</em> (1997b)</td>
</tr>
<tr>
<td>Barramundi</td>
<td><em>Lates calcarifer</em></td>
<td>Australia</td>
<td>Glazenko et al. (1990)</td>
</tr>
<tr>
<td>Barfin flounder</td>
<td><em>Verasper moser</em></td>
<td>Japan</td>
<td>Muroga (1990)</td>
</tr>
<tr>
<td>European seabass</td>
<td><em>Dicentrarchus labrax</em></td>
<td>Greece</td>
<td>Le Breton <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>Greasy grouper</td>
<td><em>Epinephelus tauvina</em></td>
<td>Singapore</td>
<td>Chew-Lim <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>Humpback grouper</td>
<td><em>Cromileptes altivelis</em></td>
<td>Indonesia</td>
<td>Zafar <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>Japanese parrotfish</td>
<td><em>Oplegnathus fasciatus</em></td>
<td>Japan</td>
<td>Yoshikoshi and Inoue (1990)</td>
</tr>
<tr>
<td>Japanese flounder</td>
<td><em>Paralichthys olivaceus</em></td>
<td>Japan</td>
<td>Nguyen <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>Kelp grouper</td>
<td><em>Epinephelus moara</em></td>
<td>Japan</td>
<td>Nakai <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>Purplish amberjack</td>
<td><em>Seriola aurata</em></td>
<td>Japan</td>
<td>Muroga (1995)</td>
</tr>
<tr>
<td>Redspotted grouper</td>
<td><em>Epinephelus akaara</em></td>
<td>Japan</td>
<td>Mori <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>Seabass</td>
<td><em>Dicentrarchus labrax</em></td>
<td>Martinique</td>
<td>Bellance and Galett de Saint-Aurin (1988)</td>
</tr>
<tr>
<td>Seabass</td>
<td><em>D. labrax</em></td>
<td>France</td>
<td>Breuil <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>Seabass</td>
<td><em>D. labrax</em></td>
<td>Italy</td>
<td>Bovo <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Sevenband grouper</td>
<td><em>Epinephelus septemfasciatus</em></td>
<td>Japan</td>
<td>Fukuda <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Striped snakehead</td>
<td><em>Channa striatus</em></td>
<td>Greece</td>
<td>Freichs <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Striped jack</td>
<td><em>Pseudocaranx dentex</em></td>
<td>Japan</td>
<td>Nguyen <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>Tiger puffer</td>
<td><em>Takifugu rubripes</em></td>
<td>Japan</td>
<td>Nakai <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>Turbot</td>
<td><em>Scophthalmus maximus</em></td>
<td>Norway</td>
<td>Bloch <em>et al.</em> (1991)</td>
</tr>
<tr>
<td>Yellow grouper</td>
<td><em>Epinephelus awoara</em></td>
<td>Taiwan</td>
<td>Lai <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>Yellowtail</td>
<td><em>Seriola quinquerostrata</em></td>
<td>Japan</td>
<td>Muroga (1995)</td>
</tr>
</tbody>
</table>
suggested the development of a carrier state in this species.

Significant differences in viral pathogenicity have been observed between two nodavirus strains when their natural host species were cross-infected (Totland et al., 1999).

**Clinical signs**

Moribund fish are dark, with abnormal swimming behaviour, which includes a characteristic corkscrew or whirling movement (Mori et al., 1991; Nguyen et al., 1994). PNN is recognized by vacuolation and necrosis of the central nervous tissues and the retina (Fig. 9.5). In Japanese seabass, *Lateolabrax japonicus*, there was conspicuous coagulative necrosis in the brain and spinal cord, with changes in the retina accompanied by formation of intracytoplasmic periodic acid–Schiffs (PAS)-positive inclusions and vacuoles (Jung et al., 1996). The necrotic cells were replaced by spaces. Nervous necrosis was marked in the diencephalon and medulla oblongata of the brain, the ganglion cell layer and nuclear layer in the retina. Vacuolated cells and vacuoles were also present in the bipolar and ganglionic nuclear layer of the brain and retina of natural and experimentally infected juvenile and adult seabass (Breuil et al., 1991; Le Breton et al., 1997; Péducasse et al., 1999). Similar lesions occurred in brain tissue of larval turbot, *Scophthalmus maximus*, larval barramundi and juvenile Japanese flounder with vacuolated cells that contained pleomorphic bodies or membrane-bound viral particles (Glazenbrook et al., 1990; Bloch et al., 1991; Nguyen et al., 1994). Necrosis and vacuolation of the nerve cells were first observed in the spinal cord, particularly just above the swim-bladder of striped jack and then later in the brain and retina (Nguyen et al., 1996). Yoshikoshi and Inoue (1990) noted numerous virus particles in the cytoplasm of neurones and oligodendrocytes forming the myelin sheath of juvenile Japanese parrotfish. Le Breton et al. (1997) described an increase in ocular lesions in adult seabass compared with larvae and juveniles, with necrosis involving the upper jaw.

![Characteristic vacuolation due to piscine neuropathy nodavirus in the retina of juvenile redspotted grouper.](image)

**Fig. 9.5.** Characteristic vacuolation due to piscine neuropathy nodavirus in the retina of juvenile redspotted grouper.
Cardiac myopathy syndrome (CMS) in Atlantic salmon, Salmo salar (Ferguson et al., 1990), has been reported to be associated with a nodavirus-like agent. Grotmol et al. (1997b) initially reported a positive reaction in the endocardium, myocytes with hypercellular lesions after immunolabelling using a primary antibody against SJNNV. Viral replication peaks during the early stages of development in salmon with primary viral replication in the heart. A specific immunolabel is present in the mesothelial lining, hypercellular lesions and necrotic myocytes, and is therefore distinct from the lesions noted by several groups investigating nodavirus infections in non-salmonids (Yoshikoshi and Inoue, 1990; Arimoto et al., 1993; Nguyen et al., 1996; Le Breton et al., 1997). However, Grotmol et al. (1997a) reported that in Atlantic halibut a vacuolating encephalopathy and retinopathy as well as endocardial lesions followed extensive losses in juvenile fish. Grotmol et al. (1997a) suggested virus-associated endocardial lesions may precede infection of the nervous system in halibut and later spread to the eye via the optic nerve.

**Diagnostic techniques**

A presumptive diagnosis of piscine nodavirus is made using light microscopy where there is vacuolating encephalopathy and retinopathy, but generally electron microscopy is considered necessary for confirmation. Arimoto et al. (1993) and Nguyen et al. (1994) also confirmed the characteristic histopathological signs of VNN. Initial attempts to isolate PNN in cell culture were unsuccessful. However, a specific cytopathic effect (CPE) was established in fathead minnow (FHM) and epithelioma papulosum cyprini (EPC) cells lines by Nguyen et al. (1994) and in an SSN-1 cell line derived from a striped snakehead fish by Frerichs et al. (1996). After 3 days of culture, localized rounding, granular, refractive cells were noted, which spread to form a network of degenerating cells before complete disintegration of the monolayer cell culture. Iwamoto et al. (1999) examined 17 isolates of piscine nodavirus for infectivity towards SSN-1 cells. Based on CPE and antigen detection with a fluorescent antibody technique (FAT), these were divided into groups according to their infectivity towards SSN-1 cells. A good correlation was reported between infectiveness towards SSN-1 cells and the coat protein gene genotypes of the isolates. A new fibroblast and epithelial-like cell line from yellow grouper brain (GB) was established to study yellow grouper nervous necrosis virus (YGNV) from yellow grouper, Epinephelus awara, with potential to study other nodaviruses (Lai et al., 2001).

Other methods for detecting the nodavirus include ELISA developed for SJNNV by Arimoto et al. (1992) and immunohistochemistry, used by Grotmol et al. (1997b) to monitor the course of infection in Atlantic halibut. Preliminary results from a study by Breuil and Romestand (1999) indicated that an ELISA screening of serum from brood seabass enabled seropositive and seronegative individuals to be differentiated. This approach will allow animals to be selected as broodstock to reduce or prevent vertical transmission. Nishizawa et al. (1995b) developed an ELISA using monoclonal mAbs to SJNNV and infective larvae as an immunogen. Cloned hybridoma cells reacted in an ELISA test and were reported as recognizing epitopes on the viral coat protein, or the secondary or tertiary structure of the coat protein. Additional specific diagnostic techniques have been developed for VNN including an RT–PCR. This is based on the sequence data of SJNNV coat protein gene (RNA2) from the gonad of adult striped jack at spawning, demonstrating an important source of virus reservoir (Arimoto et al., 1992; Mushiake et al., 1992; Nishizawa et al., 1994). This approach is useful for the detection of the virus not detected using ELISA. PCR amplification has also been used by Nishizawa et al. (1997) to study the phylology of nodaviruses infecting different fish species and is effective for detecting the virus from
asymptomatic spawners of striped jack (Mushiake et al., 1994). Sequence analysis of different nodavirus isolates from seabass allowed Thiéry et al. (1999) to design a new primer set, which amplified the coat protein gene from the Atlantic nodavirus isolates, whereas earlier primers only detected the Mediterranean isolates. Grotmol et al. (2000) also found a significant level of nucleotide sequence identity between RNA2 of the Atlantic halibut nodavirus and SJNNV.

In striped jack, viral antigens are located in the gonads (Nguyen et al., 1997) and in the degenerated nervous tissue in young sevenband grouper, Epinephelus septemfasciatus (Tanaka et al., 1998). There are antigens common to VNN agents in different fish species, which suggests a degree of relatedness between the various viral isolates (Nakai et al., 1994; Nguyen et al., 1994).

**Prevention**

There is no treatment and therefore control depends upon husbandry practices that prevent contact between naive and infected fish within farms and also between different geographical areas. Furthermore, there is a general lack of information on the epizootiology of this group and at present it is unknown if the virus is present on the surface or inside the eggs (Arimoto et al., 1992); however, spawning fish are considered reservoirs (Mushiake et al., 1994). Chi et al. (1998) recorded specific viral particles in grouper fry reared from iodine-disinfected eggs and they concluded that vertical transmission of VNN could occur. There is limited work on the effectiveness of chemical or physical treatments for nodavirus control, although Arimoto et al. (1996) examined ozone, heat, hydrogen ions, ultraviolet (UV) and a range of chemicals. Ozone quickly inactivated SJNNV and heat treatment was effective at 60°C, and Grotmol and Totland (2000) reported that larvae from virus-exposed eggs washed with ozonated seawater developed viral encephalopathy and retinopathy (VER). The virus is inactivated at pH 12 and UV is useful at high intensity. The effective concentrations of sodium, calcium hypochlorite and iodine required to inactivate SJNNV are 50 mg l⁻¹ for 10 min. Arimoto et al. (1996) concluded that treatment of seawater with ozone reduced the occurrence of the virus, and washing eggs in seawater also delayed the onset of viral infection as determined using ELISA. Mushiake et al. (1994) reported that PCR-based elimination of virus-positive striped jack broodstock prior to spawning is useful for controlling VNN in the larvae. The study revealed the RNA2 gene could be detected in the gonads and eggs, and the elimination of these fish prevented disease in the larvae.

Le Breton et al. (1997) confirmed VNN in the bipolar and ganglionic layer of the retina and the telencephalon, cerebellum and diencephalon of adult seabass using a peroxidase reaction.

**Recommendations**

An increasing number of commercially important marine fish reared in cage culture are apparently susceptible to nodavirus infection and this has resulted in extensive, if not total, losses during outbreaks. Avoidance of the virus is a prime objective, although vertical transmission of the virus will make control measures difficult. The detection and elimination of carriers may be practical using sensitive PCR techniques, thus offering a degree of confidence in choosing virus-free broodstock. Characterization of the viruses from different geographical areas will assist in determining relatedness, and this may be important in developing a vaccination strategy.

**Fish Mycobacteriosis**

Fish tuberculosis or mycobacteriosis is a systemic disease caused by Mycobacterium spp. Periodic outbreaks contribute to a chronic mortality in marine and freshwater fish species, which include farmed Pacific...
salmon, yellowtail, gilthead seabream, seabass, striped bass, red drum and tilapia (Nigrelli and Vogel, 1963; Arakawa and Fryer, 1984). Infection may involve one of the main species associated with disease in fish stocks: *Mycobacterium marinum, Mycobacterium chelonae* or *Mycobacterium fortuitum*. Disease signs include listlessness, skin discoloration, distended abdomen and exophthalmos, with lateral ulcers and scale loss being prevalent in progressive infections (Gómez et al., 1993). Internally, variable sized greyish-white lesions are associated with the spleen, kidney or liver. Outbreaks involving *M. chelonae* are rare in farmed Atlantic salmon and in the context of this chapter are classified as sporadic.

Infection in Atlantic salmon

During periods of low water temperature, *M. chelonae* was isolated from farmed Atlantic salmon in the Shetland Isles, Scotland, and associated with significant losses (Bruno et al., 1998a). Moribund fish (2–3 kg) were dark with slight abdominal distension.

Other clinical signs are splenomegaly, ascites and the gills are pale with occasional blood flecks in the musculature. Multiple, grouped or single, greyish-white miliary granuloma-like nodules occur throughout the enlarged kidney. Smaller granulomatous-like lesions are also within the spleen and liver (Fig. 9.6). Tissues examined using light microscopy show the formation of granulomatous lesions with various stages of necrosis, sometimes involving a fibrous response. Abundant, acid-fast, rod-shaped bacteria in densely packed nodules are found in the kidney and liver with no surrounding capsule, fibrin deposition or central necrosis (Fig. 9.7). Circular, smooth, pale cream colonies were isolated on conventional media (e.g. TSA), although other species require a more defined media. Disease outbreaks in this case primarily in fish reared in warmer waters may also occur in cold sea temperatures (Bruno et al., 1998a). We are placing salmon mycobacteriosis into a sporadic or emerging category until it becomes problematic.

Winter Ulcers – *Vibrio viscosus* (*Moritella viscosa*)

Winter ulcers first emerged as a new problem for the aquaculture industry 18 years ago in marine-reared Atlantic salmon in Norway (Lunder, 1992; Salte et al., 1994).

![Multiple, miliary granuloma-like nodules attributed to *Mycobacterium chelonae* throughout the enlarged kidney. Smaller granulomatous-like lesions are also present in the spleen.](image.png)
The term ‘winter ulcers’ relates to the lateral lesions and the occurrence of the condition during cold water temperatures (Salte et al., 1994). The condition is now known to be associated with a psychrotrophic Vibrio sp. On the basis of infrequent records, this disease is currently considered sporadic. Vibriosis due to Listonella anguillarum and Vibrio salmonicida is discussed in Chapter 3.

Characterization

Outbreaks have been recorded from marine-cultured Atlantic salmon in Norway, Iceland and Scotland (Salte et al., 1994; Benediksdottir et al., 1998; Bruno et al., 1998b; Laidler et al., 1999). The bacterium is a short, curved, motile, Gram-negative rod, which is fermentative, and oxidase and catalase positive (Lunder et al., 1995; Bruno et al., 1998b). Two new species have been described, Vibrio viscous and Vibrio wodanis (Lunder et al., 2000), but it is now proposed to reclassify them as Moritella viscosa comb. nov. (Benediksdottir et al., 2000).

Impact

Outbreaks occur in Atlantic salmon, and Lunder et al. (1995) reported mortality of less than 10% in an outbreak, although in some cases up to 50% of the population might be affected at slaughter (Lunder, 1992). In Scotland, recorded total fish losses in one farm amounted to 2.5% over 4 months (Bruno et al., 1998b). There is some laboratory evidence that outbreaks are increasing (D.W. Bruno, unpublished results).

Clinical signs

Infected fish are dark in colour and show gill anaemia, with exophthalmos but no apparent haemorrhage in the eye chamber.

Fig. 9.7. Abundant Mycobacterium chelonae in densely packed nodules in the liver. There is no surrounding capsule, fibrin deposition or central necrosis.
Circular or oval, epidermal ulcers occur on scale-covered areas. Ulcers occur at various sites over the body and range from superficial to the exposure of the stratum compactum and the musculature. Internally, there are dark-brown, almost black, areas across the uncut surface of the normal or pale-coloured liver, in some cases confined to dark petechiae or ecchymotic haemorrhage 1 mm in diameter, whereas in other fish larger areas of haemorrhage were recorded (Fig. 9.8). Splenomegaly is common, occasionally with ascites within the abdominal cavity.

A moderate to marked congestion occurs in the liver resulting in the formation of blood-filled cavities. There is little evidence of inflammation and the hepatocyte matrix is generally intact with only scattered single-cell necrosis. Minor skin ulcers show subepidermal infiltration, a lack of cellular reaction and some patchy necrosis. More severe lesions are associated with increased numbers of inflammatory cells and localized oedema (Bruno et al., 1998b). The spleen is congested, with associated necrosis resulting in the destruction of large areas in moribund fish. Necrosis of the glomeruli and tubule degeneration are apparent in the kidney, with scattered hypertrophic cells.

**Diagnostic techniques**

*M. viscosa* (*V. viscosus*) can be isolated between 4 and 15 days following incubation at 15°C on tryptone soya agar with 2% NaCl (TSA + NaCl) with 10% horse blood or modified Anacker and Ordals medium.

**Piscirickettsiosis and *Pisrickettsia*-like Organisms**

*Piscirickettsia salmonis* is recognized as the cause of an emerging systemic disease of farmed marine trout and salmon, and follows from the first outbreak to impact on aquaculture in southern Chile during 1989 (Bravo and Campos, 1989; García et al., 1991). A rickettsia was isolated on a chinook salmon (*Oncorhynchus tshawytscha*) embryo cell line CHSE-214.

![Fig. 9.8. Gross lesion in liver infected with *Vibrio viscosus* showing dark petechiae or ecchymotic haemorrhage 1 mm in diameter, spreading to larger areas of haemorrhage.](image)
and shown to be the cause of epizootics in marine netpen-reared coho salmon (*Oncorhynchus kisutch*). The causative agent, *P. salmonis*, is an obligate bacterium, which causes a serious, systemic infection of salmonids in seawater (Fryer et al., 1992) and is discussed in Chapter 3.

Affected fish are lethargic, dark in colour and anaemic with mottled focal lesions within the liver (Bravo and Campos, 1989).

**Infectious agent**

*P. salmonis* causes an epizootic disease of fish called piscirickettsiosis (Cvitanich et al., 1991), although it has also been known as coho salmon syndrome, Huito disease and salmonid rickettsial septicaemia (SRS). It is an obligate, intracellular, Gram-negative pathogen, predominantly a coccoid, non-motile, often pleomorphic, non-encapsulated organism ranging in size from 0.5 to 1.8 µm in diameter (Cvitanich et al., 1991; Fryer et al., 1992). *P. salmonis* replicates within membrane-bound cytoplasmic vacuoles in selected fish cell lines and in the cells of tissues throughout infected fish. The type species LF-89T, ATCC (R) VR 1361 was recovered from diseased coho salmon in Chile (Fryer et al., 1992).

At present there are no reports of *P. salmonis* published from feral salmonids, although Kent (1992) considered the agent was of marine origin. However, isolation from freshwater cages of coho salmon and trout in Chile suggests its presence in this environment (Gaggero et al., 1995). In seawater the agent survives for several weeks between 5 and 20°C (Lannan and Fryer, 1994). However, one freeze–thaw cycle decreased the TCID₅₀ titre of *P. salmonis* to virtually nil (Fryer et al., 1990).

**Hosts and geographic distribution**

Piscirickettsiosis was initially confined to coho salmon in seawater. However, it is known that all marine-cultured salmonids in southern Chile, including Atlantic, chinook and masou salmon and rainbow trout, are susceptible (Fryer et al., 1990; Lannan and Fryer, 1993). In Norway, *P. salmonis* has been detected in farmed Atlantic salmon (Olsen et al., 1997). Morphologically and serologically similar pathogenic rickettsial organisms have also been reported in British Columbia in farmed chinook, pink, coho and Atlantic salmon in seawater (Brokelbank et al., 1992; Kent, 1992; Jones et al., 1998) and in Ireland and Scotland from Atlantic salmon (Rodger and Drinan, 1993; Grant et al., 1996). In the latter case, outbreaks have been sporadic and apparently limited in their impact. The geographical distribution of *P. salmonis* and related organisms is therefore widespread and all cultured salmonid species are considered susceptible, although coho salmon are the most susceptible. Several reports describing rickettsial infections in non-salmonid finfish have been published. A rickettsia-like organism (RLO) has been identified as the causative agent of an outbreak with mass mortality among pond-reared tilapia in Taiwan (Chern and Chao, 1994). Furthermore, mortalities in juvenile seabass from floating sea cages at 12–15°C have been reported along the French Mediterranean coast (Comps et al., 1996). Similarly, an RLO has been found in blue-eyed plecostomus, *Panaque suttoni*, imported as an ornamental fish from Colombia, Nile tilapia (*Oreochromis niloticus*) and feral dragonet (*Callionymus lyra*) caught off the Welsh coast (UK) (Davies, 1986; Chen et al., 1994; Khoo et al., 1995). Recently, Chen et al., (2000) found a *P. salmonis*-like bacterium associated with mortality of white seabass, *Atractoscion nobilis*.

**Gross pathology**

The clinical signs and gross lesions reported for piscirickettsiosis from natural and experimentally infected fish from Chile include lethargy, anorexia, respiratory distress, darkening of the skin and
swimming near the water surface (Branson and Nieto Díaz Muñoz, 1991; Cvitanich et al., 1991). Skin lesions include perianal and periocular haemorrhage, petechiae on the abdomen and shallow haemorrhagic ulcers, varying between 0.5 and 1.5 cm in diameter. Characteristic ring-shaped, yellow/cream-coloured subcapsular nodules are present throughout the livers of chronically infected fish (Cvitanich et al., 1991). The abdomen is frequently distended and splenomegaly is common with white spots in the skin. Petechiae on the serosa surfaces of the pyloric caeca, swim bladder and intestine have been observed in Atlantic salmon. In other organs, macroscopic changes include ascites, general pallor, diffuse swelling and multifocal pale areas in the kidney and spleen. Additionally, bilateral exophthalmia and an ulcerative inflammatory reaction around the mouth have been described. In Canada and Ireland, a similar gross pathology is reported from RLOs among farmed Atlantic salmon (Brockelbank et al., 1992; Rodger and Drinan, 1993). In acute cases, external lesions may be absent.

**Histopathology**

Histological changes have been classified into the broad category of necrosis and inflammation. Inflammatory cells, fibrosis, a generalized coagulative necrosis, tubular degeneration and necrosis of the endothelium infiltrate the liver, spleen, intestine and haematopoietic cells of the kidney. Moribund fish are anaemic and haematocrit is 50–80% below the normal.

The rickettsial organism infects a variety of cells including circulating macrophages, in which they can replicate and cause cell lysis (Fig. 9.9). The mechanisms by which *P. salmonis* can enter target cells, avoid intracellular killing and survive inside the host are unclear.

**Economic significance**

Piscirickettsiosis has caused substantial economic losses to the salmon aquaculture industry of southern Chile. During 1989, this disease was considered to be the cause of death of an estimated preharvest 1.5 million coho salmon, ranging from 200 g to market-sized fish. In the following year, major losses were also attributed to *P. salmonis* among farmed Atlantic salmon. Mortalities above 90% occurred during the 1989 outbreak and piscirickettsiosis remains a significant problem for this industry (Bravo and Campos, 1989). Mortalities typically develop 10–12 weeks after the transfer of fish to seawater, generally

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**Fig. 9.9.** *Piscirickettsia salmonis* infecting a variety of cells including circulating macrophages, in which they can replicate and cause cell lysis.
occur between March and August, and last approximately 10 weeks before they diminish. Outbreaks of piscirickettsiosis in other countries have not reached the importance and prevalence of the Chilean outbreaks. For example, 0.6–15% mortality has been reported in Canada and Norway (Brockelbank et al., 1992; Olesen et al., 1993). The agents producing these outbreaks elsewhere may not be identical to *P. salmonis* and are typically reported as a related RLO. However, RLOs reported from Canada and Ireland reacted positively with a polyclonal antibody made against *P. salmonis* demonstrating their relatedness (Brockelbank et al., 1993). House et al. (1999) investigated the relative virulence of three isolates of *P. salmonis* for coho salmon. Significant differences in relative virulence between the Chilean type strain (LF-89), a Canadian (ATL-4-91) strain and a Norwegian (NOR-92) strain were demonstrated. Phylogenetic analysis demonstrated that strains from different geographic locations form a tight monophyletic cluster with 16S rDNA similarities ranging from 98.5 to 99.7% (Mauel et al., 1999). However, the isolate EM-90 (cultured from Atlantic salmon, British Columbia, Canada) has apparently diverged genetically from the other *P. salmonis* isolates examined and appears unique.

**Isolation and diagnosis**

A presumptive diagnosis of piscirickettsiosis can be made from gross lesions, and is supported by the examination of tissue sections stained with haematoxylin and eosin (H&E), Gram, Giemsa, PAS, Machiavello and Gieméz (Branson and Nieto Diaz Muñoz, 1991; Lannan and Fryer, 1991). The use of hydrogen peroxide combined with microwave irradiation to remove melanin from sections and smears has been effective with subsequent staining properties unchanged (Larenas et al., 1996a).

A specific diagnosis requires isolation of the causative organism. Kidney tissue from affected fish is aseptically removed, homogenized and inoculated on a cell monolayer with an antibiotic-free growth media. *P. salmonis* has been cultured in six fish cell lines maintained in buffered Eagle’s minimum essential medium (MEM) supplemented with 10% fetal bovine serum (Fryer et al., 1990). Four of these susceptible fish cell lines have been derived from salmonid species, chinook salmon embryo (CHSE-214), chum salmon heart (CHH-1), coho salmon embryo (CSE-119) and rainbow trout gonad (RTG-2). The other cell lines are from warmwater fish species (Almendras and Fuentelsaías, 1997). Optimal *in vitro* growth occurs at 15–18°C, but is retarded above 20°C and below 10°C.

Typically, piscirickettsial growth is determined by the gradual appearance of a CPE in cell monolayers. The first signs of a CPE consist of the formation of cell clusters around 10 days post-inoculation and include cell rounding and the development of one or more large vacuoles within the cytoplasm (Garcés et al., 1991). Inoculated cultures should be observed for up to 28 days before they are considered negative.

An indirect fluorescent antibody technique (IFAT) (Lannan et al., 1991) and immunohistochemistry (Alday-Sanz et al., 1994) have been developed as alternative procedures to detect *P. salmonis*. These latter techniques are faster and more specific than histochemical staining. However, they require additional specialized equipment and are more expensive.

The detection of *P. salmonis* in cultivated salmonids via a nested-PCR using universal 16S rDNA bacterial outer primers and *P. salmonis* internal primers have been developed by Mauel et al. (1996). Shortly afterwards, this approach was refined by Marshall et al. (1998), who directed the assay towards a more variable region of the rDNA operon. The development of several monoclonal antibodies against *P. salmonis* and their evaluation by ELISA and indirect fluorescence microscopy have shown that they react with several antigens and will be important for diagnosis of this disease (Jameett et al., 2001).
Transmission

Piscirickettsiosis was initially described from fish in the marine environment. Horizontal transmission has been reported in marine-farmed salmon 2 weeks after the introduction of clean fish into infected sites. The extended extracellular survival time of this organism in salt water may be of sufficient duration to permit horizontal transmission without a vector (Lannan and Fryer, 1994). Natural outbreaks of piscirickettsiosis occur a few weeks after smolts are transferred to the sea, suggesting that the oral route of infection might be important. Successful transmission of the disease through cohabitation of healthy and infected coho salmon was achieved by Cvitanich et al. (1991). However, evidence of horizontal transmission in the wild is speculative. Pérez et al. (1998) recorded P. salmonis in asymptomatic migrating coho salmon suggesting they may play a role in the spread of piscirickettsiosis. The possibility of vertical transmission of P. salmonis was postulated by Fryer et al. (1992) in coho salmon, as mortality occurred within 6–12 weeks post-transfer. This was confirmed by Bustos et al. (1994) and Larenas et al. (1996b) who showed that 10% of the fertilized ova from one or both parents with rickettsia were positive for P. salmonis by IFAT after 18 days of incubation. The organism was mainly located inside the yolk. Currently, no alternative host has been identified and the source, reservoir and means of transmission of P. salmonis remain important areas of study.

The first report of P. salmonis in rainbow trout from fresh water was made by Bravo (1994), and later isolated from diseased fish during their freshwater phase (Gaggero et al., 1995). Experimental transmission to freshwater-reared Atlantic salmon is also reported (Almendras and Fuentealba, 1997) and the presence of P. salmonis confirmed using a fluorescent antibody technique. The lesions noted in moribund trout are similar to those observed in saltwater outbreaks of piscirickettsiosis.

Management practices

The occurrence of piscirickettsiosis in a variety of locations and host species suggests that this is an important emerging fish pathogen (Fryer and Lannan, 1996). Outbreaks frequently occur after smolt transfer, but good management practices are effective in reducing outbreaks. Such approaches include the early removal of mortalities and clinically diseased fish with appropriate disposal of blood from harvested fish, reducing fish stocking density and providing periods of site fallowing. Other strategic measures include an examination and diagnosis of infected broodstock, rejection of eggs from positive fish and individual incubation of egg batches.

Chemotherapy

In vitro, P. salmonis is sensitive to a variety of antibiotics including streptomycin, gentamicin, erythromycin, chloramphenicol and oxytetracycline, but shows resistance to penicillin, penicillin G and spectinomycin (Cvitanich et al., 1991; Fryer et al., 1992).

However, the use of medicated feed to control intracellular pathogens including P. salmonis has been unsuccessful (Cvitanich et al., 1991). The intraperitoneal injection of broodstock with antibiotics 30–60 days before spawning and the incorporation of antibiotics into the water during hardening of the eggs after fertilization have been used as preventive measures (Bustos et al., 1994).

Vaccine development

Several research programmes are directed towards developing a vaccine, and the results of an early trial have been published (Smith et al., 1995). During an initial field trial, an immunoprotective effect in vaccinated fish compared with controls was achieved. However, this result is tentative as the natural challenge may have been low and the bacterium causing bacterial kidney
disease, *Renibacterium salmoninarum*, was also detected in the experimental fish. Although commercial vaccines are now available, there is little published information on their efficacy.

**Recommendations**

Piscirickettsiosis was first described in 1989, and under certain conditions the agent causes high mortality in marine-farmed salmon. Further information regarding horizontal and vertical transmission, pathogenesis, intracellular survival and immunogenesis is needed to support future control strategies.

In addition, information on the geographic location and species distribution of *P. salmonis* among isolates and stocks of fish will be helpful in developing management and control strategies in the future.

**Prevention**

Control may be achieved through the use of antibiotics. In addition, Greger and Goodrich (1997, 1999) have developed a vaccine that has shown efficacy following intraperitoneal injection in rainbow trout.

**Cryptobia (Trypanoplasma) salmositica**

Salmonid cryptobiosis is caused by the haemoflagellate *Cryptobia salmositica* (Fig. 9.10). The parasite is an elongated flagellate with a prominent kinetoplast close to its round nucleus. It has two flagella that arise from the anterior end; the anterior flagellum is free while the recurrent flagellum is attached to the body and ends as a free flagellum at the posterior end (Woo, 1994).

*C. salmositica* has been recorded from all Pacific salmon (*Oncorhynchus* spp.) and sculpins (*Cottus* spp.) from California to Alaska. The parasite multiples readily in the blood of salmonids to cause disease/mortality, and the severity of the disease is related directly to the parasitaemia. It is not known to cause disease in sculpins and consequently these are reservoir hosts for the pathogen. Freshwater leeches (*Piscicola salmositica*) normally transmit the parasite in streams and rivers (Woo and Poynton, 1995). Direct transmission from infected to uninfected fish occurs when fish are held in the same tank with or without direct physical contact (Woo and Wehnert, 1983) or when fish are brought together in nets during transfer or during weighing (Bower and Margolis, 1983).

**Impact**

The parasite is a recognized pathogen in semi-natural and intensive salmon culture facilities on the Pacific coast of North America (Bower and Thompson, 1987). However, the overall impact of cryptobiosis on feral fish populations is not known. There have been sporadic outbreaks of the disease in hatcheries, and an annual 50% mortality of spring chinook salmon brood stock (*O. tshawytscha*) in a hatchery in Washington, USA (Woo, 1998).

In 1997 the parasite caused significant morbidity and mortality in smolts and pre-harvest chinook salmon in sea cages in a hatchery on Vancouver Island, Canada. There was a small mortality spike (about 1%) in post-smolts in the first 10–15 weeks after transfer to salt water. Re-emergence of the disease as a significant cause of morbidity and mortality occurred later in preharvest fish (P.T.K. Woo, unpublished results). According to the hatchery management, the outbreak was confined to fish exposed to unfiltered surface water and did not appear to be linked to handling. Also, mortality seemed to be associated with age and major stressors such as marine mammal harassment.

In early 2001, another outbreak occurred in preharvest chinook salmon (weighing about 3 kg) in sea cages in the same hatchery. *Cryptobia* were in large numbers in the blood and ascites fluid of moribund fish, and clinical signs (e.g. exophthalmia, anaemia, anorexia) were...
evident in many fish. Fish mortality varied between cages (e.g. 3.3% in one cage and 24.9% in another); they were transferred to sea cages in August–September 1999, and the parasite was detected in the blood of some fish while they were in fresh water in the hatchery. Parasites from moribund fish were morphologically similar to *C. salmositica*, and these isolates caused clinical disease in experimentally infected fish. Also, fish vaccinated using the attenuated *C. salmositica* strain (Woo and Li, 1990) were protected against the isolates from the outbreak (P.T.K. Woo, unpublished results). Since *C. salmositica* is normally transmitted indirectly by freshwater leeches, we suggest this outbreak was initiated because of relapse in some infected fish (possibly due to ‘stress’), and the pathogen was later transmitted directly to other fish, e.g. during weighing when fish are brought together in nets. Direct transmission can occur with or without physical contact between infected and uninfected fish (Woo and Wehnert, 1983) and is very efficient in seawater (Bower and Margolis, 1983).

**Clinical signs and gross pathology**

Clinical signs include exophthalmia, general oedema, abdominal distension with ascites, splenomegaly, a microcytic and hypochromic anaemia (Woo, 1979), anorexia (Li and Woo, 1991) and red blood cells that give a positive anti-globulin reaction (Thomas and Woo, 1988). The immune system is depressed during acute disease (Jones *et al.*, 1986), and anorexia contributes to the immunodepression (Thomas and Woo, 1992). Also, infected fish are susceptible to environmental hypoxia because of the anaemia and high parasitaemia (Woo and Wehnert, 1986). Metabolism and swimming performance are significantly reduced (Kumaraguru *et al.*, 1995) and bioenergetic cost of the disease to fish is significant (Beamish *et al.*, 1996).

**Diagnosis**

During acute disease, the parasite can be readily found using wet-mount preparations or Giemsa-stained smears of blood/ascites fluid. In chronic infections, it can be detected using the haematocrit centrifuge technique (Woo and Wehnert, 1983). Serological techniques are available and antibodies against the pathogen can be detected about 2 weeks after infection using either MISET (Woo, 1990) or ELISA (Sitjà-Bobadilla and Woo, 1994). Also, an antigen-capture ELISA has been developed to detect a secreted parasite glycoprotein (74 kDa) in the blood of fish (Verity and Woo, 1996). The test is positive 1 week after infection or vaccination.
Control and treatment

Protective strategies have been developed and they include:

1. Immunization with a live vaccine. 
   *C. salmositica* has been attenuated and used routinely as an experimental vaccine to protect salmonids from cryptobiosis (e.g. Woo and Li, 1990; Sitjà-Bobadilla and Woo, 1994; Li and Woo, 1995, 1997; Staines and Woo, 1997). This acquired immunity lasts at least 2 years, and the protection is due to the production of complement-fixing antibodies, enhanced phagocytosis and cell-mediated cytotoxicity (Li and Woo, 1995).

2. Selective breeding of resistant fish. Some brook char (*Salvelinus fontinalis*) are naturally resistant to *C. salmositica* infection. The resistant factor(s) is controlled by a dominant Mendelian locus and is inherited by progeny of resistant fish (Forward et al., 1995). The parasite is lysed in the blood of resistant char via the alternative pathway of complement activation (Forward and Woo, 1996). Both *Cryptobia*-resistant and *Cryptobia*-susceptible char respond equally well (humoral and cell-mediated immunity) to a commercially available *Aeromonas salmonicida* vaccine (Ardelli and Woo, 1995).

3. Chemotherapy. Under experimental conditions, the trypanocidal drug, isometamidium chloride (Samorin), is effective against the parasite when injected intramuscularly (1.0 mg kg\(^{-1}\) body weight) into adult rainbow trout and juvenile chinook salmon (Ardelli and Woo, 1999). The drug significantly lowered the parasitaemia, and its level in the blood peaked at 2 weeks after injection. All infected and treated chinook salmon survived while 100% of untreated fish died from cryptobiosis; the drug also had prophylactic effects in salmon (Ardelli and Woo, 2001). An antigen-capture ELISA has been developed to monitor drug levels in the blood of fish (Ardelli and Woo, 2000).

*Sphaerospora dicentrarchi* (Myxosoma)

The mature spore of *Sphaerospora dicentrarchi* (Sphaerosporidae) is subspherical with a slightly flattened base and bluntly pointed pole. Its surface is smooth and the suture line is straight and thin. The piriform polar capsules are equal in size and coils of the polar filament are not visible under light microscopy. The sporoplasm has two nuclei (Figs 9.11 and 9.12). The parasite occurs in the gonads, gall bladder, intestine, stomach, head kidneys, spleen, liver, pancreas and serosa, and was initially described from feral and cultured seabass, *D. labrax*, in Spain (Sitjà-Bobadilla and Alvarez-Pellitero, 1992).

It has a very high prevalence (up to 100%) in both cultured and feral seabass in the Mediterranean (Sitjà-Bobadilla and Alvarez-Pellitero, 1993a) and the Atlantic (Santos, 1996). The histopathology due to this histozoic parasite has been described in fish with high parasitaemia (Sitjà-Bobadilla and Alvarez-Pellitero, 1993b). In general, older fish have higher rates of infections but with no clinical signs. *S. dicentrarchi* has been associated with high mortality in young fish from fish farms in Greece and Italy (A. Sitjà-Bobadilla, personal communication) but it has only been reported occasionally in sea cages. It is most likely that fish were infected while in fresh water.

The first *S. dicentrarchi*-associated mortality of seabass (about 1–2 g) was in 1994 in indoor tanks in Rhodes, Greece. Mortality was high with clinical signs. It was diagnosed as a bacterial infection, and mortality was reduced after fish were treated with antibiotics (A. Sitjà-Bobadilla, personal communication). During the outbreak, *S. dicentrarchi* was detected in the trunk, kidney and gut (A. Coloni, Israel, personal communication), and was confirmed later (A. Sitjà-Bobadilla, personal communication). A disease with similar clinical signs was seen in two nearby farms about 2 weeks after the fish (2–2.7 g) were transferred to sea cages. Antibiotics also reduced the mortality to 10 and 40%. However, the diagnosis was not confirmed (A. Sitjà-Bobadilla, personal communication).

Another outbreak occurred in seabass (about 70 g) in 1997 in a fish farm in Sicily, Italy. Mortality reached 1000 fish per day in indoor tanks, with many fish showing...
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Fig. 9.11. Mature spore of *Sphaerospora dicentrarchi* showing a subspherical and slightly flattened base and bluntly pointed pole. Its surface is smooth and the suture line is straight and thin (from Sitjà-Bobadilla and Alvarez-Pellitero, 1992; courtesy of *Journal of Protozoology*).

Clinical signs and treatment

Clinical signs are only seen in young fish, and they include whirling movements, curved spine, whitish faeces, haemorrhages at the base of the fins, anorexia and slowed growth. Weights of infected fish are quite variable, but the parasite has been found in fish (about 2 g) in both indoor tanks and sea cages (A. Sitjà-Bobadilla, personal communication).

Concomitant bacterial infections were detected in some outbreaks. As antibiotics reduced mortality, it would seem that bacteria were involved. The myxospora could induce a weakened or immuno-depressed state, which together with stressful conditions (high water temperatures, low oxygen, high densities, handling or transport) could favour opportunistic bacteria. *S. dicentrarchi* could also have a direct pathogenic effect on young seabass. The curved spine and the whirling movement could be due to the parasite in the brain. Also, poor growth could be due to the anorexia and the massive numbers of spores in the gut epithelium. In addition, mortality occurred in young fish, whose immune systems are not fully developed.

Fat Cell Necrosis Syndrome in Farmed Atlantic Halibut

Atlantic halibut, *H. hippoglossus*, is a relatively new species for aquaculture in northern Europe. Despite early difficulties, production is moving from research and development to one that is commercially viable, and this species will allow the much needed diversification within this industry. In excess of 120,000 t were produced in Scotland in 1999. Health monitoring and experimental studies have continued...
Clinical signs

FCNS in cultured halibut has been described by Bricknell et al. (1996). Gross lesions typical of FCNS generally appear in the pterogophial region of the dorsal surface as multiple white to pale yellow-coloured spreading areas (Fig. 9.13). These are confined to the dorsal surface and may develop over approximately 7 days to involve most of this region with a consequent loss of the epidermis and associated localized haemorrhaging. No significant melanization of the skin occurs around these lesions.

Within the skin lesions, a diffusing necrosis of individual fat cells within the stratum compactum and an increase in infiltrating granulocytes and capillary dilation are noted. There is erosion of the epidermis and dermis with the formation of occasional scattered giant cells. No granuloma formation has been recorded.

FCNS in cultured halibut is attributed to the effect of UV radiation and is similar to a condition reported in feral common dab (Begg, 1994). However, the pathology (Bricknell et al., 1996) does not support this observation, or that typical of sunburn-induced lesions (Bullock and Roberts, 1981). The bacteria isolated from the lesions of the affected halibut were considered by Bricknell et al. (1996) to be secondary infections invading the lesions after tissue necrosis had occurred.

A dietary component may be connected to this condition in common dab (Begg, 1994) due to an uncontrolled lipid peroxidation (Demopoulos, 1973). This lipid peroxidation may result from an imbalance between oxidants and antioxidants resulting in membrane damage. Such oxidative stress may occur through exposure to UV radiation, the liberation of free radicals from necrotic fat cells, or by the production of hydroperoxidases following the release of lipases (Porter, 1989). At present the cause of FCNS in Atlantic halibut has not been fully determined. However, Bricknell et al. (1996) noted from observations on a second, Scottish halibut farm that fish kept in lightly covered 2 m tanks in direct sunshine and fed...
on a lipid-rich commercial diet (24% lipids) developed identical lesions. The fish that developed the lesions were malpigmented. These lesions resolved spontaneously when the halibut were put on to a diet containing lower levels of lipids (12%) and the exposure to direct sunlight was reduced (S. Wadsworth, 1998, personal communication). If this is the case, then reducing dietary lipids and exposure to sunlight could control outbreaks of FCNS. It is possible that increasing the levels of antioxidants, such as $\alpha$-tocopherol, in the diet may also induce resistance to FCNS (Smith, 1979).

Further research is required to decide if FCNS in halibut is related to a lipid-rich diet, an imbalance of oxidants and antioxidants and exposure to UV, and if malpigmented halibut are more susceptible than normally pigmented animals.

**Disorders Due to Vaccination**

Although there are no commercial vaccines against parasitic diseases in fish (Woo, 1995) there are numerous microbial vaccines (Woo and Bruno, 1999). Vaccination to prevent diseases has become a very important strategy in salmonid culture (Press and Lillehaug, 1995), and this approach has reduced the reliance on chemotherapy. Most commercial vaccines (e.g. against *Vibrio* spp., *Yersinia ruckeri*, *A. salmonicida*) contain immuno-potentiating chemicals (adjuvants), which enhance the efficacy of the antigen (Ellis, 1988; Anderson, 1992; Robertson *et al.*, 1994). The scope for development of new vaccines has greatly increased with the advent and use of genetic engineering (Gudding *et al.*, 1997). This technology provides an efficient and convenient method to readily produce antigens from organisms, especially from those pathogens that are difficult to culture. Recombinant vaccines usually contain only protective antigens, and are normally less immunogenic than when they are part of the pathogen. Injectable adjuvants (e.g. Freund’s complete and incomplete adjuvants, alum adjuvants and aluminium hydroxide gel with saponin) are used to increase the immunogenicity of both recombinant and the more traditional whole-cell vaccines.

**Fig. 9.13.** Gross lesions typical of fat cell necrosis syndrome (FCNS) generally appear in the pterogophial region of the dorsal surface as multiple white to pale yellow-coloured spreading areas.
The most effective way to immunize a fish is by either intraperitoneal or intramuscular injection. This approach has many disadvantages and they include the use of anaesthesia and handling followed by injection. These procedures are ‘stressful’ and may reduce growth of fish (e.g. Pickering, 1990; Lillehaug, 1991; Lillehaug et al., 1992). The reduction in growth may be as high as 4.4% in Atlantic salmon (Lillehaug, 1991) and it has been correlated to cortisol production in fish after handling and injection (Pickering, 1990).

Injectable adjuvants to enhance the immunogenicity of vaccines have numerous side effects. Freund’s complete adjuvant induces sterile abscesses with extensive local muscle necrosis when injected intramuscularly into rainbow trout, while intraperitoneal injection may result in granuloma formation and visceral adhesions. The side effects due to potassium aluminium sulphate adjuvant include peritonitis, depressed growth rate and substantial mortalities (Horne et al., 1984). Oil-based adjuvants also cause intra-abdominal adhesions in other species of salmonids (e.g. Midtlyng et al., 1996; Press et al., 1996; Poppe and Breck, 1997; Bruno and Brown, 1999), and the effects of adjuvants on non-salmonid species are less well studied.

Macrophages containing vaccine components occur in several tissues following the initial injection of vaccine (Press et al., 1996). The continued presence of these cells within the abdominal cavity indicates uptake from the vaccine depot. An outpouring of a fibrin-rich exudate occurs in response to the adjuvant insult and the exudate deposits fibrin within the abdominal cavity. Finally, the coagulum forms fibrinous adhesions between neighbouring viscera (Fig. 9.14).

Fig. 9.14. Macrophages containing vaccine components occur in several tissues following the initial injection of vaccine. The continued presence of these cells within the abdominal cavity indicates uptake from the vaccine depot. An outpouring of a fibrin-rich exudate occurs in response to the adjuvant insult and the exudate deposits fibrin within the abdominal cavity. Finally, the coagulum forms fibrinous adhesions between neighbouring viscera.
The stress on fish associated with handling/injection of vaccine, and the side effects due to the adjuvant in the vaccine are bound to be more noticeable with increased reliance on immunization as a strategy against infectious diseases.

Note Added at Proof Stage

Parvicapsula minibicornus (Myxosporea)

Parvicapsula is an internal parasite that has caused high mortality among late-run sockeye salmon in the fish-farming industry in British Columbia since the mid-1960s. The parasite was first isolated from a sea farm in Puget Sound in 1981 and caused 30% mortality in the coho salmon. Since then, it has been found in fish farms from Oregon to Alaska. Recently, similar Parvicapsula outbreaks have occurred in Norway, with significant impact on Atlantic salmon (E. Sterud, 2002, personal communication).

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