Alexandra Adams   *Editor*

Fish Vaccines

Springer

Birkhäuser Advances in Infectious Diseases

Series editors

Stefan H.E. Kaufmann
Max Planck Institute for Infection Biology, Berlin, Germany

Andrew A. Mercer
Department of Microbiology and Immunology, University of Otago
Dunedin, New Zealand

Olaf Weber
Bonn, Germany

More information about this series at
http://www.springer.com/series/5444

Alexandra Adams

Editor

Fish Vaccines

Editor
Alexandra Adams
University of Stirling
Institute of Aquaculture, Faculty of Natural Sciences
Stirling
UK

Contents

Chapter 1
Fish Disease Causing Economic Impact in Global Aquaculture

Hamish D. Rodger

Abstract One of the main hurdles to sustainable finfish aquaculture in many regions has been the management and control of infectious disease. The most significant diseases of salmonid, carp, catfish, tilapia and marine finfish farming are considered in this chapter by viral, bacterial, parasitic and fungal group. The level of impact caused by disease and methods for control or management are outlined.

Introduction

As finfish aquaculture expands globally, in terms of numbers and biomass production, species diversification, geographic regions and rearing methods, the challenges faced by the sector from disease and health issues also diversify and emerge. Climate change and evolving fish husbandry may also contribute to the balance or imbalance of pathogen, host, and environment interaction with novel pathogens being observed or isolated annually and more familiar diseases emerging in different global regions and species. Many of these diseases or pathogens have no recommended treatments, vaccines or management methods established or developed and hence remain a significant hurdle for the economic viability of aquaculture in certain regions and species. For many of the established aquaculture species such as carp, tilapia, salmonids as well as some of the marine species (sea bass, sea bream, grouper), there are commercial vaccines for a limited number of diseases and authorised treatments for specific pathogens, although there is considerable variation from country to country even within a geographic region. Many of the diseases causing significant economic impact in aquaculture are viral conditions with no treatments available and vaccines, if developed, of only partial efficacy in protection. The bacterial, parasitic and fungal diseases also have examples that can cause major economic and welfare challenges to aquaculture globally, although there are a number of effective vaccines against bacterial diseases. In this chapter

H.D. Rodger
Vet-Aqua International, Oranmore, Co.Galway, Ireland
e-mail: hamish.rodger@fishvetgroup.com

the viral, bacterial, parasitic and fungal diseases of most significant impact for global aquaculture are discussed and outlined. Non-infectious diseases can also cause significant health challenges in aquaculture; however, these are out with the scope of this communication. This chapter is organised by aquaculture species group in the first instance (salmonids, carp, tilapia, catfish and marine species) and then by pathogen group (viruses, bacteria, parasites and fungi) within each aquaculture species group, where appropriate.

Salmonids

Global salmonid production continues to increase annually year-on-year and remains in the top ten species in terms of volume but is the number one finfish species (Atlantic salmon, *Salmo salar*) in terms of economic value (FAO 2012).

Viral Diseases of Salmonids

Infectious salmon anaemia virus (ISAV) is an enveloped single-stranded RNA virus in the family *Orthomyxoviridae* and has been classified as the type species of the genus *Isavirus* (OIE 2012). There are genetic variants of ISAV with two major groups, one European and one North American group, plus a non-pathogenic suggested precursor, the non-deleted highly polymorphic region (HPR) or HPR0. The HPR0 ISAV strain appears to be present periodically in both healthy farmed and wild salmon in the majority of salmon-farming regions (OIE 2012); however, infection with an HPR-deleted ISAV strain can result in a severe anaemic disease resulting in high mortality which can continue for many months. The impact from outbreaks of clinical ISA has been highly significant in the past where the reduction in Atlantic salmon production in Chile declined from 400,000 tonnes in 2005 to just above 100,000 in 2010 and in the Faroe Islands where the decline was from 47,000 tonnes in 2004 to 12,000 tonnes in 2006 (Asche et al. 2010). In both cases the production has increased again (or surpassed previous levels) since the institution of improved biosecurity, marked changes in fish husbandry and increased surveillance.

Outbreaks of ISA have been predominantly recorded in Atlantic salmon (*S. salar*), although coho salmon (*Oncorhynchus kisutch*) in Chile have also been affected (Kibenge et al. 2001). Asymptomatic infections of farmed rainbow trout (*O. mykiss*) in Ireland as well as detection by RT-PCR in sea trout (*S. trutta*), pollack (*Pollachius virens*) and cod (*Gadus morhua*) have also been confirmed, although in the cases of the gadoid fish, these were in pens of salmon affected by clinical ISA (Kibenge et al. 2004).

ISA is predominantly a disease of marine-farmed Atlantic salmon, and mortality may start at a very low level in one pen but then can spread from pen to pen with

Fig. 1.1 Atlantic salmon affected by ISA presenting with dark red (congested) liver and numerous petechiae in the pyloric caecal fat


rising mortalities, and in the worst case, pens can reach 90 % cumulative mortality if no action is taken. Clinical signs of the disease include lethargy, pale gills, exophthalmia, ascites and sometimes haemorrhages in the eye or ventrum. Internally fish may present with blood-tinged ascitic fluid in the abdomen, petechiae in the viscera or diffuse dark red, almost black liver and splenomegaly as well as congestion of the gastrointestinal tract (Fig. 1.1). Packed red blood cell volume is often less than 10, and histopathology may reveal characteristic multifocal and confluent hepatic haemorrhage and necrosis distant from hepatic vessels. The diagnosis of ISA is based on the presence of clinical signs, pathological changes consistent with ISA with detection of ISAV in tissues by the use of ISAV-specific antibodies on fixed sections or tissue imprints, plus isolation of ISAV in cell culture or detection by RT-PCR.

Control of clinical disease in most countries is through culling and disinfection on either a pen-by-pen or farm basis and complimented by strict biosecurity, livestock movement restrictions and site or waterbody fallowing. There are no treatments for the disease; however, commercial vaccines are in use in Chile, Faroe Islands, Eastern Canada and to a limited extent in Norway.

Salmonid alphaviruses (SAVs) have emerged to become one of the most significant viral groups of pathogens affecting salmonid farming in Northern Europe. Salmonid alphaviruses are associated with the condition known as pancreas disease (PD), affecting predominantly marine-stage Atlantic salmon, and sleeping disease (SD) of mainly freshwater-reared rainbow trout (McLoughlin and Graham 2007). From the first descriptions of cases from 1976 in Scotland, PD now affects salmon in Ireland, Norway and Scotland, and SD affects trout throughout mainland Europe and the UK. PD can have a major impact on salmon farms with the Irish industry estimated to have experienced a loss of turnover of 35 million euro in 2003 to 2004 (Ruane et al. 2008) due to the virus. The number of sites diagnosed with PD in Norway has increased from 10 in 1999 to 137 in 2012 (NVI 2012). Direct costs associated with a PD outbreak in a site in Norway stocked with 500,000 salmon (vs. a similar site without the disease) have been estimated at 14.4 million Norwegian kroner (Aunsmo et al. 2010). Alphaviruses have a single-stranded RNA genome and are enveloped, and it has been demonstrated that the salmonid alphaviruses have at least six subtypes with some distinct geographical distributions (Fringuelli et al. 2008). SAV has also

Fig. 1.2 Atlantic salmon
affected by SAV infection
and presenting with *pale
yellow* casts in the lower
intestine
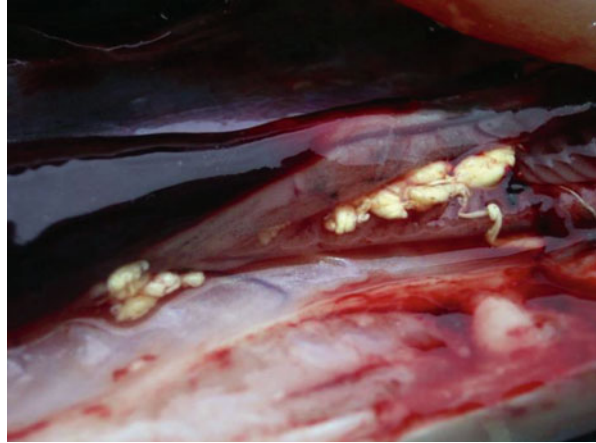
been detected in wild flatfish fish distant from aquaculture operations off the coast of
Scotland (SAV subtype 5) and Ireland (SAV subtype 1) (Snow et al. 2010; McCleary
et al. 2014).

Clinical SAV infection in marine salmon causes lethargy, anorexia, tetany, vari-
able mortality (from less than 1 % but up to 50 %) and in chronic cases the develop-
ment of a subpopulation of poor condition, thin fish which have failed to thrive. In
freshwater, trout mortalities can also be variable, and clinically high numbers of fish
may appear lying on their sides (hence the use of the name sleeping disease) but
following a period of weeks may fully recover with no mortalities. Internally fish
can present with petechiae in the caecal fat in the early stages of the disease and
cream, pale yellow to white-coloured casts in the intestine (Fig. 1.2). Histopathology
in the early stages consists of acinar pancreatic necrosis, and in the majority of fish,
the pancreatic tissue appears to regenerate; however, a population of fish are left
with pancreatic fibrosis and no functional acinar tissue. Focal to diffuse myopathy
of the myocardium is also present, usually concurrent with pancreatic pathology.
Skeletal myopathies, initially in the red (aerobic) muscle fibres, then later in the
white (anaerobic) bundles, develop to varying degrees. These myopathies may then
have an impact on fillet quality, increasing further the economic impact of a case of
PD in a farm (Lerfall et al. 2012).

Diagnosis is through a combination of clinical signs, gross pathology and labora-
tory tests including histopathology, serology, virology and RT-PCR. Routine moni-
toring of fish stocks in marine sites is often centred on monthly blood or tissue
samples for SAV screening by virology and/or PCR. Control of the disease varies
from country and region from Northern Norway where a cull and disinfect regime
is in place to Ireland and Scotland where the virus appears endemic, and biosecurity
and low-stress management can minimise the clinical impact. One commercial vac-
cine is available and in use in salmon farms in Ireland, Norway and Scotland.

Cardiomyopathy syndrome (CMS) was first described in farmed Atlantic salmon
in Norway (Ferguson et al. 1990) and then subsequently in the Faroe Islands

(Poppe and Seierstad 2003), Scotland (Rodger and Turnbull 2000) and Ireland (Rodger et al. 2013). The infectious nature of the disease has been demonstrated by Fritsvold et al. (2009), and a *Totivirus*, the piscine myocarditis virus (PMCV), has been identified as the infectious agent involved (Løvoll et al. 2010; Haugland et al. 2011). The disease primarily affects farmed Atlantic salmon in their second year at sea where it has a significant economic impact due to mortality associated with large-size fish (Brun et al. 2003). CMS has been one of the major diseases in Norwegian aquaculture for the last decade, and viral RNA can be detected in farmed fish for months without any signs of clinical disease (Wiik-Nielsen et al. 2012). Although the disease has only been described in farmed Atlantic salmon, histopathology consistent with CMS was described in wild Atlantic salmon (Poppe and Seierstad 2003), and, in a recent study, PCMV was detected and sequenced from wild Atlantic salmon in Norway (Garseth et al. 2012).

Moribund fish affected by CMS present with congestion and oedema in the skin, occasional exophthalmia (Fig. 1.3), lethargy and internally with blood-tinged ascites, purple to grey livers with diphtheritic fibrinous membranes, petechiae in caecal fat as well as swollen, blood-engorged atria of the heart and haemopericardium. Histopathology reveals a severe diffuse myopathy of the spongey layer of the heart ventricle and multifocal hepatic necrosis. Diagnosis is through clinical signs, gross pathology plus histopathology and RT-PCR. There is no treatment nor is there any commercial vaccine for the condition, and control is through biosecurity and early or accelerated harvest in significantly affected pens.

Heart and skeletal muscle inflammation (HSMI) was first observed in Norway in 1999 (Kongtorp et al. 2004a), and since then the number of cases diagnosed peaked at 162 per annum (pa) in 2007 and 2011 (NVI 2012) but has remained above 130 cases pa since 2007. HSMI is usually observed as a clinical disease in marine-stage salmon 5–9 months after transfer to sea. Affected fish exhibit anorexia, lethargy and increased mortality which can vary from less than 1–20 % in affected pens. The pathology associated with the disease appears limited to the
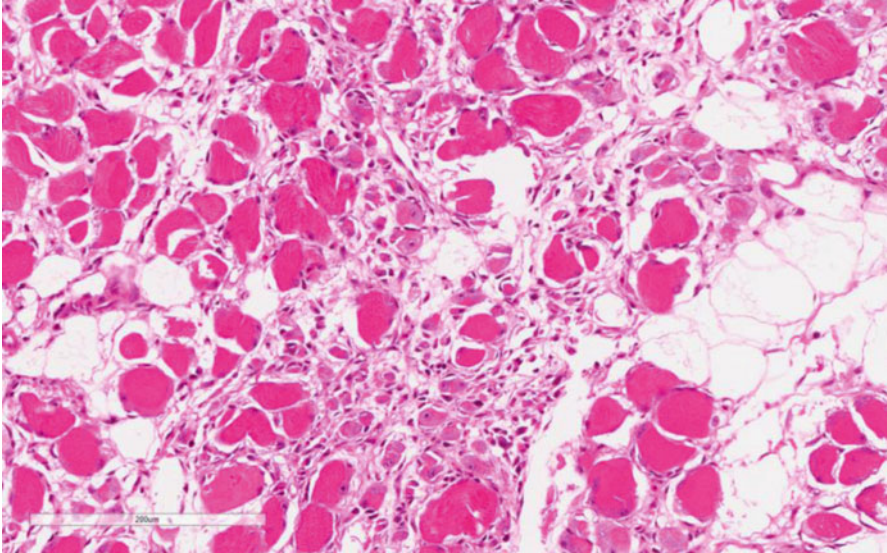
Fig. 1.4 Histopathological section of *red skeletal* muscle from Atlantic salmon affected by heart and skeletal muscle inflammation (HSMI)

heart and skeletal muscle where epicarditis, myocarditis, inflammation and degeneration of myocytes in the red skeletal muscle are the main findings (Fig. 1.4) (Kongtorp et al. 2004b).

HSMI has been demonstrated as an infectious disease and is strongly associated with the piscine *Orthoreovirus* (PRV), a recently discovered virus identified through high-throughput pyrosequencing of serum from HSMI-affected fish (Palacios et al. 2010). PRV is a double-stranded RNA virus and belongs to the *Reoviridae* family and appears to be most close to the genus *Orthoreovirus*. HSMI as a disease has also been reported in Scotland (Ferguson et al. 2005) and Ireland but PRV appears widespread in farmed fish in Norway, Scotland, Ireland and Chile but is present in healthy farmed and wild salmon as well as the cases of HSMI in Norway and Scotland. As shown by Løvoll et al. (2012), the PRV load increases after transfer of smolts to sea, and the cases of HSMI in Norway are associated with high levels of virus, but the observations indicate that environmental factors associated with the seawater locations may be more important than PRV status. Recent research has also demonstrated that PRV resides in the erythrocytes of salmon with up to 50 % PRV positive in individual fish (Finstad et al. 2014). The appearance and investigations of PRV in the erythrocytes have shown strong similarities to the viral disease previously described as erythrocytic inclusion body syndrome (EIBS) (Leek 1987). There are no vaccines for HSMI nor any specific treatment, although there is one report of a reduction in impact of clinical disease through the feeding of tetradecylthioacetic acid (a synthetic fatty acid) (Alne et al. 2009).

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease of young fish of salmonid species held under intensive rearing conditions. Susceptibility

Fig. 1.5 Salmon parr affected by IPN displaying a swollen intestine filled with mucus and catarrhal exudate

generally decreases with age and with resistance to the disease being reached at 1500° days except for Atlantic salmon smolts (Smail et al. 1992).

The first clinical sign in salmonid fry is frequently a sudden and usually progressive increase in daily mortality, particularly in the faster-growing individuals. Clinical signs include darkening pigmentation, a pronounced distended abdomen and a corkscrewing/spiral swimming motion. Cumulative mortalities may vary from less than 10 % to more than 90 % depending on a combination of various factors such as virus strain, host and environment. Internally the fish can display swollen intestine and catarrhal exudates in the lumen (Fig. 1.5). There may also be petechiae on the caecal fat and a pale liver. Histopathology of IPN affected fish involves focal necrosis of the acinar pancreatic tissue with necrotic areas replaced by a loose fibrous network and fat degeneration. Macrophages and leucocytes may infiltrate pancreatic and hepatic tissues. There may be necrosis and sloughing of the caecal endothelium. The causal agent is a double-stranded RNA virus of the family *Birnaviridae*, and there appear to be at least seven genogroups described so far based on molecular phylogenetic analyses (Munro and Midtlyng 2011). Histopathology and clinical signs can be diagnostic with confirmation conducted by cell culture and/or PCR. IPN historically emerged in North America and Europe and has been confirmed in most salmonids throughout the world; however, *Birnaviruses* appear to have a global distribution in both farmed and wild fish species and further in many non-salmonids and shellfish (Munro and Midtlyng 2011). There is a measurable antibody response to viral challenge, although in young fish this is limited. Commercial vaccines are available and are utilised in Norway, Scotland, Ireland and Chile. Prevention can be achieved by avoidance of fertilised eggs originating from IPN virus-carrier broodstock and the use of protected water supply. In outbreaks, a reduction in the stocking density may help reduce the overall mortality, or alternatively a short period of increased water temperature (>18 °C) also appears to be of benefit. Significant benefits have been observed through the use of genetics in recent years following the identification of two genomic quantitative trait loci (QTL) for IPN susceptibility or resistance (Houston et al. 2008).

Infectious haematopoietic necrosis (IHN) is an infectious disease, caused by a *Rhabdovirus*, of salmonids. It is of concern due to its clinical and economic consequences in trout and salmon farming and its effects in wild stocks. The disease was first reported in the early 1940s in North America (Pacific Rim states) but later spread to central and eastern USA, Canada, Japan and southern Europe. The economic impact from IHN can be very significant, and in the outbreak in British Columbia in 2001–2003, the cumulative mortality attributed to the disease in the 36 farms surveyed averaged 58 % with over 12 million Atlantic salmon lost either through mortality or culling (Saksida 2006).

Natural outbreaks of IHN are rare above 15 °C. Diseased fry are usually lethargic and hang at the areas of low water current. Whirling or flashing may also be seen. In older fish these signs may not be seen. Pale gills, dark skin, swollen abdomens, haemorrhages at the fin bases and opaque faecal pseudocasts trailing from the vent are frequently reported. Caecal fat petechiae and peritoneal haemorrhages may also be observed. Subdermal haemorrhage between the head and dorsal fin and spinal deformities in surviving sockeyes are quite common (Plumb and Hanson 2011). Histopathology of IHN-affected fish reveals multifocal degeneration and necrosis in the spleen and interstitial tissue of the kidney. Necrosis of the eosinophilic granular layer in the digestive tract is considered pathognomic. The aetiological agent is an enveloped RNA virus belonging to the family *Rhabdoviridae.* IHN affects members of the family *Salmonidae* in North America, Asia and Europe, but not in the Southern hemisphere, and in both fresh- and seawater. Reservoirs of IHNV are clinically infected fish and covert carriers from either cultured, feral or wild fish. The transmission of IHNV is horizontal and possibly vertical or egg associated. Strong antibody response in survivors is mounted to IHNV. Vaccination is widespread in salmon farming in British Columbia, Canada, since 2006, using a nucleic acid-based vaccine. Control methods in most countries for IHNV currently lie in official health surveillance schemes coupled with control policy measures. Thorough disinfection of eggs and incubation of eggs and rearing of fry and alevins in virus-free water supplies in premises completely separated from those harbouring possible virus carriers and free from possible contact with fomites are critical for preventing the occurrence of IHNV in a defined fish production site.

Viral haemorrhagic septicaemia (VHS) is an infectious disease caused by a coldwater *Rhabdovirus* which is of clinical and economic importance in rainbow trout and turbot farming in Europe. In North America VHS primarily affects wild fish species with several die-offs observed in recent years in the Great Lakes region with at least 28 species affected (OIE 2012).

Typical outbreaks result in acute to chronic disease among fingerling rainbow trout at temperatures generally below 14 °C. A wide range of possible disease signs are recorded including a profuse haemorrhaging, but in many fish a less dramatic pathology is noted. Fish may be lethargic and congregate at tank/pond sides or outlets, have pale gills, dark body colour, exophthalmos and in some cases intermittent periods of erratic spiralling swimming. Haemorrhage may be visible in the eyes and skin, within the muscle and internally in the viscera and intestine. In more chronic cases, some of the above signs may be obvious with abdominal distension due to oedema in visceral organs and ascites. The causal agent is an enveloped RNA virus belonging to the family *Rhabdoviridae* (Fig. 1.6), genus *Novirhabdovirus.* There
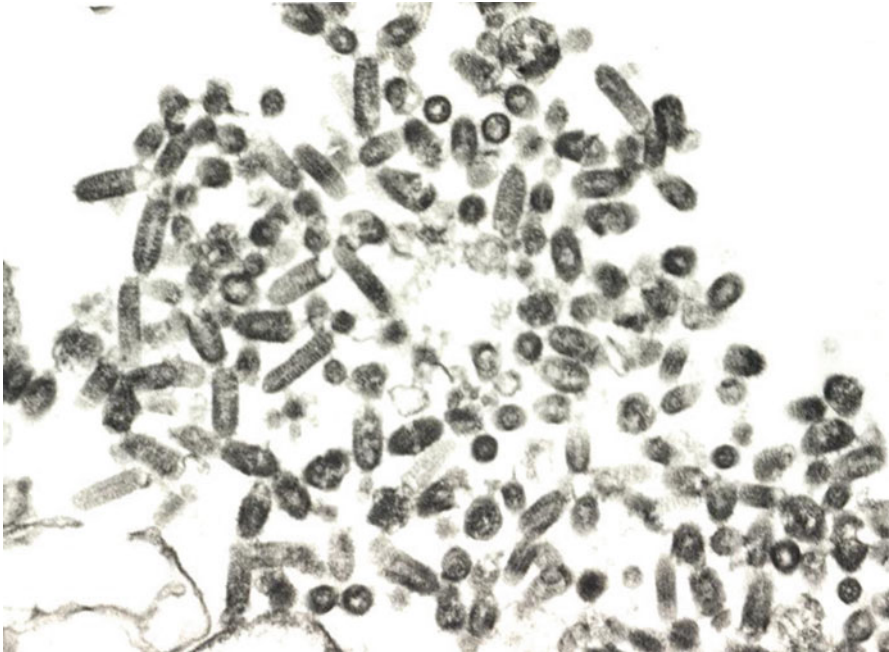
Fig. 1.6 Transmission electron micrograph of viral haemorrhagic septicaemia virus isolated from turbot (*Scophthalmus maximus*) in Scotland. Note the classical bullet-shaped virions (70–180 nm in size) (×43,000)

are four major genotypes (I to IV) of the virus, and these appear more associated with geographic origin than fish species; genotype I contains the European freshwater VHSV isolates and a group of marine isolates, and genotype IV contains the North American and Japanese/Korean isolates. Antibody response mounted to VHSV and fish serology could be of importance for detecting the carrier state among fish stocks, but has yet to be validated. Vaccine development has been ongoing for many years; however, no commercial vaccine is available. Control methods for VHS currently lie in official health surveillance schemes coupled with control policy measures, such as stamping-out procedures, and have resulted in eradication of the disease from several parts of Europe. Genetic approaches to selection of disease-resistant stock and intergeneric hybridisation are also being pursued.

Bacterial Diseases of Salmonids

Diseases caused by *Flavobacterium* spp. affect many farmed fish species in freshwater and conditions in salmonids are known as either:

(a) Bacterial coldwater disease (CWD), which is a serious septicaemic infection of hatchery-reared salmonids, also referred to as peduncle disease, is prevalent in

northwest American hatcheries during colder months of the year. *F. psychrophilum* is the bacterial species associated with this condition.

(b) Rainbow trout fry syndrome (RTFS) or rainbow trout fry anaemia is a freshwater systemic disease affecting trout (and to a lesser extent salmon) in Europe that results in high mortalities. *F. psychrophilum* is the causal agent of this disease.

(c) Bacterial gill disease which is commonly observed in freshwater salmonids and is associated with *F. branchiophilum*.

(d) Columnaris disease as induced by *F. columnare* is usually associated with warm water (20–25 °C) but can be observed in trout at lower temperatures (12–14 °C) (Starliper and Schill 2011).

CWD was first described in the USA in rainbow trout; however, juvenile coho salmon appear most susceptible. RTFS was described throughout Europe in the 1990s where due to its level of impact and persistent nature it has risen to become the most important disease problem for freshwater rainbow trout farming in Europe (Starliper and Schill 2011). Clinical signs of CWD include haemorrhage at the base of fins, pale gills, haemorrhagic ulceration in the muscle and tail rot. The disease usually appears in the spring with water temperatures 4–10 °C. If alevins are affected by yolk-sac erosions, mortalities can be 30–50 %. Coagulated yolk sac may precede the disease. In RTFS high mortalities occur in trout fry with pale gills, swollen spleens, with blood-tinged caecal fat around the spleen, lethargy, darkened skin, ascites and exophthalmos (Fig. 1.7). Skin ulcerations or eroded/dissolving jaw may present in older affected fish. Bacterial gill disease presents with mortality and pale patches on the gills. These bacteria are Gram negative and filamentous and require extended growth (14 days) on Anaker and Ordal's media (or equivalent low-nutrient agar) at 15 °C. The diagnosis is based on clinical observations, fresh microscopy and histopathology with biochemical or serological characterisation of the isolated bacteria.

Natural reservoirs of the bacteria are uncertain; however, the disease can be transmitted vertically and horizontally. The bacterium is very robust, resisting some disinfectants which are normally used for egg cleaning (iodophors). Protection has
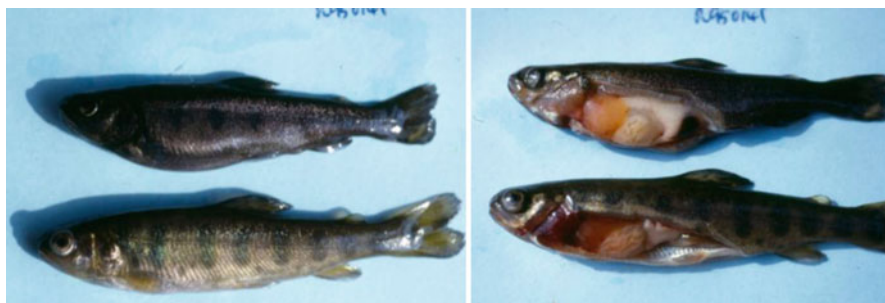
Fig. 1.7 Rainbow trout affected by *Flavobacterium psychrophilum* infection (*top fish in both images*) displaying dark colouration and abdominal swelling (*left upper*) and pale gills and swollen spleen typical in RTFS (*right upper*) with healthy trout for comparison