ICES COOPERATIVE RESEARCH REPORT

RAPPORT DES RECHERCHES COLLECTIVES

NO. 265

Trends in important diseases affecting the culture of fish and molluscs in the ICES area 1998–2002

Prepared and edited by

The Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK-1261 Copenhagen K Denmark

February 2004

Recommended format for purposes of citation:

ICES. 2004. Trends in important diseases affecting fish and molluscs in the ICES area 1998–2002. Prepared and edited by the Working Group on Pathology and Diseases of Marine Organisms. ICES Cooperative Research Report No. 265. 26 pp.

ISBN 87-7482-011-7
ISSN 1017-6195

Table of Contents

Section **Page**

Table of Contents

Section **Page**

Table of Contents

Section **Page**

Table of Contents

Section **Page**

1 Introduction

One of the regular Terms of Reference of the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) since its inception in 1976 is to provide and review annual national reports submitted by ICES Member Countries on the disease status of farmed fish and shellfish and to highlight new disease trends.

At its 2000 meeting, the WGPDMO emphasized that these reports could be made even more informative if presented as trends over a five-year period. This would make it possible to obtain an impression of diseases that might create problems in the near future. Such information on emerging disease problems, especially in new fish and mollusc species brought into aquaculture, is considered to be of importance for countries planning the development of production of these species.

In order to facilitate a wide dissemination, the WGPDMO agreed that such a report should be brought to the attention of ICES Member Countries, national and international organizations involved in diseases of farmed marine organisms, and interested scientists and managers by means of appropriate publications. The basic idea is to present this information in the *ICES Cooperative Research Report* series and on the ICES website as an internet publication which may be updated biannually.

It is the intention for the future that this report will address long-term trends in disease development in mariculture.

In order to obtain the most updated information possible, the present report provides data from the period 1998–2002 on diseases in common farmed finfish and shellfish species, which have occurred during this period. The WGPDMO has decided to present information on each disease by species and, in order to facilitate reading, each disease is presented with a short general description. Since the objective of this first report is to focus on the most significant diseases in common marine fish and mollusc species, the report does not cover all diseases or cultured species that may be of interest to ICES Member Countries and is, therefore, *per se*, far from being complete.

WGPDMO members that contributed to this report are J. Barja (Spain), S. Bower (Canada), D. Bruno (UK), A. Figueras (Spain), S. Ford (USA), B. Hjeltnes (Norway), S. Jones (Canada), T. Lang (Germany), S. Mellergaard (Denmark), T. Renault (France), T. Wiklund (Finland), and M. Vigneulle (France).

2 Diseases of Farmed Fish

2.1 *Atlantic salmon (Salmo salar)*

2.1.1 Viral diseases

2.1.1.1 Infectious Salmon Anaemia (ISA)

2.1.1.1.1 Description of agent

ISA is caused by a virus, ISAV, which is an enveloped single-stranded RNA virus belonging to the Orthomyxoviridae family (Falk *et al.*, 1997; Krossøyr *et al.*, 1999; Mjaaland *et al.*, 1997). The virus is pleomorphic, with a diameter ranging from 100 nm to 140 nm. The genome consists of eight segments and the virus contains four major structural polypeptides.

2.1.1.1.2 Geographical distribution

ISAV has been confirmed from Norway, Scotland, Faroe Islands, USA (Maine), Canada (East Coast), and Chile.

2.1.1.1.3 Short description of clinical signs

The fish are lethargic, congregate in the upper water level, gasp at the surface, go off feed, and hang motionless at the sides of the cage. Affected fish may exhibit exophthalmia, ocular haemorrhage, distended abdomen and/or skin haemorrhage. Internal pathology may include dark, pale or yellow liver, ascites, pale gill and heart, enlarged spleen, petechial haemorrhage in visceral fat, dark foregut. Low haematocrit values (< 10) are typical findings. Typical histological findings are multifocal haemorrhagic hepatic necroses that may become confluent to give the changes a "zonal" appearance, leaving areas around large veins intact (late stage of disease development). Focal congestion and dilatation of hepatic sinusoids, sometimes with distribution as described for necroses (early stage), and rupture of the sinusoidal endothelium with the presence of erythrocytes within the space of Disse (early sign) are also observed (Thorud and Djupvik, 1988; Evensen *et al.*, 1991).

2.1.1.1.4 Indications of impact/severity at stock level

ISA is a serious disease that has caused significant losses in salmon-producing countries. Clinical disease in the field due to ISAV has been recorded in Atlantic salmon and more recently in coho salmon (*Onchorhynchus kisutch*).

2.1.1.1.5 Control/preventive measures

Restrictions on the movement of live fish include sanitary slaughtering, disinfection of offal/wastewater

from fish slaughterhouses, cleaning and disinfection of farm premises and equipment, fallowing, rotation of farm sites, and vaccination.

2.1.1.1.6 Temporal trends

ISAV was previously restricted to Norway, but has now been confirmed from all the main salmon-producing countries. This represents a major new extension of the known distribution of this disease and its viral causative agent. The origin of the infection in all affected areas remains unknown, but there is increasing evidence for a local natural presence. Zoosanitary precautions, whenever possible to enforce, have proven to be very effective in controlling the disease. During recent years, an increasing number of new outbreaks in Norway have been observed. However, in 2002, a decrease in the number of new outbreaks was reported.

2.1.1.1.7 Other host species

Evidence for persistence of the virus has been obtained in sea trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Nylund *et al.*, 1995). ISAV has been isolated from eel (*Anguilla anguilla*), saith (*Pollachius virens*) (Snow *et al.*, 2002), herring (*Clupea harengus*), and cod (*Gadus morhua*). There is evidence that sea lice (*Lepeophtheirus salmonis*) may serve as a vector for ISAV (Nylund *et al.*, 1993).

References

Evensen, Ø., Thorud, K.E., and Olsen, Y.A. 1991. A morphological study of the gross and light microscopic lesions of infectious anaemia in Atlantic salmon (*Salmo salar*). Research in Veterinary Science, 51: 215–222.

Falk, K., Namork, E., and Dannevig, B.H. 1997. Morphology and morphogenesis of infectious salmon anaemia virus replicating in the endothelium of Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 29: 99–109.

Gattuso, A., Mazza, R., Imbrogno, S., Sverdrup, A., Tota, B., and Nylund, A. 2002. Cardiac performance in *Salmo salar* with infectious salmon anaemia (ISA): putative role of nitric oxide. Diseases of Aquatic Organisms, 52: 11–20.

Gregory, A. 2002. Detection of infectious salmon anaemia virus (ISAV) by *in situ* hybridisation. Diseases of Aquatic Organisms, 50: 105–110.

Krossøyr, B., Hordvik, I., Nilsen, F., Nylund, A., and Endresen, C. 1999. The putative polymerase sequence of infectious salmon anaemia virus suggests a new genus within the Orthomyxoviridae. Journal of Virology, 73: 2136–2142.

Mjaaland, S., Rimstad, E., Falk, K., and Dannevig, B.H. 1997. Genomic characterization of the virus causing infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.): an orthomyxovirus-like virus in a teleost. Journal of Virology, 71: 7681–7686.

Murray, A.G. 2002. Shipping and the Spread of Infectious Salmon Anaemia in Scottish Aquaculture. Emerging Infectious Diseases, 8: 1–5.

Nylund, A., Alexandersen, S., and Rolland, J.B. 1995. Infectious salmon anaemia virus (ISAV) in brown trout. Journal of Aquatic Animal Health, 7: 236–240.

Nylund, A., Wallace, C., and Hovland, T. 1993. The possible role of *Lepeophtheirus salmoni* (Kroyer) in the transmission of infectious salmon anaemia. *In* Pathogens of Wild and Farmed Fish, 28: 367–373. Ed. by G.A. Boxshall and D. Defaye. Ellis Horwood Ltd., New York.

Snow, M., Raynard, R.S., Bruno, D.W., van Nieuwstadt, A.P., Olesen, N., Løvold, T., and Wallace, C. 2002. An investigation into the susceptibility of saithe (*Pollachius virens*) to Infectious Salmon Anaemia Virus (ISAV) and their potential role as a vector for viral transmission. Diseases of Aquatic Organisms, 50: 13–18.

Thorud, K., and Djupvik, H.O. 1988. Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.). Bulletin of the European Association of Fish Pathologists, 8: 109–111.

Wergeland, H.I., and Jakobsen, R.A. 2001. A salmonid cell line (TO) for production of infectious salmon anaemia virus (ISAV). Diseases of Aquatic Organisms, 44: 183–190.

2.1.1.2 Infectious Pancreatic Necrosis (IPN)

2.1.1.2.1 Description of agent

IPN is caused by a virus, IPNV, which is un-enveloped with icosahedral capsid (~ 60 nm in diameter) and a bi-segmented, double-stranded RNA genome, belonging to the genus *Aquabirnavirus*. The capsid consists of two structural proteins, VP2 and VP3. Three main serotypes have been recognized: one American (VR 299) and two European (Ab and Sp) (Dobos, 1995; Reno, 1999; Smail and Munro, 2001).

2.1.1.2.2 Geographical distribution

The disease has a wide geographical distribution, occurring in most, if not all, major salmonid farming countries of North and South America, Europe, and Asia.

2.1.1.2.3 Short description of clinical signs

Diseased fish usually appear dark, with an abnormal, lethargic swimming behaviour. Spinning movement (whirling around the longitudinal axis) is characteristic. Abdominal swelling is also characteristic, especially in fry. At necropsy, petechiae in the perivisceral adipose tissue are the most consistent lesions. Fry often show pronounced ascites, whereas post-smolts usually have little or no ascitic fluid and a remarkably dry body muscle. A whitish liver is most commonly found in fry. In the acute stage, extensive necrosis of exocrine pancreatic tissue is the most prominent lesion at histopathological examination. In these lesions, individual acinar cells are seen at different stages of degeneration and cell death. Also, foci with necrotic remnants of exocrine pancreatic cells appearing as an amorphous eosinophilic mass are often found. In liver tissue, similar foci of necrotic eosinophilic hepatocytes may be found. Usually there are small necrotic foci in the pyloric intestinal epithelium and cell debris in the lumen of the pyloric caeca. Some individuals develop a chronic disease with fibroplasias of pancreatic tissue and emaciation.

2.1.1.2.4 Indications of impact/severity at stock level

IPN is a highly contagious viral disease, principally of young salmonid species. Cumulative mortality may vary from less than 10 % to more than 90 %. Furthermore, IPN is known to cause significant losses in early post-smolts of Atlantic salmon.

2.1.1.2.5 Control/preventive measures

Fertilized eggs originating from IPNV carrier broodstock should be avoided. Vaccination is possible, but the efficacy of existing vaccines remains to be proven.

2.1.1.2.6 Temporal trends

In Norway and Scotland, infections with IPNV are considered to be a major problem in the post-smolt phase of Atlantic salmon. In Scotland, IPNV showed strong regional variation in marine waters between 1996 and 2001. An annual increase in the prevalence of this virus was found in seawater (10 %) and freshwater sites (2 % to 3 %), with a greater increase (6.5 %) in the Shetland Isles. Trends in clinical IPN in Scotland over the past five years have increased from 1.1 % to 12.5 %, but with prevalence changes between years. Similarly, in the Shetland Isles, IPN prevalence has increased from 5.2 % to 14.4 %. In Norway, there are indications that high density, increasing use of oxygenation and low water flow may increase losses in post smolts. Furthermore, there is increasing evidence that IPN isolates belonging to the same serotype show variations in virulence. Vaccines against IPN have been introduced in Norway, but current field data are conflicting regarding vaccine efficacy.

2.1.1.2.7 Other host species

IPNV is found in a great variety of non-salmonid fish including halibut (*Hippoglossus hippoglossus*), cod, haddock (*Melanogrammus aeglefinus*), and turbot (*Scophthalmus maximus*) as well as in bivalves, gastropods, and crustaceans.

References

Dobos, P. 1995. The molecular biology of infectious pancreatic necrosis virus (IPNV). Annual Review of Fish Diseases, 5: 25–54.

Reno, P.W. 1999. Infectious pancreatic necrosis and associated aquatic birnaviruses. *In* Fish Diseases and Disorders, Vol. 3. Viral, Bacterial and Fungal Infections, pp. 1–56. Ed. by P.T.K. Woo and D.W. Bruno. CAB International, Wallinford, Oxon.

Smail, D.A., and Munro, A.L.S. 2001. The Virology of Teleosts. *In* Fish Pathology, Third Edition, pp. 169–253. Ed. by R.J. Roberts. W.B. Saunders, London.

2.1.1.3 Salmon Pancreas Disease (SPD)

Salmon Pancreas Disease is a serious infectious viral disease of farmed Atlantic salmon that primarily occurs during their first year at sea, but other year classes are also susceptible.

2.1.1.3.1 Description of agent

A toga-like virus termed salmon pancreas disease virus (SPDV) causes SPD. SPDV and sleeping disease virus (SDV) affecting trout in fresh water exhibit minor biological differences. There is a close genetic similarity between SPDV and SDV and there is indication that they are closely related isolates of the same viral species for which the name *salmonid alphavirus* has been proposed.

2.1.1.3.2 Geographical distribution

SPD is reported from Atlantic salmon in Scotland, Ireland, Norway, France, and North America.

2.1.1.3.3 Short description of clinical signs

Affected fish cease shoaling and are cachectic in appearance. There is a decreased feeding response; the fish become anorexic and are unable to maintain their position in the water. During the acute phase, there is a rapid and dramatic necrosis of the entire acinar tissue with eosinophilic debris voided through the ducts. SPD has also been associated with an acute cardiomyopathy characterized by a coagulative necrosis, and myodegeneration and phagocytosis of fibre remnants affecting both spongy and compact layers with an increased eosinophilia and loss of striation.

2.1.1.3.4 Indications of impact/severity at stock level

SPD is an economically important disease and yearly losses of 10–50 % are reported for individual farms; however, SPD and Infectious Pancreatic Necrosis Virus (IPNV) can occur concurrently and total losses may be caused by the combined infections.

2.1.1.3.5 Control/preventive measures

A reduction in common stressors, e.g., transport and handling, during the acute phase may enhance recovery. Some farmers have reported that keeping fish on a reduced pellet size feed reduces anorexia and the overall mortality associated with SPD. Vaccine trials are continuing and a commercial vaccine may be available in the near future.

2.1.1.3.6 Temporal trends

In Scotland and Ireland the number of recorded outbreaks of SPD are low.

References

Desvignes, L., Quentel, C., Lamour, F., and Le, V.A. 2002. Pathogenesis and immune response in Atlantic salmon (*Salmo salar* L.) parr experimentally infected with salmon pancreas disease virus (SPDV). Fish and Shellfish Immunology, 12: 77–95.

Weston, J., Villoing, S., Bremont, M., Castric, J., Pfeffer, M., Jewhurst, V., McLoughlin, M., Rodseth, O., Christie, K.E., Koumans, J., and Todd, D. 2002. Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection. Journal of Virology, 76: 6155–6163.

2.1.2 Bacterial diseases

2.1.2.1 Vibriosis (*Vibrio* sp.)

2.1.2.1.1 Description of agent

Atlantic salmon bacterial pathogens belonging to the genus *Vibrio* are ubiquitous within the marine environment. These are Gram-negative, non-spore-forming bacteria that appear as straight or slightly curved, motile rods. All species are facultative anaerobes and ferment carbohydrates producing acid rather than gas. They are distinguished from the aeromonads by their sensitivity to the pteridine vibriostat 0/129, which inhibits cell division. Pathogenic vibrios are cultured on marine-based media containing 1–2 % sodium chloride. Among the species, the mol % G+C of the DNA is 45.6 % (*V. anguillarum*), and 44 % (*V. salmonicida*). *Vibrio* (= *Listonella*) *anguillarum* is a serologically heterogeneous species, each serotype defined by antigenically distinct lipopolysaccharide O-antigens. In contrast, *V. salmonicida* is a relatively homogeneous species, represented by only two serotypes.

2.1.2.1.2 Geographical distribution

Vibrio anguillarum has been responsible for most outbreaks of vibriosis in farmed salmon worldwide (Kent and Poppe, 2002). *Vibrio salmonicida*, the cause of cold-water vibriosis or Hitra Disease, is a psychrophilic species reported from salmon-producing countries bordering the North Atlantic. *Vibrio viscosus* (= *Moritella viscosa*) and *V. wodanis* have been associated with "winter ulcer" in farmed salmon in Scotland, Norway, and Iceland (Bruno *et al.*, 1998; Benediktsdottir *et al.*, 2000; Lunder *et al.*, 2000). A winter-ulcer-like syndrome has been observed in farmed salmon in eastern Canada and the USA and was associated with *V. wodani* (Whitman *et al.*, 2001).

2.1.2.1.3 Short description of clinical signs

The clinical signs of vibriosis are typical of a haemorrhagic septicaemia and may be manifested as peracute, acute, or chronic. In peracute vibriosis, particularly in juvenile salmon, mortality occurs in the absence of clinical signs other than the occasional oedema. Acute vibriosis is associated with anaemia, skin darkening with ulcerative swellings, red necrotic lesions in the abdominal muscle and erythema at the base of the fins, around the vent, and within the mouth. Bacteraemia occurs in most cases. In chronic vibriosis, anaemia remains a prominent sign accompanied by granulating lesions in the muscle and fibrous peritoneal adhesions. Oedema associated with acute and chronic vibriosis results in exophthalmia and abdominal distension. Mortality associated with vibriosis can be severe. Fish affected with *V. salmonicida* generally exhibit an extensive haemorrhaging of the visceral organs with oedema, pale gills, and erythema, and associated swelling of the vent. The pathogenicity of this organism is less than that of *V. anguillarum*. Winter ulcers may be associated with elevated mortality and, as the name suggests, are most commonly observed during the first winter at sea. Moribund fish become lethargic and cease feeding. Ulcers ranging in size from a few millimetres to several centimetres occur on the flanks and are surrounded by hyperaemia or haemorrhage.

2.1.2.1.4 Indications of impact/severity at stock level

Vibriosis in farmed salmon has largely become a disease of historical interest because of the widespread application of effective vaccines. The present impact of vibriosis caused by *V. anguillarum* and *V. salmonicida* is minimal and when outbreaks do occur they are associated with failure to vaccinate or use of an inappropriate vaccine. Winter ulcer continues to have some impact on the industry in Norway.

2.1.2.1.5 Control/preventive measures

Adequate vaccination, along with the maintenance of optimal husbandry practices, has provided the single most important means of preventing vibriosis. The serological heterogeneity among *Vibrio* species and serotypes requires that vaccines must contain representative antigens of each as cross protection is not elicited. In the event of an outbreak, the rapid development of disease often reduces the effectiveness of oral antibiotic treatment since affected fish refuse to eat. Therefore, where rapid diagnosis and effective treatment is not possible, isolation or removal of diseased fish remains the most effective control. Recent indications from Norway are that, as with other vibrios, vaccination against *V. viscosus* is beneficial.

2.1.2.1.6 Temporal trends

Vibriosis has been effectively controlled through the widespread use of vaccination. Diseases caused by *V. anguillarum* and *V. salmonicida* have been virtually non-existent in salmon culture for several years. In 2001, an increasing trend in cases of *V. viscosus* was reported from Scotland, and disease outbreaks were observed in Norway despite vaccination.

2.1.2.1.7 Other host species

Vibriosis has been reported from a wide range of fish species in marine and brackish waters. *Vibrio anguillarum* has been associated with disease outbreaks in numerous farmed non-salmonid and salmonid fish species (Toranzo and Barja, 1990). *Vibrio salmonicida* is primarily associated with Atlantic salmon, but the bacteria has also been isolated from diseased rainbow trout and Atlantic cod. *Vibrio viscosus* has also been isolated from rainbow trout (Larsen and Pedersen, 1999).

References

Benediktsdottir, E., Verdonck, L., Sproer, C., Helgason, S., and Swings, J. 2000. Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographic locations: a proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. International Journal of Systematic and Evolutionary Microbiology, 50: 479–488.

Bruno, D.W., Griffiths, J., Petrie, J., and Hastings, T.S. 1998. *Vibrio viscosus* in farmed Atlantic salmon *Salmo salar* in Scotland: field and experimental observations. Diseases of Aquatic Organisms, 34: 161–166.

Kent, M.L., and Poppe, T.T. 2002. Infectious diseases of coldwater fish in marine and brackish water. *In* Diseases and disorders of finfish in cage culture, pp. 61–105. Ed. by P.T.K. Woo, D.W. Bruno, and L.H.S. Lim. CABI Publishing, Wallingford, Oxon.

Larsen, J.L., and Pedersen, K. 1999. Infeksjoner med Vibrio-bakterier. *In* Fiskehelse og fiskesygdommer, pp. 68–83. Ed. by T. Poppe. Universitetsforlaget AS, Oslo.

Lunder, T., Sorum, H., Holstad, G., Steigerwalt, A.G., Mowinckel, P., and Brenner, D.J. 2000. Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with "winter ulcer". International Journal of Systematic and Evolutionary Microbiology, 50: 427–450.

Pedersen, K. 1999. The fish pathogen *Vibrio anguillarum* with special emphasis on ecology, epizootiology, pathogenicity, serology and typing methods. PhD-thesis, The Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, Laboratory of Fish Diseases, Copenhagen, 192 pp.

Toranzo, A.E., and Barja, J.L. 1990. A review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special refernce to aquaculture in the northwest of Spain. Diseases of Aquatic Organisms, 9: 73–82.

Whitman, K.A., Backman, S., Bendiktsdottir, E., Coles, M., and Johnson. G. 2001. Isolation and characterization of a new *Vibrio* spp. (*Vibrio wodanis*) associated with "Winter Ulcer Disease" in sea water raised Atlantic salmon (*Salmo salar* L.) in New Brunswick. Aquaculture Association Canada Special Publication, 4: 115–117.

2.1.2.2 Furunculosis (*Aeromonas salmonicida*)

Classical furunculosis derives its name from the boil-like lesions observed on the skin and in the musculature of infected fish. However, development of "furuncles" on the dorsal body is the exception rather than the rule and tends only to occur in older fish suffering from the chronic form of the disease.

2.1.2.2.1 Description of agent

Aeromonas salmonicida is a non-motile Gram-negative, facultative anaerobe, rod-shaped bacterium. Classification of *Aeromonas salmonicida* into several subspecies has been discussed for a long time and a number of subspecies have been proposed (Austin and Austin, 1999). *A. salmonicida* subsp. *salmonicida* strains associated with classical furunculosis in salmonids are termed "typical", while all other strains which do not fit into this description are called "atypical" including *A. salmonicida* subsp. *achromogenes*, *A. salmonicida* subsp. *nova*, and *A. salmonicida* subsp. *smithia*. This overview will concentrate on the classical furunculosis caused by the "typical" *A. salmonicida* subsp. *salmonicida*.

2.1.2.2.2 Geographical distribution

Classical furunculosis is present worldwide. However, *A. salmonicida* subsp. *salmonicida* has so far not been reported from Australia.

2.1.2.2.3 Short description of clinical signs

The clinical manifestations of classical furunculosis are often divided into peracute (usually restricted to young fish), acute (in growing fish), and subacute or chronic (in older fish) forms. Fish may darken and go off feed. Fish may show slight exophthalmia, focal haemorrhage in the gills with dilated blood vessels and punctate haemorrhage. Internally, the viscera are hemorrhagic, the kidney is very soft, the spleen enlarged, and the liver pale or mottled with petechiae. In chronic cases, there is a more gradual onset of mortality. Externally, fish have skin lesions or furuncules, along with internal lesions (Brown and Bruno, 2002).

2.1.2.2.4 Indications of impact/severity at stock level

Due to vaccination programmes, the impact of *A. salmonicida* on stock levels has considerably decreased and furunculosis is no longer perceived as a problem in the commercial sector in Europe. However, the oil-based adjuvants added to the vaccines are causing side effects in vaccinated fish including granuloma formation and visceral adhesions.

2.1.2.2.5 Control/preventive measures

Control of furunculosis has been achieved through successful vaccination programmes using oil-based vaccines and through improved management.

2.1.2.2.6 Temporal trends

After the introduction of oil-based vaccines, disease outbreaks due to "typical" *A. salmonicida* have declined drastically and the last five years of data indicate that clinical outbreaks have remained low, with a possible slight decline.

2.1.2.2.7 Other host species

"Typical" *A. salmonicida* has been associated with clinical disease outbreaks in a number of farmed salmonids and non-salmonids as well as in wild fish from sea water (Bernoth, 1997). Atypical variants of *A. salmonicida* have been introduced into Australia with the importation of diseased goldfish (Trust *et al.*, 1980) and have been isolated from other non-salmonids, including cod and spotted wolffish (*Anarhichas minor*) (Cornick *et al.*, 1984).

References

Austin, B., and Austin, D.A. 1999. Bacterial fish pathogens. Disease of farmed and wild fish. Praxis Publishing Ltd, Chichester, UK, 457 pp.

Bernoth, E.-M. 1997. Furunculosis: the history of the disease and of disease research. *In* Furunculosis: Multidisciplinary fish disease research, pp. 1–20. Ed. by E.-M. Bernoth, A.E. Ellis, P.J. Midtlyng, G. Olivier, and P. Smith. Academic Press, London.

Brown, L.L., and Bruno, D.W. 2002. Infectious diseases of coldwater fish in fresh water. *In* Diseases and disorders of finfish in cage culture, pp. 107–169. Ed. by P.T.K. Woo, D.W. Bruno, and L.H.S. Lim. CABI Publishing, Wallingford, Oxon.

Cornick, J.W., Morrison, C.M., Zwicker, B., and Shum, G. 1984. Atypical *Aeromonas salmonicida* infection in Atlantic cod, *Gadus morhua* L. Journal of Fish Diseases, 7: 495–499.

Lillehaug, A., Lunestad, B.T., and Grave, K. 2003. Epidemiology of bacterial diseases in Norwegian aquaculture—a description based on antibiotic prescription data for the ten-year period 1991 to 2000. Diseases of Aquatic Organisms, 53: 115–125.

Trust, T.J., Khouri, A.G., Austen, R.A., and Ashburner, L.D. 1980. First isolation in Australia of atypical *Aeromonas salmonicida*. FEMS Microbiology Letters, 9: 39–42.

2.1.2.3 Piscirickettsiosis (*Piscirickettsia salmonis*)

Piscirickettsia salmonis was reported for the first time from coho salmon in Chile, but it is now also common in both Atlantic salmon and rainbow trout (Kent and Poppe, 2002). The disease is occurring mainly in marine water although some outbreaks have been reported from fresh water, probably due to the transport of infected eggs from salt water to fresh water (Gaggero *et al.*, 1995). It is suggested that *P. salmonis* infects the host in the sea water; however, a marine reservoir has not yet been identified.

2.1.2.3.1 Description of agent

P. salmonis is a pleomorphic, Gram-negative, non-motile, coccoid bacterium occurring intracellularly as individuals, pairs or groups.

2.1.2.3.2 Geographical distribution

P. salmonis has been found from the east and west coasts of Canada, Chile, Ireland, Norway, Scotland, and the west coast of the USA (Fryer and Hedrick, 2003).

2.1.2.3.3 Short description of clinical signs

Severely affected fish are lethargic, dark in colour, and show pale gills and inappetance. Less affected fish may show no abnormal external signs. Internally, the kidney is swollen and discoloured, and the spleen is enlarged. Ascites and haemorrhages on internal organs and body musculature may be present. The liver might be pale and exhibit cream-coloured circular opaque nodules (Olsen, 1999; Fryer and Hedrick, 2003).

2.1.2.3.4 Indications of impact/severity at stock level

The impact of the pathogen on Atlantic salmon has been rather moderate compared to the mortalities observed in coho salmon, which might reach up to 90 % (Olsen, 1999).

2.1.2.3.5 Control/preventive measures

Improved husbandry practices appear to aid in minimizing infections caused by *P. salmonis*. Antimicrobials have been used but the efficacy of these treatments is inconsistent, probably due to the difficulty in obtaining intracellular concentrations of drug sufficient to kill the bacteria. Promising results of vaccination trials indicate that the disease can be better controlled in the near future.

2.1.2.3.6 Temporal trends

An increased incidence of the disease has recently (2002) been observed in Norway, and in 2002, *P. salmonis* was detected in Scotland for the first time since 1996.

2.1.2.3.7 Other host species

P. salmonis has been associated with disease in coho salmon, rainbow trout, cherry salmon (*Oncorhynchus masou*), chinook salmon (*O. tshawytscha*), pink salmon (*O. gorbuscha*), and white sea bass (*Atractoscion nobilis*) (Fryer and Hedrick, 2003).

References

Fryer, J.L., and Hedrick, R.P. 2003. *Piscirickettsia salmonis*: a gram-negatvie intracellular bacterial pathogen of fish. Journal of Fish Diseases, 26: 251–262.

Gaggero, A., Castro, H., and Sandino, A.M., 1995. First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle. Journal of Fish Diseases, 18: 277–279.

Kent, M.L., and Poppe, T.T. 2002. Infectious diseases of coldwater fish in marine and brackish water. *In* Diseases and disorders of finfish in cage culture, pp. 61–105. Ed. by P.T.K. Woo, D.W. Bruno, and L.H.S. Lim. CABI Publishing, Wallingford, Oxon.

Olsen, A.B. 1999. Piscirickettsiose. *In* Fiskehelse og fiskesygdommer, pp. 110–112. Ed. by T. Poppe. Universitetsforlaget AS, Oslo.

2.1.3 Parasitic diseases

2.1.3.1 Sea lice (*Lepeophtheirus salmonis*)

Sea lice are the most economically important parasites affecting salmon in cage culture. The group encompasses several species of marine ectoparasitic copepods of the genera *Lepeophtheirus* and *Caligus*, family Caligidae, that infect marine fishes, particularly salmonids.

2.1.3.1.1 Description of agent

Sea lice have ten developmental stages: two free-living planktonic nauplius stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two pre-adult stages, and one adult stage. The copepodid, chalimus, pre-adult, and adult stages all feed on mucus, skin, and blood of fish. Copepodids and chalimus larvae of sea lice are small (< 4 mm in length) and can occur on the body surface and fins as well as in the buccal cavity and on the gills. Pre-adult and adult sea lice are visible to the naked eye. They usually occur on the body surface, especially on the head, back, and the perianal region. These stages usually cause the most damage to the fish. Pre-adult and adult stages of *Caligus* species can be distinguished from *Lepeophtheirus* species by the presence of lunules on their anterior margin.

2.1.3.1.2 Geographical distribution

Lepeophtheirus salmonis has a circumpolar distribution and is restricted to salmonids, except as a result of accidental transfer from salmonids. In contrast, *Caligus* species infecting salmon have broad host ranges that include both non-salmonid teleost and elasmobranch hosts.

2.1.3.1.3 Short description of clinical signs

Attack by sea lice can cause skin lesions that range from minor to severe. Fish will show a tendency to go off feed, thus reducing growth and condition. Heavy infections greatly reduce the market value of the fish and ultimately result in death. Mortality may occur due to the development of secondary diseases (e.g., vibriosis, furunculosis) fostered by the high levels of accompanying stress. In severe cases where the epidermis is breached, death may be due to a loss of physiological homeostasis including osmotic stress, anaemia, and hypoproteinaemia. Pre-adult and adult parasites actively move on the surface of fish, and lesions caused by these stages may be severe and widespread. In contrast, damage by the non-motile copepodid and chalimus larvae is generally localized. Infected salmon commonly show mild to extensive areas of skin erosion and haemorrhaging on the head and back and exhibit distinct areas of erosion, dark colouration, and subepidermal haemorrhage in the perianal region. Severely infected salmon show ulcers in which the epidermis is breached and the underlying tissues are exposed. These lesions often occur on the head and behind the dorsal fin.

2.1.3.1.4 Indications of impact/severity at stock level

Gordon Rae of Scottish Quality Salmon (SQS) estimated (June 2001) the cost of sea lice to the Scottish industry at over 43 million EURO, composed thus:

Loss of growth in fish: 5 % per annum - value in 2000:	EURO 21 million
Cost of medicines	EURO 8 million +
Additional labour and hardware	EURO 3 million
Accidental mortality during treatment	EURO 3 million +
Mortality and downgrades at harvest	EURO 8 million +

2.1.3.1.5 Control/preventive measures

The main therapeutant products for control of sea lice currently licensed for use in the UK are as follows:

Product name	Type	Active constituent	Manufacturer
Excis	Bath	Cypermethrin	Novartis
Salmosan	Bath	Azamethipos	Novartis
Calicide	In-feed	Teflubenzuron	Nutreco/Trouw
Slice	In-feed	Emamectin benzoate	Schering-Plough

Bath treatments are usually administered by placing tarpaulin skirts around the cages. In-feed treatments are administered over a seven-day period, to cover the varying appetites and feeding patterns in individual fish. The advantages to farmers of in-feed treatments are that they are easy to administer, non-weather-dependent and, in the case of Slice, 100 % effective against all parasitical stages of the louse. Calicide exerts its effects at the moulting stage, and so must be used before adult lice appear.

With the use of bath treatments, re-infestation can occur within a very short period, particularly if not all the lice are killed. Such treatments are effective only against the mobile stages of lice, and their administration stresses the fish. A critical period exists during which the use of these products is most efficacious. Hydrogen peroxide, which was widely used in the industry until the late 1990s, is a less popular choice due to reduced efficacy that has resulted from growing resistance to the product in lice.

In Norway, the following quantities of lice treatment products were prescribed in 2000. Provisional figures suggest a reduction in amounts for 2001.

Cypermetrin (Exis, Betamax):	68.7 kg
Deltamethrin (Alpha Max):	17.6 kg
Diflubenzuron (Lepsidon):	12.4 kg
Emamectin benzoate (Slice):	11.0 kg
Teflubenzuron (Ektobann):	61.5 kg

In Norway, wrasse are frequently used to control sea lice numbers. However, this practice has declined recently as it is difficult to combine with an almost zero-tolerance for lice, which requires delousing at low infestation levels. Other control methods are organized delousing at low temperatures and synchronized delousing.

In Ireland, Slice is being widely used as an in-feed treatment, particularly for the first year in the sea. It is proving very effective.

2.1.3.1.6 Temporal trends

During the past several years, sea lice infestations in farmed fish have been controlled by chemotherapeutics in Scotland and Norway. However, in Norway, the main problem is an apparent increase in infestation of wild fish.

In Ireland, the trend in lice numbers is downwards. The national mean for ovigerous lice in 2001 was less than 0.5 per fish. The spring levels as measured in May were lower for both ovigerous and total mobile lice than in the previous year and continue a downward trend begun in 1998. Management protocols are in place nationally that trigger treatments once levels exceed 0.3–0.5 ovigerous lice per fish during the spring and 2.0 ovigerous lice during the remainder of the year.

2.1.3.1.7 Other host species

See Section 2.1.3.1.2, above.

References

Rae, G.H. 2002. Sea louse control in Scotland, past and present. Pest Management Science, 58: 515–520.

Ramstad, A., Colquhoun, D.J., Nordmo, R., Sutherland, I.H., and Simmons, R. 2002. Field trials in Norway with SLICE (0.2 % emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 50: 29–33.

Revie, C.W., Gettinby, G., Treasurer, J.W., Grant, A.N., and Reid, S.W. 2002. Sea lice infestations on farmed Atlantic salmon in Scotland and the use of ectoparasitic treatments. The Veterinary Record, 151: 753–757.

Ritchie, G., Ronsberg, S.S., Hoff, K.A., and Branson, E.J. 2002. Clinical efficacy of teflubenzuron (Calicide) for the treatment of *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. Diseases of Aquatic Organisms, 51: 101–106.

2.2 *Other salmonids*

2.2.1 Viral diseases

2.2.1.1 Viral Haemorrhagic Septicaemia (VHS)

2.2.1.1.1 Description of agent

Viral Haemorrhagic Septicaemia (VHS) is caused by a Novirhabdovirus (VHS Virus – VHSV) belonging to the rhabdovirus family. The size of the virus particle is approximately 70 nm × 180 nm and the virus is sensitive to a number of organic solvents as well as acids and bases. The virus has five structural proteins of which the G-protein is the most important with regard to antigenicity and virulence.

VHS is the most important viral disease of farmed salmonid fishes in Europe (McAllister et al 1985). VHS was regarded as a freshwater trout disease until 1988, when VHSV was isolated for the first time in North America from returning chinook salmon (*Oncorhynchus tshawytscha*) (Hopper, 1989) and coho salmon (*O. kisutch*) (Brunson *et al.*, 1989).

So far, VHSV has been isolated from twelve different species in the North American Pacific area, three different species in the North American Atlantic area, one species in Japan, and fifteen species in the North Sea area and adjacent areas including the Baltic Sea, but the number of host species still seems to be increasing.

Serological analyses were not able to distinguish the North American VHSV isolates from the typical European reference strains (Winton *et al.*, 1989). Nucleotide sequencing and phylogenetic analysis demonstrated four distinct genotypes (Snow *et al.*, 1999). Genotype I comprised European freshwater VHSV isolates causing mortality in rainbow trout and a group of marine isolates from the Baltic Sea as well as a German turbot isolate (Schlotfeldt *et al.*, 1991). Genotype II comprised a group of marine isolates circulating in the Baltic Sea. Genotype III comprised a Scottish turbot VHSV isolate (Ross *et al.*, 1994) and isolates from wild marine fishes in the adjacent sea areas. The North American Pacific herring VHSV isolates comprised a fourth distinct type.

2.2.1.1.2 Geographical distribution

VHS is widely distributed in the continental parts of Europe having intensive production of rainbow trout in freshwater aquaculture. Finland, Sweden, Norway, UK, and Ireland are considered free of VHS although a few outbreaks have been observed in marine fish farms in Finland and Sweden in recent years. In Spain, the virus has not been diagnosed for more than ten years and it has never been diagnosed in Portugal or Greece (Olesen, 1998). In Pacific USA and Canada, VHSV has been

isolated from Pacific salmon species ascending the rivers for spawning. In the marine environment, isolates have been made from wild fishes in the Pacific and Atlantic coastal areas of the USA, Canada, and Japan. In Europe, marine VHSV has been isolated from fish in the North Sea, the English Channel, the Skagerrak, the Kattegat, and the Baltic Sea.

2.2.1.1.3 Short description of clinical signs

The clinical signs of classical VHS in freshwater salmonids are dark skin and pale gills, petechiae in the gills and in the skin, and haemorrhages in the orbits and exopthalmus. Widespread petechiae developing to haemorrhages are observed in the peritoneal surfaces, in the swimbladder, in the skeletal muscles, and in the meninges. The liver is pale with haemorrhages, and the spleen is often enlarged and reddish. High mortality is observed in the acute phase of the disease.

The clinical signs in marine fishes are quite varying. In Pacific salmonids, VHSV was isolated from fish without clinical signs. In Pacific cod (*Gadus macrocephalus*) and herring (*Clupea harengus pallasi*), infection appeared to be associated with skin lesions, while other Pacific fish species from which VHSV was isolated did not display any gross symptoms. In European waters, the clinical signs in farmed salmonids and turbot were almost identical: classical signs were observed in freshwater salmonids, whereas VHSV in most other fish species was isolated from specimens showing no clinical signs.

The North American VHSV isolates were found to be less pathogenic to rainbow trout than the European reference strains (Winton *et al.*, 1991).

2.2.1.1.4 Indications of impact/severity at stock level

So far, two mass mortality events have been reported from the Pacific USA and Canada. In August 1998, mass mortalities in Pacific herring, Pacific hake (*Merluccius productus*), and in Walleye pollock (*Theragra chalcogramma*) were reported in Lisianski Inlet, Alaska. Marine VHSV could be isolated from samples of dead fish of all three species (Meyers *et al.*, 1999). None of the affected fish showed any gross external or internal lesions and the histology displayed unspecific changes of which some could be referred to as autolytic changes.

During the period November 1998 to February 1999 another mass mortality occurred a little further south in Queen Charlotte Strait, on the northeast coast of Vancouver Island. In particular, pilchards (*Sardinops sagax*) but also Pacific herring, blackcod (*Anaplopoma fimbria*), ratfish (*Hydrolagus colliei*) and shinner perch (*Cytomaster aggregata*) were reported floating on the surface moribund or dead (Traxler *et al.*, 1999). On this occasion, marine VHSV was isolated from pilchard, Pacific herring, and blackcod.

2.2.1.1.5 Control/preventive measures

According to the OIE International Aquatic Animal Health Code, Viral Haemorrhagic Septicaemia is a notifiable disease. This means that, in case of outbreaks of VHS in areas having a disease-free status, OIE has to be informed and specific eradication measures have to be implemented to reduce the risk of spread of the disease. Affected farmed fish stocks must be destroyed. Similar measures have to be taken within EU countries according to EU Council Directive 91/67. However, the widespread distribution of VHSV in marine fish species may cause serious problems for marine aquaculture.

2.2.1.1.6 Temporal trends

An occurrence of VHS, with no associated pathology, was reported in 1998 from a hatchery in western Norway. This is the first record of this disease in rainbow trout in Norway since 1974. Following stock eradication, no new cases have been reported. In 1998, VHS was recorded in a small rainbow trout, on a fattening farm on the Swedish west coast. This was the first record of this disease in Sweden since 1972. The disease recurred at the site in 2000. The fish stock was destroyed on both occasions. In 2000, clinical VHS was diagnosed in sea-reared rainbow trout from four farms in two areas in Finland. Exclusion orders and destruction of the stock were implemented at three farms. Wild fish are strongly suspected as the source of infection. It has not been possible to trace the source of the infection for any of these outbreaks.

2.2.1.1.7 Other host species

Since 1998, VHS-like virus has been isolated from an increasing number of wild fish species along the US and Canadian Pacific and Atlantic coasts, in the North Sea, the Skagerrak, the Kattegat, and the Baltic Sea.

References

Brunson, R., True, K., and Yancey, J. 1989. VHS virus isolated at Makah National Fish Hatchery. ASF News Letter, 17: 3–4.

Hopper, K. 1989. The isolation of VHSV from chinook salmon at Glenwood Springs, Orcas Island, Washington. Fish Health Section, American Fisheries Society Newsletter, 17: 1

Meyers, T.R., Short, S., and Lipson, K. 1999. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fish. Diseases of Aquatic Organisms, 38: 81–86.

Mortensen, H.F., Heuer, O.E., Lorentzen, N., Otte, L., and Olesen, N.J. 1999. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. Virus Research, 63: 95–106.

Olesen, N.J. 1998. Sanitation of viral haemorrhagic septicaemia (VHS). Journal of Applied Ichthyology, 14: 173–177.

Ross, K., McCarthy, U., Huntley, P.J., Wood, B.P., Stuart, D., Rough, E.I., Smail, D.A., and Bruno, D.W. 1994. An outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. Bulletin of the European Association of Fish Pathologists, 14: 213–214.

Schlotfeldt, H.-J., Ahne, W., Jørgensen, P.E.V., and Glende, W. 1991. Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) - a natural outbreak. Bulletin of the European Association of Fish Pathologists, 11: 105–107.

Snow, M., Cunningham, C.O., Melvin, W.T., and Kurath, G. 1999. Analysis of the nucleoprotein gene identifiers distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. Virus Research, 63: 35–44.

Traxler, G.S., Kieser, D., and Richard, J. 1999. Mass mortality of pilchard and herring associated with viral hemorrhagic septicemia virus in British Columbia, Canada. Fish Health Newsletter, 27: 3–4.

Winton, J.R., Nishizawa, T., and Stehr, C.M. 1989. Characterization of the first North American isolates of viral hemorrhagic septicemia virus. ASF News Letter, 17: 2–3.

Winton, J.R., Batts, W.N., Deering, R.E., Brunson, R., Hopper, K., Nishizawa, T., and Stehr, C. 1991. Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. *In* Proceedings of the Second International Symposium on Viruses of Lower Vertebrates, pp. 43–50. Oregon State University, Corvallis, OR.

2.2.2 Bacterial diseases

2.2.2.1 Vibriosis (*Vibrio* sp.)

See Section 2.1.2.1, above.

2.2.2.2 Furunculosis (*Aeromonas salmonicida*)

See Section 2.1.2.2, above.

2.3 *Sea Bass (Dicentrarchus labrax)/ Seabream (Sparus aurata)*

2.3.1 Viral diseases

2.3.1.1 Viral Encephalopathy and Retinopathy (Nodavirus)

2.3.1.1.1 Description of agent

The causative agent belongs to the Nodaviridae family. A description is provided in Section 2.4.1.1, below, on nodavirus in halibut.

2.3.1.1.2 Geographical distribution

Nodavirus infection in sea bass and seabream has been described in France and also in most of the Mediterranean countries.

2.3.1.1.3 Short description of clinical signs

Most often no external signs are associated with nodavirus infection, which causes Viral Encephalopathy and Retinopathy (VER), although some whitish discolouration on the cranial epithelium and reddening of cephalic area may be visible. Clinical signs are for the most part abnormal swimming behaviour: fish swim quickly in circles or stay in a vertical position with the head or caudal peduncle above the water surface. Microscopic observation shows spongiosis of the nervous system.

2.3.1.1.4 Indications of impact/severity at stock level

Nodavirus infections in commercial hatcheries affect mainly larvae and juveniles, but also one-year-old fish. Mortality ranging from 15–60 % has been recorded. Seabream seems to be less susceptible to the disease than sea bass.

2.3.1.1.5 Control/preventive measures

No completely effective control measures for nodavirus have been established, however, screening of broodstock (gonads, sera) in order to obtain nodavirus-free broodstock is recommended. The best preventative action is application of strict general hygienic measures on farms.

2.3.1.1.6 Temporal trends

Nodavirus infection has become the major viral disease problem in sea bass farms, where it causes important economic losses.

2.3.1.1.7 Other host species

See Section 2.4.1.1, below.

References

Aranguren, R., Tafalla, C., Novoa, B., and Figueras, A. 2002. Experimental transmission of encephalopathy and retinopathy induced by nodavirus to seabream (*Sparus aurata* L.) using different infection models. Journal of Fish Diseases, 25: 317–324.

Breuil, G., Pépin, J.F., Boscher, S., and Thiéry, R. 2002. Experimental vertical transmission of nodavirus from broodfish to eggs and larvae of the sea bass, *Dicentrarchus labrax* (L.). Journal of Fish Diseases, 25: 697–702.

Breuil, G., Pépin, J.F., Castric, J., Fauvel, C., and Thiéry, R. 2000. Detection of serum antibodies against nodavirus in wild and farmed adult sea bass: application to the selection of broodstock in sea bass hatcheries. Bulletin of the European Association of Fish Pathologists, 20(3): 95–100.

Castric, J., Thiéry, R., Jeffroy, J., de Kinkelin, P., and Raymond, J.C. 2001. Seabream (*Sparus aurata*), an asymptomatic contagious fish host for nodavirus. Diseases of Aquatic Organisms, 47: 33–38.

2.3.2 Bacterial diseases

2.3.2.1 Pasteurellosis (*Photobacterium damselae* subsp. *piscicida*)

2.3.2.1.1 Description of agent

Pasteurellosis is caused by the halophilic bacterium *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*), which was originally isolated during 1963 mortality episodes in natural populations of white perch (*Morone americanus*) and striped bass (*M. saxatilis*) in Chesapeake Bay. The pathogen is a Gram-negative non-motile rod-shaped to cocco-bacilli, oxidase and catalase positive, fermentative, arginine dihydrolase positive and lysine and ornithine decarboxilase negative. The bacterium is sensitive to the vibriostatic agent O/129. It grows between 15–32 °C with an optimum temperature of 22–30 °C.

Regardless of the geographic origin and source of isolation, all strains of this pathogen are biochemically and serologically homogeneous (Magariños *et al.*, 1992, 1996). However, DNA fingerprinting methods, such as ribotyping (Magariños *et al.*, 1997), AFLP (Thyssen *et al.*, 2000) and RAPD (Magariños *et al.*, 2000), have proven to be valuable epidemiological tools since they demonstrate two clearly separate clonal lineages within *Ph. damselae* subsp. *piscicida*, i.e., the European and Japanese isolates.

The presumptive identification of the pathogen is based on standard biochemical tests. In addition, although *Ph. damselae* subsp. *piscicida* is not included in the API-20 E code index, this miniaturized system can also be useful for a rapid presumptive diagnosis of the disease because all strains display the same profile (2005004) (Magariños *et al.*, 1992). A slide agglutination test using specific antiserum is needed to confirm identity of the microorganism.

In the last few years, the Norwegian company Bionor AS has marketed different diagnostic kits based not only on direct bacterial agglutination (Mono-Pp) but also on ELISA (Aquarapid-Pp or Aquaeia-Pp), which allow rapid detection of *Ph. damselae* subsp. *piscicida* in fish tissues. The evaluation of these ELISA kits in the field demonstrated that the sensitivity of the Aquaeia-Pp (magnetic beads-EIA based method) was 100 to 1000 times higher than that of the standard ELISA Kit (Aquarapid-Pp) (Romalde *et al.*, 1995, 1999b), which indicates its usefulness for the detection of asymptomatic carrier fish.

Since 1997, a variety of DNA-based protocols have also been developed for rapid and specific detection of the causative agent of pasteurellosis. Only a multiplex PCR approach using as target the 16S ribosomal (16S rDNA) and ureC genes allowed the specific discrimination between *Ph. damselae* subsp. *piscicida* and *Ph. damselae* subsp. *damselae* (formerly, *Vibrio damsela*) (Osorio *et al.*, 1999, 2000; Osorio and Toranzo, 2002).

2.3.2.1.2 Geographical distribution

Since 1969, pasteurellosis has been one of the most important diseases of farmed fish in Japan, affecting mainly yellowtail (*Seriola quinqueradiata*). From 1990 to present, it has caused important economic losses in the marine culture of gilthead seabream (*Sparus aurata*) and sea bass in the Mediterranean countries of Europe, and hybrid striped bass (*M. saxatilis* × *M. chrysops*) in the USA (Toranzo *et al.*, 1991; Magariños *et al.*, 1996; Romalde and Magariños, 1997; Zorrilla *et al.*, 1999; Romalde *et al.*, 1999a). Pasteurellosis outbreaks have been reported recently in the newly developed culture of common sole (*Solea solea* and *S. senegalensis*) in several Mediterranean countries.

2.3.2.1.3 Short description of clinical signs

Fish pasteurellosis was also known as pseudotuberculosis because it is characterized by the presence of white nodules on the internal viscera, particularly the spleen and kidney. Severe mortalities usually occur when water temperatures are above 18–20 °C. Below this temperature, fish can harbour the pathogen as sub-clinical infections for long periods (Magariños *et al.*, 2001).

2.3.2.1.4 Indications of impact/severity at stock level

Differences in susceptibility to pasteurellosis based on age have been demonstrated in seabream, with adult fish being highly resistant to the infection. The disease is most severe from the larval stages to 10 g fish. Histological observations and *in vitro* killing assays have demonstrated that neutrophils and macrophages of older seabream efficiently phagocytose and kill the bacteria, while these cell types are not functional in small fish (Noya *et al.*, 1995).

2.3.2.1.5 Control/preventive measures

The application of rapid, specific, and sensitive serological and molecular tools such as those based on ELISA or PCR is of crucial importance in the case of pasteurellosis since it has been demonstrated that the pathogen can be transmitted through the ovarian and seminal fluids from apparently healthy broodstock (Romalde *et al.*, 1999b). Moreover, this microorganism undergoes a viable but non-culturable state that makes its detection in the farm environment very difficult.

Several commercial vaccines against *Ph. damselae* subsp. *piscicida* are available but their efficacy is dependent on the fish species, fish size, vaccine formulation, and use of immuno-stimulants. Only the toxoid enriched-whole-cell bacterin (DI vaccine) developed by the University of Santiago (Spain) and produced by Hipra Veterinary Laboratories (Spain) was effective in 50-day-old gilthead seabream larvae. As the majority of the pasteurellosis outbreaks occur in larval to fingerling (10–30 g) stages, a vaccination programme

comprising a first dip immunization at the larval stage (0.03–0.05 g) and a booster vaccination when fish reach about 1–2 g is recommended to avoid the high economic losses caused by this disease (Magariños *et al.*, 1994, 1999).

Despite the heavy impact on aquaculture of some species, the disease is not included in the OIE International Aquatic Animal Health Code nor in the EU legislation.

2.3.2.1.6 Temporal trends

Since 1990, pasteurellosis has caused very important losses to Mediterranean aquaculture, basically in the production of alevins and juvenile stages. With the introduction of vaccines, and improving management practices in hatcheries, there is a slowly declining trend in disease outbreaks.

2.3.2.1.7 Other host species

See Section 2.3.2.1.2, above.

References

Magariños, B., Romalde, J.L., Bandín, I., Fouz, B., and Toranzo, A.E. 1992. Phenotypic, antigenic and molecular characterization of *Pasteurella piscicida* isolated from fish. Applied and Environmental Microbiology, 58: 3316–3322.

Magariños, B., Romalde, J.L., Santos, Y., Casal, J.F., Barja, J.L., and Toranzo, A.E. 1994. Vaccination trials on giltheaad seabream against *Pasteurella piscicida*. Aquaculture, 120: 201–208.

Magariños, B., Toranzo, A.E., and Romalde, J.L. 1996. Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. Annual Review of Fish Diseases, 6: 41–64.

Magariños, B., Osorio, C.R., Toranzo, A.E., and Romalde, J.L. 1997. Applicability of ribotyping for intraspecific classification and epidemiological studies of *Pasteurella piscicida*. Systematic and Applied Microbiology, 20: 634–639.

Magariños, B., Romalde, J.L. Barja, J.L., Núñez, S., and Toranzo, A.E. 1999. Protection of gilthead seabream against pasteurellosis at the larval stages. Bulletin of the European Association of Fish Pathologists, 19: 159–161.

Magariños, B., Toranzo, A.E., Barja, J.L., and Romalde, J.L. 2000. Existence of two geographically linked clonal lineages in the bacterial pathogen *Photobacterium damselae* subsp. *piscicida*. Epidemiology and Infection, 125: 213–219.

Magariños, B., Couso, N., Noya, M., Merino, P., Toranzo, A.E., and Lamas, J. 2001. Effect of temperature on the development of pasteurellosis in carrier gilthead seabream (*Sparus aurata*). Aquaculture, 195: 17–21.

Noya, N., Magariños, B., and Lamas, J. 1995. Interactions between peritoneal exudate cells (PECs) of gilthead seabream (*Sparus aurata*) and *Pasteurella piscicida*. A morphological study. Aquaculture, 131: 11–21.

Osorio, C., and Toranzo, A.E. 2001. DNA-based diagnostics in seafarming. *In* Recent Advances in Marine Biotechnology Series, Vol. 7. Seafood Safety and Human Health, pp. 253–310. Ed. by M. Fingerman and R. Nagabhushanam. Science Publishers, Inc., Plymouth, UK.

Osorio, C.R., Collins, M.D., Toranzo, A.E., Barja, J.L., and Romalde, J.L. 1999. 16S rRNA sequence analysis of *Photobacterium damselae* ssp. *piscicida* and nested PCR method for the rapid detection of the causative agent of fish pasteurellosis. Applied and Environmental Microbiology, 65: 2942–2946.

Osorio, C.R., Toranzo, A.E., Romalde, J.L., and Barja, J.L. 2000. Multiplex PCR assay for ureC and 16S rRNA genes clearly discriminates between both subspecies of *Photobacterium damselae*. Diseases of Aquatic Organisms, 40: 177–183.

Romalde, J.L., and Magariños, B. 1997. Immunization with bacterial antigens: Pasteurellosis. *In* Fish Vaccinology, pp. 167–177. Ed. by R. Gudding, A. Lillehaug, P.J. Midtlyng, and F. Brown. Development of Biological Standardization, Vol. 90. Karger, Basel.

Romalde, J.L., Magariños, B., Fouz, B., Bandín, I., Nuñez, S., and Toranzo, A.E. 1995. Evaluation of Bionor mono-kits for rapid detection of bacterial fish pathogens. Diseases of Aquatic Organisms, 21: 25–34

Romalde, J.L., Magariños, B., and Toranzo, A.E. 1999a. Pasteurellosis. *In* ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, No. 54.

Romalde, J.L., Magariños, B., Lores, F., and Toranzo, A.E. 1999b. Assessment of a magnetic bead-EIA based kit for rapid diagnosis of fish pasteurellosis. Journal of Microbiological Methods, 38: 147–154.

Thyssen, A., van Eygen, S., Hauben, L., Goris, J., Swings, J., and Ollivier, F. 2000. Application of AFLP for taxonomic and epidemiological studies of *Photobacterium damselae* ssp. *piscicida*. International Journal of Systematic and Evolutionary Microbiology, 50: 1013–1019.

Toranzo, A.E., Barreiro, S., Casal, J.F., Figueras, A., Magariños, B., and Barja, J.L. 1991. Pasteurellosis in cultured gilthead seabream (*Sparus aurata*): first report in Spain. Aquaculture, 99: 1–15.

Zorrilla, I., Balebona, M.C., Moriñigo, M.A., Sarasquete, C., and Borrego, J.J. 1999. Isolation and characterization of the causative agent of pasteurellosis, *Photobacterium damsela* ssp. *piscicida*, from sole *Solea senegalensis* (Kaup). Journal of Fish Diseases, 22: 167–171.

2.4 *Halibut (Hippoglossus hippoglossus)*

2.4.1 Viral diseases

2.4.1.1 Viral Encephalopathy and Retinopathy (Nodavirus)

2.4.1.1.1 Description of agent

Viruses belonging to the Nodaviridae are the causative agents of Viral Encephalopathy and Retinopathy (VER). These viruses are un-enveloped and icosahedral, with diameters of approximately 25 nm. The bipartite genomes consist of single-stranded, positive-sense non-polyadenylated RNA molecules, encoding the putative RNA-dependent RNA polymerase (RNA1) and the capsid protein precursor (RNA2) (Grotmol *et al.*, 2000).

2.4.1.1.2 Geographical distribution

Nodavirus infections have been confirmed in halibut aquaculture systems in Norway and Scotland.

2.4.1.1.3 Short description of clinical signs

Common clinical signs of VER include lack of appetite, changes in pigmentation, hyperinflation of the gas-bladder, hyperreactivity and abnormal swimming behaviour. Gross pathological changes are uncommon, while characteristic microscopic lesions include cellular vacuolation and neuronal degeneration in the central nervous system (CNS) and the retina and ganglia of the peripheral nervous system (Grotmol *et al.*, 1997).

2.4.1.1.4 Indications of impact/severity at stock level

Nodavirus infections have occurred in commercial hatcheries in Norway causing high mortalities and are believed to be one of the main obstacles to the production of halibut juveniles.

2.4.1.1.5 Control/preventive measures

The most effective control and preventive measures include optimization of production factors and improved hatchery hygiene, use of nodavirus-free broodstock, and treatment of hatchery production water. Recently, vaccinations against nodavirus infections are being developed (Husgard *et al.*, 2001).

2.4.1.1.6 Temporal trends

Nodavirus infections are a potential emerging major disease problem in the farming of Atlantic halibut.

2.4.1.1.7 Other host species

Nodavirus has been isolated from and reported to cause disease in an increasing number of marine fish species (Munday *et al.*, 2002). It has been isolated in more than 35 species belonging to at least fourteen families (including wild fish) throughout the world where aquaculture has developed. Most of the affected species are warm-water fish, having an optimal growth at 20 °C to 30 °C, but the disease also occurs in cold-water species. In 2002, nodavirus infections caused significant mortalities in Atlantic cod (Canada and USA) and in haddock (Canada). In the Mediterranean area, sea bass and seabream are affected (see Section 2.3.1.1, above).

References

Grotmol, S., Nerland, A.H., Biering, E., Totland, G.K., and Nishizawa, T. 2000. Characterisation of the capsid protein gene from a nodavirus strain affecting the Atlantic halibut *Hippoglossus hippoglossus* and design of an optimal reverse-transcriptase polymerase chain reaction (RT-PCR) detection assay. Diseases of Aquatic Organisms, 39: 79–88.

Grotmol, S., Totland, G.K., Thorud, K., and Hjeltnes, B.K. 1997. Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut, *Hippoglossus hippoglossus*. Diseases of Aquatic Organisms, 36: 95–106.

Husgard, S., Grotmol, S., Hjeltnes, B.K., Rødseth, O.M., and Biering, E. 2001. Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV. Diseases of Aquatic Organisms, 45: 33–44.

Munday, B.L, Kwang, J., and Moody, N. 2002. Betanodavirus infections of teleost fish: a review. Journal of Fish Diseases, 25: 127–142.

2.5 *Turbot (Scophthalmus maximus)*

2.5.1 Bacterial diseases

2.5.1.1 Flexibacteriosis (*Flexibacter maritimus*)

2.5.1.1.1 Description of agent

Flexibacter maritimus (formerly, *Cytophaga marina* and *Flexibacter marinus*) is the causative agent of flexibacteriosis in marine fish (Wakabayashi *et al.*, 1986; Bernardet and Grimont, 1989). Several other names, including "gliding bacterial diseases of sea fish", "eroded mouth syndrome", and "black patch necrosis", have been used to designate the disease caused by this bacterium. On the basis of recent phylogenetic, chemotaxonomic and phenotypic studies, it was proposed that *Flexibacter maritimus* should be transferred to the new genus *Tenacibaculum*, as *Tenacibaculum maritinum* (Sukui *et al.*, 2001).

F. maritimus is a Gram-negative bacterium that forms long slender rods (0.5 µm by 30 µm to up to 100 µm). The pathogen is oxidase- and catalase-positive and displays an oxidative metabolism. Gliding motility is a characteristic feature of this bacterium. Generally, growth occurs between 15–34 °C (optimum at 25–30 °C).

The preliminary diagnosis must be supported by an isolation of the pathogen in the appropriate medium or by the use of specific molecular DNA-based methods applied directly to fish tissues. This bacterium grows only in specific media since it has an absolute requirement for sea water as well as a low concentration of nutrients. Although several media (i.e., Anacker and Ordal, Marine Agar, FMM) have been devised to isolate *F. maritimus*, the FMM medium has proven to be the most effective for recovery of this pathogen from fish tissues (Pazos *et al.*, 1996). Typical colonies of *F. maritimus* are pale yellow and flat, with uneven edges. Although the bacterium is biochemically homogeneous, two main O antigenic groups can be detected in marine fishes (Pazos *et al.*, 1993; Ostland *et al.*, 1999; Avendaño *et al.*, 2003).

One of the major problems in the study of this bacterium is the difficulty of distinguishing it from other phylogenetically and phenotypically similar species, particularly those of the genera *Flavobacterium* and *Cytophaga*. Therefore, the application of the PCR methodology is very important for an accurate identification of the pathogen. Different PCR protocols have been published using the 16S rRNA gene as target (Toyama *et al.*, 1996; Bader and Shotts, 1998; Osorio and Toranzo, 2002) which demonstrated its efficacy in field conditions.

2.5.1.1.2 Geographical distribution

Marine flexibacteriosis is widely distributed in cultured and wild fish in Europe, Japan, and the USA (McVicar and White, 1979; Wakabayashi *et al.*, 1986; Pazos *et al.*, 1993; Devesa *et al.*, 1989; Santos *et al.*, 1999). In Europe, the disease has been reported in sole, sea bass, turbot, and coho salmon (*Oncorhynchus kisutch*). In Japan, *F. maritimus* has been isolated from red seabream (*Pagrus major*), black seabream (*Acanthopagrus schlegeli*), and flounder (*Paralichthys olivaceus*). In the USA, marine flexibacteriosis has been described in white sea bass, Pacific sardine, and Pacific anchovy (*Engraulis mordax*).

2.5.1.1.3 Short description of clinical signs

In general, the affected fish have eroded and haemorrhagic mouths, ulcerative skin lesions, frayed fins, and tail rot. A systemic disease can also be established involving different internal organs. The loss of epithelial integrity, typical of this disease, is also a portal of entry for secondary bacterial or parasitic pathogens.

The clinical signs, along with the microscopical observation of accumulations of very long rods in wet mounts or Gram-stained preparations obtained from gills or lesions, can be used as an initial step for the presumptive diagnosis of marine flexibacteriosis.

2.5.1.1.4 Indications of impact/severity at stock level

Although both adults and juveniles may be affected by marine flexibacteriosis, younger fish suffer a more severe form of the disease. An increased prevalence and severity of the disease have been reported at higher temperatures (above 15 ^{o}C). In addition to water temperature, the disease is influenced by a multiplicity of environmental (stress) and host-related factors.

2.5.1.1.5 Control/preventive measures

Until recently, no vaccines were available to prevent the disease (Bernardet, 1997), however, a flexibacteriosis vaccine has now been developed by the University of Santiago (Spain) and produced by Hipra (Spain). It is the only bacterin currently on the market to prevent mortalities caused by *F. maritimus* in marine fishes. Divalent formulations to prevent simultaneous flexibacteriosis/vibriosis or flexibacteriosis/ streptoccocosis are also available.

Marine flexibacteriosis is not listed in OIE or EU legislation.

2.5.1.1.6 Temporal trends

Flexibacteriosis in turbot showed an increasing trend from 1996 until the middle of 1999. Since then, vaccination has helped to reduce losses.

2.5.1.1.7 Other host species

See Section 2.5.1.1.2, above.

References

Avendaño, R., Magariños, B., Romalde, J.L., and Torranzo, A.E. 2003. An update on the antigenic diversity in *Tenacibaculum maritimum* strains isolated from marine fishes. Fish Health Section/American Fisheries Scociety News Letters 31 (2) (In press).

Bader, J.A., and Shotts, E.B. 1998. Identification of *Flavobacterium* and *Flexibacter* species by species—polymerase chain reaction primers to the 16S ribosomal RNA gene. Journal of Aquatic Animal Health, 10: 311–319.

Bernadet, J.F. 1997. Immunization with bacterial antigens: *Flavobacterium* and *Flexibacter* infections. *In* Fish Vaccinology, pp. 179–188. Ed. by R. Gudding, A. Lillehaug, P.J. Midtlyng, and F. Brown. Development of Biological Standardization, Vol. 90. Karger, Basel.

Bernardet, J.F., and Grimont, P.A.D. 1989. Deoxyribonucleic acid relatedness and phenotypic characteristics of *Flexibacter columnaris* sp. nov., nom. rev. *Flexibacter psychrophilus* sp. nov. nom rev. and *Flexibacter maritimus* Wakabayashi, Hikida and Masamura, 1986. International Journal of Systematic Bacteriology, 39: 346–354.

Devesa, S., Barja, J.L., and Toranzo, A.E. 1989. Ulcerative and skin and fin lesions in reared turbot (*Scophthalmus maximus* L.). Journal of Fish Diseases, 12: 323–333.

McVicar, A.H., and White, P.G. 1979. Fin and skin necrosis of Dover sole *Solea solea* L.). Journal of Fish Diseases, 2: 557–562.

Pazos, F., Santos, Y., Núñez, S., and Toranzo, A.E. 1993. Increasing occurrence of *Flexibacter maritimus* in marine aquaculture of Spain. Fish Health Section/American Fisheries Society News Letter, 21: 1–2.

Pazos, F., Santos, Y., Macías, A.R., Núñez, S., and Toranzo, A.E. 1996. Evaluation of media for the successful culture of *Flexibacter maritimus*. Journal of Fish Diseases, 19: 193–197.

Osorio, C., and Toranzo, A.E. 2002. DNA-based diagnostics in sea farming. *In* Recent Advances in Marine Biotechnology Series, Vol. 7. Seafood Safety and Human Health, pp. 253–310. Ed. by M. Fingerman, and R. Nagabhushanam. Science Pyblishers, Inc., Plymouth, UK.

Ostland, V.E., La Trace, C., Morrison, D., and Ferguson, H.W. 1999. *Flexibacter maritimus* associated with a bacterial stomatitis in Atlantic salmon smolts reared in net-pens in British Columbia. Journal of Aquatic Animal Health, 11: 35–44.

Santos, Y., Pazos, F., and Barja, J.L. 1999. *Flexibacter maritimus*, causal agent of flexibacteriosis in marine fish. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, No. 55.

Sukui, M., Nakagawa, Y., Harayama, S., and Yamamoto, S. 2001. Phylogenetic analysis and taxonomic study of marine Cytophaga-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 51: 1639–1652.

Toyama, T., Tsukamoto, K.K., and Wakabayashi, H. 1996. Identification of *Flexibacter maritimus*, *Flavobacterium branchiophilum* and *Cytophaga columnaris* by PCR targeted 16S ribosomal DNA. Fish Pathology, 31: 25–31.

Wakabayashi, H., Hikida, H., and Masumura, K. 1986. *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. International Journal of Systematic Bacteriology, 36: 396–398.

3 Diseases of Farmed Molluscs

3.1 *European flat oyster (Ostrea edulis)*

3.1.1 Viral diseases

3.1.1.1 Herpesvirus

3.1.1.1.1 Description of agent

See Section 3.2.1.1.1, below.

3.1.1.1.2 Geographical distribution

Herpes-like virus infections have been reported in spat and larvae of the European flat oyster in France (Comps and Cochennec, 1993; Renault *et al.*, 2000). Concomitant mortalities were observed among larvae and spat of Pacific oyster (*Crassostrea gigas*) and *O. edulis* in 1994 and 1995, with the detection of herpes-like virus particles by transmission electron microscopy (Renault *et al.*, 2000). In 2000, the viral infection of *O. edulis* was also reported in one larval sample in the United Kingdom originating from a commercial hatchery.

Replication of herpes-like viruses has been described in adults of the Australian flat oyster (*O. angasi*) in Australia (Hine and Thorne, 1997) and in larval *O.* (= *Tiostrea*) *chilensis* in New Zealand (Hine, 1997; Hine *et al.*, 1998).

3.1.1.1.3 Short description of clinical signs

See Section 3.2.1.1.3, below.

3.1.1.1.4 Indications of impact/severity at stock level

So far, there have been no indications of impacts at stock level.

3.1.1.1.5 Control/preventive measures

See Section 3.2.1.1.5, below.

3.1.1.1.6 Temporal trends

Herpes-like viral infections have been reported regularly in *Ostrea edulis* spat and larvae in France since 1993.

3.1.1.1.7 Other host species

So far, herpes-like viruses have been reported from nine different bivalve species around the world on the basis of transmission electron microscopy and molecular techniques: Pacific oyster, American oyster (*C. virginica*), Portuguese oyster (*C. angulata*), European flat oyster, Australian flat oyster, *O.* (= *Tiostrea*) *chilensis*, Manila clam (*Ruditapes philippinarum*), carpet shell clam (*R. decussatus*), and scallop (*Pecten maximus*). The number of host species seems to be still increasing.

References

Comps, M., and Cochennec, N. 1993. A herpes-like virus from the European oyster *Ostrea edulis* L. Journal of Invertebrate Pathology, 62: 201–203.

Hine, P.M. 1997. Trends in research on diseases of bivalve molluscs. Bulletin of the European Association of Fish Pathologists, 17(6): 181–183.

Hine, P.M., and Thorne, T. 1997. Replication of herpes-like viruses in haemocytes of adult flat oysters *Ostrea angasi* (Sowerby, 1871): an ultrastructural study. Diseases of Aquatic Organisms, 29(3): 197–204.

Hine, P.M., Wesney, B., and Besant, P. 1998. Replication of herpes-like viruses in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures. Diseases of Aquatic Organisms, 32(3): 161–171.

Renault, T., Le Deuff, R.-M., Chollet, B., Cochennec, N., and Gérard, A. 2000. Concomitant herpes-like virus infections in hatchery-reared larvae and nursery-cultured spat *Crassostrea gigas* and *Ostrea edulis*. Diseases of Aquatic Organisms, 42(3): 173–183.

3.1.2 Parasitic diseases

3.1.2.1 Bonamiosis (*Bonamia* sp.)

3.1.2.1.1 Description of agent

When stained with a commercial bloodstaining kit, the parasite (2–5 µm in size) has a basophilic cytoplasm and an eosinophilic nucleus (colours may vary with the stain used). It may be observed inside or outside the haemocytes. At the electron microscope level, most of the information available in the literature concerns dense forms (Pichot *et al.*, 1979; Grizel *et al.*, 1983; Montes *et*

al., 1994) or stages slightly developed from it (Hervio *et al.*, 1991). Dense forms of *B. ostreae* (2.5 µm in diameter) present a central circular nucleus, dense ribosomes, vacuous mitochondria, few haplosporosomes and clear margin. Multi-nucleated and diplokaryotic plasmodia are also reported (Bonami *et al.*, 1985).

3.1.2.1.2 Geographical distribution

Bonamia ostreae has been reported in Denmark, France, Ireland, Italy, the Netherlands, Spain, the United Kingdom (excluding Scotland) (Pichot *et al.*, 1979; Comps *et al.*, 1980; Grizel and Tigé, 1982; Bannister and Key, 1982; van Banning, 1982; Polanco *et al.*, 1984), and the USA (Elston *et al.*, 1986) (California, Maine, and Washington State). A related species, *B. exitiosus*, has been described from New Zealand (around South Island and lower North Island) (Hine *et al.*, 2001) and an unidentified species has been detected in Australia (Western Australia, Victoria, and Tasmania) (OIE, 2000).

3.1.2.1.3 Short description of clinical signs

Most infected oysters show no obvious macroscopic symptoms, although infections are sometimes accompanied by yellow discolouration and extensive lesions in the gills and mantle. Microscopic lesions (cellular infiltration) occur in the connective tissue of the gills, mantle, and digestive gland.

3.1.2.1.4 Indications of impact/severity at stock level

In highly susceptible hosts, bonamiosis is a lethal infection. These intrahaemocytic protistans quickly become systemic, with overwhelming numbers of parasites coinciding with the death of the oysters.

3.1.2.1.5 Control/preventive measures

Importation of molluscs should occur only from countries where no outbreak caused by *B. ostreae* has occurred for at least the previous two years and no *B. ostreae* has been detected in any mollusc tested during an official mollusc health surveillance programme, using the procedures described in the OIE Manual, for a period of at least two years.

When the imported molluscs are going to be immersed in local waters, the Competent Authority of the importing country should require that the consignment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country or a certifying official approved by the importing country. When the imported molluscs are going to be stored for a short period before consumption, the containment tank should be isolated from the local environment (e.g., in quarantine) to avoid the potential introduction of the pathogen.

3.1.2.1.6 Temporal trends

For the last fifteen years, the prevalence of *B. ostreae* has remained stable in France and Spain.

3.1.2.1.7 Other host species

See Section 3.1.2.1.2, above.

References

Bannister, C., and Key, D. 1982. *Bonamia*, a new threat to the native oyster fishery. Fish Note, MAFF Direction of Fishery Resources, Lowestoft. 9 pp.

Bonami, J.R., Vivarès, C.P., and Brehelin M. 1985. Etude d'une nouvelle Haplosporidie parasite de l'huître plate *Ostrea edulis* L.: morphologie et cytologie de différents stades. Protistologica, 21: 161–173.

Comps, M., Tigé, G., and Grizel, H. 1980. Etude ultrastructurale d'un protiste parasite de l'huître *Ostrea edulis*. Compte Rendu de l'Académie des Sciences de Paris Série III, 290: 383–384.

Elston, R.A., Farley, C.A., and Kent, M.L. 1986. Occurrence and significance of Bonamiosis in European flat oyster, *Ostrea edulis*, in North America. Diseases of Aquatic Organisms, 2: 49–54.

Grizel, H., and Tigé, G. 1982. Evolution of the haemocytic disease caused by *Bonamia ostreae*. *In* Proceedings of the 3rd International Colloqium of Invertebrate Pathology, pp. 258–260. 6–10 September, Brighton, Great Britain.

Grizel, H., Comps, M., Raguenes, D., Leborgne, Y., Tigé, G., and Martin, A.G. 1983. Bilans des essais d'acclimatation d'*Ostrea chilensis* sur les côtes de Bretagne. Revue des Travaux de l'Institute des Pêches Maritimes, 46: 209–225.

Hervio, D., Chagot, D., Godin, P., Grizel, H., and Miahle, E. 1991. Localization and characterization of acid phosphatase activity in *Bonamia ostreae* (Ascetospora), an intrahomocytic protozoan parasite of the flat oyster *Ostrea edulis*. Diseases of Aquatic Organisms, 12: 67–72.

Hine, P.M., Cochennec-Laureau, N., and Berthe, F.C.J. 2001. *Bonamia exitiosus* n.sp. (Haplosporidia) infecting flat oysters *Ostrea chilensis* in New Zealand. Diseases of Aquatic Organisms, 47: 63–72.

Montes, J., Anadon, R., and Azevedo, C. 1994. A possible life cycle for *Bonamia ostreae* on the basis of electron microscopy. Journal of Invertebrate Pathology, 63: 1–6.

OIE. 2000. Diagnostic Manual for Aquatic Animal Diseases. Third Edition. Office International des Epizooties, Paris. pp. 143–146.

Pichot, Y., Comps, M., Tigé, G., Grizel, H., and Rabouin, M.A. 1979. Recherches sur *Bonamia ostreae* gen. N. sp., parasite nouveau de l'huître plate *Ostrea edulis* L. Revue des Travaux de l'Institute des Pêches Maritimes, 43: 131–140.

Polanco, E., Montes, J., Outon, M.J., and Melendez, M.I. 1984. Situation pathologique du stock d'huîtres plates en Galice (Espagne) en relation avec *Bonamia ostreae*. Haliotis, 14: 91–95.

van Banning, P. 1982. Some aspects of the occurrence, importance and control of the oyster pathogen *Bonamia ostreae* in the Dutch oyster culture. *In* Proceedings of the 3rd International Colloqium of Invertebrate Pathology, pp. 261–265. 6–10 September, Brighton, Great Britain.

3.1.2.2 Marteiliosis (*Marteilia refringens*)

3.1.2.2.1 Description of agent

Marteilia refringens is currently classified as a member of the Phylum Paramyxea (Berthe *et al.*, 2000). The parasite is 5–8 µm in size in the early stages and may reach up to 40 µm during sporulation. The cytoplasm of the cells stain basophilic and the nucleus is eosinophilic. The secondary cells or sporoblasts are surrounded by a bright halo (colour may vary slightly with the stain used).

Although the sequence of SS rRNA gene does not allow discrimination among *Marteilia* isolates (Berthe *et al.*, 2000), it is possible to find genetic polymorphisms in the ITS region linked to the host shellfish species, oysters and mussels (Le Roux *et al.*, 2001). Based on RFLP of the parasite internal transcriber spacer –1 (ITS-1) PCR products, Le Roux *et al.,* (2001) defined the existence of two *Marteilia* profiles: an M type found in the mussel and an O type found in the flat oyster. These types were always associated with the same molluscan species and were seldom found in another species. The authors suggested the existence of two *Marteilia* species in Europe: *M. maurini* (not notifiable pathogen) infecting mussels and *M. refringens* (notifiable pathogen) infecting oysters. This would indicate that mussel movements would not be affected by the legal regulations of the notifiable diseases.

3.1.2.2.2 Geographical distribution

Currently, *Marteilia refringens* has been found in France, Greece, Italy, Morocco, Portugal, and Spain.

3.1.2.2.3 Short description of clinical signs

Marteilia refringens develops mainly in the epithelia of the digestive gland (digestive tubules, stomach, and intestine) where it produces a discolouration. It causes cessation of growth, loss of tissue condition, and emaciation due to exhaustion of glycogen reserves.

3.1.2.2.4 Indications of impact/severity at stock level

M. refringens has caused serious and recurring mortalities, with a significant negative impact on the European flat oyster industry. Mortality appears to be related to the sporulation of the parasite. Earlier stages occur in the epithelia of the palps, stomach, digestive ducts, and possibly the gills.

3.1.2.2.5 Control/preventive measures

Importation of molluscs should occur only from a country where no outbreak of disease caused by *M. refringens* has occurred for at least the previous two years and no *M. refringens* has been detected in any mollusc tested during an official health surveillance programme, using the procedures described in the OIE Manual, for a period of at least two years.

When the imported molluscs are to be immersed in local waters, the Competent Authority of the importing country should require that the consignment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country or a certifying official approved by the importing country. When the imported molluscs are going to be stored for a short period before consumption, the containment tank should be isolated from the local environment (e.g., in quarantine) to avoid the potential introduction of different strains of the pathogen. This document must certify, on the basis of an official mollusc health surveillance scheme comprising inspection and laboratory tests on susceptible host species conducted according to the procedures described in the Manual of the OIE, whether or not the place of harvest of the consignment is a country officially declared marteiliosis-free. If the place of harvest of the consignment is not a country officially declared marteiliosis-free, the certificate must state whether the place of harvest of the consignment is a zone or an aquaculture establishment officially declared marteiliosis-free.

3.1.2.2.6 Temporal trends

Due to the trends in water temperature, the situations in France and Spain are totally different. Whilst in France there is a clear seasonality, this does not apply to Spain. The prevalence of marteiliosis has remained more or less stable in France, whereas the disease has almost disappeared in Spain.

3.1.2.2.7 Other host species

M. refringens has been recorded from mussels (*Mytilus edulis*), cockles (*Cardium edule*), American oyster (only a single case; Renault *et al.*, 1995), *Ostrea* (= *Tiostrea*) *chilensis*, and Australian flat oyster (experimental infection). Putative other *Marteilia* species have been described from Pacific oyster (Montes *et al.*, 1998) and Calico scallop (*Argopecten gibbus*), mussels (*M. edulis* and *M. galloprovincialis*) from the Atlantic coasts of Spain and France, and from the Persian Gulf (Comps *et al.*, 1981), and *Saccostrea* (= *Crassostrea*) *cucullata* from the Persian Gulf (Comps, 1976), as well as from *Scrobicularia piperata* in France (Comps, 1985). However, due to the near impossibility to distinguish between these marteilias, the identification of these species remains to be resolved (Bowers and McGladdery, 2003).

References

Berthe, F.C., Renas, M., Zerabib, M., Haffner, P., Thébault, A., and Figueras, A. 1998. Experimental transmission of *Marteilia refringens* with special consideration of its life cycle. Diseases of Aquatic Organisms, 34: 135–144.

Berthe, F.C.J., Le Roux, F., Peyretaillade, E., Peyret, P., Rodriguez, D., Goy, M., and Vivarès, C.P. 2000.

Phylogenetic analysis of the small subunit ribosomal RNA of *Marteilia refringens* validates the existence of Phylum Paramyxea (Desportes and Perkins, 1990). The Journal of Eukaryotic Microbiology, 47: 288–293.

Bowers, S., and McGladdery, S. 2003. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish. (URL: http://www-sci.pac.dfo-mpo.gc.ca/shelldis/title_e.htm).

Comps, M. 1976. *Marteilia lengehi* n. sp., parasite of the oyster *Crassostrea cucullata* Born. Revue des Travaux de l'Institute des Pêches Maritimes, 40: 347–349. (In French, with English summary).

Comps, M. 1985. Morphological study of *Marteilia christenseni* sp. n., parasite of *Scrobicularia piperata* P. (Mollusc Pelecypod). Revue des Travaux de l'Institute des Pêches Maritimes, 47: 99–104. (In French, with English summary).

Comps, M., Pichot, Y., and Papagianni, P. 1981. Research on *Marteilia maurini* n. sp. parasite of the mussel *Mytilus galloprovincialis* Lmk.). Revue des Travaux de l'Institute des Pêches Maritimes, 45: 211–214. (In French, with English summary).

Le Roux, F., Lorenzo, G., Peyret, P., Audemard, C., Figueras, A., Vivarès, C., Gouy, M., and Berthe, F. 2001. Molecular evidence for the existence of two species of *Marteilia* in Europe. The Journal of Eukaryotic Microbiology, 48: 449–454.

Montes, J., Longa, M.A., Lama, A., and Guerra, A. 1998. Marteiliosis of Japanese oyster (*Crassostrea gigas*) reared in Galicia NW Spain. Bulletin of the European Association of Fish Pathologists, 18: 124–126.

Renault, T., Cochennec, N., and Collet, B. 1995. Marteiliosis in American oyster *Crassostrea virginica* reared in France. Diseases of Aquatic Organisms, 23: 161–164.

3.2 *Crassostrea* species

3.2.1 Viral diseases

3.2.1.1 Herpesvirus

3.2.1.1.1 Description of agent

Herpes-like virus infections in Pacific oysters are caused by viruses presenting morphological features, cellular locations, and size ranges characteristic of virions belonging to the Herpesviridae family. The virogenesis begins in the nucleus of infected cells where capsids and nucleocapsids appear. Then virions pass into the cytoplasm and are released at the cell surface or by cytolysis. The diameter of enveloped virus particles is approximately 120 nm.

Virus particles have been purified from fresh infected *C. gigas* larvae, DNA has been extracted from purified virions (Le Deuff and Renault, 1999), and the viral genome sequenced. The overall genome structure is: TR_L - U_L - IR_L - X - IR_S - U_S - TR_S with a 207439 bp total genome size. TR_L and IR_L (7584 bp) are inverted repeats flanking a unique region (U_L, 167843 bp). TR_S and IR_S (9774 bp) are inverted repeats flanking a unique region (U_S, 3370 bp), and X (1510 bp) is located between IR_L and IR_S. A similar genome structure has evolved independently in certain vertebrate herpesviruses (e.g., herpes simplex virus and human cytomegalovirus). The presence of several isomers described in the OsHV-1 genome is also a feature reported in vertebrate herpesvirus genomes.

The sequence data demonstrate that the Ostreid Herpes Virus type 1 (OsHV-1) is not closely related to herpesviruses with vertebrate hosts (including fish). Amino acid sequence comparisons failed to identify a single protein which has homologues only in other herpesviruses. Several OsHV-1 proteins have homologues that are distributed widely in nature (e.g., DNA polymerase), but these are no more closely related to homologues in other herpesviruses than to homologues in other organisms. However, a genetic indication of a common origin between OsHV-1 and vertebrate herpesviruses resides with the ATPase subunit of the terminase. Homologous genes are present in all herpesviruses, and the only non-herpesvirus counterparts are specified by T4 and related bacteriophages. The T4 and OsHV-1 genes are unspliced, whereas those in herpesviruses of mammals and birds contain one intron and those in herpesviruses of fish and amphibians contain two introns.

OsHV-1 is the sole recognized viral disease of Pacific oysters in Europe. So far, herpes-like viruses have been reported from nine different bivalve species (see Section 3.1.1.1.7, above) around the world on the basis of transmission electron microscopy and molecular techniques. The number of host species seems to be still increasing.

Recent data (Arzul *et al.*, 2001a, 2001b) show that OsHV-1 can infect several bivalve species. This contrasts with vertebrate herpesviruses, which are generally confined to a single species in nature. Consequently, the true host of OsHV-1 is unknown. The apparent loss of several gene functions in OsHV-1 prompts the speculation that this may have promoted interspecies transmission in the context of introduction of non-native bivalve species and the use of modern aquaculture techniques. It is possible that the parental virus still resides in its natural host.

3.2.1.1.2 Geographical distribution

In 1991, viruses interpreted as belonging to the Herpesviridae were associated with high mortalities of hatchery-reared larval *Crassostrea gigas* in France (Nicolas *et al.*, 1992) and in New Zealand (Hine *et al.*, 1992). Since 1992 and 1993, sporadic high mortalities of larval and juvenile *C. gigas* have been regularly observed in some commercial French hatcheries. They occur each summer in association with a herpes-like virus (Renault *et al.*, 1994b). Since 1993, sporadic high mortalities have also occurred in some *C. gigas* spat cultured in several French locations (Renault *et al.*, 1994a, 1994b). Replication of herpes-like viruses was also described in larval Manila clams and scallops (*P. maximus*) in France (Renault, 1998; Arzul *et al.*, 2001a, 2001b; Renault *et al.*, 2001a, 2001b).

PCR was used to investigate the presence of OsHV-1 DNA in larvae of several bivalve species from different geographical origins in Europe. Positive samples were observed in several hatcheries from three different European countries (France, Spain, and the United Kingdom).

Herpes-like virus infections in bivalves seem to be ubiquitous and are associated with substantial mortalities in hatcheries.

3.2.1.1.3 Short description of clinical signs

Infected larvae show a reduction in feeding and swimming a few days after spawning. Significant mortalities occur by Day 6, with peak of mortality (80–100 %) by Day 8 to Day 12 in most affected batches. Moribund larvae show a less extended velum and detached parts of this velum are often observed free in water. High mortalities occur in spat during summer periods, with 80–90 % mortality occurring within a few days.

3.2.1.1.4 Indications of impact/severity at stock level

In 2000, PCR permitted the detection of herpes-like viral infection in Pacific oyster larvae from the field (Arcachon Bay, France). This is the first record of a herpes-like infection of oyster larvae from natural spawning.

3.2.1.1.5 Control/preventive measures

According to the OIE International Aquatic Animal Health Code, OsHV-1 infection is not a notifiable disease. However, the widespread distribution of herpes-like viruses in marine bivalve species may cause serious problems for marine aquaculture, especially hatcheries.

The availability of sensitive and reliable PCR techniques (Renault and Lipart, 1998; Renault *et al.*, 2000) to detect OsHV-1 DNA in less than 24 hours may be useful to manage larvae and juveniles reared in commercial hatcheries and nurseries. The detection of viral DNA by PCR in bivalves presenting mortalities may help to avoid the spread of viral infections among broodstocks.

3.2.1.1.6 Temporal trends

During the period 1998–2002, herpes-like viruses were reported from an increasing number of larval bivalve species around the world, especially in Europe: *C. gigas* and Manila clams in France in 1997, and in Spain and the United Kingdom in 1999; carpet shell clams in France in 1999; and scallops (*P. maximus*) in France in 2000.

3.2.1.1.7 Other host species

See Section 3.1.1.1.7, above.

References

Arzul, I., Renault, T., and Lipart, C. 2001a. Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission. Diseases of Aquatic Organisms, 46: 1–6.

Arzul, I., Renault, T., Lipart, C., and Davison, A.J. 2001b. Evidence for interspecies transmission of oyster herpesvirus in marine bivalves. Journal of General Virology, 82: 865–870.

Culloty, S.C., and Mulcahy, M.F. 1995. Mortalities in Pacific oyster *Crassostrea gigas* with particular reference to Ireland. Dept. of Zoology, U.C.C. 27 pp.

Hine, P.M., Wesney, B., and Hay, B.E. 1992. Herpesvirus associated with mortalities among hatchery-reared larval Pacific oysters, *C. gigas*. Diseases of Aquatic Organisms, 12(2): 135–142.

Le Deuff, R.-M., and Renault, T. 1999. Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. Journal of General Virology, 80: 1317–1322.

Nicolas, J.L., Comps, M., and Cochennec, N. 1992. Herpes-like virus infecting Pacific oyster larvae, *C. gigas*. Bulletin of the European Association of Fish Pathologists, 12(1): 11–13.

Renault, T. 1998. Infections herpétiques chez les invertébrés: détection de virus de type herpès chez les mollusques bivalves marins. Virologie, 2: 401–403.

Renault, T., and Arzul, I. 2001. Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR. Journal of Fish Diseases, 24: 161–167.

Renault, T., and Lipart, C. 1998. Diagnosis of herpes-like virus infections in oysters using molecular techniques. EAS Special Publication, 26: 235–236.

Renault, T., Lipart, C., and Arzul, I. 2001a. A herpes-like virus infects a non-ostreid bivalve species: virus replication in *Ruditapes philippinarum* larvae. Diseases of Aquatic Organisms, 45: 1–7.

Renault, T., Lipart, C., and Arzul, I. 2001b. A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France. Journal of Fish Diseases, 24: 369–376.

Renault, T., Cochennec, N., Le Deuff, R.-M., and Chollet, B. 1994a. Herpes-like virus infecting Japanese oyster (*C. gigas*) spat. Bulletin of the European Association of Fish Pathologists, 14(2): 64–66.

Renault, T., Le Deuff, R.-M., Cochennec, N., and Maffart, P. 1994b. Herpesviruses associated with mortalities among Pacific oyster, *C. gigas*, in France - Comparative study. Revue de Médicine Vétérinaire, 145(10): 735–742.

Renault, T., Le Deuff, R.-M., Lipart C., and Delsert C. 2000. Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. Journal of Virological Methods, 88: 41–50.

3.2.2 Parasitic diseases

3.2.2.1 Perkinsosis (*Perkinsus marinus*)

3.2.2.1.1 Description of agent

Perkinsus marinus (= *Labyrinthomyxa marina* = *Dermocystidium marinum*) infects American oysters (*C. virginica*). It is currently classified as a member of the Phylum Apicomplexa, however, genetic sequencing places it closer to the dinoflagellates (Mackin *et al.*, 1950; Goggin and Barker, 1993; Reece *et al.*, 1997).

Unicellular trophont (also termed trophozoite and merozoite) stages (4–12 µm) are often found inside haemocytes, where they survive, proliferate by schizogony, and are distributed throughout the oyster. Trophonts are characterized by a single vacuole containing a vacuoplast, which is refractile at the light microscope level. Multicellular schizont (15–45 µm) stages may also be intracellular or encapsulated by haemocytes. All stages found in oysters appear to be infective. Transmission is direct; infection occurs through the digestive tract and external epithelia. A zoospore stage can be obtained in culture, but its role in transmission has not been demonstrated (Perkins, 1976).

3.2.2.1.2 Geographical distribution

The parasite is found along the east coast of the United States from Maine to Florida and around the Gulf of Mexico to Mexico and Venezuela. It has also been reported from Jamaica, Puerto Rico, Cuba, and Brazil, and was apparently introduced to the Pacific in Hawaii, USA (Andrews, 1988).

3.2.2.1.3 Short description of clinical signs

The disease is associated with loss of tissue condition and inhibition of shell growth; however, these are not unique to Perkinsosis (Mackin, 1951).

3.2.2.1.4 Indications of impact/severity at stock level

Mortalities range from 5–30 % during the first year of an epizootic, and total from 60–80 % by the end of the second year. Chronic mortalities of 50 % annually are reported from enzootic regions. Fecundity is reduced in oysters with advanced infections (Ford and Tripp, 1996).

3.2.2.1.5 Control/preventive measures

Oysters from an enzootic area should not be introduced to an area where the pathogen is absent. Oysters should be maintained at reduced salinities (<15 ppt). If final conditioning at higher salinity is needed for market, this should be done as late in the growing season as possible to avoid the acquisition and development of infections. It generally takes two years before serious mortalities occur; therefore, early harvesting of oysters will avoid considerable losses (Andrews and Ray, 1988). Uninfected hatchery-produced seed deployed in late summer can avoid serious infection until the following summer and can be harvested that autumn with little or no mortality. Selective breeding has produced strains with modestly improved survival. Particle filtration (1 µm filters) and UV irradiation of water coming into hatcheries or nurseries can eliminate infective stages (Ford *et al.*, 2001).

3.2.2.1.6 Temporal trends

After a major range extension into the northeastern United States in the early 1990s, associated with a warming trend, *P. marinus* is now well established in this new range (Ford, 1996). The parasite is inhibited by low temperature and salinity, and thus is highly influenced by local conditions (Burreson and Ragone Calvo, 1996). There have been no major changes in distribution or prevalence in the past five years.

3.2.2.1.7 Other host species

DNA analysis indicates that *P. marinus* can infect clam species (Baltic clam (*Macoma balthica*), *M. mitchelli* and hard-shell calm (*Mercenaria mercenaria*) (Coss *et al.* 2001)).

References

Andrews, J.D. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. *In* Disease Processes in Marine Bivalve Molluscs, pp. 47–63. Ed. by W.S. Fisher. American Fisheries Society, Bethesda, MD.

Andrews, J.D., and Ray, S.M. 1988. Management strategies to control the disease caused by *Perkinsus marinus*. *In* Disease Processes in Marine Bivalve Molluscs, pp. 257–264. Ed. by W.S. Fisher. American Fisheries Society, Bethesda, MD.

Burreson, E.M., and Ragone Calvo, L.M. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research., 15: 17–34.

Coss, C.A., Robledo, J.A.F., and Vasta, G.R. 2001. Fine structure of clonally propagated *in vitro* life stages of a *Perkinsus* sp. isolated from the Baltic clam *Macoma balthica*. The Journal of Eukaryotic Microbiology, 48: 38–51.

Ford, S.E. 1996. Range extension by the oyster parasite *Perkinsus marinus* into the northeastern US: Response to climate change? Journal of Shellfish Research., 15: 45–56.

Ford, S.E., and Tripp, M.R. 1996. Diseases and defense mechanisms. *In* The Eastern Oyster *Crassostrea virginica*, pp. 383–450. Ed. by R.I.E. Newell, V.S. Kennedy, and A.F. Eble. Maryland Sea Grant College, College Park, Maryland.

Ford, S.E., Xu, Z., and DeBrosse, G. 2001. Use of particle filtration and UV irradiation to prevent infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in hatchery-reared larval and juvenile oysters. Aquaculture, 194: 37–49.

Goggin, C.L., and Barker, S.C. 1993. Phylogenetic position of the genus *Perkinsus* (Protista, Apicomplexa) based on small subunit ribosomal RNA. Molecular and Biochemical Parasitology, 60: 65–70.

Mackin, J.G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. Bulletin of Marine Science of the Gulf and Caribbean, 1: 72–87.

Mackin, J.G., Owen, H.M., and Collier, A. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). Science, 111: 328–329.

Perkins, F.O. 1976. Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan-like organelles. Journal of Parasitology, 62: 959–974.

Reece, K.S., Siddall, M.E., Burreson, E.M., and Graves, J.E. 1997. Phylogenetic analysis of *Perkinsus* based on actin gene sequences. Journal of Parasitology, 83: 417–423.

3.2.2.2 Haplosporidiosis (*Haplosporidium nelsoni*)

3.2.2.2.1 Description of agent

Haplosporidium (= *Minchinia*) *nelsoni*, Phylum Haplosporidia, causes MSX disease in the American oyster (*C. virginica*) (Haskin *et al.*, 1966). It is present extracellularly, mostly as plasmodia in the tissues of infected oysters. Production of spore stages occurs primarily in juvenile oysters (Barber *et al.*, 1991; Burreson, 1994). Plasmodia are from 5–>50 µm in diameter, depending on the number and size of nuclei they contain (Perkins, 1968). Nuclei are spherical, 1.5–3 µm in diameter with a peripheral endosome, or elongated (up to 7.5 µm). During sporulation, plasmodia develop into sporocysts, with spore walls forming around each nucleus. Spores are approximately 5.5 µm × 7.5 µm, operculated, and have a cap with an overhanging lid.

Initial infections occur in the gill epithelium. Parasites multiply along the basal lamina of the epithelium and eventually break through into the circulatory system, where they are circulated throughout the oyster. Spores, when produced, are formed only in the epithelium of the digestive diverticula. The mode of transmission is unknown (Haskin and Andrews, 1988).

3.2.2.2.2 Geographical distribution

Haplosporidium nelsoni is found along the east coast of the United States from Maine to Florida in *C. virginica*. Persistent epizootics with high mortality have been restricted to the mid-Atlantic states and New England (Ford and Tripp, 1996). The parasite is present in Korea, Japan, the west coast of the United States, and possibly France in the Pacific oyster, *C. gigas*, where it is rare and has not caused noticeable mortality (Kern, 1976; Burreson *et al.*, 2000; Renault *et al.*, 2000). In 2002, *H. nelsoni* associated with mortality was reported from *C. virginica* in the Bras d'Or Lakes Region of Nova Scotia. This represented the first report of the parasite in Canada, and its most northerly outbreak to date.

3.2.2.2.3 Short description of clinical signs

The disease is associated with loss of tissue condition and inhibition of shell growth; however, these are not unique to Haplosporidian-caused diseases (Farley, 1968).

3.2.2.2.4 Indications of impact/severity at stock level

Haplosporidium nelsoni causes annual mortalities of up to 90 % during an initial outbreak and chronic losses of 50 % per year thereafter, with reductions in shell growth, meat quality, and reproductive capabilities in survivors. Oyster production in Chesapeake and Delaware Bays has been severely depressed since the appearance of *H. nelsoni* in the late 1950s (Ford and Tripp, 1996). An outbreak in Long Island Sound in 1997–1998 also caused heavy losses.

3.2.2.2.5 Control/preventive measures

Selective breeding has been successful in producing highly resistant strains of oysters (Ford and Tripp, 1996). These are useful when hatchery-produced seed are economically practical. Development of resistance in the natural population is reported in Delaware Bay and parts of lower Chesapeake Bay, and resistance appears to be developing in Long Island Sound. Management strategies include maintaining oysters at reduced salinities (< 15 ppt) as long as possible. If final conditioning at higher salinity is needed for market, the conditioning should be done late in the season to avoid the major early-summer infection period (Ford and Haskin, 1988). Immersion of oysters at ≤ 10 ppt salinity for 2–3 weeks at ≥ 20 ^{o}C or more should eliminate the parasite from infected oysters (Ford, 1985). Particle filtration (1 µm cartridge filter) and UV irradiation will eliminate infective stages from water coming into hatcheries or nurseries (Ford *et al.*, 2001). Although direct transmission has not been demonstrated, it should be avoided to introduce oysters, especially juveniles that may contain spores, from an enzootic area to an area where the pathogen is not present.

3.2.2.2.6 Temporal trends

An outbreak of *H. nelsoni*-caused disease occurred in Long Island Sound in 1997–1998, causing heavy losses (Sunila *et al.*, 1999). This was the first such reported, large-scale epizootic in this water body, although there is anecdotal evidence that the parasite caused mortalities in the Sound in the mid-1980s. The reason for the outbreak is not known. In 2002, a localized mortality caused by *H. nelsoni* was reported in the Bras d'Or Lakes Region of Nova Scotia. This represented the first report of the parasite in Canada, and its most northerly outbreak to date. It has become clear over the past five years that the continued low prevalence of *H. nelsoni* in Delaware Bay (where the first outbreak was discovered in 1957) is not due to the absence of the parasite, but to the development of a very high level of resistance in the wild population.

3.2.2.2.7 Other host species

Haplosporidium nelsoni infects the Pacific oyster in Korea, Japan, the west coast of the United States, and possibly, France; however, prevalences are typically very low (< 2 %) and not associated with measurable mortality (Kern, 1976; Burreson *et al.*, 2000; Renault *et al.*, 2000; Kamaishi and Yoshinaga, 2002).

References

Barber, R.D., Kanaley, S.A., and Ford, S.E. 1991. Evidence for regular sporulation by *Haplosporidium nelsoni* (MSX) (Ascetospora: Haplosporidiidae) in spat of the American oyster, *Crassostrea virginica*. Journal of Protozoology, 38: 305–306.

Burreson, E.M. 1994. Further evidence of regular sporulation by *Haplosporidium nelsoni* in small oysters, *Crassostrea virginica*. Journal of Parasitology, 80: 1036–1038.

Burreson, E.M., Stokes, N.A., and Friedman, C.S. 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. Journal of Aquatic Animal Health, 12: 1–8.

Farley, C.A. 1968. *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *Crassostrea virginica*. Journal of Protozoology, 15: 585–599.

Ford, S.E. 1985. Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Mackin) in oysters. Journal of Shellfish Research, 2: 85–90.

Ford, S.E., and Haskin, H.H. 1988. Management strategies for MSX (*Haplosporidium nelsoni*) disease in eastern oysters. *In* Disease Processes in Marine Bivalve Molluscs, pp. 249–256. Ed. by W.S. Fisher. American Fisheries Society, Bethesda, MD.

Ford, S.E., and Tripp, M.R. 1996. Diseases and defense mechanisms. *In* The Eastern Oyster *Crassostrea virginica*, pp. 383–450. Ed. by R.I.E. Newell, V.S. Kennedy, and A.F. Eble. Maryland Sea Grant College, College Park, MD.

Ford, S.E., Xu, Z., and DeBrosse, G. 2001. Use of particle filtration and UV irradiation to prevent infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in hatchery-reared larval and juvenile oysters. Aquaculture, 194: 37–49.

Haskin, H.H., and Andrews, J.D. 1988. Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). *In* Disease Processes in Marine Bivalve Molluscs, pp. 5–22. Ed. by W.S. Fisher. American Fisheries Society, Bethesda, MD.

Haskin, H.H., Stauber, L.A., and Mackin, J.A. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causative agent of the Delaware Bay oyster epizootic. Science, 153: 1414–1416.

Kamaishi, T., and Yoshinaga, T. 2002. Detection of *Haplosporidium nelsoni* in Pacific oyster *Crassostrea gigas* in Japan. Fish Pathology, 37: 193–195.

Kern, F.G. 1976. Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the Pacific oyster *Crassostrea gigas* (Thunberg) from the republic of Korea. Journal of Protozoology, 23: 498–500.

Office Internationale des Epizooties. 2002. MSX disease (*Haplosporidium nelsoni*) in Canada. [Online]. Available: http://www.oie.int/eng/info/hebdo/AIS_48.HTM#Sec1 [2003, April 18].

Perkins, F.O. 1968. Fine structure of the oyster pathogen *Minchinia nelsoni* (Haplosporida, Haplosporidiidae). Journal of Invertebrate Pathology, 10: 287–307.

Renault, T., Stokes, N.A., Chollet, B., Cochennec, N., Berthe, F., Gérard, A., and Burreson, E.M. 2000. Haplosporidiosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. Diseases of Aquatic Organisms, 42: 207–214.

Sunila, I., Karolus, J., and Volk, J., 1999. A new epizootic of *Haplosporidium nelsoni* (MSX), a Haplosporidian oyster parasite, in Long Island Sound, Connecticut. Journal of Shellfish Research, 18: 169–174.

3.2.2.3 Haplosporidiosis (*Haplosporidium costale*)

3.2.2.3.1 Description of agent

Haplosporidium costale (= *Minchinia costalis*), Phylum Haplosporidia, infects the American oyster (*C. virginica*) (Wood and Andrews, 1962). It is found extracellularly as both plasmodial and spore stages in oysters (Perkins, 1969). Plasmodia are typically < 10 µm in diameter, and nuclei average about 1.6 µm in diameter. During sporulation, plasmodia develop into sporocysts, with spore walls forming around each nucleus. Spores are approximately 2.6 µm × 3.1 µm, operculate, and have a cap with an overhanging lid. Sporulation is common and occurs in all tissues except the epithelia. Plasmodia, nuclei, and spores of *H. costale* are smaller than those of *H. nelsoni*. The mode of transmission is unknown.

3.2.2.3.2 Geographical distribution

Infected oysters have been reported from Maine to the mouth of Chesapeake Bay in locations where salinities are > 25 ppt. Prevalence is low in most areas; associated mortalities, although rare, have been reported in Massachusetts and Virginia. In 2002, during the intense surveillance that followed the first report of *H. nelsoni* in Canada, *H. costale* was detected in several oysters on the north shore of Cape Breton and in the Gulf of St. Lawrence (OIE, 2003). This marks the first report of *H. costale* in Canada.

3.2.2.3.3 Short description of clinical signs

H. costale infections inhibit oyster growth, although this is a non-specific symptom (Andrews and Castagna, 1978).

3.2.2.3.4 Indications of impact/severity at stock level

H. costale inhibits the growth of infected oysters and kills them one to two months after infections become patent. The parasite can kill 20–50 % of affected stocks annually (Ford and Tripp, 1996).

3.2.2.3.5 Control/preventive measures

Maintaining oysters at salinities < 25 ppt should minimize the acquisition of new infections. Transfer of infected oysters to salinities < 25 ppt is likely to eliminate, or at least control, the proliferation of parasites in infected oysters (Ford and Tripp, 1996). Particle filtration (1 µm filters) and UV irradiation of water coming into hatcheries or nurseries should eliminate infective stages, as they do for the related pathogen, *H. nelsoni*.

3.2.2.3.6 Temporal trends

Infections of *H. costale* seem to be sporadic, or at least sporadically detected, and associated with mortality

< 20 %. Recent examination of tissue slides with a DNA probe has revealed *H. costale* infection, and sporulation, in the autumn in both Long Island Sound and coastal Virginia (Stokes and Burreson, 2001; Sunila *et al.*, 2002). Previously, it was thought that histologically detectable infections were present only in spring and early summer (Andrews and Castagna, 1978). In 2002, during the intense surveillance that followed the first report of *H. nelsoni* in Canada, *H. costale* was detected in several oysters on the north shore of Cape Breton and in the Gulf of St. Lawrence (OIE, 2003). The infections were light and without evidence of pathology. This marks the first report of *H. costale* in Canada.

References

Andrews, J.D., and Castagna, M. 1978. Epizootiology of *Minchinia costalis* in susceptible oysters in seaside bays of Virginia's Eastern Shore, 1959–1976. Journal of Invertebrate Pathology, 32: 124–138.

Ford, S.E., and Tripp, M.R. 1996. Diseases and defense mechanisms. *In* The Eastern Oyster *Crassostrea virginica*, pp. 383–450. Ed. by R.I.E. Newell, V.S. Kennedy, and A.F. Eble. Maryland Sea Grant College, College Park, MD.

Office Internationale des Epizooties. 2003. SS0 disease (*Haplosporidium costale*) in Canada, [Online]. Available: http://www.oie.int/eng/info/hebdo/AIS_26.HTM#Sec2 [2003, April 18].

Perkins, F.O. 1969. Electron microscope studies of sporulation in the oyster pathogen, *Minchinia costalis* (Sporozoa; Haplosporida). Journal of Parasitology, 55: 897–920.

Stokes, N.A., and Burreson. E.M. 2001. Differential diagnosis of mixed *Haplosporidium costale* and *Haplosporidium nelsoni* infections in the eastern oyster, *Crassostrea virginia*, using DNA probes. Journal of Shellfish Research, 20: 207–213.

Sunila, I., Stokes, N.A., Smolowitz, R., Karney, R.C., and Burreson, E.M. 2002. *Haplosporidium costale* (seaside organism), a parasite of the eastern oyster, is present in Long Island Sound. Journal of Shellfish Research, 21: 113–118.

Wood, J.L., and Andrews, J.D. 1962. *Haplosporidium costale* (Sporozoa) associated with a disease of Virginia oysters. Science, 136: 710–711.

3.2.2.4 Mikrocytosis (Denman Island Disease) (*Mikrocytos mackini*)

3.2.2.4.1 Description of agent

Mikrocytos mackini is a small (2–3 µm diameter), amitochondriate, intracellular, protistan parasite of unknown taxonomic affiliation that causes mikrocytosis, commonly known as Denman Island disease, in oysters (Farley *et al.*, 1988; Hine *et al.*, 2001). The disease was first detected in Pacific oysters in British Columbia, Canada in 1960 (Quayle, 1961, 1982).

3.2.2.4.2 Geographical distribution

Mikrocytos mackini is reported from the west coast of Canada, where it is probably ubiquitous throughout the Strait of Georgia and confined to other specific localities around Vancouver Island. Recently, the parasite was detected in a few old oysters from two relic populations in northern Washington State, USA.

3.2.2.4.3 Short description of clinical signs

Focal green to yellow pustules or abscess-like lesions up to 5 mm in diameter occur within the body wall, on the surface of the labial palps or mantle, or in the adductor muscle. A brown scar often occurs on the shell, adjacent to abscess on the mantle surface. These lesions are not unique to mikrocytosis. The pustules are the result of haemocyte infiltration and tissue necrosis at the sites of intracellular infection of *M. mackini* in vesicular connective tissue cells, heart and adductor muscle myocytes and haemocytes within the lesions (Hervio *et al.*, 1996). In the field, severe infections appear to be restricted to older oysters (over two years old). However, small juvenile oysters (seed, about 1 cm in shell length) were susceptible to infection when challenged by bath exposure in the laboratory.

3.2.2.4.4 Indications of impact/severity at stock level

Lesions and mortalities occur predominantly in April and May following a three- to four-month period when water temperatures are less than 10 °C (Bower and Meyer, 1999). Mortality due to Denman Island disease is most significant in older oysters at low tide levels. Over a period of thirty years, the prevalence of mikrocytosis in older oysters on one beach has varied between 10–40 %. Approximately 10 % of infected C. gigas appear to recover. In most cases, mortalities are not significant to the oyster culture industry within the enzootic area because oysters are normally cultured and marked using methods that circumvent the disease. However, in addition to potential losses due to mortalities, impact to the industry occurs in loss of export markets for seed (stock for grow-out), possible rejection of product at the shucking plant, and increased production costs arising from the implementation of varying degrees of management practices to minimize disease impact.

3.2.2.4.5 Control/preventive measures

The effect of the disease on oyster populations in enzootic areas can be reduced to a manageable level by harvesting or moving large oysters to locations high in the intertidal zone prior to March and not planting oysters at lower tide levels before June (Bower, 1988). Seed stock for hanging culture should not be stored on the beach in enzootic areas between March and June. Oysters from infected areas (currently or historically) should not be moved to areas where mikrocytosis has not been recorded.

3.2.2.4.6 Temporal trends

Since the early 1990s, losses due to mikrocytosis in enzootic areas have been insignificant. This trend can be attributed to current oyster culture practices of remote setting of hatchery-produced eyed larvae, the use of

hanging culture techniques, and the trend towards short production cycles. The detection of *M. mackini* at new locations (i.e., in northern Washington State in 2002) was attributed to the screening of relic oyster populations and not indicative of the spread of the disease.

3.2.2.4.7 Other host species

In addition to the usual host, *C. gigas*, other oysters (e.g., American oyster, European flat oyster, and *Ostrea conchaphila*) are susceptible to infection and the resulting disease. *C. gigas* seemed to be more resistant to the disease than these other species of oysters when challenged experimentally under laboratory and field conditions (Bower *et al.*, 1997).

References

Bower, S.M. 1988. Circumvention of mortalities caused by Denman Island Oyster Disease during mariculture of Pacific Oysters. American Fisheries Society Special Publication, 18: 246–248.

Bower, S.M., and Meyer, G.R. 1999. Effects of cold water on limiting or exacerbating some oyster diseases. Journal of Shellfish Research, 18: 296. (Abstract).

Bower, S.M., Hervio, D., and Meyer, G.R. 1997. Infectivity of *Mikrocytos mackini*, the causative agent of Denman Island disease in Pacific oysters *Crassostrea gigas*, to various species of oysters. Diseases of Aquatic Organisms, 29: 111–116.

Farley, C.A., Wolf, P.H., and Elston, R.A. 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g.n.) and two new species *Mikrocytos mackini* (sp.n.) and *Mikrocytos roughleyi* (sp.n.). United States National Marine Fisheries Service Bulletin, 86: 581–593.

Hervio, D., Bower, S.M., and Meyer, G.R. 1995a. Life cycle, distribution and lack of host specificity of *Mikrocytos mackini*, the cause of Denman Island disease in Pacific oysters, *Crassostrea gigas*. Journal of Shellfish Research, 14: 228. (Abstract).

Hervio, D., Meyer, G.R., Bower, S.M., and Adlard, R.D. 1995b. Development of specific molecular probes for serological and PCR assays for the identification and diagnosis of *Mikrocytos mackini*, the cause of Denman Island disease in the Pacific oyster *Crassostrea gigas*. Journal of Shellfish Research, 14: 268. (Abstract).

Hervio, D., Bower, S.M., and Meyer, G.R. 1996. Detection, isolation and experimental transmission of *Mikrocytos mackini*, a microcell parasite of Pacific oysters *Crassostrea gigas* (Thunberg). Journal of Invertebrate Pathology, 67: 72–79.

Hine, P.M., Bower, S.M., Meyer, G.R., Cochennec-Laureau, N., and Berthe, F.C.J. 2001. Ultrastructure of *Mikrocytos mackini*, the cause of Denman Island disease in oysters *Crassostrea* spp. and *Ostrea* spp. in British Columbia, Canada. Diseases of Aquatic Organisms, 45: 215–227.

Joly, J.-P., Bower, S.M., and Meyer, G.R.. 2001. A simple technique to concentrate the protozoan *Mikrocytos mackini*, causative agent of Denman Island disease in oysters. Journal of Parasitology, 87: 432–434.

Quayle, D.B. 1961. Denman Island disease and mortality, 1960. Fisheries Research Board of Canada Manuscript Report Ottawa Series Number, 713: 1–9.

Quayle, D.B. 1982. Denman Island oyster disease 1960–1980. British Columbia Shellfish Mariculture Newsletter (Victoria, Canada), 2(2): 1–5.

3.2.3 Diseases of unknown aetiology

3.2.3.1 Juvenile Oyster Disease (JOD)

3.2.3.1.1 Description of agent

The aetiological agent of Juvenile Oyster Disease is unknown, but bacterial involvement is strongly indicated (Lee *et al.*, 1996; Paillard *et al.*, 1996; Boettcher *et al.*, 1999, 2000). The disease primarily affects cultured American oysters (*C. virginica*) during their first summer of growth.

3.2.3.1.2 Geographical distribution

Major outbreaks have been reported from New York to Maine (USA), with few or no reports from more southern sites (Bricelj *et al.*, 1992; Barber *et al.*, 1996). A similar syndrome has been reported in *C. virginica* experimentally kept in a French hatchery (Renault *et al.,* 2002).

3.2.3.1.3 Short description of clinical signs

Oysters affected by JOD exhibit two symptoms: 1) extreme cupping of the lower valve and loss of the growing edge of the upper valve, leaving a band of exposed inner shell on the lower valve; and 2) secretion of a conchiolin layer, on both valves, that surrounds soft tissues, which retract well into the shell cavity. The conchiolin layer is raised into a ridge at the periphery of the mantle, resulting in the appearance of a distinct "brown ring" on the shells of affected oysters. The conchiolin layer frequently disrupts the adductor muscle attachment, causing the soft tissues to fall out of the shell (Ford and Borrero, 2001).

3.2.3.1.4 Indications of impact/severity at stock level

Mortalities of up to 90 % occur in animals < 25 mm; larger juveniles show shell deposit symptoms, but have much lower mortalities (Ford and Borrero, 2001).

3.2.3.1.5 Control/preventive measures

Selective breeding has produced oyster strains with significantly better survival than unselected controls (Barber *et al.*, 1998; Lewis *et al.*, 1996). Additional control measures include early production and

deployment of juveniles so that they reach the critical 25 mm size "threshold" before the onset of disease during mid-summer. Decreasing density in trays and bags, increasing mesh size, and increasing flow rate through upwellers have all proven effective in reducing losses (Ford and Borrero, 2001).

3.2.3.1.6 Temporal trends

For nearly ten years, JOD was a major impediment to oyster aquaculture in the northeastern United States, but the prevalence of the disease has diminished significantly since about 1997. A low prevalence of JOD symptoms can still be found in a New York aquaculture facility that experienced very heavy losses in the early 1990s, but losses are no longer economically significant. The only recent outbreaks have occurred in Maine.

References

Barber, B.J., Carnegie, R.B., and Davis, C.V. 1996. Effect of timing of seed deployment on growth and mortality of oysters, *Crassostrea virginica*, affected by Juvenile Oyster Disease (JOD). Journal of the World Aquaculture Society, 27: 443–448.

Barber, B.J., Davis, C.V., and Crosby, M.A. 1998. Cultured oysters, *Crassostrea virginica*, genetically selected for fast growth in the Damariscotta River, Maine, are resistant to mortality caused by Juvenile Oyster Disease (JOD). Journal of Shellfish Research, 17: 1171–1175.

Boettcher, K.J., Barber, B., and Singer, J.T. 1999. Use of antibacterial agents to elucidate the etiology of Juvenile Oyster Disease (JOD) in *Crassostrea virginica* and numerical dominance of an a-proteobacterium in JOD-affected animals. Applied Environmental Microbiology, 65: 2534–2539.

Boettcher, K.J., Barber, B.J., and Singer, J.T. 2000. Additional evidence that juvenile oyster disease is caused by a member of the roseobacter group and colonization of nonaffected animals by *Stappion stellulate*-like strains. Applied Environmental Microbiology, 66: 3924–3930.

Bricelj, V.M., Ford, S.E., Borrero, F.J., Perkins, F.O., Rivara, G., Hillman, R.E., Elston, R.A., and Chang, J. 1992. Unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica* (Gmelin). Journal of Shellfish Research., 11: 331–347.

Ford, S.E., and Borrero, F.J. 2001. Epizootiology and pathology of Juvenile Oyster Disease (JOD) in the Eastern oyster, Crassostrea virginica. Journal of Invertebrate Pathology, 78: 141–154.

Lee, M., Taylor, G.T., Bricelj, V.M., Ford, S.E., and Zahn, S. 1996. Evaluation of *Vibrio* spp. and microplankton blooms as causative agents of Juvenile Oyster Disease in *Crassostrea virginica* (Gmelin). Journal of Shellfish Research, 15: 319–329.

Lewis, E.J., Farley, C.A., Small, E.B., and Baya, A.M. 1996. A synopsis of juvenile oyster disease (JOD) experimental studies in *Crassostrea virginica*. Aquatic Living Resources, 9: 169–178.

Paillard, C., Ashton-Alcox, K., and Ford, S.E. 1996. Changes in bacterial densities and haemocyte parameters in oysters affected by Juvenile Oyster Disease. Aquatic Living Resources, 9: 145–158.

Renault T., Chollet B., Cochennec N., and Gérard, A. 2002. Shell disease in American oysters, *Crassostrea virginica*, reared in France. Journal of Invertebrate Pathology, 79: 1–6.

3.3 *Other mollusc species*

3.3.1 Parasitic diseases

3.3.1.1 QPX (Quahog Parasite X)

3.3.1.1.1 Description of agent

QPX is a member of the Labyrinthulomycota that infects the hard-shell clam (*Mercenaria mercenaria*) (Ragan *et al.*, 2000). It is probably an opportunistic, facultative pathogen that primarily affects cultured stocks. Thalli, ranging from about 2 µm to 20 µm in diameter, are usually the most common forms found in clam tissues (Whyte *et al.*, 1994; Ragone Calvo *et al.*, 1998). Larger (10–48 µm) cells (sporangia) each contain 20 to 40 endospores with diameters of 2–5 µm. The parasite is found most commonly in mantle and gill tissues.

3.3.1.1.2 Geographical distribution

QPX is reported from the east coast of Canada to Virginia, on the east coast of the United States (Smolowitz *et al.*, 1998; Ragone Calvo *et al.*, 1998; MacCallum and McGladdery, 2000).

3.3.1.1.3 Short description of clinical signs

Heavy lesions appear as a swollen mantle edge or nodules on the mantle. Chipping of the shell edge may occur in clams living in sandy sediment. Infected clams also experience reduced shell growth and loss of tissue condition (Smolowitz *et al.*, 1998).

3.3.1.1.4 Indications of impact/severity at stock level

Mortalities of up to 90 % have been reported in susceptible stocks, although some of the mortality may not be directly caused by QPX, but by unfavourable conditions that also facilitate infection by the pathogen (Ford *et al.*, 2002). The disease has been a serious impediment to clam culture in some sites in Massachusetts, although the problems appear to be at least partly related to poor husbandry (Smolowitz *et al.*, 1998).

3.3.1.1.5 Control/preventive measures

QPX has never been found in hatchery seed and all evidence indicates that clams become infected after planting (Ford *et al.*, 1997). There is strong evidence, however, that southern stocks planted in northern regions are more susceptible to QPX. Therefore, the use of local stocks is recommended. High-density plantings also seem to favour the parasite, so reducing planting density is also recommended (Ford *et al.*, 2002).

3.3.1.1.6 Temporal trends

The parasite has been known since the late 1950s, when it was found in Canada's Prince Edward Island (Drinnan and Henderson, 1963). Recent epizootics in the United States appear to be associated with the increase in hard clam aquaculture, poor animal husbandry, and the use of southern stocks, which grow faster than northern stocks, but also appear to be more susceptible to QPX infection.

References

Drinnan, R.E., and Henderson, E.B. 1963. 1962 mortalities and a possible disease organism in Neguac quahaugs. Annual Report B11. Biological Station, St. Andrews, New Brunswick, Canada.

Ford, S.E., Kraeuter, J.N., Barber, R.D, and Mathis, D. 2002. Aquaculture-associated factors in QPX disease of hard clams: density and seed-source. Aquaculture, 208: 23–38.

Ford, S.E., Smolowitz, R., Ragone-Calvo, L., Barber, R.D., and Kraeuter, J.N. 1997. Evidence that QPX (Quahog Parasite Unknown) is not present in hatchery-produced hard clam seed. Journal of Shellfish Research, 16: 519–521.

MacCallum, G.S., and McGladdery, S.E. 2000. Quahog Parasite Unknown (QPX) in the northern quahog *Mercenaria mercenaria* (Linnaeus, 1758) and *M. mercenaria* var. notata from Atlantic Canada, survey results from three maritime provinces. Journal of Shellfish Research, 19: 43–50.

Ragan, M.A., MacCallum, G.S., Murphy, C.A., Cannone, J.J., Gutell, R.R., and McGladdery, S.E. 2000. Protistant parasite QPX of hard-shell clam *Mercenaria mercenaria* is a member of Labyrinthulomycota. Diseases of Aquatic Organisms, 42: 185–190.

Ragone Calvo, L.M., Walker, J.G., and Burreson, E.M. 1998. Prevalence and distribution of QPX, Quahog Parasite Unknown, in hard clams, *Mercenaria mercenaria*, in Virginia, USA. Diseases of Aquatic Organisms, 33: 209–219.

Smolowitz, R., Leavitt, D., and Perkins, F. 1998. Observations of a protistan disease similar to QPX in *Mercenaria mercenaria* (hard clams) from the coast of Massachusetts. Journal of Invertebrate Pathology, 71: 9–25.

Whyte, S.K., Cawthorn, R.J., and McGladdery, S.E. 1994. QPX (Quahaug Parasite X) a pathogen of northern quahaug *Mercenaria mercenaria* from the Gulf of St. Lawrence, Canada. Diseases of Aquatic Organisms, 19: 129–136.

Titles Published in the *ICES Cooperative Research Report* Series

No.	Title	Price (Danish Kroner)
264	Alien Species Alert: *Rapana venosa* (veined whelk)	
263	Report of the ICES Advisory Committee on the Marine Environment, 2003. 227 pp.	150
262	Report of the ICES Advisory Committee on Ecosystems, 2003. 229 pp.	170
261	Report of the ICES Advisory Committee on Fishery Management, 2004 (Parts 1–3). 975 pp.	430
260	Stockholm 1999 Centenary Lectures. 48 pp.	170
259	The 2002/2003 ICES Annual Ocean Climate Status Summary. 29 pp.	150
258	Seabirds as Monitors of the Marine Environment. 73 pp.	200
257	Proceedings of the Baltic Marine Science Conference. 334 pp.	420
256	Report of the ICES Advisory Committee on the Marine Environment, 2002. 155 pp.	270
255	Report of the ICES Advisory Committee on Fishery Management, 2002. (Parts 1–3). 948 pp.	1200
254	Report of the ICES Advisory Committee on Ecosystems, 2002. 129 pp.	250
253	ICES Science 1979–1999: The View from a Younger Generation. 39 pp.	170
252	Report of the ICES/GLOBEC Workshop on the Dynamics of Growth in Cod. 97 pp. (including CD-Rom from ICES ASC 2001)	220
251	The Annual ICES Ocean Climate Status Summary 2001/2002. 27 pp.	180
250	ICES/GLOBEC Sea-going Workshop for Intercalibration of Plankton Samplers. A compilation of data, metadata and visual material, 2002. 34 pp.	190
249	Report of the ICES Advisory Committee on Ecosystems, 2001. 75 pp.	200
248	Report of the ICES Advisory Committee on the Marine Environment, 2001. 203 pp.	310
247	Effects of Extraction of Marine Sediments on the Marine Ecosystem. 80 pp.	200
246	Report of the ICES Advisory Committee on Fishery Management, 2001. (Parts 1–3). 895 pp.	1170
245	The Annual ICES Ocean Climate Status Summary 2000/2001. 21 pp.	150
244	Workshop on Gadoid Stocks in the North Sea during the 1960s and 1970s. The Fourth ICES/GLOBEC Backward-Facing Workshop. 55 pp.	160
243	Report of the 12th ICES Dialogue Meeting (First Environmental Dialogue Meeting). 28 pp.	130
242	Report of the ICES Advisory Committee on Fishery Management, 2000. (Parts 1–3). 940 pp.	1100
241	Report of the ICES Advisory Committee on the Marine Environment, 2000. 263 pp.	370
240	Report of the Young Scientists Conference on Marine Ecosystem Perspectives. 73 pp.	170
239	Report of the ICES Advisory Committee on the Marine Environment, 1999. 277 pp.	350
238	Report on Echo Trace Classification. 107 pp.	200
237	Seventh Intercomparison Exercise on Trace Metals in Sea Water. 95 pp.	190
236	Report of the ICES Advisory Committee on Fishery Management, 1999. (Part 1 and Part 2). 821 pp.	920

235	Methodology for Target Strength Measurements (With special reference to *in situ* techniques for fish and mikronekton). 59 pp.	160
234	Report of the Workshop on Ocean Climate of the NW Atlantic during the 1960s and 1970s and Consequences for Gadoid Populations. 81 pp.	180
233	Report of the ICES Advisory Committee on the Marine Environment, 1998. 375 pp.	440
232	Diets of Seabirds and Consequences of Changes in Food Supply. 66 pp.	170
231	Status of Introductions of Non-Indigenous Marine Species to North Atlantic Waters 1981–1991. 91 pp.	190
230	Working Group on Methods of Fish Stock Assessment. Reports of Meetings in 1993 and 1995. 259 pp.	330
229	Report of the ICES Advisory Committee on Fishery Management, 1998. (Part 1 and Part 2). 776 pp.	900
228	Report of the 11th ICES Dialogue Meeting on the Relationship Between Scientific Advice and Fisheries Management. 37 pp.	140
227	Tenth ICES Dialogue Meeting (Fisheries and Environment in the Bay of Biscay and Iberian Region: Can the Living Resources Be Better Utilized). 30 pp.	130
226	Report on the Results of the ICES/IOC/OSPARCOM Intercomparison Programme on the Determination of Chlorobiphenyl Congeners in Marine Media – Steps 3a, 3b, 4 and Assessment. 159 pp.	250
225	North Atlantic – Norwegian Sea Exchanges: The ICES NANSEN Project. 246 pp.	320
224	Ballast Water: Ecological and Fisheries Implications. 146 pp.	230
223	Report of the ICES Advisory Committee on Fishery Management, 1997. (Part 1 and Part 2). 780 pp.	760
222	Report of the ICES Advisory Committee on the Marine Environment, 1997. 210 pp.	250
221	Report of the ICES Advisory Committee on Fishery Management, 1996. (Part 1 and Part 2). 642 pp.	660
220	Guide to the Identification of North Sea Fish Using Premaxillae and Vertebrae. 231 pp (including 300 photographs)	560
219	Database Report of the Stomach Sampling Project, 1991. 422 pp.	410
218	http://www.ices.dk/pubs/crr/crr218/crr218.htmAtlas of North Sea Benthic Infauna. 86 pp. (plus two diskettes containing relevant data)	210
217	Report of the ICES Advisory Committee on the Marine Environment, 1996. 159 pp.	210
216	Seabird/Fish Interactions, with Particular Reference to Seabirds in the North Sea. 87 pp.	140
215	Manual of Methods of Measuring the Selectivity of Towed Fishing Gears. 126 pp.	160
214	Report of the ICES Advisory Committee on Fishery Management, 1995. (Part 1 and Part 2). 636 pp.	1200
213	Report of the Results of the Fifth ICES Intercomparison Exercise for Nutrients in Sea Water. 79 pp.	125
212	Report of the ICES Advisory Committee on the Marine Environment, 1995. 135 pp.	175
211	Intercalibration Exercise on the Qualitative and Quantitative Analysis of Fatty Acids in Artemia and Marine Samples Used in Mariculture. 30 pp.	90
210	Report of the ICES Advisory Committee on Fishery Management, 1994. (Part 1 and Part 2). 534 pp.	575
209	Underwater Noise of Research Vessels: Review and Recommendations. 61 pp.	115
208	Results of the 1990/1991 Baseline Study of Contaminants in North Sea Sediments. 193 pp.	225

These publications may be ordered from: ICES Secretariat, Palægade 2–4, DK-1261 Copenhagen K, Denmark, fax: +45 33 93 42 15, e-mail: info@ices.dk. An invoice including the cost of postage and handling will be sent. Publications are usually dispatched within one week of receipt of payment. Further information about ICES publications, including ordering and payment by credit card, cheques and bank transfer, may be found at: www.ices.dk/pubs.htm.