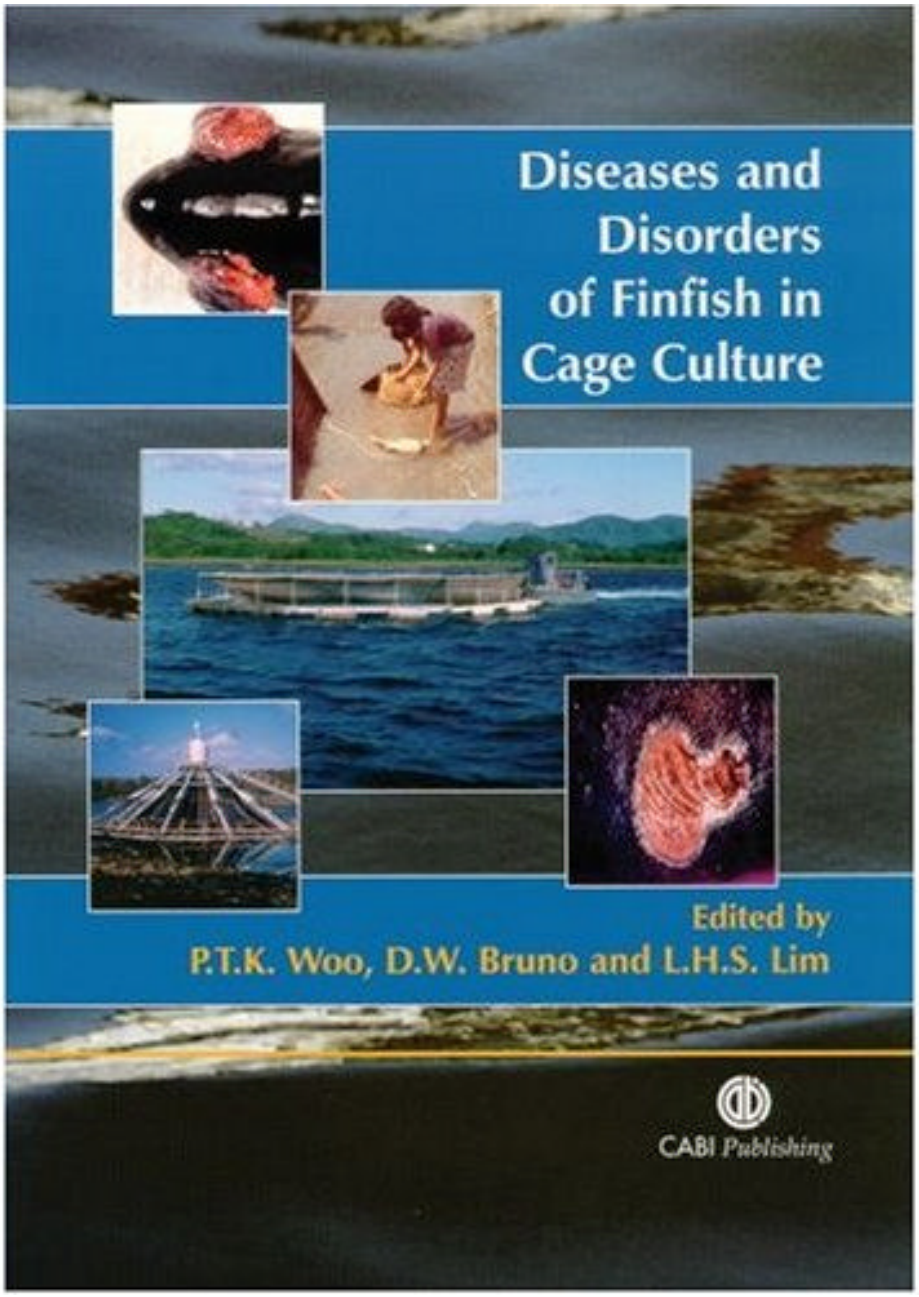

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Edited by
P.T.K. Woo, D.W. Bruno and L.H.S. Lim
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Diseases and Disorders of Finfish in Cage Culture

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Preface

In many parts of the world the primary source of animal protein for humans is finfish. The intensive culture of finfish has grown significantly since the 1980s partly because of the dramatic decline in the natural fish stocks and the increase in fish consumption by the ever-increasing population. For example, the worldwide consumption of fish between 1990 and 1997 increased by 30% while the capture fisheries increased only by 9%. The demand for fish is expected to continue to increase, especially as the more affluent consumers in the developed countries become more aware of the beneficial effects of fish (e.g. marine fish are an excellent source of polyunsaturated omega-3 fatty acids). Aquaculture is the only solution to the demand as it can provide consistently high quality fish protein year round. The industry is already considered the single fastest-growing food production process in the world.

The cage culture of finfish, especially mariculture, is becoming more popular because there are many economic advantages associated with this approach. However, it also has problems and one of them is disease. Disease outbreaks tend to occur more often when fish are raised under intensive culture conditions, and consequently both infectious and non-infectious diseases are important constraints to the industry.

Our primary objective is to produce an authoritative and practical volume on diseases and disorders of finfish in cage culture. We hope the book will also alert the industry to potential and/or emerging disease problems in specific regions of the world, and to point out gaps in our knowledge so as to stimulate further research. This book is designed for aquaculturalists who are using or intend to use cage culture. It will also be useful to fish health consultants (e.g. veterinarians), microbiologists, parasitologists, fish pathologists, and managers and directors of diagnostic laboratories. Each chapter is written by international experts who have personal experience or expertise on diseases and their diagnosis, and/or solutions to problems associated with the cage culture of finfish.

This book is divided into four parts – the first part is on the cage culture system, the second and third are on diseases/disorders in warmwater fish (water temperature above 15°C) and in coldwater fish, respectively. In each of these parts, there are three chapters – one on infectious diseases in fresh water (zero salinity), one on estuarine and marine diseases and one on non-infectious disorders. The final part on emerging diseases is to alert the industry to potential problems. We hope this division of the book will make it easier for the reader to access information on known diseases/disorders within a group of fish. The arrangement will also help to highlight similarities and differences in disease problems between groups of fish (e.g. between marine warmwater and marine coldwater fish). However, such divisions also create some minor problems, e.g. a few pathogens have been

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isolated from both seawater and freshwater fish, so our authors and editors have worked closely to avoid extensive overlaps in coverage. For example, furunculosis is in Chapter 4, with only brief reference to it in Chapter 3, because it is often seen in freshwater fish. Similarly, important infectious agents (e.g. *Piscirickettsia salmonis*) of marine fish (Chapter 3) are only briefly mentioned in Chapter 4 because of their lesser importance to freshwater fish.

There are books on infectious and on non-infectious diseases/disorders of fish (e.g. *Fish Diseases and Disorders*, Volumes 1–3, CAB International), but there are none devoted specifically to problems associated with cage culture of finfish. Problems encountered in cage culture are in some ways different from those using other rearing methods. In cage culture, fish may be exposed constantly to ubiquitous pathogens. Also, the stress associated with captive rearing creates opportunities for disease, and to a lesser extent non-infectious disorders, to become significant causes of morbidity and mortality. Transmissions of infectious agents are also enhanced, and fish become more susceptible to disease partly because their immune system may be compromised due to prolonged exposure to pollutants in the water and/or crowding stress. The impact and spread of new and/or emerging diseases are also important, and are influenced by factors that include international trade in eggs or fry, unauthorized transportation of fish, and contact with migratory or naive fish species. Under natural conditions these agents in their natural hosts may not be considered important pathogens, but in an expanded geographical and/or host range, under different environmental conditions or temperatures, they may lead to epizootics with serious consequential economic impact.

As the demand for animal protein increases in the new millennium, we expect a significant increase in cage culture activity in many countries. This will be true especially in countries with limited usable land mass but with relatively long coastlines and/or extensive river–lake systems. We hope this book will fill a niche and be useful to colleagues who are active in the industry.

Patrick T.K. Woo
David W. Bruno
L.H. Susan Lim

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1 Introduction and History of Cage Culture

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History of Cage Culture

Open sea activities, such as cage and pen culture, are viewed by many stakeholders in the industry as the aquaculture system of the millennium. Fish production from cages and pens (both in freshwater and marine environments) contributes significantly to total foodfish produced. Cage culture has made possible the large-scale production of commercial finfish and will probably be the most efficient and economical way of raising fish.

Aquaculturists realize the need to limit further conversion of wetlands and mangroves into traditional aquaculture farms. We face a situation where even freshwater ecosystems have reached critical levels with respect to their carrying capacities. The depletion of ocean and coastal fishery resources in some areas has led to the development of marine cage culture.

The earliest record of cage culture practices dates back to the late 1800s in Southeast Asia, particularly in the freshwater lakes and river systems of Kampuchea (Coche, 1976; Pantulu, 1979; Beveridge, 1987). The fish cultured included snakeheads (*Channa* spp.), catfishes (*Pangasius* spp.) and gobies (*Oxycleotris* spp.). By 1995, more than 5000 fish farmers were engaged in cage culture in the Mekong river system around Phnom Penh (Thana, 1995). There were also reports of similar culture practices in Indonesia in the 1920s and 1940s (Hickling, 1962).

Marine fish farming in cages traces its beginnings to the 1950s in Japan where fish farming research at the Fisheries Laboratory of the Kinki University led to the commercial culture of the yellowtail, *Seriola quinqueradiata*. Takashima and Arimoto (2000), however, traced back a history of 200 years where wooden farm net cages were being operated for anchovies or sardines or bait for skipjack. Similar cages were later used for yellowtail culture in Japan and developed into a significant industry as early as 1960. The cage culture of common carp (*Cyprinus carpio*) in lakes also started at this time (Kuronuma, 1968). Since the 1970s, Thailand has developed cage culture techniques for two important marine finfish: the seabream (*Pagrus major*) and grouper (*Epinephelus* spp.) (Coche, 1976). Chua and Teng (1978) pioneered the development of cage culture methods/designs for groupers in Malaysia, although large-scale cage farming in marine waters really gained ground in the 1980s and in inland waters in the 1990s (Shariff and Nagaraj, 2000). Korea started growing a European variety of common carp and maintained yellowtail in holding cage enclosures in the late 1970s. By the end of 1980, cage culture of the olive flounder (*Paralichthys olivacens*) and black rockfish

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(*Sebastes schlegeli*) was established, and developed into a successful aquaculture industry in the 1990s (Kim, 2000). Cage culture of groupers (*Epinephelus* spp.) in the Philippines has been practised since the 1980s. Mariculture of milkfish in the 1990s led to the further growth and development of the industry (Marte *et al.*, 2000).

In Europe, cage culture of rainbow trout (*Oncorhynchus mykiss*) in fresh water began in the late 1950s and, in Norway, Atlantic salmon (*Salmo salar*) followed in the 1960s. More than 40% of its rainbow trout comes from freshwater cages (Beveridge, 1987). Salmonid culture is currently dominated by production from Norway, Scotland and Chile. Cage culture of fish was adopted in the USA in 1964 (Coche, 1976). Records show commercial production of channel catfish (*Ictalurus punctatus*) in freshwater cages (Collins, 1970a,b, 1972; Trotter, 1970; Bennet, 1971; Brett, 1974; Novotny, 1975). Cage culture in Africa, however, is still in its infant stage (ADB/NACA, 1998). In Central Africa, there was no real practical experience in cage culture before 1974. Very limited observations were recorded for *Clarias lazera* (de Kimpe and Micha, 1974). Semi-intensive rearing was done in Lake Victoria, Tanzania, using Nile tilapia (*Tilapia zillii*) (Ibrahim *et al.*, 1974). Research initiatives on intensive production of commercial sized *Tilapia nilotica* were carried out in Lake Kossou, Ivory Coast (Coche, 1974, 1975; Shehadeh, 1974). Cook (1995) reported that it was only in the 1980s that the potential of aquaculture in South Africa gained grounds with respect to becoming a viable commercial industry. Freshwater aquaculture was limited to availability of water while mariculture had to rely on only 3000 km of coastlines (the majority of which did not have sheltered bays or lagoons). In the years that followed, efforts were geared towards improvement in the culture of tilapia and cage design (Coche, 1976).

Currently many fish species have been cultivated in various designs and sizes of cages in Asia, Europe and other parts of the world (Table 1.1). Tilapia and carp predominate in freshwater cage culture in Asia, while salmonids are commonly farmed in Europe and the Americas.

Table 1.1a. Major species of freshwater finfishes cultured in cages.

Species cultured	Country	Reference
Anguillidae		
Anguilla japonica (eel)	China	Yuan (1991)
Bagridae		
Mystus nemerus (mystid catfish)	Malaysia	Shariff and Nagaraj (2000)
Chanidae		
Chanos chanos (milkfish)	Philippines	Guerrero (1996); Ramos (1996); Bagarinao (1998); Marte *et al.* (2000)
Channidae		
Channa macrocephalus	Thailand	Lin (1990)
Channa micropeltes (snakehead)	Cambodia	Thana (1995)
	Vietnam	Pantulu (1976); Thuoc (1995)
Channa striatus	Vietnam	Pantulu (1976); Thuoc (1995)
Giant snakehead	Malaysia	Ang *et al.* (1988)
Characidae		
Colossoma macropomum (Amazonian fish tambaqui)	Brazil	Chellappa *et al.* (1995)
Cichlidae		
Black tilapia	Malaysia	Ang *et al.* (1988)
Oreochromis mortimeri	Zimbabwe	Norberg and Stenstroem (1993)
Oreochromis niloticus (Nile tilapia)	Zimbabwe	Norberg and Stenstroem (1993)
	Bangladesh	Mazid (1995)
	Malaysia	Shariff and Nagaraj (2000)

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Species cultured	Country	Reference
	Philippines	Santiago and Arcilla (1993); Lopez (1995)
	Thailand	Chiayvareesajja *et al.* (1990); Lin (1990)
	Egypt	Ishak and Hassanen (1987)
Red tilapia	Malaysia	Ang *et al.* (1988)
Sarotherodon aureus	USA	Schmittou (1969); Perry and Avault (1972)
	El Salvador	Bayne *et al.* (1976); Ramirez (1977); Sanchez (1978); Street (1978)
	Puerto Rico	Jordan and Pagan (1973); Miller and Ballantine (1974)
	USA	Williams *et al.* (1974)
Sarotherodon esculentus	Tanzania	Ibrahim *et al.* (1976)
Sarotherodon galilaeus	Nigeria	Konikoff (1975); Ita (1976)
Sarotherodon mossambicus	Philippines	Guerrero (1975); IFP (1976); Pantastico and Baldia (1979)
	Taiwan	Maruyama and Ishida (1976)
	Guatemala	Bardach *et al.* (1972)
Sarotherodon mossambicus × *S. honorum* (hybrid)	USA	Suffern *et al.* (1978)
Sarotherodon niloticus	Sri Lanka	Anon. (1980); Muthukumarana and Wcerakoon (1987)
	Ivory Coast	Coche (1975, 1976, 1977, 1978); Campbell (1976); Shehadeh (1976); de Kimpe (1978); Amoikon (1987)
	Nigeria	Konikoff (1975); Campbell (1987)
Sarotherodon spilirus niger (tilapia)	Kenya	Haller (1974)
Tilapia	Philippines	PCARRD (1981); Aragon *et al.* (1985); Guerrero (1985, 1996)
Tilapia	Brazil	FAO (1977)
Tilapia	Dominican Republic	Olivo (1987)
Tilapia heudeloti	Togo	Issifou and Amegavie (1987)
Tilapia nilotica	USA	McGinty (1991)
	Sierra Leone	Iscandari (1987)
	Togo	Issifou and Amegavie (1987)
Tilapia niloticus	Dominican Republic	Olivo (1987)
	Nigeria	Ali (1987)
Tilapia rendalli	Colombia	Patino (1976); McLarney (1978); Popma (1978)
	Zimbabwe	Norberg and Stenstroem (1993)
Tilapia zillii	Tanzania	Ibrahim *et al.* (1974)
	Togo	Issifou and Amegavie (1987)
	Kenya	Haller (1974)
	Nigeria	Konikoff (1975); Campbell (1987)
Clariidae		
Clarias gariepinus	Vietnam	Tuan and Hambrey (2000)
	South Africa	Hoffman and Prinsloo (1992)
Clarias lazera (Nile catfish)	Egypt	Ishak (1987)
Clarias macrocephalus (catfish)	Thailand	Lin (1990)
	Vietnam	Tuan and Hambrey (2000)
Cyprinidae		
Abramis brana (bream)	Russia	Ziliukiene (1994)
Aristichthys nobilis (bighead carp)	Nepal	Swar and Pradhan (1992); Pradhan and Pantha (1995)

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Table 1.1a. *Continued.*

Species cultured	Country	Reference
	Malaysia	Ang *et al.* (1988)
	Philippines	Fermin (1990); Marte *et al.* (2000)
	Sri Lanka	Muthukumarana and Weerakoon (1987)
Carps	India	Basavaraja (1994)
Carps	Indonesia	Costa-Pierce and Effendi (1988)
Carps	Iran	Matinfar and Nikouyan (1995)
Cirrhinus microbis	Cambodia	Thana (1995)
Cirrhinus sp.	Cambodia	Thana (1995)
Ctenopharyngodon idella (grass carp)	Malaysia	Ang *et al.* (1988)
	Nepal	Pradhan and Pantha (1995)
	Sri Lanka	Muthukumarana and Weerakoon (1987)
	Vietnam	Lovatelli (1997)
	Egypt	Siemelink *et al.* (1982); Ishak (1987)
	Netherlands	Huisman (1979)
	India	Bandyopadhyay *et al.* (1991)
Cyprinids	Philippines	Lopez (1995)
Cyprinus carpio (common carp)	Poland	Filipiak (1991); Mamcarz (1992)
	Russia	Evtushenko (1994)
	Nepal	Pradhan and Pantha (1995)
	Indonesia	Costa-Pierce and Roem (1990); Zainal *et al.* (1990)
	Korea	Kim *et al.* (1992)
	Egypt	Hamza (1996)
	Israel	Viola and Lahav (1991); Wolhfarth and Moav (1991)
(mirror carp)	Turkey	Erden (1987)
Hypophthalmichthys molitrix (silver carp)	Nepal	Swar and Pradhan (1992); Pradhan and Pantha (1995)
	Egypt	Hamza (1996)
	India	Sivakami and Ayyappan (1991)
(Javanese carp)	Malaysia	Ang *et al.* (1988)
Leptobarbus hoeveni (slender carp/sultan fish)	Vietnam	Thuoc (1995); Lovatelli (1997)
	Malaysia	Shariff and Nagaraj (2000)
	Indonesia	Dahril and Ahmad (1990)
Nile carp	Egypt	Hamza (1996)
River carp	Malaysia	Ang *et al.* (1988)
Eleotridae		
Goby	Malaysia	Ang *et al.* (1988)
Oxyeleotris marmoratus (sand goby)	Thailand	Menasveta (2000)
	Vietnam	Lovatelli (1997)
Ictaluridae		
Ictalurus punctatus (Channel catfish)	USA	Schmittou (1969); Perry and Avault (1972); Collins and Delmendo (1979); Parker (1988); Masser and Duarte (1992); Burtle and Newton (1993); Webster *et al.* (1994)
Moronidae		
Morone chryops × *M. saxatilis* (sunshine bass)	USA	Kelly and Kohler, 1996; Pagan (1970); Suwanasart (1971); Pagan-Font (1975)
Osphronemidae		
Osphronemus gourami (giant gouramy)	Indonesia	Ang *et al.* (1988)
	Malaysia	Ang *et al.* (1988)

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Species cultured	Country	Reference
	Vietnam	Lovatelli (1997)
Pangasiidae		
Pangasius bocourti (yellow catfish)	Vietnam	Lovatelli (1997); Tuan and Hambrey (2000)
Pangasius conchophilis	Cambodia	Thana (1995)
Pangasius hypophthalmus (catfish)	Vietnam	Tuan and Hambrey (2000)
	Cambodia	Thana (1995)
Pangasius lardnaudi	Cambodia	Thana (1995)
Pangasius micronemus	Vietnam	Tuan and Hambrey (2000)
Pangasius nasutus (catfish)	Vietnam	Thuoc (1995)
Pangasius pangasius (river catfish)	Thailand	Menasveta (2000)
Pangasius sutchii (striped catfish)	Malaysia	Shariff and Nagaraj (2000)
River catfish	Malaysia	Ang *et al.* (1988)
Percidae		
Perca fluviatilis (perch)	France	Tamazouzt *et al.* (1993)
Salmonidae		
Coregonus	Germany	Marciak (1979)
Coregonus albula (vendace)	Poland	Mamcarz (1984)
Coregonus lavaretus (Baltic whitefish)	Finland	Mamcarz (1984)
	Germany	Schultz *et al.* (1993)
	Russia	Jager and Nellen (1981)
(whitefish)	France	Champigneulle and Rojas-Beltran (1990)
Coregonus peled (peled)	Canada	Mamcarz and Kozlowski (1992)
Oncorhynchus mykiss (rainbow trout)	Bolivia	Menton (1991)
	Canada	Srivastava *et al.* (1991); Cornel and Whoriskey (1993)
	Denmark	Torrissen *et al.* (1995)
	Iran	Matinfar and Nikouyan (1995)
	Sweden	Alanaerae (1992)
	Switzerland	Mamcarz and Szczerbowski (1984)
	Norway	Torrissen *et al.* (1995)
Salmo salar (Atlantic salmon)	USA	Rottiers (1994)
Salmo trutta (broom trout)	Indonesia	Goeltenboth and Krisyanto (1994)
Stenodus (whitefish)	Northern Europe	Bronisz (1979)
Sciaenidae		
Sciaenops ocellatus (red drum or red fish)	Ecuador	Benetti *et al.* (1995)
	Israel	Kissil (1996)
	Panama	Garces (1992)
	Poland	Mamcarz and Worniallo (1985)
Siluridae		
Silurus glanis (sheat fish)	Yugoslavia	Stevic *et al.* (1993)
Esox lucius (pike)	Russia	Ziliukiene (1994)
Puntius gonionotus (minnows)	Bangladesh	Mazid (1995)
	Vietnam	Thuoc (1995)
Puntius schwanenfeldii (tinfoil barb)	Indonesia	Christensen (1993)
(minnows)	Vietnam	Thuoc (1995)
Puntius spp.	Cambodia	Thana (1995)

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Table 1.1b. Major species of brackish water finfishes cultured in cages.

Species cultured	Country	Reference
Chanidae		
Chanos chanos (milkfish)	Philippines	Guerrero (1996); Ramos (1996); Bagarinao (1998); Marte *et al.* (2000)
Cichlidae		
Oreochromis urolepsis hornorum × *O. mossambicus* male (Florida red tilapia)	USA	Rust *et al.* (1991)
Moronidae		
Morone chryops × *M. saxatilis* (sunshine bass)	USA	Pagan (1970); Suwanasart (1971); Pagan-Font (1975); Kelly and Kohler (1996)
Pisodonophis		
Pisodonophis boro (brackishwater eel)	Vietnam	Lovatelli (1997)
Salmonidae		
Coregonus lavaretus (Baltic whitefish)	Germany	Schultz *et al.* (1993)
Oncorhynchus mason rhodurus (Amago salmon)	Yugoslavia	Teskeredzic and Teskeredzic (1990)
Salmo salar (Atlantic salmon)	USA	Rottiers (1994)

Table 1.1c. Major species of marine finfishes cultured in cages.

Species cultured	Country	Reference
Carangidae		
Longirostrum/Caranx delicatissimus (striped jack)	Japan	Watanabe (1988a,b)
Seriola dumerili	Taiwan	Su *et al.* (2000)
Seriola magatlana (Pacific yellowtail)	Ecuador	Benetti *et al.* (1995)
Seriola purpurescens (amberjack)	Hong Kong	Wong (1995)
Seriola quinqueradiata (yellowtail)	Japan	Fujiya (1976); Mitani (1979); Kafuku and Ikenoue (1983); Shepherd and Bromage (1988); Fukumoto (1989); Watanabe *et al.* (1996)
	China	Lin (1997)
	Korea	Shepherd and Bromage (1988); Fukumoto (1989); Jeon *et al.* (1992); Kim (1995)
Sturgeon	Iran	Matinfar and Nikouyan (1995)
Sturgeon (beluga × sterlet, 'bestir')	Russia	Romanycheva and Salnikov (1979)
Trachinotus carolinus (pompano)	USA	Smith (1973)
Trachinotus oaitensis (pompano)	Ecuador	Benetti *et al.* (1995)
Trachinotus teraia	France	Trebaol (1991)
Centropomidae		
Centropomus nigrescens (snook)	Ecuador	Benetti *et al.* (1995)
Lates calcarifer (seabass)	China	Yongjia *et al.* (1996)
	Hong Kong	Wong (1995)
	Indonesia	Sakaras (1982); Kungvankij (1987b)
	Malaysia	Singh (1991); Hannafi *et al.* (1995)
	Philippines	Toledo *et al.* (1991); Fermin *et al.* (1993); Alcantara *et al.* (1995); Lopez (1995)
	Singapore	Anon. (1986); Cheong and Lee (1987)
	Thailand	Sakaras (1984); Kungvankij (1987a); Tookwinas (1990b); Chaitanawisuti and Piyatiratitivorakul (1994a)
	Vietnam	Lovatelli (1997)
	Australia	Barlow *et al.* (1995); Rimmer (1998)

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Species cultured	Country	Reference
Characidae		
Piaractus mesopotamicus (pacu)	Brazil	Ferraz de Lima *et al.* (1992)
Cichlidae		
Oreochromis spilirus (tilapia)	Kuwait	Cruz and Ridha (1990b)
Oreochromis urolepsis hornorum × *O. mossambicus* male (Florida red tilapia)	USA	Rust *et al.* (1991)
Cyprinidae		
Barbus gonionotus (silver barb)	Vietnam	Lovatelli (1997)
Cirrhina (rohu)	Nepal	Pradhan and Pantha (1995)
Gadidae		
Cod	Norway	Kaspruk and Tvejte (1994); Hjelt (2000)
Gadus morhua (Atlantic cod)	Canada	Jones and Iwama (1990)
Lutjanidae		
Lutjanus argentimaculatus (red snapper)	China	Yongjia *et al.* (1996)
	Malaysia	Ali (1987); Hannafi *et al.* (1995)
	Philippines	Emata (1996)
	Singapore	Cheong (1988)
	Thailand	Doi and Singhagraiwan (1993); Chaitanawisuti and Piyatiratitivorakul (1994b)
Lutjanus erythropeterus	Taiwan	Su *et al.* (2000)
Lutjanus johni (golden snapper)	Malaysia	Hannafi *et al.* (1995)
	Singapore	Lee (1982); Anon. (1986)
Lutjanus russelli (Russell's snapper)	China	Yongjia *et al.* (1996)
	Hong Kong	Wong (1995)
	Malaysia	Rahim (1982)
Lutjanus sebae	Thailand	Tanomkiat (1982)
Lutjanus stellatus	Taiwan	Su *et al.* (2000)
Pagrus major (Japanese red seabream/red seabream)	Israel	Kissil (1996)
	Japan	Fukumoto (1989
	Korea	Kim (1995)
	Taiwan	Su *et al.* (2000)
Moronidae		
Dicentrarchus labrax (seabass)	Egypt	Ishak and Hassanen (1987)
	Italy	Barbato *et al.* (1991)
(European seabass)	Israel	Kissil (1996)
Oplegnathidae		
Oplegnathus fasciatus (rock bream)	Korea	Kim (1995)
Paralichthyidae		
Paralichthys olivaceus (bastard halibut/flounder)	Japan	Watanabe (1988a,b)
	Japan	Hiraishi *et al.* (1995)
	Japan	Kikuchi *et al.* (1993)
	Korea	Kim (1995)
(olive flounder)	Korea	Jeon *et al.* (1992)
Percichthyidae		
Lateolabrax japonicus (Japanese seabass)	Korea	Kim (1995)
Percidae		
Stizostedion lucioperca (wild zander)	Finland	Salminen *et al.* (1992)
Pleuronectidae		
Hippoglossus hippoglossus (Atlantic halibut)	UK	Martinez-Cordero *et al.* (1994)

Continued

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Table 1.1c. *Continued.*

Species cultured	Country	Reference
Limanda herzentein (brown sole)	Korea	Kim (1995)
Limanda punctatissima (longsnout flounder)	Japan	Hiraishi *et al.* (1995)
Rachycentridae		
Rachycentron canadum	Taiwan	Su *et al.* (2000)
Salmonidae		
Caspian salmon	Iran	Matinfar and Nikouyan (1995)
Onchorynchus kisutch (Coho salmon)	Chile	Jelvez-Flores (1992)
Oncorhynchus mason rhodurus (Amago salmon)	Yugoslavia	Teskeredzic and Teskeredzic (1990)
Oncorhynchus mykiss (rainbow trout)	Canada	Srivastava *et al.* (1991); Cornel and Whoriskey (1993)
Oncorhynchus tshavytocha (Chinook salmon)	Canada	Jones and Iwama (1990)
Prosopium	Germany	Marciak (1979)
Salmo salar (Atlantic salmon)	Canada	Egan and Kenney (1990); Menton and Allen (1991); Duston and Saunders (1994)
	Scotland	Glen (1974); Went (1980); Worniallo and Mamcarz (1985); Sangster and Munro (1991); Smith *et al.* (1993)
	Norway	Kraakenes *et al.* (1991)
	USA	Rottiers (1994)
Salmo trutta (broom trout)	France	Arzel *et al.* (1993)
Salvelinus alpinus (Arctic charr)	Norway	Torrissen *et al.* (1995)
Sciaenidae		
Cynoscion stolzmanni (corvina)	Ecuador	Benetti *et al.* (1995)
Ophicephalus sp. (serpent head)	Thailand	Menasveta (2000)
Scianops teraia (Western African pompano)	France	Trebaol (1991)
Sebastidae		
Sebastes schlegeli (Schlegel's black rock fish)	China	Liu *et al.* (1991)
	Korea	Kim (1995)
Scophthalmidae		
Scophthalmus maximus (turbot)	France	Vigneulle and Laurencin (1995)
Serranidae		
Cephalopholis mimata	Vietnam	Tuan and Hambrey (2000)
Cephalopholis pachycenteron	Philippines	Sayong (1981)
Epinephelus akaara	Hong Kong	Chao and Lim (1991); Wong (1995)
	Japan	Ukawa *et al.* (1966); Chao and Lim (1991)
	Vietnam	Tuan and Hambrey (2000)
	China	Chao and Lim (1991); Wong (1995)
Epinephelus alwaora (grouper)	China	Chao and Lim (1991)
Epinephelus amblycephalus	Taiwan	Maruyama and Ishida (1976)
Epinephelus areolatus (spotted grouper)	Hong Kong	Wong (1995)
Epinephelus bleeker	Philippines	Kohno *et al.* (1988)
Epinephelus bleekeri	Vietnam	Tuan and Hambrey (2000)
Epinephelus coioides	Philippines	Quinitio *et al.* (1997)
	Taiwan	Su *et al.* (2000)
	Vietnam	Tuan and Hambrey (2000)
Epinephelus fario	Sri Lanka	Chao and Lim (1991)
Epinephelus fuscoguttatus	Singapore	Lim *et al.* (1990); Chao and Lim (1991)
	Indonesia	Chao and Lim (1991)

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Species cultured	Country	Reference
	Japan	Chao and Lim (1991)
	Philippines	PCARRD (1986); Quinitio and Toledo (1991)
Epinephelus hexagonatus	India	Hamsa and Kasim (1992)
Epinephelus macrospilos	Philippines	PCARRD (1986); Quinitio and Toledo (1991)
Epinephelus malabaricus	China	Yongjia *et al.* (1996)
	Philippines	Kohno *et al.* (1988)
Epinephelus merra	Philippines	Sayong (1981)
	Vietnam	Tuan and Hambrey (2000)
Epinephelus microdon	Japan	Chao and Lim (1991)
Epinephelus moara	Japan	Chao and Lim (1991)
(kelp bass)	China	Yongjia *et al.* (1996)
Epinephelus salmonoides	Philippines	Kungvankij *et al.* (1986)
	Sri Lanka	Chao and Lim (1991)
	Japan	Chao and Lim (1991)
	Malaysia	Chua (1979); Chua and Teng (1979, 1980)
Epinephelus sexfaciatus	Philippines	Kohno *et al.* (1988)
	Vietnam	Tuan and Hambrey (2000)
Epinephelus spp.	Malaysia	Leong (1998)
	Philippines	Quinitio and Toledo (1991)
	Singapore	Anon. (1986)
	Thailand	Tookwinas (1990a); Menasveta (2000)
Epinephelus suillus	Philippines	Toledo *et al.* (1993)
	Taiwan	Maruyama and Ishida (1976)
Epinephelus summana	Philippines	Sayong (1981)
Epinephelus tauvina (green grouper, estuarine grouper)	Hong Kong	Wong (1995)
	India	Hamsa and Kasim (1992)
	Indonesia	Lanjumin (1982)
	Malaysia	Chua and Teng (1978); Rahim (1982); Ali (1987)
	Philippines	Kohno *et al.* (1988); Lopez (1995)
	Singapore	Cheong and Lee (1987)
	Singapore	Chao and Lim (1991)
	Kuwait	Hussain *et al.* (1975); Chao and Lim (1991)
Siganidae		
Siganus canaliculatus (rabbit fish)	Indonesia	Tacon *et al.* (1990)
Siganus guttatus (siganid)	Philippines	Lopez (1995); Soriano *et al.* (1995)
	Vietnam	Lovatelli (1997)
Sillaginidae		
Sillago sihama (sand whiting)	India	James *et al.* (1985)
Sparidae		
Acanthopagrus schlegeli (black seabream)	Korea	Kim (1995)
Chrysophrys major	China	Yongjia *et al.* (1996)
(red pargo)	Hong Kong	Wong (1995)
Mylio latus (yellow finned seabream)	Hong Kong	Wong (1995)
Puntazzo puntazzo (sheepshead bream)	Israel	Kissil (1996)
Rhabdosargus sarba (goldlined seabream)	Hong Kong	Wong (1995)
Sparrus aurata (gilthead seabream)	Israel	Kissil (1996)
	Israel	Porter *et al.* (1991)
Sparrus macrocephalus	China	Yongjia *et al.* (1996)
Tetraodontidae		
Takifugu rubripes (tiger puffer)	Japan	Shepherd and Bromage (1988)
	Korea	Moon *et al.* (1993); Kim (1995)

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The rapid growth of the industry in most countries may be attributed to: (i) suitable offshore sites for cage culture; (ii) well established breeding techniques that yield a sufficient quantity of various marine and freshwater fish juveniles; (iii) availability of supporting industries, such as feed and fishing net manufacturers, and fish processors and packers; (iv) strong research and development initiatives from institutions, government and universities; and (v) the private sector ensuring refinement and improvement of techniques/culture systems, thereby further developing the industry.

With the experiences seen in salmon farming, seabream (*Sparus auratus*) and seabass (*Dicentrarchus labrax*) cage culture activities started to move toward offshore areas. The lack or non-availability of sheltered sites in many regions because of varied coastline configurations, the build-up of organic matter in closed bays due to poor water exchange, and use conflicts between industries and tourism for sea water were the main reasons for such a shift (Lisac, 1991).

Some of the offshore cage systems that later developed include: Dunlop Tempest I (Fearn, 1991); 'SADCO' cages (Muravjev *et al.*, 1993); Ocean Spar (Loverich and Croker, 1993); Farmocean system (Gunnarson, 1993); Seacon system (Lien, 2000); and Bridgeton Hi-Seas (Gunnarson, 1993; Lien, 2000).

Muir (1998) considered the following criteria important for success in offshore cage culture: (i) location (> 2 km from shore); (ii) environment (average waves > 5 m, regularly 2–3 m oceanic swells, variable wind periods); (iii) access (about 80% of the time when cages are accessible to working staff); and (iv) operation (remote; with automated feeding devices and long-distance monitoring).

Advantages and Limitations of Cage Culture

In general, cage culture practices have numerous advantages over other culture systems. By integrating the cage culture system into the aquatic ecosystem the carrying capacity per unit area is optimized because the free flow of current brings in fresh water and removes metabolic wastes, excess feed and faecal matter (Beveridge, 1983). Operationally, this has a number of advantages. Some cage designs, especially those used in inshore cultures, are relatively easy to construct with minimal skilled labour, and cages utilize minimal physical facilities and space. Economically, cage culture is a low-input farming practice with high economic return. However, cage culture is a high risk and labour-intensive operation. The practice is vulnerable to natural hazards (strong tides, storms and typhoons) and can be affected by deteriorating water quality attributed to chronic pollution from oil and chemical spills from oil tankers and cargo vessels (Tabira, 1980; Nose, 1985). In addition, poaching and vandalism are reported by cage farmers. The advantages and limitations of cage culture are summarized in Table 1.2.

In view of the high production attainable in cage culture system and the presence of large sheltered coastal waters in many countries, marine cage farming can play a significant role in increasing fish production.

Cage culture systems vary in terms of farm size and intensity of operation. Floating cages, for instance, in Korea can reach yields exceeding 500 t ha^{-1} (ADB/NACA, 1998).

Cage Design

Cage design is determined by conditions in the culture site, as well as the ecological requirements and behaviour of the target species for culture. Each design is site-specific and knowledge of the topography, wind force, wind direction, prevalence of storms, monsoons, wave load, current velocity and water depths are important parameters for consideration. In designing cages, it is also important to consider the rate of biofouling and the species composition of the marine fauna in and around the potential site (Chua, 1982). A checklist of

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Table 1.2. Advantages and limitations of cage fish culture technique.

Advantages	Limitations
Maximizes use of available water resources	Locations restricted to sheltered areas
Reduces pressure on land resources	Requires back-up food store, hatchery and processing facilities
Combines several types of culture within one water body; treatments and harvests independent	
Ease of movement and relocation of cages	
Intensification of fish production (high densities and optimum feeding result in improved growth rates, reducing rearing period)	Needs adequate water exchange to remove metabolites and maintain high dissolved oxygen levels; rapid fouling of cage walls requires frequent cleaning
Optimum utilization of artificial food improves food conversion efficiencies	Absolute dependence on artificial feeding unless in sewage ponds; high-quality balanced rations essential; feed losses possible through cage walls
Easy control of competitors and predators	Sometimes important interference from the natural fish population, i.e. small fish enter cages and compete for food
Ease of daily observation of stocks for better management and early detection and treatment of parasites and diseases	Natural fish populations act as a potential reservoir of disease and parasites, and the likelihood of spreading disease by introducing new cultured stocks is increased
Reduces fish handling and mortalities	Increased difficulties of disease and parasite treatment
Easy fish harvest	Risks of theft are increased
Storage and transport of live fish facilitated	Amortization of capital investment may be short
Initial investment is relatively small	Increased labour costs for handling, stocking, feeding and maintenance

fish species popularly cultured in Asia with cage and culture specifications is provided in Table 1.3.

Types of cages

A fish cage is usually made up of netting with an opening at the surface to facilitate feeding, removal of debris and dead fish, and for harvesting. The netcage system consists of a netcage proper and the frame, which supports the nets. The frame is normally kept afloat by buoys, usually metal (or traditionally plastic drums), and held in position by anchors. Cages may be classified as follows.

Fixed

A stationary cage is fastened to a fixed bamboo or wooden pole at its corners. It consists of a net bag supported by posts driven into the bottom of a lake or river. It is traditionally used in tropical countries like the Philippines for raising fish fingerlings. It is inexpensive and simple to build. This type of cage is normally restricted to shallow areas with suitable substrates usually in freshwater systems.

Floating

A floating cage consists of a floating unit from which a single cage or a battery of netcages is suspended. Floating cages are widely used for fish rearing in both fresh and coastal waters. They are less restrictive in terms of site selection compared with the stationary fixed types. Surface floating cages are used in lakes, protected bays and lagoons, sheltered coves and inland seas. The surface-floating unit consists of floats, framework and netcage. Most floating cages

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Table 1.3. Fish species and culture specifics of fish in Asia and Australia. (From Buendia, 1998).

Species	Cage/pen dimension	Culture specifics	Country/ references
Milkfish (*Chanos chanos*)	Rectangular marine pen, 20 × 50 × 60 m (1000 m^2 × 6 m); wood, bamboo, polythene net	Stocking density is 30,000 fingerlings weighing 10 g; feeding with commercial pellets or crumbles, given 3× daily to satiation, and with FCR of 1.77; 138 days culture period, 94% survival; production about 5–7 days	Philippines (Ramos, 1996; Bagarinao, 1998)
Seabass (*Lates calcarifer*)	Cylindrical floating netcage, 2 m diameter × 2 m depth (6 m^3); wood, bamboo, polythene and 200 l plastic drums for floats	Stocking density is 40 fish m^{-3} of size 18 cm; feeding with trash fish, once daily; 9 months culture period, 95.4% survival; production of 490 g per fish	Thailand (Chaitanawisuti and Piyatiratitivorakul, 1994a)
	Box-shaped floating netcage, 5 × 5 × 3 m; wood and plastic drums	Stocking density is 44 fish m^{-3} of size 80–100 g; feeding with trash fish, cooked rice bran and aquatic vegetation, with FCR of 4.5:1; 6–7 months of culture, 90% survival; production of 600 g per fish	Singapore (Anon., 1986)
	Rectangular broodstock floating netcage 4 × 4 × 3 m, installed with a hapa net of the same dimension with mesh size of 0.4–0.6 mm as egg collector; made of bamboo, wood and 200 l plastic drums	Stocking density is 60–80 fish per cage, sex ratio is 13–28 female:male fish; feeding with trash fish daily at 3–5% of body weight; culture period of 4 years; fish matured and naturally spawned; also demonstrates an efficient, simple and cheap egg collector (116 million eggs in one breeding season)	Philippines (Toledo *et al.*, 1991)
	Circular or rectangular broodstock floating netcages, 4 × 4 × 3 m or 10 × 10 × 2 m nylon mesh of size 4–8 cm	Stocking density is 1 fish m^{-3}, sex ratio of 1:1 female:male fish; feeding with trash fish and commercial bait fish (the pilchard *Sardinops neopilchardus*) and vitamin supplement	Australia (Rimmer, 1998)
	2 × 2 × 1.5 m or 10 × 5 × 1.5 m floating netcage	Stocking density is 100 kg m^{-2} of size 15 cm; feeding with floating pellets twice daily (warm months) or once daily (winter) to satiation, with FCR of 1.6–1.8:1; 8 months to 2 years culture period; production of 350–600 g to 2–3 kg per fish	Australia (Barlow *et al.*, 1995)
	3 × 3 × 2 m floating netcage	Stocking density is 15–25 fish m^{-3} of size 2–3 inches; feeding with trash fish once daily; 6–8 months of culture; production of 500–600 g per fish	Malaysia (Singh, 1991)
	2.5 × 2.5 × 1.5 m bamboo and polythene netting	Stocked with juveniles; feeding with trash fish at 5% of body weight twice daily, with FCR of 3.6:1; 4 months culture period; growth rate of 4 g per day	Philippines (Alcantara *et al.*, 1995)
	5 × 5 × 2 m, galvanized iron pipe and bamboo, concrete weight	Stocking density of 12–300 fish m^{-3}; feeding fresh trash fish twice daily, with FCR of 4–10:1; 12 months culture period; production of 1 kg per fish, 80–95% survival	Thailand (Tookwinas, 1990b)

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Grouper (*Epinephelus* spp.)	$3 \times 3 \times 2$ m, $4 \times 4 \times 2$ m or $5 \times 5 \times 2$ m cages, bamboo or wood, with plastic carboys and 2–5 cm mesh net	Stocking density is 20–30 fish m^{-3} measuring 9–10 cm, feeding with commercial feeds; 7–8 or 12–14 months of culture; production of 600–800 g per fish or 1.2–1.4 g per fish	Malaysia (Leong, 1998)
	$5 \times 5 \times 3$ m, wood and plastic drums	Stocking density is 44 fish m^{-3} of size 80–100 g; feeding with trash fish at 3–5% of body weight twice daily; 6–7 months of culture; production of 600 g per fish, 90% survival	Singapore (Anon., 1986)
	$2 \times 2 \times 2.5$ m or coco lumber, with empty 200 l plastic drums	Stocking density is 120 fish m^{-3} of size 13–15 cm (grow-out), 5–13 cm (transition), or 2–3 cm (nursery); feeding with dry pellets and minced trash fish (grow-out) or *Chlorella, Brachionus* and *Artemia* (nursery); FCR of 2.5–2.8:1 for dry pellets and 6.3:1 for trash fish; culture period of 1 month (nursery), 3 months (transition) or 8 months (grow-out); production of 500–800 g per fish	Philippines (Quinitio and Toledo, 1991)
	$5 \times 5 \times 2$ m or $3 \times 3 \times 3$ m, galvanized iron, wood, bamboo, empty plastic drums, carboys, concrete weight	Stocking density is 10–100 m^{-3} of size 7.5–10 cm; feeding with artificial feeds and live or frozen trash fish and crustaceans, feeds given at 10% body weight during the first 2 months, 5% thereafter until harvest; 8 months culture period; production of 580 g per fish, 80% survival	Thailand (Tookwinas, 1990a)
	$7 \times 8 \times 2$ m	Stocking density is 12–100 m^{-3} of size 12 cm or 20 g; feeds given at 10% of body weight on the first 2 months, then at 5% on the third month; 10–18 months culture period; production of 700–900 g per fish	Thailand (Tookwinas, 1990a)
Red snapper (*Lutjanus argentimaculatus*)	$3 \times 3 \times 2$ m, bamboo frame, polythene net and 200 l plastic drums	Stocking density is 90 fish m^{-3} of size 12 cm or 20 g; feeding with chopped carangids (*Seloroides* spp.), feed given twice daily to satiation; 10 months culture period; production of 890 g per fish, 83% survival	Thailand (Chaitanawisuti and Piyatiratitivorakul, 1994b)
	$1 \times 1 \times 1$ m (for juveniles), $2.5 \times 2.5 \times 4$ m (grow-out)	Stocking density is 6 fish m^{-3} of size 30 g or 100 fish m^{-3} of size 10 cm or 20 g; feeding with trash fish once or twice a day; 9–10 months culture period; production of 500–960 g per fish, 95% survival	Thailand (Doi and Singhagraiwan, 1993)

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Table 1.3. *Continued.*

Species	Cage/pen dimension	Culture specifics	Country/ references
Golden snapper (*Lutjanus jobni*)	5 × 5 × 3 m, wood and plastic drums	Stocking density is 44 fish m^{-3} of size 80–100 g; feeding with trash fish at 3–5% of body weight once or twice daily; 6–7 months of culture; production of 600 g per fish	Singapore (Anon., 1986)
Red seabream (*Pagrus major*)	Square, circle cages of size 4 × 4 × 3 m, 4 × 4 × 4 m, 5 × 5 × 5 m, 7 × 7 × 7 m or 20 × 20 × 5 m, cages may be synthetic, nylon-coated wire or bamboo with styrofoam as buoy	Stocking density is 100 fish m^{-3} (1-year-old fish); feeding with trash fish (anchovy and sardines) and moist pellets; 1–7 years culture period; production of 800 g to 1.4 kg per fish	Japan (Shepherd and Bromage, 1988; Fukumoto, 1989)
Yellowtail (*Seriola quinqueradiata*)	Square, circle net enclosures made of bamboo, wood, 50 mm steel pipes; also big open sea cages of sizes 1600–2400 m^2 with 1–6 cm mesh nets	Stocking density is 115–340 fish m^{-3} of size 200–500 g or 5 fish m^{-3} for size 1 kg; feeding with trash fish (anchovy, sardines, sand lance) and moist pellets; feed given 1–4× daily at 1–3% of body weight or at 4–8% of body weight for fish less than 100 g; FCR of about 5–9:1; 1–2 years culture period; production of 2.5–6 kg per fish	Japan (Shepherd and Bromage, 1988; Fukomoto, 1989)
	Square broodstock floating netcages 5 × 5 × 5 m	Stocking density is 25 fish of size 0.89 g per cage; feeding with moist pellets once every 2 days at 3% of body weight; 20 months culture period or until fish reach maturity and spawning (about 3.7 kg size)	Japan (Watanabe *et al.*, 1996)
Rabbitfish (*Siganus canalculatus*)	1 × 1 × 1.5 m cages housed in 6 × 6 m floating raft	Stocking density is 15 fish of size 48–68 g per cage; feeding with formulated diet, given 2× daily to satiation; 100 days culture period; production of 119 g per fish, 100% survival	Indonesia (Tacon *et al.*, 1990)
Carp	Bamboo cages 3 × 4 × 0.5 m	Stocking density is 1 kg m^{-3} (8–10 fish per kg); no feeding; 6 months of culture in sewage canal; production of 800 g per fish	Indonesia (Costa-Pierce and Effendi, 1988)
	2000 × 5000 m^3 pens made of casuarina poles and bamboo and with monofilament nylon fabric (30 cm mesh)	Stocking density is 4–5 million fish ha^{-1} (3-day-old hatchery-reared); feeding with a mixture of groundnut, oil cake and rice bran; with periodic dressing of organic (manure) and inorganic fertilizers; 3–4 months of culture	India (Basavaraja, 1994)
Giant gourami (*Osphronemus goramy*)	1.5 × 2 × 1 m cage made of bamboo and wood	Stocking density is 15 fish m^{-3} of size 14 g or 9 cm; feeding with yam and formulated diet, 3× daily at 5% of body weight; 18 weeks of culture; production of 180 g fish, 99% survival	Malaysia (Ang *et al.*, 1988)

FCR, food conversion ratio.

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have a rigid wooden or metal framework surrounded by a catwalk to facilitate operation and maintenance. The net bag is supported by a buoyant collar or a frame, and can be designed in various shapes and sizes.

Floats. Common flotation materials include metal, plastic drums, PVC pipes, Styrofoam, cement blocks, rubber tyres with polystyrene, bamboo and logs. Metal drums coated with tar or fibreglass are popular because they are cheap, but they corrode easily in seawater and have a life span ranging from 0.5 to 3 years (IDRC, 1979). Fibreglass drums or buoys are preferred by commercial fish farmers as they can last for many years in seawater although the initial cost is comparatively higher. Styrofoam blocks, covered with polythene sheets provide good buoyancy and may last for as long as 5 years under tropical conditions. Cement floats, though promising, require skill in construction and are presently not widely used. Bamboo and logs widely used in freshwater cages are also used in constructing brackish and marine cages, but they are easily attacked by fouling and boring organisms. Their life span in seawater is relatively short (1–2 years).

Catwalk. For cages designed with a catwalk, the framework from which a single net or a battery of nets is suspended is usually large to provide a stable and rigid platform for workers. Some marine cages do not have catwalks and the surface unit consists of floats from which each cage is suspended.

Netcage proper. The netcage (Fig. 1.1) is normally flexible, and made up of synthetic netting of nylon or polythene fibres reinforced at the corners with polythene ropes. The nets are kept stretched vertically with weights at the bottom of the cage or fastened by rope to the framework (Kennedy, 1975). The net can also be stretched with rectangular, round or square steel or PVC pipes depending on the shape of the cage. Rigid cages, made of metal netting (galvanized mesh, copper–nickel mesh or vinyl-coated mesh) mounted on rigid metal or wooden frameworks, are also commonly used in sea farming (Swingle, 1971; Powell, 1976; Milne, 1979). The relative merits of flexible and rigid cages are discussed by Hugenin and Ansuini (1978). The choice of flexible or rigid types is dependent on economics. Flexible cages are more widely used in developing countries because of lower cost.

Mesh size. This is determined by the size of the fish to be stocked. Small mesh size nets become clogged, especially in tropical areas, and easily damaged by floating objects and increased drag force and hence affect the morning load of the cages. As the fish grow, a larger mesh size should be used (Chua, 1979).

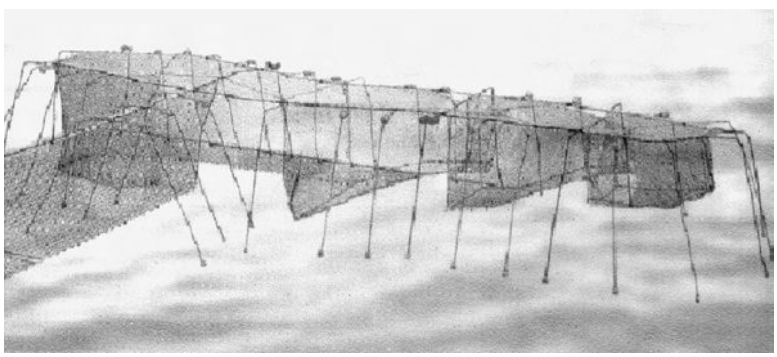


Fig. 1.1. Set net showing typical netcage structure (King Chou Fish Net Manufacturing Co., Ltd).

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Rotating and non-rotating floating cages

Rotating cages have been designed primarily to reduce the impact of fouling organisms and insects. The cage rotates from a central axis attached to a solid floating framework (Christensen, 1995). Non-rotating types are widely used and may be designed with narrow or wide collars. Rigid narrow collars made of non-wooden materials (glass fibre and steel) and buoys have been used in Western Europe.

Submersible cages

Submersible cages (Figs 1.2–1.4) have also been developed. This type of cage design has no collar, and the bag rests on a frame to maintain its shape. The position of submersible cages (with reference to the water column) can be adjusted by means of buoys. The cages are designed for deep waters, to overcome strong waves, and strong and rough seas. The disadvantage of submersible cages is that it is difficult to maintain the bag shape under water and not many species adapt to this condition (Beveridge, 1987). Submerged cages are also used in shallow water in Indonesia and Russia (Vass and Sachlan, 1957; Martyshev, 1983; Beveridge, 1987).

Shape and size of cages

In general, square or rectangular cages are preferred because they are easy to construct and maintain. They have been widely used for the culture of yellowtail (Harada, 1970; Fujiya, 1979), salmonids (Kennedy, 1975; Møller, 1979) and groupers (Chua, 1979). Cylindrical cages are also used for marine or brackish water species such as milkfish (Yu *et al.*, 1979) and rainbow trout (Tatum, 1973). Cylindrical cages are designed to rotate so as to delay biofouling (Caillouet, 1972). Other forms of cages such as orthogonal (Anon., 1976; Milne, 1979) and octagonal (Møller, 1979) have been used for salmonid culture in Scotland, Norway and France.

The size of cages ranges from less than 1 m^3 to 50,000 m^3. Freshwater cages for tilapia in the Philippines and Indonesia are

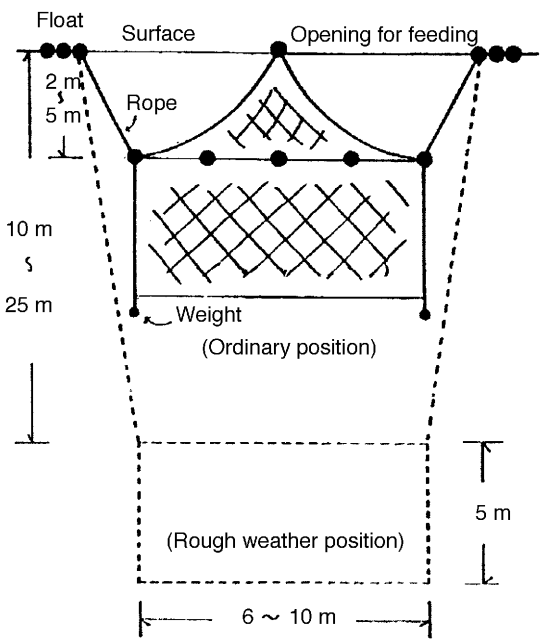


Fig. 1.2. Submersible cage for yellowtail (from Fujiya, 1979).

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usually very large (exceeding 100 m^3) and are installed in calm shallow lakes (Chua, 1982). Currently, dimensions for marine cages are usually smaller, even in relatively calm waters, because large nets are difficult to maintain due to biofouling problems in the marine environment. Although large size cages reduce construction costs, the optimum size must be within the physical capacity of the fish farmer(s) to manage and maintain. For tropical conditions where biofouling can be rapid and heavy, net cage sizes are between 20 and 50 m^3. Various shapes and sizes of traditional cage structures are shown in Figs 1.5 and 1.6. Figures 1.7–1.14 show other modifications in cage structures and set-ups that have been developed through the years.

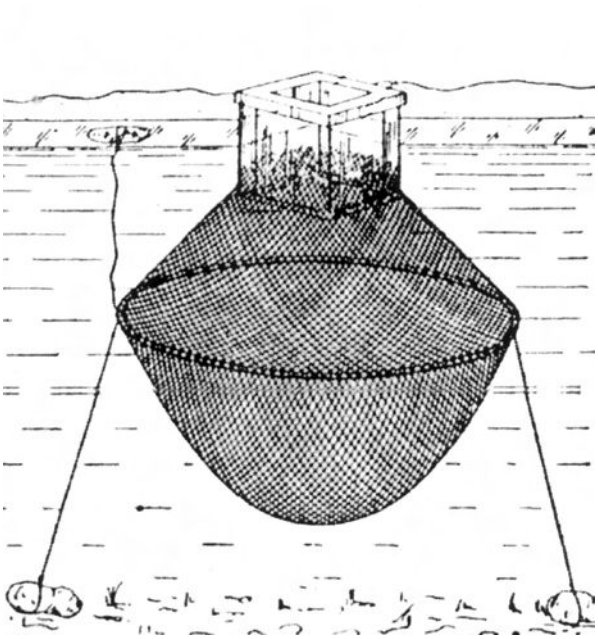


Fig. 1.3. Submerged trout cage, Russia (Martyshev, 1983).



Fig. 1.4. Submerged cage (King Chou Fish Net Manufacturing Co., Ltd).

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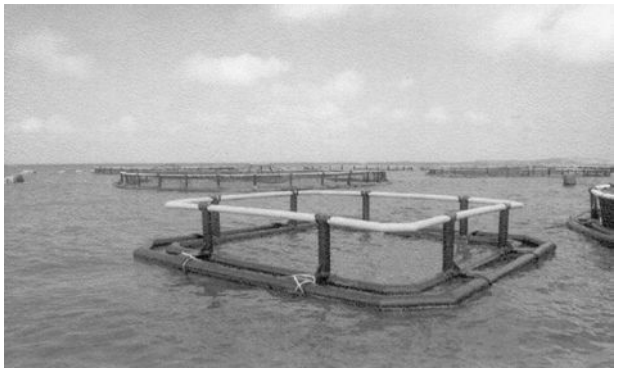


Fig. 1.5. Square cage (Fong Yu).

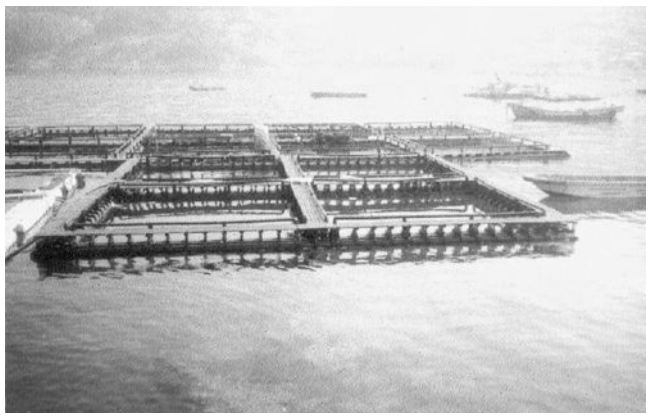


Fig. 1.6. Square cage (Water Diamond Equipment Co., Ltd).

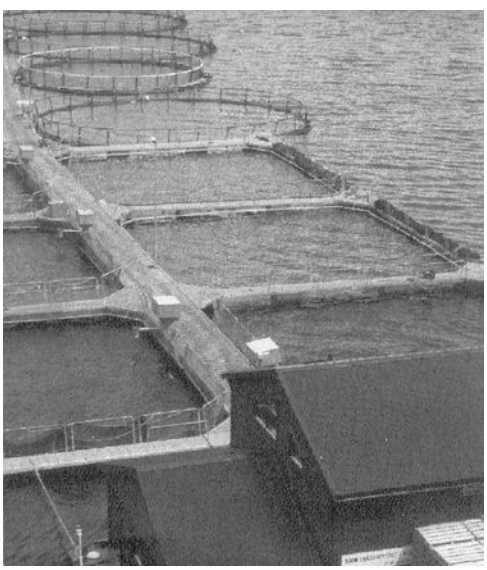


Fig. 1.7. Cage structures by EKSPORTFINANS ASA.

Cage Culture Operations

Stocking

The stocking density depends on the carrying capacity of the cages and the feeding habits of the cultured species. For fish such as bighead (*Aristichthys mobilis*) and silver carp (*Hypophthalmicthys molitrix*), which are low in the food chain, stocking will also depend on the primary and secondary productivity of the sites. When feeding is required, the rate of water exchange is an important consideration. Studies have shown that optimal stocking density varies with species and size of fish (Brown, 1946; Chua and Teng, 1979). As stocking density affects the growth rate of fish (Stickney *et al.*, 1972; Allen, 1974; Kilambi *et al.*, 1977), determination of the optimal stocking density is important in

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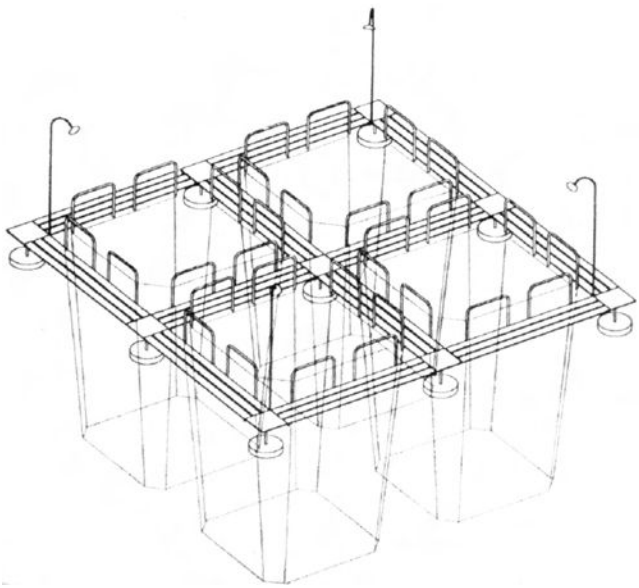


Fig. 1.8. Prototype of modular steel cage: 8 × 8 m module can be divided into four 4 × 4 m cages (Cruz, 1998).



Fig. 1.9. Main cage structure with security chain.

cage culture. High stocking density may create group effects resulting in high mortality, as in estuary grouper (*Epinephelus salmoides*) (Chua and Teng, 1978). Optimal stocking density ensures optimum yield for food conversion, and low disease prevalence with good survival rate.

Feeding

Feeding is a vital operational function and is affected by the interplay of many biological, climatic, environmental (water quality) and economic factors. Growth rate is affected by feeding intensity and feeding time (Chua, 1982). Each fish species varies in maximum food intake, feeding frequency, digestibility and conversion efficiency. These in turn affect the net yield, survival rates, size of fish and overall production of the cage. Trash fish is the main feed for yellowtail, grouper, bream, snapper and other carnivorous species cultured in marine cages (Anon., 1986; Quinitio and Toledo, 1991; Doi and Singhagraiwan, 1993; Leong, 1998). The shortage of trash fish as feed is a serious problem in Thailand, where the large-scale development of catfish farming has resulted in increased demand for trash fish (Chua,

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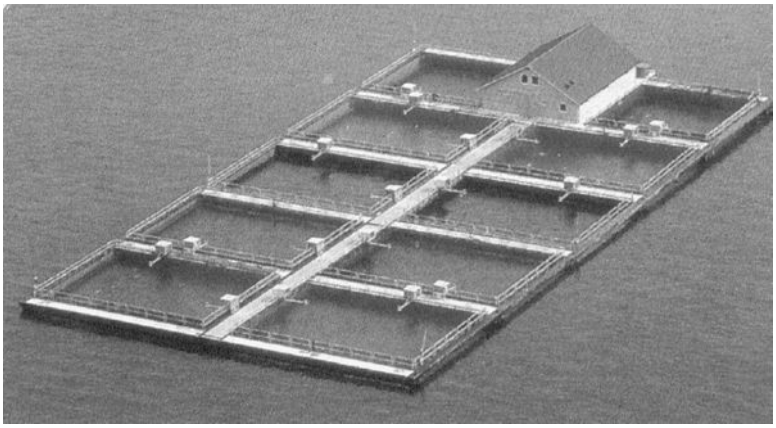


Fig. 1.10. Ocean catamaran fish farm.

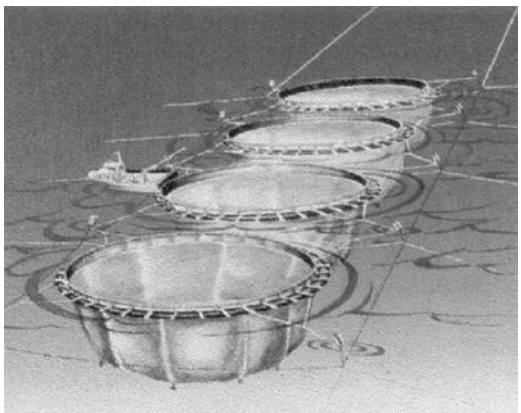


Fig. 1.11. Aqualine prefabricated mooring system.

1982; Shepherd and Bromage, 1988; Fukumoto, 1989). The shortage of trash fish and fishmeal is recognized as an increasing problem in aquaculture (Chua, 1982). This concern has been addressed by research institutions and the private sector, bringing forth developments and innovations in formulated diet research.

Farm management

Farm management must optimize production at minimum cost. Efficient management depends heavily on the competence and experience of the farm operator. The operator has to ensure that the cultured fish grow at the expected rate with respect to feeding rate and stocking density, minimize losses due to disease and predators, monitor environmental parameters and maintain efficiency of the technical facilities (Chua, 1982).

Maintenance work is also of vital importance. The entire structure (raft and netcages) must be routinely inspected. Necessary repairs and adjustments to anchor ropes and netcages should be carried out immediately.

Plastic drum floats have to be regularly painted with non-toxic antifouling paints. Scraping accumulated fouling organisms may be carried out by rotating the drums regularly. Monthly replacement of net

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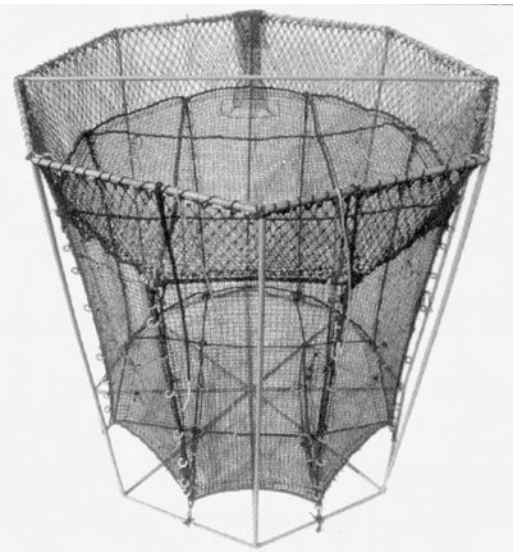


Fig. 1.12. Model cage structure (King Chou Fish Net Manufacturing Co., Ltd).

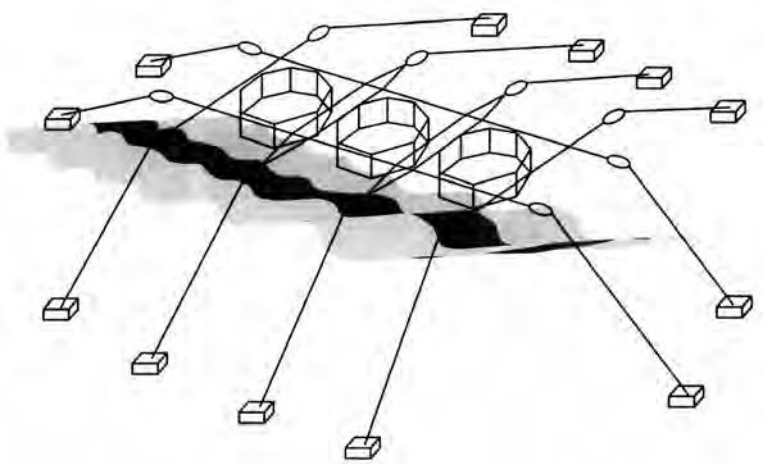


Fig. 1.13. Mooring system (Ching Fa flexible system).

structures should also be considered, as this ensures a good water exchange in the net, thereby washing away faeces and uneaten food.

Biofouling

Biofouling is an important and common problem in cage culture. The rate of biofouling in tropical waters is faster than in subtropical and temperate regions. The net walls, as well as the firm structures such as the floats, can be covered with biofouling organisms. Common fouling organisms include barnacles (*Balanus* spp.), green mussels (*Perna* spp.), oysters (*Crassostrea* spp.), algae and tunicates. Biofouling clogs the mesh of the net, reducing the rate of

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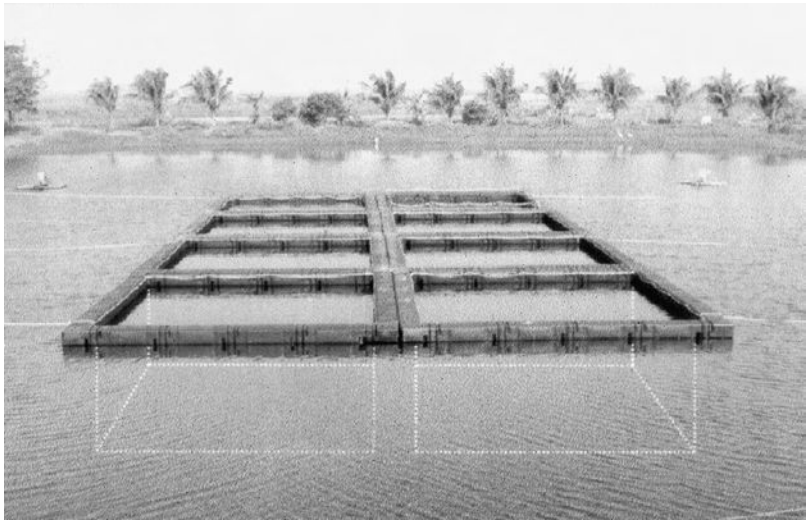


Fig. 1.14. Typical inland cage (Team Aqua Corporation).

water exchange, thereby causing stress to the fish due to low oxygen and accumulated wastes. The rate of fouling depends on the mesh size of the nets, temperature of the water and productivity of the site. Small mesh size nets (0.7–1.3 cm) can easily be fouled within 7–14 days, while larger mesh-size nets (2.5–3.8 cm) may be fouled in 1–2 months (Cheah and Chua, 1979). In tropical waters, netcages have to be replaced at least once a month. Boring organisms such as *Martesia* spp. frequently damage the wooden structures of the cage unit (Milne, 1970; Chua, 1979).

A fouled net will be heavier, thereby increasing drag, and this results in loss of nets and fish (Milne, 1970). Mechanical cleaning of fouled nets is still the most efficient and cheapest method of removing biofouling organisms, using hard bristle brushes above and below the waterline to dislodge weeds and accumulated debris (Beveridge, 1987). Antifouling paints and the use of copper–nickel alloy delay the establishment of biofoulers. Herbivorous fish such as rabbitfish (*Siganus* spp.), pearlspots (*Etroplus* spp.) and scat (*Scatophagus argus*) can be used to control biofoulers (Beveridge, 1987), but their application on a large scale needs to be assessed.

Routine checking of moorings will normally require a diver. In addition, regular lifting of the nets to check for predators and damage caused by poachers has to be carried out. Air- or sun-drying of nets at regular intervals will allow removal of debris and other materials that clog the mesh and block water exchange. Checking and cleaning of walkways should be routine procedures to avoid accumulation of slime, which makes walkways slippery. Boats used for monitoring should also be regularly serviced and equipped with back-up motor engines and emergency flares.

Regulation of fish growth and production

An important farm management strategy is the skilful manipulation of operational functions such as stocking density and feeding. The main purpose is to regulate fish growth to attain the desired size for the targeted market and season. For the estuary grouper (*Epinephelus tauvina*), at water temperatures between 29 and 30°C and feeding to satiation daily, a fingerling of 15–16 g will attain marketable size of 500 g in 7–8 months at stocking densities of 30–60 fish m^{-3}, and 11–12 months at stocking densities of 90–120 fish m^{-3} (Chua and Teng, 1979).

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Grading

Grading of fish is a management process to maintain uniform size of the product (Brown, 1957). Greater size uniformity improves feed conversion rates and overall growth, especially in meeting marketing and processing requirements (Hugenin and Ansuini, 1978). Frequency of grading depends on individual fish species and size requirement at harvest (Aquacop, 1975; Fujiya, 1979).

Disease

Disease outbreaks can occur and are problems in cage culture (see later chapters). Increased production, pressures on faster growth, high density and structure efficiency can create conditions conducive to outbreaks of infectious diseases and an increase in prevalence of parasites. Infectious diseases in fish culture are not only accentuated by waste pollution, but exacerbated by crowding, handling, temperature and biofouling. The most common fish disease in cages is vibriosis caused by *Vibrio* spp. Furthermore, abrasions cause fin and skin damage to cultured stocks (Moring, 1982). Occurrence of infection/disease may be minimized by selecting good sites, proper mooring and observance of optimal stocking densities and careful handling of stocks (Boydstun and Hopelain, 1977). Adequate spacing between cages and farms is also an essential management tool to reduce the spread of disease (Wong, 1995).

Monitoring of water quality

Constant monitoring of water quality is an essential routine. The farmer should be sensitive to and aware of threats such as pollutants caused by industrial discharge or indicators of the occurrence of algae blooms such as red tides. The routine monitoring of water quality is a useful practice.

Predation

Information on the actual extent of problems related to predation is scanty (Beveridge, 1987). Mills' (1979) survey in Scotland recorded a nearly 90% predation caused by piscivorous birds in fish farms. Some, like cormorants (*Phalacracorax carbo*) and herons (*Ardea cinerea*), are seasonal visitors. Other types reported include squid, predatory fishes, turtles, sea snakes and mammals. Salmon and trout cages in the USA reportedly have been attacked by spiny dogfish (*Squalus acanthias*), monks and otters (Mahnken, 1975; Lindberg, 1979), while those in Scotland and England were attacked by seals, otters, cormorants, gannets and gulls (Mills, 1979). Salmon farms in Canada experienced severe losses due to predation by gulls and herons (Henderson, 1980) and losses in seabream farms in Israel were attributed to Moray eel and squid predation (Pitt *et al.*, 1977). Chua and Teng (1980) reported predation in grouper cages by otters (*Lutra sumatrana*), gulls and puffer fish (*Sphaeroides* spp.). Preventive measures such as extra outer net enclosures, guarddogs and night guards have proven useful.

Cleaning

Cleaning of the seabed by dredging is undertaken once a month in some cage farms in Korea (Kim, 2000). However, this is a costly operation and is usually not undertaken by most cage farmers in other countries.

Government role

Cage farms should be licensed and farm sizes limited. For example, a cage farm in Korea is limited to 0.5 and 1 ha for each culture bed, with 300 m waterway between cages with the cage area exceeding 20% of the licensed area (Kim, 1995). In May 1997, the central government in Korea ruled that all inland water cage farms be dismantled upon expiration of the 10 year licence agreement (Kim, 2000). Size restrictions have also been implemented in most parts of Scandinavia (Beveridge, 1987). In Finland, small units with annual production of less than 3 t need to notify the authorities, while those producing over 40 t require

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government permits (Beveridge, 1987). Before 1983, Norwegian salmon farms observed size restrictions (at a volume of 8000 m^3), and by 1985, 150 new licenses were issued. Certain countries have allocated specific areas for cage culture development. In Hong Kong and Singapore, for instance, legislations apply to marine cage farms, while in the Philippines, laws were passed in the early 1980s to control cage and pen farming developments in overexploited Laguna Lake (Beveridge, 1984) and later in Lingayen Gulf (Marte, 2000). In Nepal, management measures call for a balance between cage culture and overall management of lakes, particularly with respect to carrying capacity. Regular environmental monitoring is the function of the lake authority (Pradhan and Pantha, 1995).

It is also the duty of the government to protect wild fish populations from any ecological threat (i.e. escape of exotic species or the introduction of fish disease organisms). Suitable legal and institutional frameworks will have to be established so as not to deter efforts to establish fish farms and also prejudice or limit the chances of obtaining financial assistance.

Special Considerations

Site selection

The choice of a site contributes significantly to the success of a cage farm. A number of factors directly or indirectly affect the viability of cage culture.

The quality of water in the farm culture site is one important factor that merits investigation together with other considerations indicated in Table 1.4. This information is needed to plan the scale of the venture, design and size of cages, assessment of carrying capacity for stocking rate, feeding strategy and other operational and management purposes (Chua, 1982).

Lakes, bays, lagoons, straits and inland seas are ideal sites for cage culture provided these sites are protected from strong winds and rough weather and have sufficient water movements. Vertical stratification in deep water areas may pose problems with respect to wide fluctuations in oxygen and pH levels. Before the start of the venture it is also important to have baseline data on the seasonal variation of salinity, temperature, water current, turbidity, dissolved oxygen and primary productivity. Whenever possible, suitable areas should also be free from potential predators.

Selection of species for culture

Knowledge of the biology of each fish species is crucial in optimizing production from cages. The selection of fish should be based on a number of biological criteria (omnivore or carnivore, hardiness, fast-growing, efficient food conversion ability, availability of eggs and juveniles, and

Table 1.4. Site selection criteria for cage farming.

Factor	Condition to be satisfied
Water current	Site should be sheltered from strong waves and away from strong currents
Depth	Nets should be kept at least 2 m off the ground to facilitate water flow
Dissolved oxygen levels	Should remain generally high and stable the whole year round (7–8 ppm)
Salinity	Salinity levels should not vary significantly, with little stratification
Temperature	Should be nearly constant throughout the year
Pollution	Site should be situated far from areas with high loads of sewage discharge and/or industrial effluents

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disease resistance); economic marketability and demand, as well as the prevailing conditions of the culture site, influence the species selected for aquaculture. Environmental factors such as water current, salinity, temperature, dissolved oxygen and pH affect the target species, and hence are also important considerations.

Pollution and impact

Cage culture systems contribute wastes (solid and soluble) to the aquatic environment from uneaten food, dust, fish faeces, scales, mucus and other debris. These may accumulate beneath the cages or downstream, and result in a reduction in dissolved oxygen, and a build-up of wastes in the water (Beveridge *et al.*, 1982; Penczak *et al.*, 1982; Beveridge, 1984; Phillips *et al.*, 1985; Phillips and Beveridge, 1986; Heping, 1995; Yusuf *et al.*, 1995). The accumulation of uneaten food and waste leads to the formation of hydrogen sulphide, and high levels may cause fish mortality. Improper use of antibiotics and their release into the aquatic environment may result in the development of antibiotic-resistant bacteria.

Cage culture can introduce and/or disrupt disease and parasite transmission, cause changes to the aquatic flora and fauna, and may even alter the behaviour and distribution of local fish communities (Loyacano and Smith, 1976; McGuigan and Sommerville, 1985; Phillips *et al.*, 1985). The escape of non-indigenous species from culture cages may alter the species composition of indigenous fish, especially in enclosed aquatic environments, as reported in a freshwater lake in Nepal (Swar and Gurung, 1988).

Socio-economic aspects

Cage farming is not as costly as raceways or enclosure methods (Collins and Delmendo, 1979). The cost of floats, platform, mooring facilities, a motor boat and a floating hut is usually less than 10% of the total operational cost. The life span of nets is estimated at 4 years. Takashima and Arimoto (2000) reported an average life span of 3 years based on experience in Japan. Cage farming is the most economically feasible method of intensive farming of salmonids, yellowtail and grouper in marine waters (Beveridge, 1987). The main operating expenses are feed (30–50%) and ova (30–35%), which make up 60–85% of the total operational cost (Arroyo, 1973; Collins and Delmendo, 1979; Chua and Teng, 1980). Other expenses include labour, fuel, repair and maintenance.

Cage culture provides alternative employment to the inshore fishermen in many countries who are faced with significant declines in the natural fish stocks, for example due to overfishing. It also creates subsidiary employment in hatchery, fry gathering, net making and mending, cage construction and feed preparation.

Problems and Major Constraints

Usual problems reported include mooring systems, which may cause additional high loads on the cage structure, pollution from excess feed, and limited waste dispersion due to insufficient or slow water movement around cages. Furthermore, poaching and vandalism, severe damage caused by typhoons and long winter monsoon periods, and disease outbreaks are constraints for aquaculture.

Other constraints include the supply of ova, expensive feed, lack of adequate feed, congestion on existing farm sites and lack of new sites for expansion (limited resource base).

Conclusion

Fish culture using cages has proven to be technically and commercially viable in most countries. Future development in the industry should be geared towards the use of cheaper and more 'environment friendly' floating facilities, higher quality netting

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materials, ensuring a supply of quality juvenile fish, and suitable and affordable feed. The establishment of cost-effective means to reduce fouling, control degradation of water quality in cage sites and the implementation of proper management of the aquatic environment are necessary if the industry is to expand. Rosenthal (1994) indicated the importance of dealing with environmental issues, since in many instances ecological changes have become risk factors to the industry itself. Cultured species respond quickly to imposed stress as expressed by reduced growth, increased incidence of disease and occurrence of mortality. To reduce environmental degradation and unpredictable environmental catastrophe, it will be necessary to shift from focusing solely on increasing yields to paying attention to the importance of inputs or the generation of unwanted wastes and pollutants. More attention will also be required on managing the input aspects of production systems, including resource utilization and the output aspects of production, the environmental damage (Folke and Kautsky, 1989).

The main operational challenge is the integration of cage culture into the aquatic environment. While taking full advantage of the natural environment, cage farmers should make every effort to reduce the possible negative impacts arising from the operation. Governmental agencies can help in this aspect by including cage culture in their integrated environmental management plan to ensure a holistic planning and management approach pertaining to the use of natural resources (Chua, 1997). There should also be a concerted effort to support and encourage entrepreneurs into this industry. The refinement of current review and assessment procedures for approval of cage culture sites should also be reviewed. Challenges ahead will include: (i) reduction of production costs and improving global distribution; (ii) adjustment of production scale to meet market needs; and (iii) new markets that will probably arise as the price of farmed fish becomes more competitive.

Another operational challenge is to reduce loss due to disease. Research scientists can help by developing updated information on diagnosis, treatment and prevention protocols, as well as breeding fish that are more disease-resistant and/or respond well to vaccination. Improvements in production, particularly for a well-researched commodity such as the tilapias, are expected to allow increases in the production of juvenile stock for cage farming. Research and development in various fields/areas will lead to stock improvement, for example through genetic selection and advances in biotechnology.

Sustainability of this industry will also rely on the development of technologies, such as typhoon-proof cage systems; better engineering design to improve automatic feeding systems, which can reduce labour costs and operational risks and increase productivity; and vaccines, which can reduce the impact of infectious diseases.

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2 Overview of Cage Culture

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Introduction

Aquaculture rearing systems consist of two principal types, land-based and water-based. The former includes ponds, raceways, tanks and silos, and the latter comprises enclosures, pens and cages. The prime function of any rearing system is to provide an environment in which stock can thrive – adequate space, good water quality, etc. – while minimizing the impacts of predation, stress, disease, theft, vandalism and losses through escape. Rearing systems should also readily facilitate routine management operations such as grading and harvesting. These must all be achieved as cost-effectively as possible.

Although the terms 'pen' and 'cage' are used synonymously in North America, for the sake of clarity the term 'pen' will be confined here to systems in which the bottom of the structure is formed by the seabed. This type of system is used in a number of South-east Asian countries. Cages, by contrast, are enclosed on the bottom as well as the sides by wooden, mesh or net screens (Fig. 2.1).

The present chapter provides an overview of cage aquaculture. The history and development of cage technology and the importance of cages in the context of present-day global aquaculture production are discussed in Chapter 1. This chapter is primarily concerned with cage design, site selection and culture practices. Cage culture is also examined from a disease and environment perspective, and comparisons are made with alternative aquaculture technologies.

Background

Brief historical perspective

Although aquaculture has been practised for several thousand years, cage aquaculture is a relatively recent phenomenon (Beveridge and Little, 2002). The earliest written account dates from China in 1243 (Hu, 1994). In the Great Lake region of Cambodia and in Java, Indonesia, wooden cages have

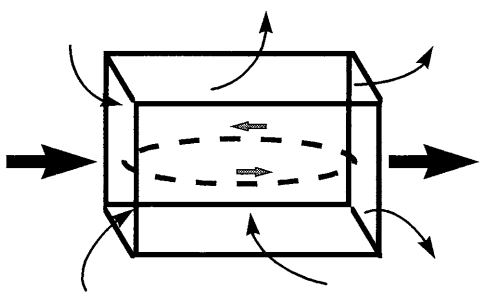


Fig. 2.1. Diagram illustrating the operating principles of cages. Arrows indicate water exchange patterns and relative importance of flow directions. Note internal water circulation is included.

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been used since the turn of the century (Vass and Sachlan, 1957; Hickling, 1962; Ling, 1977; Costa-Pierce and Effendi, 1988). Although traditional methods of cage culture are still practised in parts of Indonesia and Indo-China (Fig. 2.2), the technology has had little influence on developments elsewhere. Modern cages utilize synthetic mesh or netting materials and often have collars fabricated from synthetic polymers and metal. Japan has undoubtedly been a key influence here. Experimentation with cage mariculture began in the early 1950s and commercial cage culture of yellowtail *Seriola quinqueradiata* was well-established by the end of the decade (Milne, 1974). In Norway, cage culture of Atlantic salmon (*Salmo salar*) began in the early 1960s while, in Scotland, the White Fish Authority commenced caged salmon rearing trials around 1965.

In short, cage fish farming is a recent phenomenon. The principal impetus for its development has been the expansion of marine finfish culture in western Europe and Japan. In Asia, rapid population growth and ambitious reservoir construction programmes have also been important factors (see Beveridge, 1996; Beveridge and Little, 2002, for review).

Culture methods

Cage aquaculture may be described as extensive, semi-intensive and intensive on the basis of feed inputs. In extensive culture, fish rely solely on natural foods such as plankton and seston (suspended particulate matter), carried in the drift. Semi-intensive culture involves the use of low protein (< 10%) feedstuffs, usually compounded from locally available plants or agricultural by-products, to supplement the intake of natural food. In intensive culture operations, fish rely on an external supply of high protein (> 20%), nutritionally complete food, usually based on fishmeal (see Coche, 1982).

Extensive cage culture is largely restricted to highly productive tropical lakes, reservoirs and sewage-fed streams and rivers. Although practised on a significant scale only in the Philippines, China and Indonesia (Vass and Sachlan, 1957; Aquino and Nielsen, 1983; Gaigher and Krause, 1983; Costa-Pierce, 1993; Li, 1994; Beveridge and Stewart, 1998), where, at most, it accounts for the production of a few thousand tonnes of carp and tilapia, this method of aquaculture is of considerable interest. Caged bighead carp (*Aristichthys*

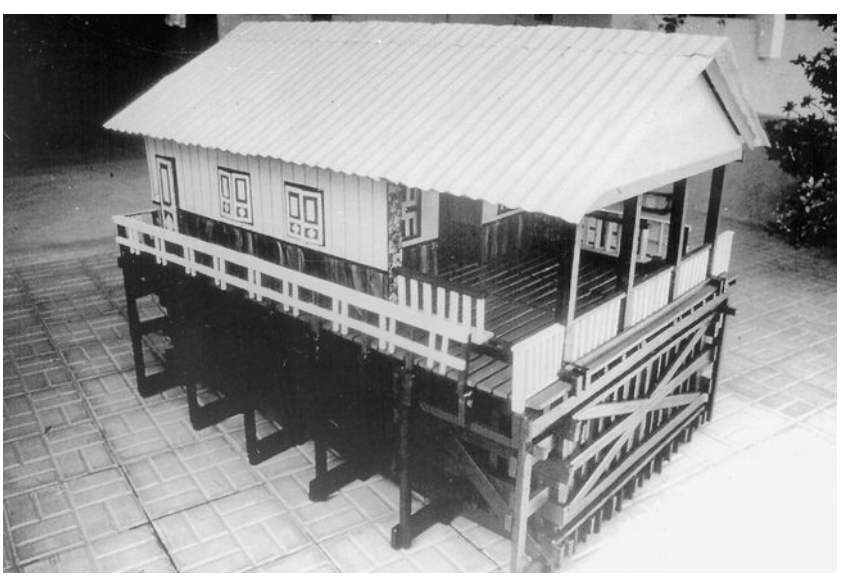


Fig. 2.2. Model of a contemporary traditional floating fish cage-cum-house, found in the upper reaches of the Mekong River, Vietnam (courtesy of M. Phillips).

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nobilis) have been used in Singapore to help control eutrophication in municipal water supplies (Yang, 1982) and there is growing interest in employing a similar approach in Brazil (Starling *et al.*, 1998). There have also been a number of studies into the technical and economic viability of rearing juvenile planktivorous stages of salmonids, coregonids and pike (*Esox lucius*) in cages fitted with lights to attract zooplankton (Holm and Moller, 1984; Jäger, 1986; Mamcarz and Nowak, 1987) (Fig. 2.3).

Semi-intensive cage culture is largely confined to tropical fresh waters. Here it is the most common method of rearing fish in cages, accounting for an estimated 0.8 Mt of farmed fish production per annum. Species that feed low in the food chain, such as tilapia (*Oreochromis niloticus, Oreochromis mossambicus, Oreochromis aureus*), and bighead, silver (*Hypophthalmichthys molitrix*) and common carp, are widely grown in this way and fed on a variety of materials including rice bran, wheat middlings, brewery and domestic wastes (Lovell, 1989; Tacon, 1990; New *et al.*, 1993). Supplementary feeding practices are largely determined by availability and feeds tend to be given on an *ad hoc* basis rather than according to predetermined feeding regimes.

Intensive cage culture is primarily associated with high-value carnivorous and omnivorous species, e.g. salmonids and channel catfish (*Ictalurus punctatus*) in fresh water and Atlantic salmon, yellowtail and seabass (*Lates calcarifer*, *Dicentrarchus labrax*) in the marine environment. Intensive rearing of caged tilapia and carp – omnivores, which typically have a low market value – is largely limited to parts of the world where such fish fetch high market prices (e.g. Europe, North America, Singapore, Taiwan) or where there are limitations in terms of growing season (Beveridge and McAndrew, 2000). However, high-density (< 200 kg m^{-3}), low-volume ($1\ m^3$), intensively managed tilapia cages are beginning to be promoted as a highly profitable business appropriate for small-scale farmers (Schmittou, 1993).

Cage Design

Introduction

Good, practical cage designs should resolve engineering and cost considerations with the requirements of the species being farmed and the farmer who must operate the system. However, designs have largely developed empirically and reflect more the capital costs involved and the limitations

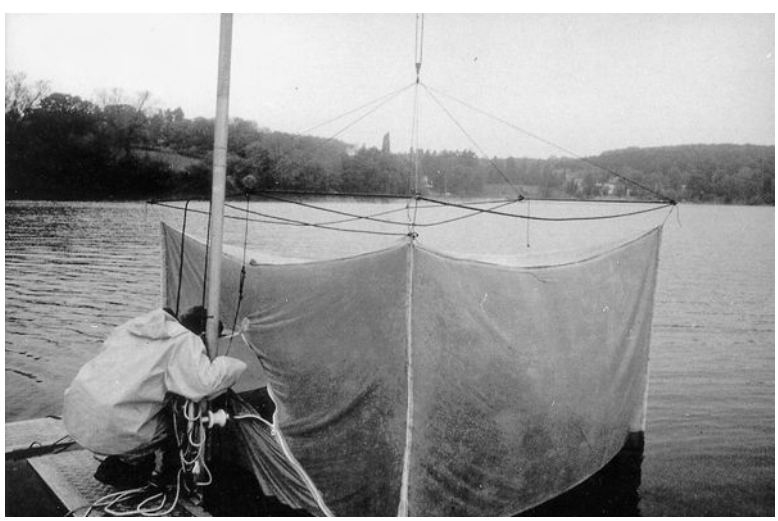


Fig. 2.3. Illuminated, submersible cage, being used to culture juvenile pike in a lake in northern Germany.

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of the materials selected rather than the requirements of the fish, site conditions or the convenience or safety of farm staff.

Shape and size

Although a circular structure is the most efficient way to enclose an area of water, circular structures are not always the most economic to build (Beveridge, 1996). Nevertheless, observations made of shoaling, actively swimming species such as salmonids and milkfish (Sutterlin *et al.*, 1979; Yu *et al.*, 1979; Srivastava *et al.*, 1991; Juell and Westerberg, 1993) suggest that structures that are circular or near circular (i.e. polygonal or square) in plan area are best in terms of space utilization.

Within the limits of the materials and construction methods used, costs per unit volume decrease with increasing cage size. From the perspective of the farmer, however, very large cages may limit stocking, grading and harvesting options, while tasks such as net changing and disease treatment also become increasingly difficult as cages increase in size. There has been no systematic study of the relationship between the size of aquaculture systems and fish growth and production. Some general principles seem intuitively obvious: certain species by virtue of their lifestyle or size may require greater living space than others. Experience has shown that larger shoaling and pelagic fish, such as salmon, tuna and yellowtail, grow faster in cages with larger surface areas.

Until recently, it had been thought that cage depths of 3–10 m would be acceptable for most species under most circumstances. However, commercial trials in Norway showed that Atlantic salmon utilize cage depths of up to 35 m (Huse and Holm, 1993). Diurnal and seasonal changes in distribution of caged rainbow trout and Atlantic salmon with depth, corresponding to changes in light levels, have been reported (Phillips, 1985; Huse and Holm, 1993; Juell and Westerberg, 1993). High light levels are stressful – even harmful – to fish, exposure to high levels of ultraviolet (UV) causing sunburn-like lesions (Bullock, 1988). Huse and Holm (1993) concluded that because of poor attenuation of light in water, cages of 20 m depth may not be sufficient for Atlantic salmon in summer in northern Europe. Shading can be used to modify behaviour and depth preferences (Huse *et al.*, 1990).

Cage types

There are four principal types: fixed, floating, submersible and submerged (Fig. 2.4). Fixed cages consist of a net bag supported by posts driven into the bottom of a lake or river (Fig. 2.4a). They are comparatively inexpensive, simple to build and are widely used in inland waters in the tropics. However, they are limited in size and shape and use is restricted to sheltered shallow sites with suitable substrates.

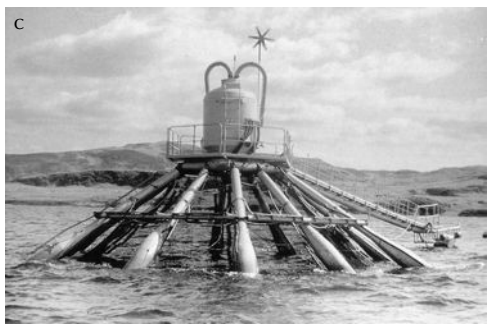
The bag of floating cages is supported by a buoyant collar or, in some instances, frame (Fig. 2.4b). This type of cage is by far the most widely used and can be designed in an enormous variety of shapes and sizes to suit the purposes of the farmer and the limitations of the site. A key design feature is the collar. Most floating cages have collars, 1–3 m wide, that additionally serve as work platforms and that facilitate routine farm operations. Where rigid, narrow collars, constructed from glass fibre or steel section and buoys, are employed, feeding and net changing must be performed from a boat or pontoon. Some offshore floating cage designs incorporate a flexible collar that deforms under wave action, thereby dissipating forces.

The advantage of submersible cages over other designs is that the position in the water column can be changed to exploit environmental conditions (Fig. 2.4c). The cages operate on the sea surface during calm weather and are submerged during bad weather or during an algal bloom or pollution incident. Some designs rely on the bag being suspended from buoys or a frame that floats on the water surface while others have variable buoyancy (see Beveridge, 1996; Huguenin, 1997, for reviews).

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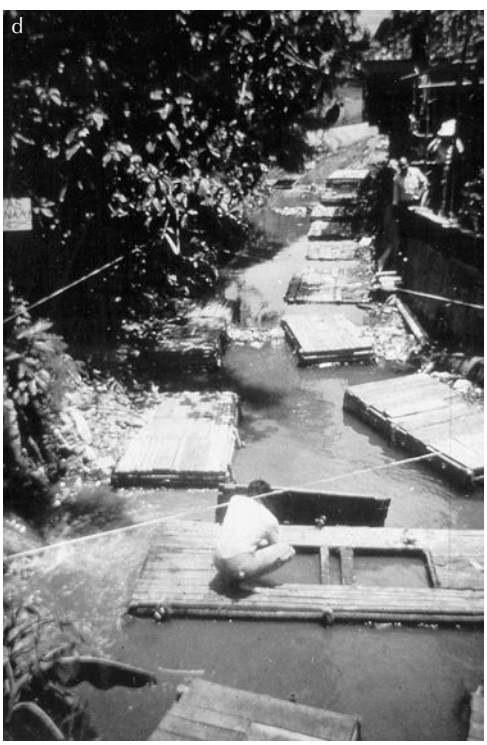


Fig. 2.4. Four principal types of cage. (a) A fixed tilapia cage from Laguna Lake, The Philippines. The net back bag is supported by the bamboo posts driven into the substrate and the structure strengthened by use of horizontal bamboos lashed to the frame. (b) Floating seabass cages, Greece. Here, walkways link the steel-collared structures from which the net bags are suspended. (c) Submersible FarmOcean© cage, used for Atlantic salmon culture, Scotland. The cage is shown in normal, semi-submerged operating conditions. The structure may be raised or lowered in the water column by changing air pressure in the buoyancy tanks, some of which can be seen. (d) Submerged (i.e. moored to the bottom) wooden cages, Bandung, Java, Indonesia. The caged common carp feed on wastes carried down the canal and some supplemental food.

The principal advantage of submerged cage designs is that being installed below the surface they are less susceptible to adverse weather or environmental conditions. Traditional submerged cages that are little more than wooden boxes are anchored to the substrate of flowing waters in some parts of Indonesia (Fig. 2.4d) and net mesh

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bag designs are used in lakes and reservoirs in the former USSR and China (Vass and Sachlan, 1957; Martyshev, 1983; Costa-Pierce and Effendi, 1988; Li, 1994). However, few of the designs periodically heralded in the trade press progress beyond the drawing-board. Moreover, it remains a moot point whether all species readily adapt to being cultured in submersible or submerged cages.

Site Selection

Site selection, through its influence on capital outlay and operational costs, production and mortality, is a key determinant of the economic viability of a cage farming operation. While farm managers have little control over economic factors such as fish and feed prices, those managing cage farms are further disadvantaged in having little control over environmental variables, there being little that can be done if sites prove too exposed and water exchange is poor or if water quality deteriorates.

There are three categories of site selection criteria that must be addressed (Table 2.1). The first is primarily concerned with the physico-chemical conditions that dictate whether a species can thrive in an environment (temperature, salinity, oxygen, currents, pollution, algal blooms, exchange), the second comprises those factors that must be considered in order to site a cage system successfully (weather, shelter, depth, substrate) while the third must be given careful consideration if a profitable business is to be established (legal aspects, access, proximity to hatcheries or other components of the company, planning regulations, security, economic, social and market considerations). However, there have been few attempts to define the relationships between site factors, development, capital and operating costs in aquaculture (Muir and Kapetsky, 1988). Is ideal depth more important than proximity to a shore base, for example, and what effects might currents of 0.3 m s^{-1} have on production costs compared with currents of 0.5 m s^{-1}? While checklists of parameters and scoring systems, such as those of Black and Truscott (1994) and Huguenin (1997), can help to guide prospective cage farmers, the relationships between site characteristics and profitability remain poorly understood.

One tool that has been used to explore these relationships at national, regional and local scales is Geographical Information Systems (GIS) (Meaden and Kapetsky, 1991; Beveridge *et al.*, 1994a; Kapetsky and Nath, 1997). Ross *et al.* (1993) used a GIS-based approach to site selection for salmon cages within a 0.2 km^2 bay on the west coast of Scotland. Detailed maps, derived from field work, were produced for bathymetry, wave height, water quality (salinity variation) and currents, and a scoring system was employed to establish the optimum location for the cages (Fig. 2.5).

Ideally, site selection should be through thorough site reconnaissance and survey. In practice, however, as Huguenin (1997) points out, the site selection process is limited because options are few, the resources too limiting or the site is already predetermined. While offshore sites are less congested and arguably provide a better environment for caged stock in terms of water quality and current regimes, the technology required is much more complex and expensive to build and service. At present, the economic viability of the marine fish culture industries in Europe, North America and Asia is such that any large-scale move to farming offshore is unlikely.

Table 2.1. Criteria for cage site selection. Note that several criteria are common to more than one category (see text for explanation). (Modified from Beveridge, 1996.)

Category 1	Category 2	Category 3
Temperature	Depth	Planning
Salinity	Shelter	Access
Suspended solids	Substrate	Security
Algal blooms	Currents	Seed and feed
Pathogens	Fouling	supplies
Water exchange		Food quality and
Currents		safety
Fouling		Markets

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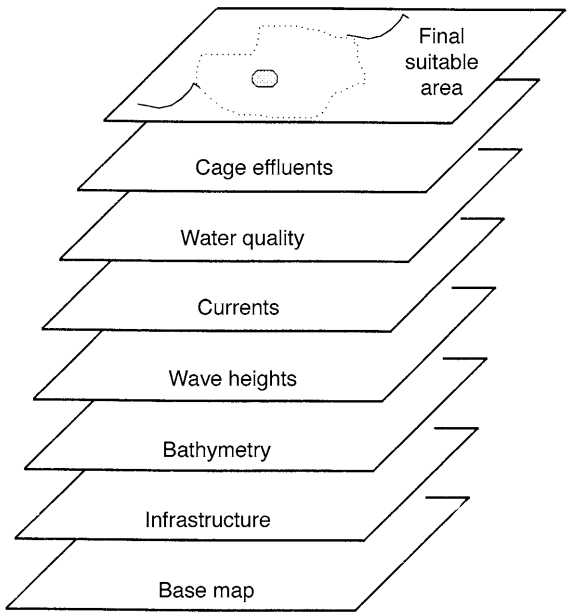


Fig. 2.5. Layers used in GIS site assessment for coastal cage aquaculture, showing the general approach that could be used for salmon cages. (Modified from Ross *et al.*, 1993; Beveridge *et al.*, 1994a.)

Cage Culture Practices

Introduction

Cage farm management strategies are directed towards minimizing stock losses and promoting good growth while controlling costs. In practice this means:

- stocking at densities appropriate to the site, species and method of rearing;
- feeding in the most cost-effective manner;
- ensuring the best possible water quality within cages;
- maintaining cages, moorings, anchors and ancillary gear; and
- regular monitoring of stocks for signs of disease, removal of mortalities and treatment of infected stock.

Managers are also responsible for ensuring that staff working conditions are safe and comfortable. It follows that choice of management and staff is crucial to any successful cage fish farming venture.

The production cycle

Stocking

Although fry and fingerlings for fishes such as milkfish or tilapia can be produced in cages, supplies are usually derived either from land-based hatcheries or from the wild. The transport and transfer of fish to cages is often long, tortuous and, inevitably, stressful, involving a number of transfer operations. Transport of fry in the holds of boats that allow sea water circulation or are fitted with aeration/oxygenation systems ('well-boats') is common in coastal areas. An alternative is to use towable cages, often fabricated on-farm by building a makeshift bow on to a small floating cage. Fish should be left for several hours (12–24 h is recommended for salmon) to recover before feeding.

Although stocking densities should be determined by species requirements and operational considerations, the influence of stocking densities on growth and production has been determined empirically. Less active, warmwater fishes such as carp and

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tilapia can be stocked at densities as high as 30–60 kg m^{-3} without any apparent adverse effects. However, while similar stocking densities were initially used for caged yellowtail and salmonids, it has become increasingly apparent that stocking at 10–20 kg m^{-3} is more appropriate. Small-scale farmers producing crops of tilapia or carp in a few months tend to stock fry in numbers that result in desired densities at harvest (e.g. stocking tilapia fry at a rate of 600 fish m^{-3} would result in 60 kg m^{-3} of 100 g fish at harvest). For more intensive operations, especially where stocking and harvesting are carried on throughout much of the year, utilization of cage space is optimized by stocking high numbers of fish and grading and redistributing fish among cages as they grow (see Schmittou, 1993; Duarte *et al.*, 1994; Boghen, 1995; Beveridge, 1996; Lazur, 1996).

Feeding

The range of feeds used in semi-intensive cage culture is enormous, being largely based on the availability of materials, and is prepared on-site. On-farm feeds predominate in freshwaters, but are also widely used in grouper, seabass and other marine finfish culture operations in Asia (Tacon, 1990; New *et al.*, 1993). Pelleted, dry complete diets have been used for years in the intensive channel catfish and salmonid industries. These are gradually replacing the moist pellets and trash fish used on intensive seabass and yellowtail farms (Beveridge *et al.*, 1997b). Although cheap to produce and highly palatable, these diets are variable in quality, expensive to transport and store and have a high wastage rate.

Fish should not be fed during extremes of cold and heat or during rough weather. Most cage farms that use supplementary feeds, trash fish or moist feeds, feed by hand as it is cheaper and easier, at least in small-scale operations. Hand-feeding results in an even distribution of food, and through assessment of feeding response, appetite and health status of the stock can be assessed. However, it is labour-intensive and is not always appropriate at large, intensive farms.

Mechanical feeders predominate at intensive cage farms for reasons of economy of labour. Demand feeders consist of a feed hopper fitted with a plate connected to a pendulum rod, which, when touched by a fish, releases small quantities of food. They are cheap and fish are able to determine for themselves when to eat. Claims of less size variability among stock, better food conversion ratios (FCR; ratio of feed used:fish harvested), faster growth, higher production, improved water quality and fewer disease problems have often not been rigorously tested or applied to particular species, circumstances and methods of culture (see Beveridge, 1996, for review). Automatic feeders that rely on a timing device to deliver a measured ration at fixed intervals preset by the farmer are well-proven and prevail at intensive cage fish farms today (Fig. 2.6).

Two other systems are worthy of note. Compressed air feeders, operated from a boat, are often used to distribute feed at large, offshore cages. While this appears to ensure good feed distribution over a large surface area and is less labour-intensive than hand-feeding, evidence suggests that a great deal of feed is wasted, resulting in higher FCR values, feed costs and a deterioration in environmental conditions. So-called 'smart' feeding systems that employ hydro-acoustic or other detectors to monitor uneaten food and adjust feeding rate accordingly are increasingly being employed in the salmon, yellowtail and barramundi industries because of improved yields and reduced feeding and environmental costs (Blyth *et al.*, 1993, 1997; Juell *et al.*, 1993; Foster *et al.*, 1995; Beveridge and Kadri, 2000).

Grading and stock management

Samples of fish should be taken at regular intervals and weighed in order to determine stocking, feeding policies and when to harvest. Current sample weighing methods are stressful and inaccurate, and considerable effort is currently being directed towards the development of video and hydroacoustic-based non-invasive counting and sizing methods (Dunn and Dalland, 1993; Beddow *et al.*, 1996).

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Fig. 2.6. A typical automatic feeder, showing the feed-filled hopper and spinning plate that distributes food, suspended above a cage.

Disease monitoring

Monitoring of fish stock health is essential and early indications can often be surmised from changes in behaviour, especially during feeding. While some indication of disease status can be gained from examination of moribund fish netted from the cage surface, studies at Canadian salmon farms have shown this to be an unreliable indicator of disease status, in terms of both incidence and type of disease (Stephen and Ribble, 1995). Rapid detection and removal of dead fish helps to prevent the spread of disease, and daily lifting of nets and removal of dead fish from the cage bottom is recommended. Divers are sometimes employed, especially where large cages are used. Recording numbers of dead fish is important as changes in the incidence of mortalities can warn of the onset of a disease outbreak and provide valuable information on the performance of different stocks or management strategies. Records are also essential for insurance claims. Dead fish should be examined, then carefully disposed of ashore, preferably in a pit and covered with lime – particularly if a disease outbreak is suspected.

Harvesting

For a day or more prior to harvesting, fish are starved in order to firm the flesh and allow the gut to evacuate. The harvesting process is straightforward: the net is lifted until the fish are concentrated in a small volume of water and can be readily netted out. For very large cages, power-assisted hoists may be necessary. Fish should be handled gently as they are easily damaged and as undue stress causes accumulation of ATP in muscle, decreasing shelf-life.

In Southeast Asia, grouper, snapper and seabream are cultured for the live fish market. However, fish are usually killed prior to leaving the farm. Leaving fish to asphyxiate may be practical when harvesting small fish such as tilapia, but is generally not recommended as it increases the lactic acid content of the muscles, thereby accelerating degenerative processes. Larger fish such as yellowtail and salmon are usually killed individually by bleeding. Fish are first anaesthetized in chilled (< 4°C) CO_2-saturated water for 3–5 min and then bled by severing the blood vessels in the gill arches and placing the fish in tanks of clean, cold flowing water until the water runs clear.

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Blood-contaminated water must be carefully disposed of. Fish should be dispatched as rapidly as possible after harvesting to ensure the freshness of the product. Many producer organizations, especially in salmon farming, have introduced quality assurance schemes detailing fish harvesting procedures as well as product specifications.

Routine maintenance

Nets must be regularly checked for damage, and repairs made immediately. At most sites, especially in the marine environment, bags become fouled and, unless a rotating design is used, have to be changed and cleaned. Nets are also often exchanged as fish grow. Care must be taken over mesh size. If too small, water exchange is restricted, and if too large, some fish, such as seabream, will chew the bars of the net. The frequency of net changing varies from once per week to once per year, depending upon site location, materials, season, and management and design of the cage. In northern Norway, one change per year may suffice if farmers delay until after the period of maximum larval tunicate and mussel settlement in July (Sutterlin and Merrill, 1978). The frequency of net changing may also be reduced if the top of the net where fouling is heaviest is periodically pulled out of the water and left to dry.

The art of net changing varies little. At least two people are required. The rigging is released at two adjacent corners, freeing one side of the bag from the cage collar. The freed side is then drawn towards the opposite side and gathered up so that the fish are concentrated in a small section near the surface, the rigging on the other two sides being released from the collar as necessary. One side of the new bag is then attached to the two free corners and drawn under the old bag. The fish are then gently transferred from the old to the new bag, prior to the fouled net being removed for cleaning. On some farms one or two cages within a group are left empty throughout the summer and each week fish are gently decanted from one cage into an adjacent empty cage so that the fouled net may be exchanged with the minimum of stress to stock. The entire net changing process usually takes from 30 min to 2 h, depending on the degree of fouling, size and design of cage, and weather. A large farm can have as many as 50 or 60 cages, therefore routine net changing can fully occupy two members of staff for much of the year. Several of the more innovative designs of industrial cages have tried to address this by the partial mechanization of net changing.

At most farms nets are hung or laid out to dry for a few days. Hard bristle brushes, sticks or high pressure hoses are then used to dislodge adhering material, the latter method being more effective at removing some of the more stubborn fouling organisms (Fig. 2.7). If high pressure hoses are used, then cleaning should be carried out in an area with a sloping concrete floor so that the debris and water can drain away. Net washing machines are also increasingly widely used and although expensive to buy can clean a net in less than 1 h, saving considerably on manpower.

Moorings must be checked regularly by divers, particularly after storms. Mooring lines should be kept relatively free from fouling and worn shackles replaced.

Cage Fish Farming and Disease

Whether caged fish are at greater risk of disease than those reared in tanks or ponds is uncertain. Fish diseases have several different origins, including genetic, nutritional, environmental (light, natural and man-made pollutants), physical injury and pathogens. In comparing cages with other systems, diseases with a genetic and nutritional basis are likely to affect fish in different systems to the same degree and can be excluded. Caged fish tend to be more subject to environmental change than those grown in other systems. Unless shaded or if the water body is particularly turbid, they are exposed to greater levels of light than tank- or pond-reared fishes and there is little opportunity to modify temperature, dissolved oxygen (DO), salinity or other water-quality conditions. Caged stock are

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Fig. 2.7. Hauling and cleaning a net from a large, offshore cage.

also vulnerable to phytoplankton blooms and to oil spills and other pollutants, about which there is little that can be done. While booms or shallow skirts, 10–20 cm deep, have been used in Singapore and elsewhere in attempts to reduce impacts of small oil spillages on caged stock, farmers inevitably have to move or temporarily sink cages below the water surface to avoid serious incidences.

Certain types of physical injury are specific to caged fish, e.g. if over-stocked they may suffer from fin and skin damage caused by net abrasion (Moring, 1982) and are susceptible to pathogenic organisms if handled without due care. Caged groupers (*Epinephilus* spp.), for example, are vulnerable to 'red boil' disease (*Vibrio anguillarum*) following routine handling operations at polluted sites (Chua and Teng, 1980).

Caged fish established in lake, reservoir and coastal environments may be exposed to a wide range of pathogens. From this perspective, the worst sites are those in which pathogenic or potentially pathogenic organisms exist prior to establishment of the farm and those in which disease organisms thrive following the installation of cages. Facultative pathogenic organisms are often associated with water bodies where a source of infection, such as untreated sewage, is present or where there is a supply of organic nutrients that the organisms can utilize. Haemorrhagic septicaemia in cultured freshwater carps is caused by the bacterium *Aeromonas punctata*, whose presence in fresh waters has been related to trophic state and organic enrichment (Rippey and Cabelli, 1980; Toor *et al.*, 1983). There is also evidence of a link between trophic state and fungal infections in fish (Grimaldi, 1973), while Chua (1979) observed that the ectoparasitic isopod *Nerocilia* sp. that attacked caged rabbit fish (*Siganus rivulatus*) was more prevalent in organically enriched waters.

Both wild fish populations and intermediate hosts in the life cycle of a fish parasite represent a risk for the fish farmer. The introduction of large numbers of caged fish to a lake or bay can have a dramatic effect on disease agents, although the mechanisms involved in the transmission of parasites from wild to cage fish and vice versa are not fully understood. Cages of salmon attract scavenging saithe (*Pollachius virens*) that often harbour the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*, and laboratory trials have clearly shown that lice can transfer between host species (Bruno and Stone, 1990). In the UK, caged fish have sometimes become severely infested with

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the cestodes *Triaenophorus nodulosus* and *Diphyllobothrium* spp. resulting in heavy mortalities and the closure of at least one farm (Wootten, 1979; Jarrams *et al.*, 1980). The source of infection was subsequently traced to the wild fish populations.

Disease risks can be minimized by avoiding sites where a pre-development survey reveals parasites or disease agents to be present in the wild fish or intermediary hosts. However, problems may still occur through the introduction of diseased stock to the farm or the attraction of birds and other opportunistic predators. Often, the cage fish farmer has little recourse but to consider treatment. Ectoparasites and other pathogenic skin conditions pose a special problem as many of the chemicals currently in use are biocides that must be used in bath treatment form. In theory, farmers have two options: to transfer fish to a special treatment bath or cage, or to carry out the treatment *in situ*. Although special treatment cages have been designed (e.g. Kleinholz and Luker, 1994), these are impractical for the treatment of the large numbers of stock typically held at intensive cage fish farms.

Epidemiological studies have also revealed the importance of management in reducing the incidence of disease and mortality. A 4 year study of disease outbreaks in 11 Irish salmon farms showed that interruption of parasite life cycles through fallowing, the separation of year classes of fish to different sites and the practice of basic hygiene methods could significantly reduce the severity of disease outbreaks (Wheatley *et al.*, 1995).

Environment

Introduction

Aquaculture is reliant on a wide range of natural resources (or environmental 'goods') and 'services'. The former include:

- space or land for siting the operation;
- materials (timber, steel) for construction;
- eggs, fry, fingerlings;
- water to support the animals; and
- feed and/or fertilizers to enhance production (Beveridge *et al.*, 1997b).

From an ecological perspective, aquaculture production takes place in 'open' systems. Materials are imported to the production site and transformed into marketable products through inputs of energy and, as a result, wastes (uneaten food, faecal and urinary wastes, chemicals and feral animals) are inevitably produced. The 'services' of the environment are then required to disperse and assimilate wastes that would otherwise accumulate in the production system and exert a deleterious effect on growth and production (ammonia, nitrite, etc.), and to replenish oxygen. Overexploitation of environmental goods, such as fishmeal, and environmental services (i.e. the capacity of the immediate environment to assimilate wastes) lies at the heart of sustainability issues in aquaculture (Beveridge *et al.*, 1997a,b; Kautsky *et al.*, 1997; Naylor *et al.*, 1998).

Cage aquaculture and consumption of environmental goods

Is cage aquaculture an efficient means of producing food or does it consume more resources than pond- or tank based aquaculture? This question will be increasingly asked as resources such as fishmeal and fish oils become increasingly scarce.

Results from energy analysis, in which all production inputs and outputs are converted into units of energy, suggest only that intensive aquaculture is much more inefficient than extensive aquaculture methods (Folke and Kautsky, 1989; Folke *et al.*, 1994; Kautsky *et al.*, 1997; Naylor *et al.*, 2000). No appropriate comparisons have been made between intensive cage and intensive tank culture of salmon, for example. Ecosystem support analysis estimates the areas required to supply fry, food and oxygen and to assimilate wastes in order to facilitate comparisons between production systems ('ecological footprints'). Berg *et al.* (1996) used this technique to compare cage and

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pond tilapia culture methods in Lake Kariba, Zimbabwe (Fig. 2.8). However, as semi-intensive pond culture was compared with intensive cage culture, it is impossible to conclude whether cage aquaculture is any less efficient than pond aquaculture.

Cage aquaculture and wastes

The relationship between waste and the environment is particularly crucial in cage aquaculture. With few exceptions, waste from cages is released directly into the environment. Any deleterious changes caused by farming activities are likely to affect not only the cage operation itself but also the amenity value for others – including fishermen, shellfish farmers, recreational users, irrigation and drinking water supplies – where cages are sited in multi-purpose, publicly owned waters. Opponents of cage aquaculture argue that it is intrinsically bad for the environment and the economy, and that if the environmental and social costs of wastes were internalized, then intensive cage culture would not be economically viable (Folke *et al.*, 1994).

While feed losses from cages have tended to be higher than from other systems (Beveridge *et al.*, 1997b), FCR values from farms using the new generation of 'smart feeders' suggest feed losses are comparable with those from tank systems (Beveridge and Kadri, 2000). Mass balance models and direct measurements mean that we have a good idea of waste feed, faecal and urinary losses. The dilution effect in most marine environments is enormous and attention has focused on the dispersion and impacts of solids on sediments (e.g. Pérez *et al.*, 2002). Models incorporate data on faecal and waste feed settling rates with local current and topographic information to produce waste contour plots of the site (Fig. 2.9). However, while such models continue to be verified and refined, the environmental capacity of sediments remains a highly contentious issue, i.e. how much loading from cage aquaculture is acceptable? An appropriate criterion might be the point at which loss of the macrobenthic community and the switch to anaerobiosis occurs. At this juncture both the essential nature and the corresponding functioning of the sediments are changed. In lakes and reservoirs, where water exchange is limited, modelling of dissolved nutrient loadings has been used to match cage culture with environmental capacity. As cage culture and productivity increase this adversely affects water quality (Fig. 2.10). The focus has been on dissolved

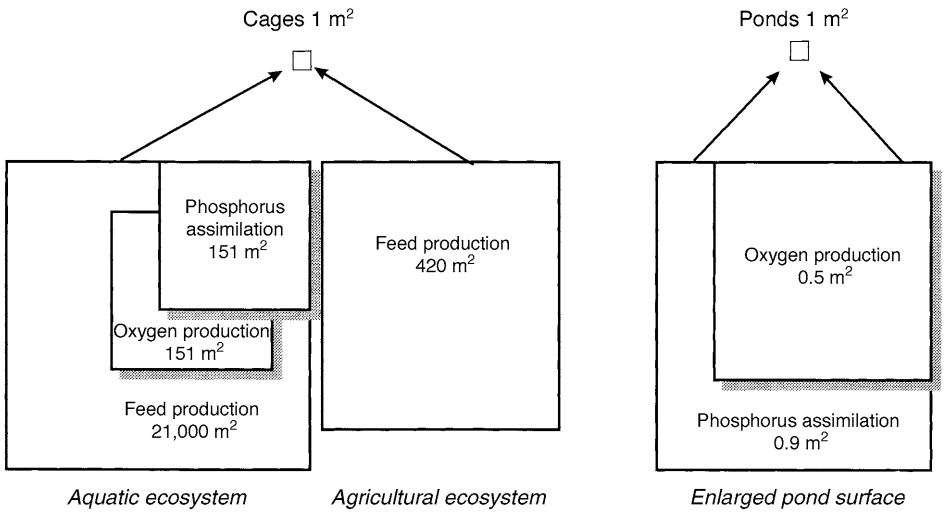


Fig. 2.8. A comparison of the 'ecological footprints' of intensive cage and semi-intensive pond tilapia culture, Lake Kariba, Zimbabwe. (After Berg *et al.*, 1996.)

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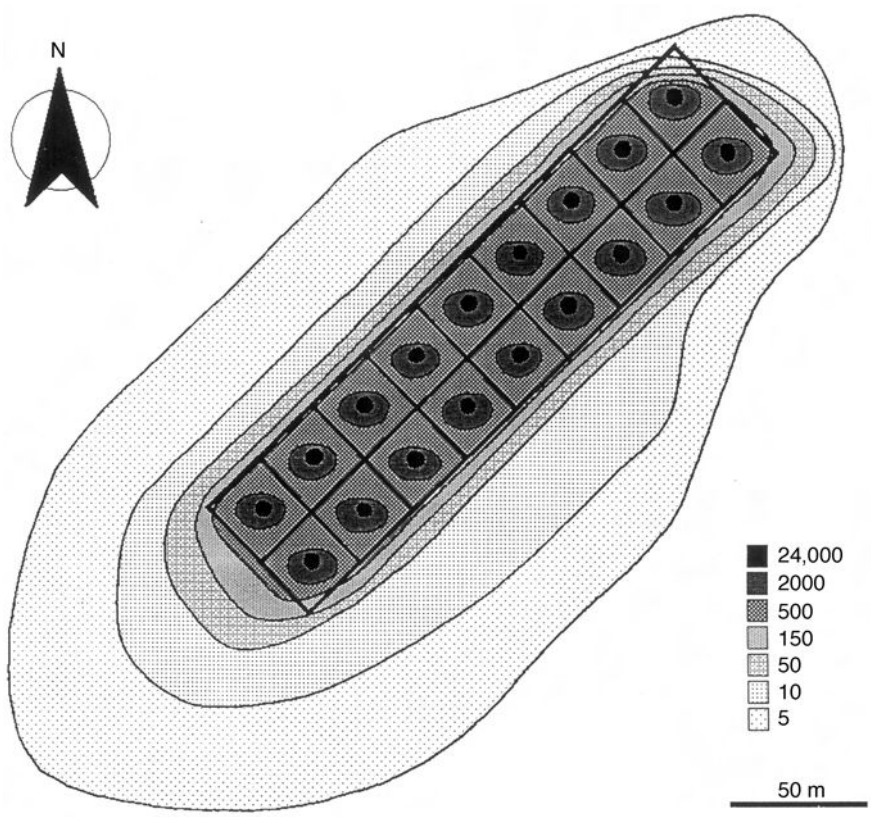


Fig. 2.9. Waste carbon contours (kg m^{-2} $year^{-1}$) predicted for a marine cage salmon site, Scotland (courtesy of T. Telfer, Stirling Environmental Services).



Fig. 2.10. Over-crowding of intensive carp cages, Zhang Hong Reservoir, China. In such conditions, water quality is likely to deteriorate.

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phosphorus, the limiting nutrient of plankton productivity in most standing fresh waters. Experience in Scotland has shown that while modelled outputs are a useful guide to development, a complementary monitoring programme is essential (Beveridge, 1984, 1996; Kelly, 1995).

Recently, attention has switched to other wastes, especially waste chemicals and feral fish. These, it is argued, can cause more profound and persistent environmental changes. In Europe, there is growing concern about use of chemical bath treatments, such as dichlorvos, to eradicate sea lice. While a number of novel in-feed products have been developed, authorities are increasingly insistent that appropriate risk assessment and post-authorization monitoring procedures be carried out before licensing will be considered.

Escapes from cages are almost inevitable and the slow trickle of escaped fish that occurs during stocking, grading and harvesting goes largely unnoticed and unrecorded. Estimates range from 2.5 to 5% of fish stocked. Occasionally, a mass release of animals, amounting to many thousands, can occur as a result of storm, predator damage or vandalism (Billington and Herbert, 1991). The fear is that feral animals become established, interbreeding with indigenous strains and thereby reducing individual fitness – as is contended with salmon in Pacific North America, Scotland and Norway – or that if a feral species is exotic to an area it will compete with or prey on the host fish community, causing serious economic impact (Welcomme, 1988; Billington and Herbert, 1991; Beveridge *et al.*, 1994b, 1997a,b). The issue remains highly contentious.

Discussion

Cage aquaculture is very much a recent phenomenon. The technology employed in the salmon industries of Europe and the Americas is based largely on innovations developed since the 1980s while even the simple artisanal cages that prevail in tropical lakes and reservoirs have evolved from a design tradition that for the most part is little older than this. Yet, despite its novelty and the fact that cage designs can surely be improved upon, further innovation in cage design is constrained at present. In mature industries, such as Atlantic salmon farming, a combination of years of practical experience and greatly reduced profit margins has resulted in the almost universal adoption of the same, 'no frills', tough-but-cheap designs that function reasonably well. There is little interest in innovation unless it can be guaranteed to bring further reductions in production costs. The development of novel species (such as flatfish) for aquaculture, or increased pressure by environmentalists to reduce wastes or move offshore, are likely to be the principal innovations in the immediate future.

The single greatest advantage that has been claimed for cages over competing systems has been in terms of capital costs (Beveridge, 1996; Huguenin, 1997). Cages undoubtedly remain the cheapest system for rearing fish in coastal areas. However, it is becoming increasingly apparent, especially in inland waters, that economic advantages can be seriously compromised by social and environmental considerations. While non-governmental organizations in Bangladesh, for example, have recently begun to appreciate that cages offer an opportunity for the poor to become involved in aquaculture, cage culture is unsustainable unless potential farmers are able to secure access through equitable arrangements with other stakeholders in the lake or river (Beveridge and Stewart, 1998; Hambrey *et al.*, 2001). It is thus essential to consider the social dimension in any cage culture development. Cage aquaculture is also heavily dependent upon the supply of environmental goods and services and, as with the social environment, where this relationship has been ignored or poorly understood, cage farming has proved unsustainable. While problems have been particularly apparent in inland waters, especially in small lakes and reservoirs with limited water exchange and where high population densities prevail, questions regarding the environmental capacity of coastal marine areas for intensive cage aquaculture are increasingly being asked. In order to try

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to match production with environmental capacity, countries such as Norway have adopted a national coastal zone management plan that takes account of both environmental and other stakeholder interests (Kryvi *et al.*, 1991). Somewhat belatedly, perhaps, Scotland and North America are beginning to consider similar approaches.

Given a scenario of static or – more probably – dwindling supplies of fish from capture fisheries, aquaculture in one or form or another is here to stay. However, the more intensive types of aquaculture face problems, especially with regard to feed supplies and wastes (Naylor *et al.*, 1998, 2000). All intensive aquaculture industries will have to come to terms with declining fishmeal supplies in the face of increasing demand, not only from fish farmers but also from other intensive animal production sectors. The issue of wastes, however, is particularly pertinent to cage farming. In an attempt to force intensive cage farms to reduce wastes, industries are likely to become increasingly regulated, either through the implementation of more stringent planning controls (see above) or through the introduction of initiatives that tax farmers for discharging wastes into the environment. The scope for reductions in wastes through further improvements in diet or feeding systems is limited (Beveridge and Kadri, 2000); any further significant advances must come from the development of waste collection systems, such as those described by Ervik *et al.* (1994) and Costa-Pierce (1996). Increases in environmental taxation may favour the adoption of alternative water-based production systems, such as floating raceways (Fast, 1991) and land-based tank and raceway systems. Some have proposed integration of intensive coastal cage aquaculture with more extensive aquaculture technologies, the cage wastes promoting the growth and production of macroalgae or filter-feeding molluscs (Folke and Kautsky, 1989). However, attempts to date in North America (Mazhari and Petrell, 1991), in the Philippines (Hurtado-Ponce, 1992) and in Chile (Buschmann, 1996) show only modest advantages and ignore food safety issues (Howgate *et al.*, 2002).

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3 Infectious Diseases of Coldwater Fish in Marine and Brackish Water

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Introduction

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

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

Viral Diseases

Several viruses are important pathogens of salmonid fishes, particularly during their early development in fresh water (Wolf, 1988). Viral diseases of fishes have historically been of great concern to fish health managers because they can cause high mortality. In addition, the presence of certain viruses in a fish population causes economic hardships to fish farmers due to restrictions on transfer or sale of these fish. At least six viral diseases are of concern for pen-reared salmon: these include infectious haematopoietic necrosis (IHN), infectious pancreatic necrosis (IPN), salmon pancreas disease (SPD), infectious salmon anaemia (ISA), salmonid herpesvirus 2 infections and erythrocytic inclusion body syndrome (EIBS). The erythrocytic necrosis virus has the potential to infect salmon in seawater,

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but has yet to be recognized as a serious problem. At least one form of plasmacytoid leukaemia is associated with a retrovirus, but this disease is described under the section on *Nucleospora salmonis*. Cardiac myopathy syndrome (CMS) has recently been associated with a nodavirus, and is thus covered in this section. IHN, IPN and EIBS are also important diseases in fresh water, and are dealt with in more depth in Chapter 4. However, the manifestations of IHN and IPN as they occur in seawater are described. Furthermore, viruses have caused disease in farmed Atlantic halibut and turbot larvae, resulting in considerable losses (Bloch *et al.*, 1991; Grotmol *et al.*, 1995; Wood *et al.*, 1996). However, to date, specific viral diseases of non-salmonids reared in coldwater sea cages have not been identified as serious problems. Vacuolating encephalopathy and retinopathy (VER) is a disease primarily of larval or juvenile marine fishes, but may be carried into cage-cultured flatfish. This nodavirus is dealt with in Chapter 9.

Infectious haematopoietic necrosis in netpens

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

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

Infectious pancreatic necrosis

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

Clinical signs of IPN in post-smolt Atlantic salmon may be minimal, but some fish stop feeding and show nervous distress. The most significant losses may

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sometimes be attributed to the long-term effects of reduced or completely ceased feeding. Fish show hyperaemia and petechial haemorrhage in the visceral fat and in the pyloric caeca.

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

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

Salmon pancreas disease

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

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

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

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

Diagnosis. Haemorrhage in tissues associated with the pyloric caeca in emaciated Atlantic salmon smolts, along with the absence of other infectious agents (e.g. IHN or IPN viruses, *Aeromonas salmonicida* or *Vibrio* spp.), is indicative of SPD. Confirmation of the disease is based on histological changes or by isolation of SPDV from



Fig. 3.1. Emaciated Atlantic salmon post-smolts with pancreas disease.

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affected fish. Isolation of the virus can be achieved by co-cultivation of kidney tissues on CHSE-214 cells at 15°C, and cultures are blind passed after 28 days (Nelson *et al.*, 1995) where the cytopathic effect (CPE) may be observed after about 10 days.

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

Infectious salmon anaemia

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

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

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

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

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Salmonid herpesvirus 2 infections

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

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

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

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

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

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

Cardiomyopathy syndrome in cage culture

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

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

Diagnosis. The diagnosis is based on the characteristic gross and pathognomonic histopathological lesions. Characteristic

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lesions are found in the spongious myocardium of the atrium and ventricle (Amin and Trasti, 1988; Ferguson *et al.*, 1990). These lesions are comprised of muscular degeneration, proliferation of the endocardial cells with macrophage infiltration and lymphocytes subendocardially and in the degenerated muscle. Blood clots are frequently found in the atrium. Focal necrosis in the hepatic parenchyma may also occur.

Diseased fish may also be diagnosed by means of ultrasound imaging (Sande and Poppe, 1995). CMS bears little resemblance to other diseases, but haemopericardium may be observed in fish dying from other diseases.

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

Bacterial Diseases

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

Vibriosis

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

V. anguillarum strains show heterogeneity in both phenotypic (Tajima *et al.*, 1985) and serotypic (Kitao *et al.*, 1984; Tajima *et al.*, 1985; Sorensen and Larsen, 1986) characteristics. The strains of *V. anguillarum* that cause vibriosis in pen-reared salmon worldwide represent only one or two serotypes (based on the 'O' antigens present), which simplifies the formulation of anti-vibriosis vaccines for controlling the disease. Vibriosis caused by *V. anguillarum* usually occurs between 15 and 21°C, and most outbreaks occur in smolts during their first summer in seawater. Vibriosis in the Pacific Northwest is mainly a problem of Pacific salmon (e.g. chinook

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and coho), although Atlantic salmon are also susceptible to the infection. Interestingly, all cases of vibriosis due to *V. ordalii* in pen-reared salmon reported to date have involved Pacific salmon in Japan and the Pacific coast of the USA (Schiewe *et al.*, 1981).

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

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

Clinical signs of coldwater vibriosis may be non-specific, but usually include lethargy and cessation of feeding. Affected fish turn dark, exhibit exophthalmos, a swollen vent and pinpoint haemorrhage along the belly and at the base of the pectoral, pelvic and anal fins. The gills are usually pale. Internally, ascites and petechial haemorrhage in the perivisceral fat, pyloric caeca, peritoneal surfaces, liver and swimbladder are typical findings (Figs 3.2 and 3.3). The latter may be filled with a blood-tinged fluid

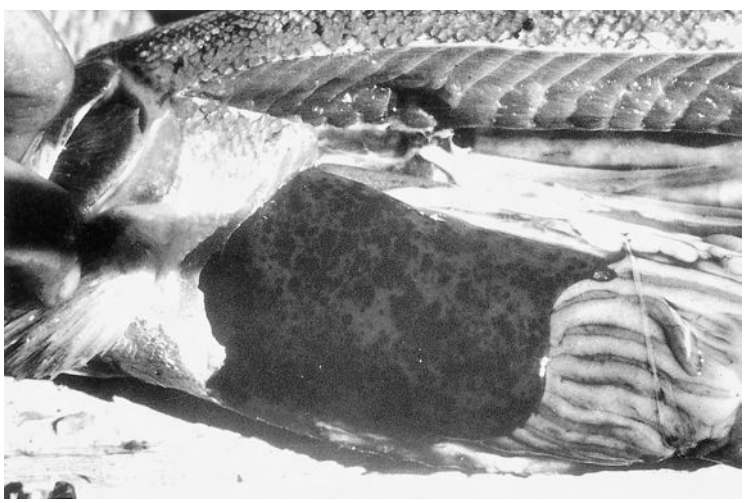


Fig. 3.2. Atlantic salmon with coldwater vibriosis. Note multiple haemorrhage in the liver.

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Fig. 3.3. *Vibrio salmonicida* infection in Atlantic salmon. Note petechial haemorrhage in the perivisceral fat between the pyloric caeca.

and the liver typically has a yellowish discoloration. In chronic cases, skin ulceration, fin rot, a pseudomembranous peritonitis and epicarditis may also be found. The spleen usually has a colour slightly lighter than normal. In cod, the pathological changes are rather diffuse and non-specific, but keratitis is frequently seen.

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

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

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

Control and treatment. Vibriosis is best conrolled by prevention, and commercial vaccines are available. With salmonids, vaccination is best carried out on fish that have attained immunocompetent size (at least 5–10 g) and before they are introduced

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to netpens. The vaccines are conveniently administered by immersion methods, and if applied properly, they afford excellent protection (Evelyn, 1984, 1988). Some farms revaccinate fish shortly after introduction to netpens, and results with revaccination in seawater have been promising. Revaccination should, however, be conducted with caution because the handling of fish shortly after their introduction to seawater is very stressful. The most recent trend is to administer the vaccine by intraperitoneal injection, usually in combination with other vaccines, e.g. with furunculosis vaccines, which are most effective when injected. Vaccines for controlling vibriosis in cod are also promising for protecting against *V. anguillarum* (Espelid *et al.*, 1991; Groman *et al.*, 1992) and *V. salmonicida* (Schroder *et al.*, 1992).

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

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

Marine myxobacteriosis

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

Proper taxonomic identifications have not been conducted on many *Cytophaga* and *Flexibacter* spp. that have been associated with disease in marine fishes. However, to date, marine *Cytophaga* and *Flexibacter* species have not been transferred to *Flavobacterium* (Bernardet *et al.*, 1996), as was proposed for certain freshwater species. Fish health workers and aquaculturists usually refer to these bacteria as 'myxobacteria'. This is technically incorrect because these bacteria belong to the order Cytophagales, and not to the order Myxobacteria. It would, therefore, be more appropriate to refer to the marine forms using collective terms such

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as 'cytophaga–flexibacter-like bacteria' or 'gliding bacteria'. However, to remain consistent with the common terminology and to avoid confusion, reference is made to these bacteria as myxobacteria in this review.

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

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

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

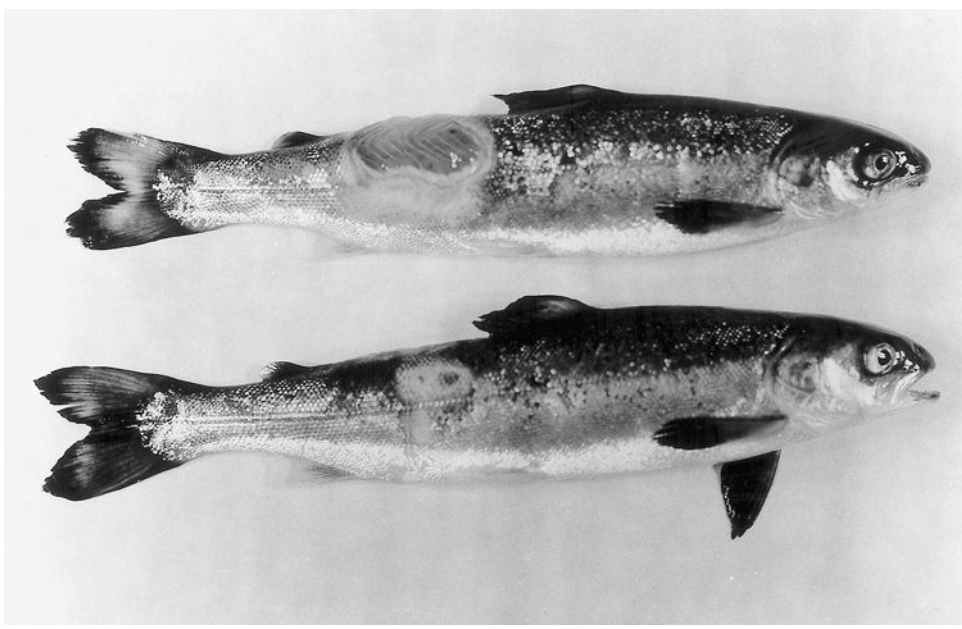


Fig. 3.4. Atlantic salmon with *Cytophaga*-associated skin lesions. Note severe lesion with exposure of underlying muscle.

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the mouth. Fish farmers in British Columbia, Canada report that the condition is particularly troublesome in waters with high salinity. Based on preliminary culture analysis, the myxobacterium from mouth lesions appears to be different from the myxobacterium causing skin lesions. A similar myxobacterial stomatitis has been observed in wild Atlantic cod in the North Sea (Hilger *et al.*, 1991).

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

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

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

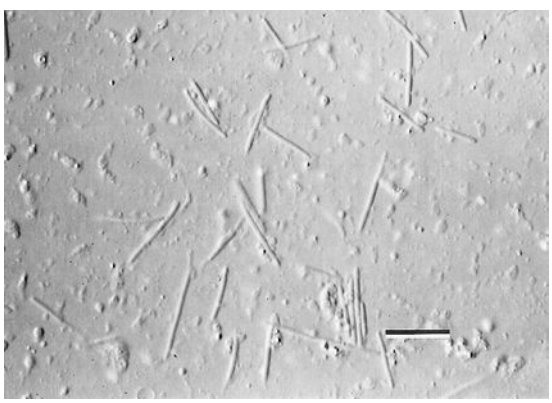


Fig. 3.5. *Cytophaga* bacteria in wet mount of skin lesions showing 'myxobacteria'. Bar, 5 µm.

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Salmonid rickettsial septicaemia

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

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

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

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

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

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

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petechiae are observed in the visceral organs and muscle, and a whitish pseudomembrane may cover the heart.

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

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

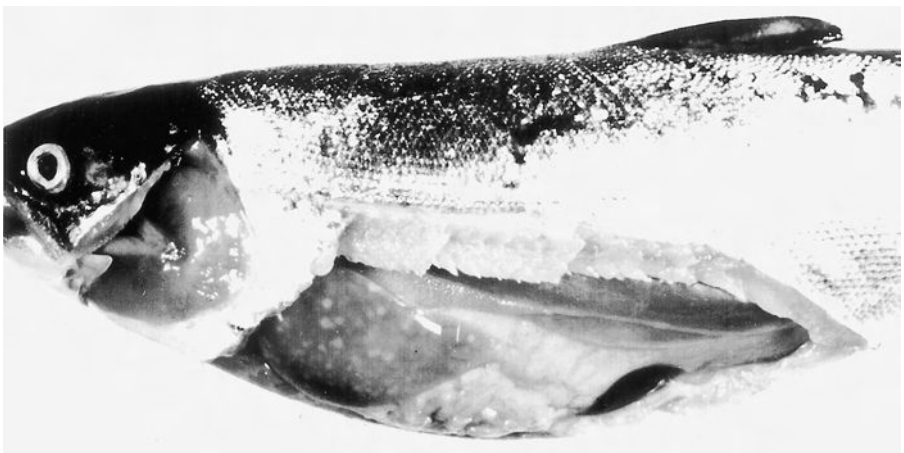


Fig. 3.6. Piscirickettsiosis in salmon. Multiple, white, crater-like lesions in the liver (courtesy of P. Bustos).

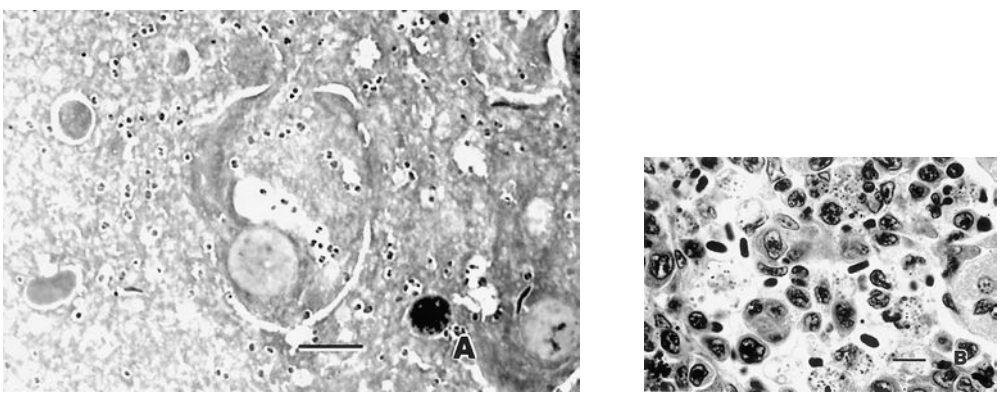


Fig. 3.7. *Piscirickettsia salmonis*. (A) Gram-stained kidney imprint. (B) Organisms in macrophages from kidney section, stained with H&E. Bar, 10 μm.

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had success with employing injectable treatments with fluoroquinolones. The microorganism is intracellular, and this probably contributes to the difficulties of treating the disease with antibiotics. Although the role of vertical transmission is unknown, the techniques used for preventing vertical transmission of bacterial kidney disease (e.g. brood stock screening) are being employed in Chile with some success.

Protozoa and Myxozoa

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

Paramoeba pemaquidensis gill infections

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

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

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

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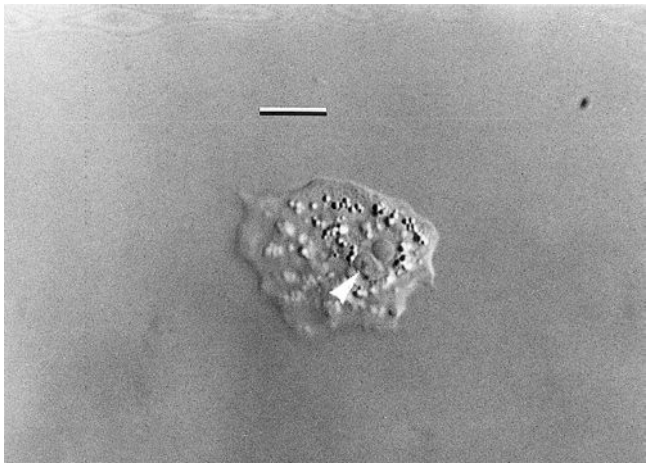


Fig. 3.8. *Paramoeba pemaquidensis* from coho salmon gill. The parasome (arrowhead) is adjacent to the nucleus. Bar, 10 μm.

polyclonal antibodies in tissue sections or imprints (Howard and Carson, 1993a).

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

Diplomonad flagellates

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

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

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observed in pen-reared salmon are morphologically indistinguishable from the relatively non-pathogenic *H. salmonis* that infects the intestinal tract of salmonids in fresh water, they probably represent new, highly invasive strains or species. Sterud *et al.* (1997) recently named the organism from pen-reared Atlantic salmon and Arctic grayling (*Thymallus arcticus*) from fresh water as *S. barkhanus*.

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

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

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

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

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

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

Kudoa thyrsites (Myxozoa)

Myxosporeans of the genus *Kudoa* and related genera infect the muscle of many marine fishes, and heavy infections can cause unsightly white cysts or soft texture in fillets (Kabata and Whitaker, 1981; Patashnik *et al.*, 1982). These parasites can lower the market value of the infected fish, although they seldom cause morbidity. *K. thyrsites* is a cosmopolitan parasite that infects many species of marine fishes (Whitaker *et al.*, 1994). Infections in pen-reared Atlantic salmon have been reported from the Pacific Northwest (Harrell and Scott, 1985; Whitaker and Kent, 1991), Spain (Barja and Toranzo, 1993) and Ireland (Palmer, 1994). In one

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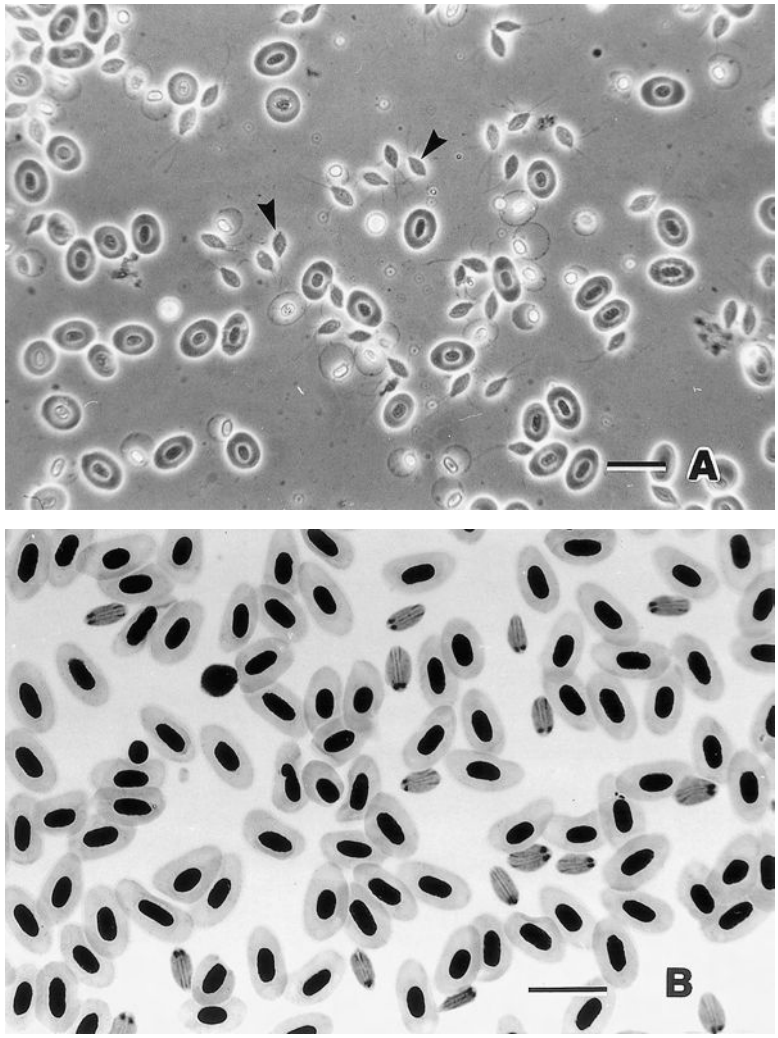


Fig. 3.9. Systemic diplomonad flagellates (arrowheads) from chinook salmon. (A) Phase-contrast wet mount. (B) Blood smear, Giemsa stain. Bar, 20 µm.

instance, Harrell and Scott (1985) attributed mortalities in Atlantic salmon smolts to this parasite. More importantly, heavy infections are associated with soft flesh in pen-reared Atlantic salmon that are either held on ice for 3–6 days or cold-smoked. *K. thyrsites* infections and associated soft flesh have also been observed in farmed coho salmon (Whitaker and Kent, 1992) and brown trout (Baudin-Laurencin and Bennassr, 1993).

The infection is much more prevalent in Atlantic salmon grilse or reconditioned grilse (i.e. fish that have reabsorbed their gonads and do not exhibit external signs of sexual maturation) than in market-size fish that have not undergone sexual maturation (St-Hilaire *et al.*, 1998). For example, infection in grilse may reach 70%, whereas market-size fish that are not sexually mature usually show infections below 10%. There is a positive correlation between intensity of infection and severity of soft flesh in Atlantic salmon held on ice (St-Hilaire *et al.*, 1997a). Heavily infected fish always have soft flesh, whereas lightly infected fish (i.e.

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fewer than 20,000 spores g^{-1}) usually do not have the condition. Soft flesh is not noticed on the processing line, and only becomes apparent after fish are held for about 3–6 days on ice or when fillets are smoked. In an investigation of *K. thyrsites* infections in Pacific hake (*Merluccius productus*), the flesh softening was apparently caused by a proteolytic enzyme produced by the parasite (Tsuyuki *et al.*, 1982). This enzyme remains active below 70°C. Therefore, tissue breakdown will continue through cold-smoking processes, which are normally conducted at about 50°C or less. Seymour *et al.* (1994) suggested that the flesh degradation is due to cathepsin L from the host inflammatory response to the parasite, instead of a proteolytic enzyme from the parasite.

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

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

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

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

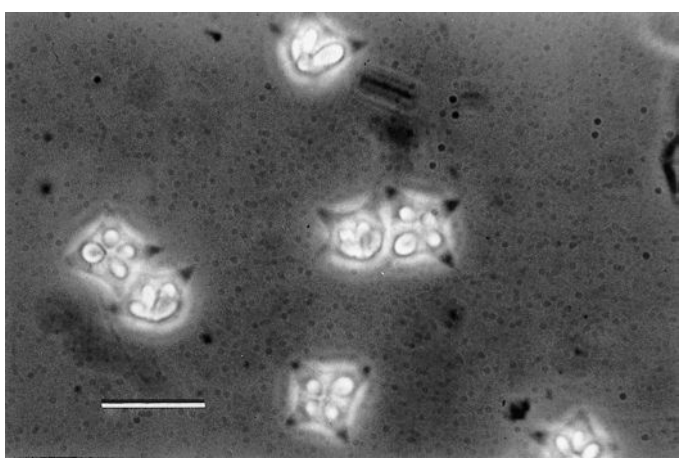


Fig. 3.10. Wet mount of *Kudoa thyrsites* spores. Bar, 15 µm.

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fluid collected from the freshly cut surface of a fillet or by crushing a small piece of muscle. The parasite shows up well in Giemsa-stained histological sections. Detection of the parasite in whole fish is a problem that is, as yet, unresolved.

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

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

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

Loma salmonae (Microsporidia)

L. salmonae is an obligate intracellular microsporidian and infects the gills and other vascularized tissues of salmonids reared in fresh water (Putz *et al.*, 1965; Putz and McLaughlin, 1970; Morrison and Sprague, 1981, 1983; Hauck, 1984; Poynton, 1986; Markey *et al.*, 1994; Bruno *et al.*, 1995). Severe gill infections have been reported in rainbow trout, steelhead trout and kokanee salmon (Wales and Wolf, 1955), and Hauck (1984) observed high mortality in chinook due to systemic infections by a *Loma* sp. (presumably *L. salmonae*).

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

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

Loma morhua is a common gill parasite of Atlantic cod (Morrison, 1983), and causes similar gill lesions to those induced by *L. salmonae* in salmon. Therefore, this parasite also has potential to cause disease in farmed cod.

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Clinical signs and gross pathology. Fish with heavy gill infections are usually lethargic, and small white cysts may be seen in the gills (Fig. 3.11). A consistent finding in pen-reared salmon is the presence of multiple petechiae in an otherwise pale gill. Infected gills may also appear nodular. Systemic infections in chinook may cause enlargement of the spleen and kidney. In Atlantic cod, obvious white cysts are also apparent in the gills and visceral organs (Morrison, 1983).

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

A sensitive PCR test for *L. salmonae* using an rDNA sequence has been described (Docker *et al.*, 1997a). This may be useful for screening fish (i.e. broodstock) for subclinical infections. Furthermore, these



Fig. 3.11. Salmon gill with *Loma salmonae* infection. Note the mottled appearance of the gills and numerous xenomas (arrowheads).

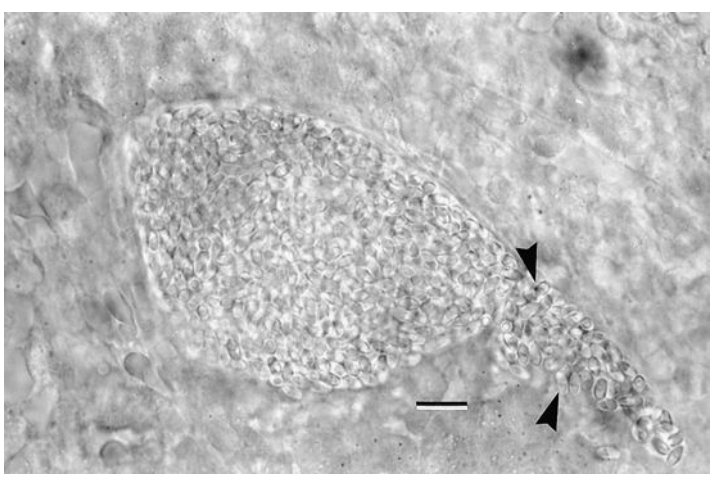


Fig. 3.12. Wet mount of a gill infected with *Loma* showing a ruptured xenoma releasing spores (arrowheads). Bar, 10 μm.

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specific primers can differentiate *L. salmonae* from other *Loma* species.

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

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

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

Nucleospora salmonis

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

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

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

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

N. salmonis is transmitted by cohabitation or feeding infected tissues to fish in fresh water (Baxa-Antonio *et al.*, 1992). These findings have been reported in our laboratory, but we were unable to transmit the infection by cohabitation in seawater. Circumstantial evidence (e.g. the occurrence of the parasite in Chile) suggests that the parasite may be transmitted via eggs.

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Clinical signs and gross pathology. Heavily infected fish are anaemic. Fish with PL are often dark, lethargic and may swim near the surface. Many of the of fish with PL exhibit severe bilateral exophthalmos (Fig. 3.13). The exophthalmos is due to massive accumulation of white or hyperaemic tissue in the orbit of the eye. The spleen and kidney are enlarged when systemic infections occur. Petechiae may occur in the liver, mesenteric fat, pancreas, heart and skeletal muscle. The lower intestinal wall may be markedly thickened. Some fish have ascites consisting of a clear or serosanguinous fluid.

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



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

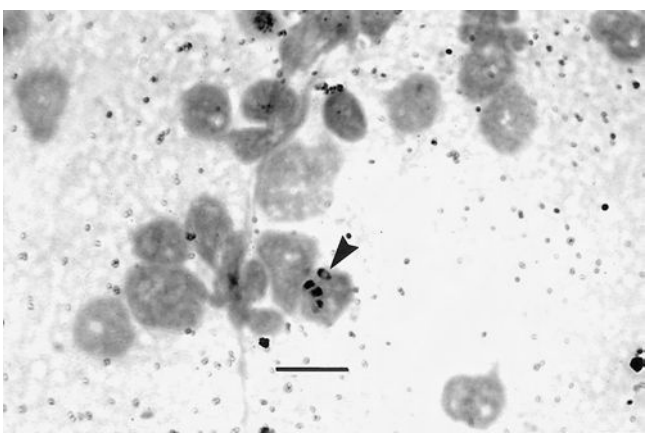


Fig. 3.14. Gram-stained kidney imprint of *Nucleospora salmonis* showing spores (arrowhead) in a remnant of a nucleus. Bar, 10 μm.

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from the small subunit region (Barlough *et al.*, 1995) or internal transcribed spacer region (Docker *et al.*, 1997b).

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

Crustacean Parasites

Sea lice – caligid copepods (family Caligidae)

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

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

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

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

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

Pre-adult and adult sea lice are visible to the naked eye. They are on the body surface, especially on the head, back and in the perianal region. It is these stages that usually cause the most damage to the fish. Pre-adult and adult stages of *Caligus* species can be distinguished from *Lepeophtheirus* species by the presence of lunules on their anterior margin (Fig. 3.15). There is a key to aid in the identification of adult sea lice of the northern hemisphere (Johnson and Margolis, 1994).

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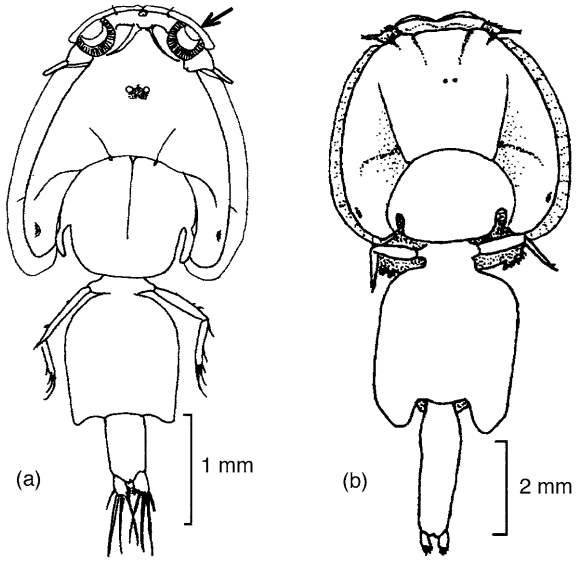


Fig. 3.15. *Caligus* (a) and *Lepeophtheirus* (b). Note lunules (arrow) on *Caligus*. (From Kabata, 1988.)

Control and treatment. There are excellent reviews on the control and treatment of sea lice (Costello, 1993; Johnson *et al.*, 1993; Roth *et al.*, 1993a; Johnson, 1998). Management strategies are useful for reducing the impact of sea lice in cage farms. Farms should be located in areas with strong water currents to flush away copepodid stages and in areas where wild fish reservoir hosts are not numerous. Fallowing of sites between production cycles and maintaining only single year classes at sites can also significantly reduce the need for treatments for *L. salmonis* (Bron *et al.*, 1993; Grant and Treasurer, 1993). This approach may be applied to several sites within a common area. In cases where farms belonging to different companies are in close proximity to each other, cooperative agreements between companies with respect to single-year class stocking, periods of fallowing and timing of sea lice treatments have been effective for controlling sea lice outbreaks (Grant and Treasurer, 1993).

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

The organophosphorus insecticides, dichlorvos, marketed as 'Nuvan 500EC' or 'Aquaguard SLT', or in its related trichlorphon form as 'Neguvon', were the first chemicals widely used to control sea lice (Brandal and Egidius, 1977; Grave *et al.*, 1991a,b). Dichlorvos and trichlorphon have been used since the 1960s as a bath treatment for parasites in pond fish culture (reviewed in Schmahl *et al.*, 1989). These treatments effectively remove both the pre-adult and adult stages of sea lice, but not the chalimus larvae, from salmon (Brandal and Egidius, 1979). Therefore, successive treatments, usually at 2–4 week intervals, are required to control infections (Wootten *et al.*, 1982). Repeated treatment with dichlorovos may induce resistance in sea lice (Jones *et al.*, 1992).

Another organophosphate, azamethiphos (marketed as Salmosan® and Alfacron®), is presently used in Europe and

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

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

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

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

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

Oral administration of diflubenzuron (a chemical that inhibits chitin synthesis) reduces infections by both adult and larval stages of sea lice (Roth *et al.*, 1993a). Treatments using Lepsidon (containing diflubenzuron) and Ektobann (containing

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

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

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

Family Pennellidae

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

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

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

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

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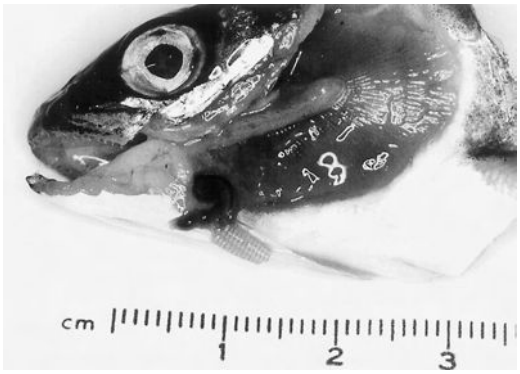


Fig. 3.16. *Haemobaphes disphaerocephalus* infecting the gills of an Atlantic salmon.

Isopods – *Ceratothoa gaudichaudii*

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

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

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

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

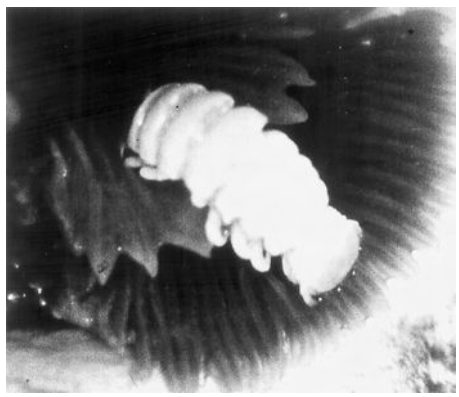


Fig. 3.17. *Ceratothoa gaudichaudii* on the gills of Atlantic salmon (courtesy of R. Inostroza).

Helminth Parasites

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

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some helminth parasites of fish can infect humans or cause unsightly lesions. The following are helminth parasites of importance in coldwater marine netpen-reared fish.

Cestodes (tapeworms)

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

Adult cestodes – Eubothrium *spp.*

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

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

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

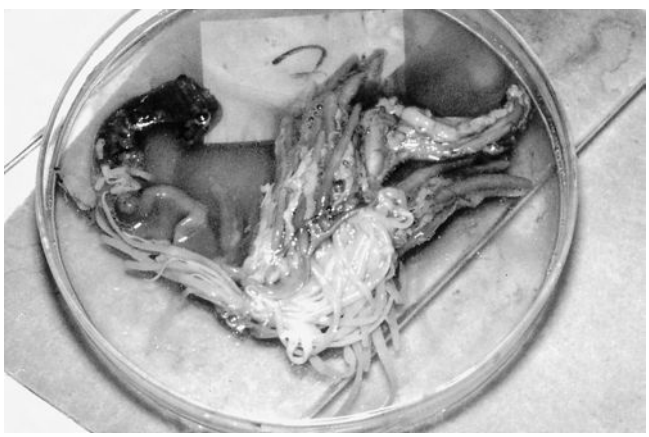


Fig. 3.18. Adult *Eubothrium* cestodes in the gut of Atlantic salmon (courtesy of B. Berland).

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infections may induce anaemia, and when extremely severe the cestodes may cause death due to blockage of the intestinal tract (Mitchell, 1993).

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

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

Gilquinia squali *metacestodes*

Eye infections by metacestodes of *G. squali* (order Trypanorhyncha) have been associated with mortality of young chinook salmon at netpen sites in British Columbia (Kent *et al.*, 1991). The definitive host for *G. squali* is the spiny dogfish (*Squalus acanthus*). The metacestode is common in the eyes of North Sea whiting, *Merlangius merlangus* (MacKenzie, 1965, 1975). Wild salmon are probably not a normal intermediate host for the worm as this infection has only been seen in cage-reared salmon (Kent *et al.*, 1991).

The adult worms are prevalent in dogfish throughout marine waters in British Columbia, and gravid adult worms are commonly found in dogfish in the spring. Dogfish are frequently found in or around netpens at this time, thus providing infective coracidia for the first intermediate host near the pens. Based on trypanorhynchs whose life cycles have been investigated (Mundry and Dailey, 1971; Overstreet, 1978; Mattis, 1986; Sakanari and Moser, 1989), it is likely that a crustacean is the first intermediate host for *G. squali*. Chinook salmon in netpens presumably acquire the infection by eating this crustacean.

Clinical signs and gross pathology. Moribund fish are lethargic and remain near the bottom of the pen. The fish can easily be captured by hand, suggesting that they are blind. However, fish are not usually emaciated and contain food in their stomachs, which indicates that they continue to feed.

The lens often appears normal, whereas the iris and vitreous chamber may be white and opaque and occasionally haemorrhagic. The lens in more severely affected eyes is also opaque, suggesting cataracts. In extreme cases, the globe is ruptured and the lens is extruded. However, many fish die with the globe of the eye still intact. The eye lesions are often bilateral.

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

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

Digenetic trematodes (flukes)

As with cestodes, fish can be intermediate hosts or definitive hosts for digenetic trematodes. Almost all flukes have either a two-host or three-host life cycle, but there is a wide variety of life cycle patterns

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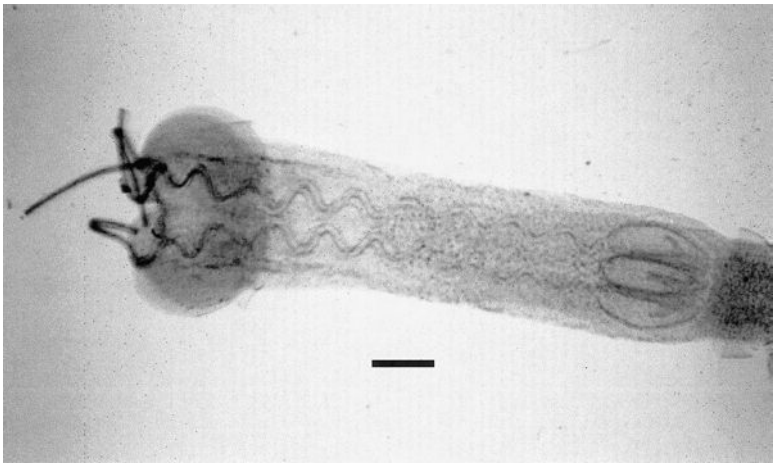


Fig. 3.19. *Gilquinia squali* metacestode from the eye of a chinook salmon. Semichon's acetocarmine. Bar, 0.2 mm.

(Williams and Jones, 1994). With a few exceptions among the marine fish blood flukes of the family Sanguinicolidae, molluscs (either snails or bivalves) are the first intermediate hosts of digeneans and second intermediate hosts may be an invertebrate or a fish. Except in occasional circumstances where the life cycle has been foreshortened, the definitive hosts are vertebrates. The species of concern in marine farming of salmon use either birds or fish as definitive hosts, with the fish serving as the second intermediate host. Cercariae of these species emerge from molluscs and infect the salmonid host by direct penetration of the skin or gills, subsequently developing into a resting stage known as a metacercaria, which may be encysted or unencysted depending upon the final site of infection in the fish.

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

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

Skin diseases caused by digenean trematodes

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

Clinical signs and gross pathology. Infected fish exhibit few to numerous raised black spots up to 1 mm in diameter in the skin,

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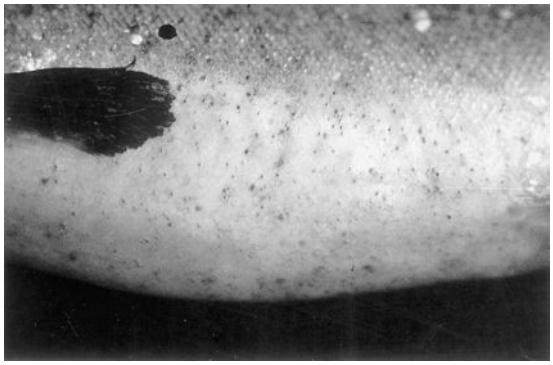


Fig. 3.20. *Cryptocotyle* (black spot) metacercariae in the skin of a Atlantic salmon.

fins, cornea and gills (Fig. 3.20). Occasionally, encysted metacercariae may be found in internal organs. Fish seldom become clinically affected unless they are heavily infected.

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

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

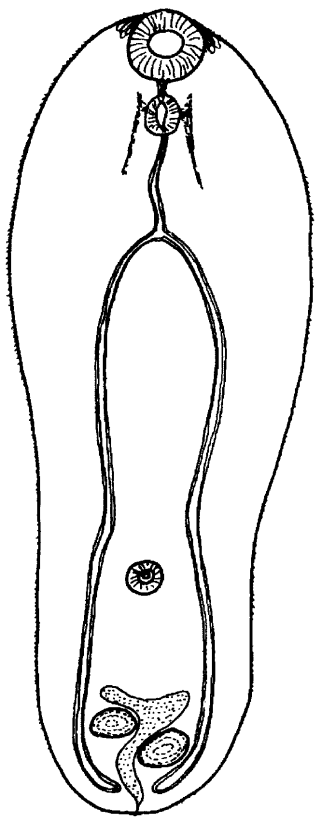


Fig. 3.21. *Cryptocotyle* metacercaria. (From Gibson, 1996.)

Stephanostomum *heart infections*

Heart (pericardial cavity) infections by metacercariae of *S. tenue* caused high

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

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

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

Nematodes

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

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

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

Calanoid copepods are the first intermediate host of *H. aduncum*, whereas fish and various invertebrates, such as polychaetes, barnacles and amphipods, act as second intermediate hosts (Svendsen, 1990).

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

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

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

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4 Infectious Diseases of Coldwater Fish in Fresh Water

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Introduction

Raising fish in fresh water is an ancient practice and the earliest records of aquaculture date from 2000 BC in China, although these relate to aquaculture in fresh warm water (Brown, 1977). The rearing of animals in a cold freshwater environment is a relatively recent phenomenon and dates from the 1930s when trout were first raised in ponds in Denmark (Shepherd, 1988). Since then, coldwater aquaculture has grown exponentially and in 1996 the global cold freshwater aquaculture production including trout, salmon, eels and sturgeon was in excess of 1.5 Mt (New, 1999).

In addition to fish that are cultured exclusively in fresh water, juvenile salmonids are raised in a freshwater environment prior to smoltification and transfer to seawater. Research was first conducted on salmonid rearing and salmonid disease in enhancement hatcheries, and the technology and information accrued were later transferred to the commercial cage culture industry.

For the purposes of this chapter, fresh water refers to zero salinity, and diseases in estuarine environments are included in Chapter 3. Freshwater fish are reared in water that originates from streams or rivers, or in groundwater, and maintained in flow-through or recirculation facilities. The book concerns diseases of finfish and we shall examine those diseases that have relevance to cage and tank culture. Diseases specific to channel or earthen pond culture will not be discussed.

To avoid excessive repetition of information given elsewhere, we have defined infectious diseases of cold fresh water as those that rarely, if ever, occur in water whose temperature exceeds 15°C. The majority of infectious diseases discussed are those that are normally associated with the dominant species cultured in cold fresh water: trout and juvenile salmonids. Many pathogens have been isolated in fish cultured both in seawater and fresh water and for some diseases it was decided that most cases are seen in fresh water and thus are in this chapter. Furunculosis, caused by *Aeromonas salmonicida*, is an example, as is viral haemorrhagic septicaemia. There are other examples where the majority of cases are seen in seawater and we have merely noted the importance of the disease in this chapter. In these cases the reader is referred to the relevant seawater chapter. In cases where the disease may occur equally in fresh or seawater, the pathogen is discussed in this chapter when transmission of the agent in fresh water either horizontally or vertically is the most important aspect. Bacterial

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kidney disease, caused by *Renibacterium salmoninarum*, is an example.

Diseases Caused by Viral Pathogens

Viral haemorrhagic septicaemia

Introduction

Viral haemorrhagic septicaemia (VHS) is the most serious, systemic, contagious disease of farmed rainbow trout (*Oncorhynchus mykiss*) in Europe. The aetiological agent, viral haemorrhagic septicaemia virus (VHSV), trout plague or Egtved virus is an enveloped RNA rhabdovirus (Jensen, 1963; Zwillenberg *et al.*, 1965). Significant and recurring losses in infected fry are reported, although surviving fish are usually immune to reinfection. Disease signs are variable, but an acute to chronic profuse internal haemorrhaging and pale viscera are typical.

Characterization

VHSV is a member of the newly accepted genus *Novirhabdovirus* (cited in Olesen *et al.*, 1999) of the family *Rhabdoviridae*. In Europe five serotypes of VHSV have been described using infectivity neutralization assays (de Kinkelin, 1988). Type 1 (strain F1 from Denmark and the most common), type 2 (Heddedam strain, also from Denmark), type 3 (French strain 23/75) and type 4 represented by the strain 02-84. Both type 3 and 4 were isolated from brown trout (*Salmo trutta*) (de Kinkelin and Le Berre, 1977; Le Berre *et al.*, 1977; Castric *et al.*, 1992). The North American strains of VHSV are genetically different from Egtved virus. An attempt to define serogroups that avoided the overlap of strains within and between these serotypes was undertaken by Olesen *et al.* (1993).

Impact

VHS still remains a serious threat to fish culture in Europe and a disease notifiable to the Office International des Épizooties (OIE). In Denmark, for example, losses in the range of £5–7 million (Sterling) were estimated for 1992–1993. Currently, annual losses are estimated to be £1.5–2.0 million (N.J. Olesen, 1997, personal communication). In Italy, losses attributed to VHS in coldwater areas have also declined over the last 10 years (G. Bovo, 1997, personal communication). In both countries eradication programmes and improved management are contributing factors to the decline of VHSV.

In general, rainbow trout are most susceptible, followed by northern pike (*Esox lucius*) (Meier and Vestergård-Jørgensen, 1980). Natural outbreaks occur in grayling (*Thymallus thymallus*) and whitefish (*Coregonus* spp.). Freshwater species reported as susceptible to VHSV are summarized in Table 4.1. The most

Table 4.1. Susceptible freshwater fish hosts of viral haemorrhagic septicaemia virus (VHSV).

Host/species	Specific name	Reference
Atlantic salmon	*Salmo salar*	Rasmussen (1965)
Brook trout	*Salvelinus fontinalis*	Rasmussen (1965)
Brown trout	*Salmo trutta*	Ghittino (1968)
Chinook salmon	*Oncorhynchus tshawytscha*	Winton *et al.* (1989)
Coho salmon	*Oncorhynchus kisutch*	Winton *et al.* (1989)
Golden trout	*Salmo aguabonita*	Ahne *et al.* (1976)
Grayling	*Thymallus thymallus*	Wizigmann *et al.* (1980)
Hybrid rainbow trout × coho salmon	*O. mykiss* × *O. kisutch*	Chevassus and Dorson (1990)
Lake trout	*Salvelinus namaycush*	Ghittino (1973)
Pike	*Esox lucius*	Meier and Vestergård-Jørgensen (1980)
Rainbow trout	*Oncorhychus mykiss*	Jensen (1963)
Whitefish	*Coregonus* sp.	Ahne and Thomsen (1985)

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pathogenic strain of VHSV is type 1, with outbreaks in freshwater fish occurring at 14°C or below (Meier and Vestergård-Jørgensen, 1979; Meier *et al.*, 1986).

Geographical distribution

VHS was first recorded in the 1930s and is now present in farmed trout throughout most of Western Europe (Wolf, 1988). Outbreaks of VHS have been reported in farmed turbot in France and Germany (Schlotfeldt *et al.*, 1991), Scotland (Ross *et al.*, 1994) and Ireland (J. McArdle, 1997, personal communication). The isolation of VHSV from returning adult chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in the Puget Sound area and Gulf of Alaska, North America (Eaton and Hulett, 1990), was the first indication there was a marine source of VHSV, possibly involving one or more fish species. Currently VHSV has been isolated from an increasing number of marine fish species (Meyers *et al.*, 1992; Ross *et al.*, 1994; Meyers and Winton, 1995; Dixon *et al.*, 1997). The use of molecular techniques including T1 nuclease fingerprinting and sequencing analyses (Oshima *et al.*, 1993; Stone *et al.*, 1997; Mortensen *et al.*, 1999; Smail, 1995; 2000) has confirmed that these North American isolates are distinct from those found in Europe. The significance of these findings to farmed salmonids and European Union (EU) fish health legislation (Directive 91/67/EEC) is reviewed in Chapter 3.

Clinical signs

VHS occurs as an acute to chronic disease. The acute phase is associated with a rapid onset of heavy mortality. Fish are lethargic, anaemic, show dark body colour and exophthalmos, and occasionally an intermittent period of erratic spiralling. Haemorrhaging may be evident in the ocular tissues, skin and the viscera, including the skeletal muscle and intestinal submucosa. Severe glomerular changes with focal necrosis and leucocytic infiltration have been recorded. The liver sinusoids become engorged with blood, with an extensive necrosis and increased pyknotic and karyolytic nuclei. Ascites is present and food is absent from the gastrointestinal tract. Clinical signs in pike fry are generally similar to those described in trout, with the exception of extravasation or bloody swelling, deposition of blood in the muscle and pancreatic necrosis (Meier and Vestergård-Jørgensen, 1980). It is possible that these signs represent a later stage of the infection.

The chronic phase is correlated with lethargy, dark skin colour and bilateral exophthalmia. A haemorrhagic anaemia is reported with a markedly distended abdomen due to oedema in the liver, spleen and kidney. In chronically infected fish the liver sinusoids remain congested, with some hyperplasia of the haematopoietic tissue.

During the latent infection, or nervous phase, there is low mortality and some fish are hyperactive, but otherwise they appear normal.

Diagnostic techniques

Cell culture. A number of serological techniques are used to tentatively identify VHSV; however, definitive diagnosis requires culture of the virus in an established susceptible cell line. A variety of established fish cell lines are susceptible to VHSV and the most sensitive for freshwater isolates from rainbow trout are bluegill fry (BF-2) (Olesen and Vestergård-Jørgensen, 1992). Other cell lines suitable for VHSV detection include chinook salmon embryo (CHSE-214), epithelioma papulosum cyprini (EPC), fathead minnow (FHM), pike gonad (PG) and rainbow trout gonad (RTG-2) (Wolf, 1988; Olesen and Vestergård-Jørgensen, 1992). Biopsy samples or sex products are homogenized, diluted and centrifuged to remove cell debris, filtered and the material added to a growth medium in flasks containing a tissue culture cell monolayer. The cytopathic effect (CPE) results in pronounced cell shrinkage with some cell rounding and in RTG-2 cells staggered edge plaques. When a viral (CPE) is recognized, the supernatant virus is harvested and identified using a serum neutralization test and a susceptible cell line.

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Enzyme-linked immunosorbent assay (ELISA). An ELISA is an accepted technique for the detection of virus in culture supernatant. Way and Dixon (1988) described a polyclonal direct antigen-capture system for VHSV. Further development by Mourton *et al.* (1990) resulted in three forms of the ELISA for virus detection: an indirect ELISA, a direct ELISA and an antigen-capture ELISA that used a variety of monoclonal antibodies to the viral glycoprotein G. Using a highly specific double sandwich protocol, they were able to detect VHSV to a sensitivity of $TCID_{50} = 10^4\ g^{-1}$ tissue (Mourton *et al.*, 1990). A comparative study of tissue culture and immunohistochemistry was reported by Evensen *et al.* (1994), who noted that tissue culture was more sensitive in rainbow trout-infected tissues. When the former was compared with PCR, tissue culture was more sensitive in most rainbow trout carriers (Bruchof *et al.*, 1995).

Virus gene probes. The nucleotide sequences of the nucleoprotein (N) gene from the Pacific Makah strain of VHSV and the virulent French 07-71 strain were compared by Bernard *et al.* (1992). Two strains showed differences of around 13% in sequence homology and enabled Batts *et al.* (1993) to design three cDNA probes. The first probe, universal to all VHSV isolates, was 29 nucleotides long and synthesized to the messenger RNA (mRNA) sequence from nucleotides 430–458 in the open reading frame (ORF) of the *N* gene. A second probe, specific to the Makah strain, was synthesized to a unique 28 base sequence that occurred after the true coding sequence in the *N* gene of the Makah strain, but not in the same region of the 07-71 strain. The third probe, specific to the 07-71 strain, was made to a 22 nucleotide sequence within the *N* gene, where six mismatches occurred with the Makah strain. Each probe was labelled with three biotin molecules at the 5′ end of the primer.

Monoclonal antibody (mAb) capture and polymerase chain reaction (PCR). A highly sensitive and specific mAb capture assay combined with PCR detection of VHSV, using sense primers to the glycoprotein (G) gene in a defined region of 379 base pairs (amino acids 64–195), was reported by Estepa *et al.* (1995). This method gave virus-specific gel electrophoresis products for VHSV with *N* and *G* gene primers.

The identification of a reverse transcriptase-dependent polymerase chain reaction (RT–PCR) specific for the detection of VHSV sequence data and differentiation of serologically similar strains was made by Bruchof *et al.* (1995) and Einer-Jensen *et al.* (1995). The latter group used two primers that amplified sequences from the *N* gene of European and Makah strains of VHSV. An additional primer, which amplified only the American strains, revealed a unique non-coding intron of 20 nucleotides near to the *N* gene. Gene probe work established that the Makah and European strain of VHSV formed two genetically distinct groups and that American and European strains of VHSV could be distinguished using this methodology.

Methods of prevention and control

Legislation introduced within the EU is designed to prevent the movement of potentially infected VHS fish to areas free of this disease. Control and prevention by avoidance and keeping stress to a minimum remain the most effective means of control. In Brittany, parts of Denmark and Italy, eradication of VHSV has been successful using systematic programmes of hatchery disinfection with chlorine, formalin, hypochlorite and iodophore, quarantine measures and restocking with specific virus-free fish and eggs (Enzmann, 1983). The use of ultraviolet (UV) radiation to inactivate virus in the inflowing water is also a practical method for VHSV control; a dose of $1–3 \times 10^3\ \mu W\ s^{-1}\ cm^{-2}$ is required to inactivate VHSV (Yoshimizu *et al.*, 1986).

Host factors in genetic control. Triploid hybrids produced using heat shock of either male coho salmon or brook trout (*Salvelinus fontinalis*) × rainbow trout females are less susceptible to VHSV than either of the

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diploid or triploid rainbow trout (Dorson and Chevassus, 1985; Dorson *et al.*, 1994). This approach shows that host susceptibility of rainbow trout to VHSV has a genetic basis, and this manifests in the uptake of virus and the rate of virus multiplication in the target organs.

Killed vaccine. The testing of a killed vaccine was evaluated by de Kinkelin (1988). β-Propriolactone and formalin at a dilution of 1/5000 and 1/2000, respectively, inactivated strain 07-71. At 10°C intraperitoneal injection was essential to promote an immune response. The range of protection in 5 g fish lasted between 30 and 100 days at 10°C or below. Cross-protection between three serotypes was shown and a high titre of virus-neutralizing antibodies in the sera of vaccinated fish reported.

Live vaccines. The use of a live VHSV vaccine was suggested by Jørgensen (1976). Later, Vestergård-Jørgensen (1982) attenuated the Reva strain (related to the F1 reference strain) through 240 successive subcultures in RTG-2 cells at 14°C and reported a genetically stable strain after 20 backpassages in rainbow trout fry. Fry immersed in 10^4 plaque-forming units (p.f.u.) ml^{-1} water for 1 h below 10°C showed a protective effect up to 150 days after vaccination.

An attenuated F1-related strain obtained by subculture at 25°C in EPC cells was produced and called the F25 strain. Immunization with F25 resulted in an average increase of 30% survival compared with non-immunized fry. Later work showed that a neutralizing antibody response was made to the immunizing variant virus; however, there was no protection against the wild-type virulent virus (de Kinkelin and Bearzotti, 1981).

Recombinant protein vaccines. Advances towards the development of subunit vaccines against VHSV have been described (Lorenzen *et al.*, 1993; Heppell *et al.*, 1998), following the cloning and sequencing of the gene encoding the G of a Danish isolate of VHSV (Benmansour *et al.*, 1997). The major part of this protein, without the leader segment, was expressed in *Escherichia coli* as a protease-cleavage fusion protein. When this protein was renatured and purified, it could stimulate VHSV-specific antibodies when injected into rainbow trout. This provides a method of mass producing viral antigens, and if additional tests are successful, a commercial VHSV vaccine may become possible.

The development of a recombinant vaccine by expressing a glycoprotein in insect cells, using a baculovirus vector, has been examined (Lecocq-Xhonneux *et al.*, 1994). The baculovirus-encoded protein was shown to induce the synthesis of virus-neutralizing antibodies in trout, but stimulates only moderate protection from viral challenge when injected intraperitoneally. A lack of protection after immersion vaccination may limit this approach as a vaccine.

Progress with vaccination. The history of VHS vaccination features a 20 year period of research into the testing of candidate strains, either killed or attenuated (de Kinkelin, 1988). Research is underway to understand more of the disease interactions and the susceptibility of new species, and to determine if the protection from DNA vaccines for VHSV correlates with the presence of neutralizing antibodies.

Infectious pancreatic necrosis

Introduction

Infectious pancreatic necrosis (IPN) is a contagious, economically important viral disease of farmed salmonids, especially juvenile fish. Infectious pancreatic necrosis virus (IPNV) is widespread and occurs in most areas where these fish are cultured. Diseased fish are characterized by acute catarrhal enteritis and are generally dark, anorexic and emaciated. IPNV is a member of the *Birnaviridae*, and particularly affects rainbow trout and brook trout in fresh water. However, this virus has also been isolated from a wide range of fish species in

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brackish water and seawater, in particular salmon post smolts.

Characterization

IPNV is assigned to the family *Birnaviridae*, which are naked icosahedral viruses. IPNV, like other birnaviruses, has a bi-segmented, double-stranded RNA genome (Dobos and Roberts, 1983). Several serotypes are known to be pathogenic to fish (reviewed by Hill and Way, 1995). The genome segment A contains two overlapping ORFs that encode a 106 kDa polyprotein (NH_2–pVP2–NS protease–VP3–COOH), which is cotranslationally cleaved by the protease to generate the major capsid proteins VP2 and VP3. The second ORF overlaps the amino end of the large ORF but in a different reading frame, and encodes a 17 kDa arginine-rich minor polypeptide (Dobos, 1995). Analyses of viral proteins showed that VP2, a capsid protein, is the major structural and immunogenic polypeptide of the virus (Christie *et al.*, 1990; Dobos, 1995).

Impact

IPNV is a disease of fry and fingerlings, with brook and rainbow trout being highly susceptible. Although they can be infected, brown trout, lake trout (*Salvelinus namaycush*), coho and Atlantic salmon (*Salmo salar*) are less likely to develop clinical disease. IPNV also infects non-salmonid species including eels, molluscs and crustaceans, all of which may act as carriers. Mortality and morbidity among marine-reared Atlantic salmon with IPNV is of increasing concern and this is discussed in Chapter 9. Reports of IPNV in *Salmo*, *Oncorhynchus* and *Salvelinus* spp. have been summarized (Reno, 1999).

Infection of susceptible species in fresh water can result in high mortality. It is estimated that over the period 1994–1997, this disease was responsible for fry, parr and presmolt losses of approximately 20 million NKr per annum (T. Poppe, personal communication, 1999). Virus-associated mortality is rapid between 10 and 14°C, and at lower temperatures is prolonged. Water temperature, fish age and the virus strain affect the severity of the disease, as well as the establishment of covert infections in fish. IPNV replicates in kidney, pancreas, gonad, spleen and intestinal epithelium and may be shed from carrier fish via faeces, as well as through the seminal and ovarian fluids. Virus shedding appears to be cyclic and dependent on temperature and/or other environmental stresses. Although stress may induce a recurrence of IPN in fish 6–11 months old, many older fish show no clinical signs.

The apparent increased incidence of IPN is considered to be the result of widespread fish and egg movements between countries, and because of increased sensitivity of diagnostic methods, resulting in improved surveillance practices. It is possible that other commercially important fish species may be infected (Reno, 1999).

IPNV is transmitted horizontally and vertically. However, vertical transmission has been confirmed only in brook trout (Wolf, 1988). The exact mechanism of egg entry or location of the virus within the egg is still unclear. Bebak *et al.* (1998) infected rainbow trout with IPNV by immersion challenge. The fish started to excrete the virus within 2 days of infection and shedding increased and then declined in less than 12 days post-exposure. From this study it was estimated that within 14 days, more than 75% of the population can be infected (Bebak *et al.*, 1998). This gives rise to a rapid spread of IPNV.

Ethanol, methanol, iodophore and chlorine inactivate IPAV (Inouye *et al.*, 1990), but it retains more than 90% of its infectivity after treatment with chloroform or ethyl ether, at pH 3.0 for 60 min. IPNV has been shown to be infective for several years at –70°C and for several months at 4°C. Smail *et al.* (1993) found that IPNV was not deactivated by an acidic pH unless the sample (silage) was heated for at least 2 h at 60°C. This confirms that IPNV is a robust virus with long survival in the environment.

Clinical signs

IPN in young fish is characterized by a sudden onset of increasing mortality. Affected

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fish become dark and rotate their bodies while swimming. The fish show a distended abdomen, exophthalmos and some petechial haemorrhaging on the ventral surface, particularly at the base of the fins. Internally, petechiae, pale liver and spleen are noted with evidence of anaemia confirmed by a reduction in haematocrit. The presence of a gelatinous material in the stomach and anterior intestine and the production of mucoid faecal casts are common.

Histologically, the principal lesions are focal or coagulative necrosis of the pancreatic acinar tissue with acute enteritis, necrosis and sloughing of the intestinal epithelium. Eventually the whole pancreas may be destroyed. Some affected acinar cells show pyknosis and basophilic inclusions and are recognized as products of cell breakdown (Hong *et al.*, 1998). This pathology may extend into the gut mucosa and the renal haematopoietic elements. Necrotic areas may be replaced with fibrous/fatty tissue, and cell sloughing in combination with increased mucous results in a pink to white exudate in the lumen. Hyaline degeneration of skeletal muscle has also been reported. Survivors of an outbreak may exhibit fibrous replacement of the exocrine pancreas. In moribund fish, necrosis of the renal tubules, haematopoietic tissue and liver has been recorded.

Diagnostic techniques

The diagnosis is based on clinical signs, histological examination of tissues and isolation and identification of the virus in kidney and other tissues using cell culture. Established cell lines for IPNV isolation include CHSE-214 (Lannan *et al.*, 1984), BF-2 (Wolf and Quimby, 1966) and RTG-2 (Wolf and Quimby, 1962). *In vivo* it is postulated that the virus causes CHSE cells to undergo apoptosis, followed by post-apoptotic necrosis (Hong *et al.*, 1998). Virus identification can also be accomplished using a staphylococcal co-agglutination (COA) test (Taksdal and Thorud, 1999), and a variety of immunodiagnostic techniques including serum neutralization (Ishiguro *et al.*, 1984), Western blotting (Williams *et al.*, 1994), immunoperoxidase (Nicholson and Henchal, 1994), immunofluorescence (Swanson and Gillespie, 1981), immunohistochemistry (Evensen and Rimstad, 1990) and immunodot (Kirsinger *et al.*, 1999) using polyclonal and monoclonal IPNV antisera. The detection of IPNV by hybridization, using either oligonucleotide DNA probes or cloned cDNA probes (Dopazo *et al.*, 1994), has been reported, although cell culture is considered a sensitive method.

Prevention

There is no effective therapeutic for the control of IPNV. Vertical transmission of the virus can be prevented or at least reduced through the testing of broodstock. This is an important procedure to limit the spread of IPNV, and the use of virus-free stock remains an essential means of eliminating or reducing IPNV. In some cases, increasing water temperature has been recommended to reduce mortality in fresh water.

Immunity against IPNV can be transferred with serum in rainbow trout (Agneil, 1975), establishing a humoral response as an important factor in protecting against IPNV. Some success with the cloning, characterization and expression in insect cells of virus-like-particles (VLPs) of IPNV has been reported (Magyar and Dobos, 1994). Such VLPs are formed by the self-assembly of the coat proteins of a virus and are ideal candidates for vaccines (referred to as subunit vaccines) because the proteins of VLPs retain the characteristic antigenicity of the live virus. However, since they consist only of the virus's protein coat they are non-pathogenic. The virulence associated with IPNV comes from gene segment A, and portions of this segment have been cloned, and recombinant VP2 (rVP2), with structures resembling these epitopes, has been a candidate vaccine; a commercial injectable IPNV vaccine is now licensed in Norway (Frost and Ness, 1997). When salmon were immunized with rVP2, a strong humoral immune response against rVP2 and a moderate response against IPNV was recorded (Frost *et al.*, 1998). The pooled antisera from

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salmon challenged with IPNV neutralized the virus, demonstrating that purified *E. coli*-expressed rVP2 of IPNV induces production of specific antibodies in salmon.

Future studies

Recombinant DNA vaccines are likely to offer long-term immunity, but have to be reliable, safe and acceptable to the regulatory authorities. The development of a subunit vaccine for IPNV, combined with studies on its potential to confer protection against vertical transmission of the virus, will make important contributions to fish health management

Sleeping disease of rainbow trout

Introduction

Sleeping disease (SD) is an infectious viral condition that has been described in farmed rainbow trout and occasionally in coho salmon (Boucher and Baudin Laurencin, 1994, 1996). The first record was in Italy (Ghittino, 1987), but an occurrence in Brittany, France, a few years later prompted more detailed observations. The disease has recently been confirmed in farmed trout in Scotland using light microscopy and PCR (Bruno, 2002, unpublished observations). SD is characterized by the unusual behaviour of affected fish, which remain on their sides at the tank bottom. A toga-like virus has been isolated from infected rainbow trout (Boucher *et al.*, 1994; Castric *et al.*, 1997) and the name sleeping disease virus (SDV) has been proposed (Castric *et al.*, 1997).

Characterization

Stained viral suspension has revealed intact enveloped particles with an external diameter between 55 and 65 nm (Castric *et al.*, 1997). Using cDNA cloning and nucleotide sequencing of part of the SDV RNA genome it has been possible to assign it to the genus *Alphavirus* (family *Togaviridae*) (Villoing *et al.*, 2000a).

Impact

Boucher and Baudin Laurencin (1994) reported that SD affected 15% of the freshwater rainbow trout in cage culture farms in Brittany, France, although mortality was low. Infected fish may stop feeding for several weeks, resulting in a loss of growth.

Farmed rainbow trout and occasionally coho salmon reared in fresh water are the only known species naturally affected by SD (Boucher and Baudin Laurencin, 1994, 1996), although experimental infection in Atlantic salmon has been successful (Boucher and Baudin Laurencin, 1994). Stress induced by *Gyrodactylus* spp. feeding on the fish was proposed as a contributing factor to SD (Ghittino, 1987). The similarity between SDV and salmon pancreas disease virus (SPDV) and the subsequent pathology (Nelson *et al.*, 1995; McLoughlin *et al.*, 1996) in marine-reared Atlantic salmon has promoted the idea that these agents might be similar (Boucher and Baudin Laurencin, 1996). Moreover, an acquired cross-protection against SDV and SPDV in laboratory studies supports this hypothesis (Boucher and Baudin Laurencin, 1996). At present, SD has been described only in France (Boucher and Baudin Laurencin, 1994) and Italy (Ghittino, 1987). A review of SPDV is given in Chapter 3.

Clinical signs

Clinical signs include fish lying or resting on the bottom of the tank or raceway. When disturbed the fish swim for short periods and then return to the tank bottom. In experimental studies, necrosis of the exocrine pancreas occurred in the first week after injection. Tissue changes included a rounding of acinar cells, and later lymphocyte and fibrocyte infiltration. Focal heart lesions can develop and these are characterized by hyalinization and loss of striation. Within the red muscle an increase in cellularity and disappearance of fibres have been recorded (Boucher and Baudin Laurencin, 1996). The histopathology observed in experimental and natural outbreaks of SD is similar (Boucher and Baudin Laurencin, 1994).

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Diagnostic techniques

Provisional diagnosis is based on the characteristic behaviour of the fish and associated pathology. Recently, a virus responsible for causing SD has been isolated on a CHSE-214 cell line (Castric *et al.*, 1997). Kidney homogenate in Glasgow minimal essential medium (GMEM) inoculated on to cell lines with fetal calf serum and antibiotics showed small groups of refringent round cells after three passages. The development of a sensitive RT–PCR-based method for the diagnosis of SD has recently been reported (Villoing *et al.*, 2000b).

Prevention

No treatment for SD is available. Boucher and Baudin Laurencin (1996) have shown cross-protection between SD and SPDV, and this may allow young fish to be exposed to SD with the benefit of protection against SPDV in seawater.

Future studies

Comparative studies of the viruses causing SD and SPD in fish will be valuable and help in our understanding of these conditions. Furthermore, long-term protection recorded in salmon parr against SPDV following transfer to seawater (Houghton, 1994) may also be similar for SD in fresh water. The cross-protection between SDV and SPDV may allow young fish to be exposed to SD while protecting against SPDV.

Infectious haematopoietic necrosis

Introduction

Infectious haematopoietic necrosis (IHN) is an acute systemic rhabdovirus infection. IHN occurs primarily among cage-cultured Pacific salmonids in fresh water from coastal North America, although outbreaks in wild stocks have been documented. The first reports of serious epizootics attributed to IHN were made in the late 1940s and 1950s (Rucker *et al.*, 1953). Losses of up to 100% have been reported (Wolf, 1988).

Characterization

The causative agent of IHN was first isolated from sockeye salmon (*Oncorhynchus nerka*) and subsequently from rainbow trout (Parisot *et al.*, 1965). IHN virus (IHNV) was isolated from salmonids in Japan (Sano *et al.*, 1977), Italy, France (Baudin-Laurencin, 1987; Bovo *et al.*, 1987) and in other parts of Europe, evidently spread through fish transportation (Miller *et al.*, 1998). However, it is possible that the virus was already present in these countries and detected because of improvements in methodology. IHNV shares the bullet shape of other members of this group (McCain, 1970). IHNV has an unsegmented, negative-sense, single-stranded RNA genome of approximately 11,130 nucleotides. These encode a nucleoprotein, a phosphoprotein, a matrix protein, a glycoprotein, a non-virion protein and a polymerase (Morzunov *et al.*, 1995; Schutze *et al.*, 1995).

Clinical signs

Clinical disease is most common in salmonid fry with extensive yolk-sac haemorrhage, swollen abdomen, darkening of the skin and petechial haemorrhage on the abdomen and around the eyes. One characteristic sign is the long gelatinous faecal casts, which can be seen trailing from the vent of affected fry. Anaemia can also be seen, as well as haemorrhage in the visceral tissues. Dark red areas may develop behind the head. Ascites and stomach distension can also be observed. However, gross clinical signs are frequently absent and the characteristic necrosis of the haematopoietic tissues from which the virus derives its name is seen only by histopathological examination.

Impact

In recently hatched fry, mortalities due to IHNV can reach 80–100% within 8–15 days at 10–12°C. Mortality in fish older than

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1 year does not usually exceed 25% (Winton, 1997). Generally, fish less than 100 g have the highest susceptibility to IHNV, such as rainbow trout and Pacific salmon, and particularly sockeye salmon. Fish that survive IHNV infections can be chronic carriers of the virus with 2–4% of surviving fish exhibiting scoliosis, which renders them unmarketable (Winton, 1997).

Husbandry

Millions of eggs from fish diagnosed for IHNV have been destroyed on the assumption that the virus is transmitted within eggs. Transmission within the yolk has not yet been conclusively determined. However, screening for infected stocks, and the use of virus-free water and eggs that are derived from certified virus-free broodstock, are common practice. Salmonid enhancement or production hatcheries generally couple these practices with iodophore surface-disinfection of the certified eggs and continuation with a programme of iodophore disinfection at regular intervals throughout egg incubation. At present, these are the only effective control measures available for IHNV (Winton, 1991).

Vaccines

Considerable progress has been made in research on vaccine development (Winton, 1997). The glycoprotein of the virus elicits neutralizing antibodies that are protective (Engelking and Leong, 1989a,b). The neutralizing epitopes of the glycoprotein have been studied and mapped (Xu *et al.*, 1991; Huang *et al.*, 1996). Subunit vaccines have been developed, using part of the IHNV glycoprotein cloned into *E. coli*. These have been reported to be safe, effective and inexpensive (Noonan *et al.*, 1995). Emmenegger *et al.* (1995) developed synthetic peptides that included amino acid sequences representing the neutralizing domains on the glycoprotein. These were coupled to bovine serum albumin carriers or synthesized on lysine cores to produce multiple, eight-branched antigenic peptides. Trout injected with these peptides (with Freund's complete adjuvant) did not produce sera with neutralizing activity (with the exception of one individual); control fish injected with an attenuated strain of IHNV demonstrated high neutralizing activity in their sera. This suggests that the peptides alone have low immunogenicity in trout.

DNA vaccines are relatively new for fish health. Anderson *et al.* (1996) produced plasmid vectors that encoded the IHNV *N* and *G* genes, with a cytomegalovirus immediate early promoter. The constructs were injected into rainbow trout fry, which were subsequently challenged with IHNV by water-borne exposure. Fish injected with the *G* or *G* + *N* constructs produced higher neutralizing antibody titres and were significantly protected against the challenge. DNA vaccines delivered by injection hold promise for large fish, although a more cost-effective delivery system is required for smaller fish.

Spring viraemia of carp

Introduction

Spring viraemia of carp (SVC) is an acute, systemic rhabdovirus disease of the common carp (*Cyprinus carpio*), its varieties and other cyprinids. Significant losses occur during the spring in young and adult fish. The causal agent of SVC is a member of the *Rhabdoviridae* and is known as rhabdovirus carpio or spring viraemia of carp virus (SVCV) (Lenoir and de Kinkelin, 1975). This disease is notifiable to the OIE.

Characterization

Several conditions are now recognized as synonyms of SVC including infectious dropsy of carp, red contagious disease, infectious ascites and haemorrhagic septicaemia (Fijan, 1972). Several groups have established the aetiology of this virus (Bucke and Finlay, 1979; Ahne, 1980). SVCV is a typical bullet-shaped virus measuring 90–180 nm in length with a regular

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array of spicules on the surface. Work on the characteristics of the large (L) gene (Björklund *et al.*, 1995), glycoprotein gene and internal gene junctions place the SVCV firmly in the genus *Vesiculovirus* of the family *Rhabdoviridae* (Björklund *et al.*, 1996). Recently, Johnson *et al.* (1999) reported the nucleotide and predicted amino acid sequences of the *G* genes of the warmwater rhabdovirus of penaeid shrimp (RPS) and snakehead rhabdovirus (SHRV), and found over 99% similarity to the *G* nucleotide sequence from SVCV. From this study they suggested that RPS and SVCV may be the same virus.

Impact

SVC is prevalent in farmed carp in Italy, France, Spain, Romania, Slovak Republic, Hungary and Israel, and is believed to be restricted to Europe and parts of Asia. In Germany, serologically positive fish have been recorded, but without clinical disease (Wizigmann *et al.*, 1980).

The main species affected by SVCV are crucian carp (*Carassius carassius*) (Kölbl, 1975), grass carp (*Ctenopharyngodon idella*) (Roudikov, 1980), bighead carp (*Hypophthalmichthys molitrix*) (Roudikov *et al.*, 1975) and sheathfish (S*iluris glanis*) (Fijan *et al.*, 1984). Young carp are the main susceptible group (Shchelkunov and Shchelkunov, 1989), followed by grass carp and bighead × silver carp hybrids. SVC is prevalent during periods of rising water temperature with mortality occurring above 7°C, and a maximum between 10 and 15°C. Above 23°C mortality is rare and clinical disease absent (Fijan, 1976). Losses attributed to SVC are in the order of 30%, but may reach as high as 70%. SVCV enters through the gill epithelium following adsorption to the plasma membrane and receptor-mediated endocytosis (Granzow *et al.*, 1997). The infection is maintained in the population through the shedding of virus during the winter months (Baudouy *et al.*, 1980). The carp louse (*Argulus foliaceus*) and leech (*Philometra geometra*) can also act as vectors (Ahne, 1985).

Clinical signs

Clinical signs vary and may even be absent, particularly during the early stages of infection. Signs in infected fish include dark pigmentation, lethargy, loss of balance, swollen abdomen, exophthalmia, pale gills, petechial haemorrhage and trailing white or yellowish faecal casts (Bachmann and Ahne, 1974; Fijan, 1975). Internally, haemorrhaging, peritonitis, copious mucus in the intestine and splenomegaly have been reported. Histologically, varying degrees of oedematous perivasculitis leading to multifocal degeneration, necrosis, clogged kidney tubules, hyaline degeneration and vacuolation have been described from experimentally infected carp (Negele, 1977). Osad haja and Rudenko (1981) also reported myocardial necrosis with lymphocyte infiltration, haemorrhagic spleen and acute enteritis.

Diagnostic techniques

The diagnosis of SVC primarily requires the isolation of the virus in cell culture. Although other diagnostic methods have been developed, virus isolation in cell culture is the most sensitive and therefore widely used. Diluted tissue samples of encephalon or ovarian fluid from brood fish are inoculated on to epithelioma papillosum of carp (EPC) or other sensitive cell lines and held at 15°C for 7 days (OIE, 1997). The CPE involves clear cell rounding and quick spreading across the cell sheet. The diagnosis of SVC by ELISA has been documented (Rodak *et al.*, 1993), but has a lower sensitivity than that achieved with cell culture. Similarly, the indirect fluorescent antibody test (IFAT) has been used (Faisal and Ahne, 1984; OIE, 1997) but this technique does not distinguish between SVCV and the closely related contagious disease, pike fry rhabdovirus (PFRV) (Jørgensen *et al.*, 1989). A competitive immunoassay for detection of SVCV fish antibodies has been tested on experimentally and naturally infected carp and is considered a sensitive method when compared

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with a neutralization test (Dixon *et al.*, 1994). Furthermore, Ahne *et al.* (1998) have described a ribonuclease protection assay (RPA) using an SVCV *G* gene probe that differentiated isolates of SVCV and PFRV.

Prevention

No vaccine is available for SVC, although trials with experimental live and attenuated strains have been encouraging in laboratory studies (Fijan *et al.*, 1977; Kölbl, 1980; Dixon *et al.*, 1994). Work by Macura *et al.* (1983) with inactivated vaccines showed increased survival rates in field trials, but less success under laboratory conditions. Unfortunately, the promising live vaccine experiments have not been followed by appropriate licensing documentation (Midtlyng, 1997). In some countries farmed carp are serologically positive for SVCV and therefore the feasibility of using live attenuated strains in these areas remains problematic (Kölbl, 1980). Wolf (1988) proposed a genetic selection programme, but this was not developed. As with many diseases, overcrowding, water quality and handling stress increase susceptibility to SVC. Currently, prevention relies on restricting the movement of infected fish, carrying out health checks, purchasing certified healthy stock, disinfecting eggs and practising good husbandry. SVC may be avoided in farms supplied with spring or well water.

Recommendations

SVC is widespread in Europe and eradication appears unlikely. Information is required to identify vaccine effectiveness under different regimes. The susceptibility of selected fish strains to SVCV and an expansion of the genetic selection programme are priorities. Future vaccination strategies involving immersion or oral vaccination of fry were suggested by Clem *et al.* (1996). Improvements to ELISA and PCR diagnosis would also be beneficial. Johnson *et al.* (1999) has strongly suggested that SVCV is identical to the rhabdovirus RPS of penaeid shrimp in Hawaii. Further studies as recommended by Johnson *et al.* (1999) are required to establish the enzootic nature of the virus in the Pacific region and the occurrence of the virus in both vertebrate and invertebrate hosts.

***Oncorhynchus masou* virus**

Introduction

Oncorhynchus masou virus (OMV) or salmonid herpesvirus is a virulent pathogen of salmonids of the genus *Oncorhynchus*, particularly affecting juvenile fish in fresh water. Infection is reported in wild and cage-cultured fish, but only from Japan. The virus typically causes an acute systemic infection in juvenile fish, especially alevins. Fish surviving infection frequently become subclinical carriers of infection. Kimura and Yoshimizu (1989) have published a review article on OMV.

Characterization

Electron microscopy shows OMV to be an enveloped Type II salmonid herpesvirus of 200–240 nm in diameter (Tanaka *et al.*, 1987). Two closely related strains of a herpesvirus have been isolated from trout in North America. These isolates are distinct from OMV and other Japanese herpesviruses, indicating they are not the result of the transfer of infected fish or eggs between continents.

Impact

Infection with OMV is listed by the OIE as a notifiable disease. OMV was first reported from ovarian fluids of a landlocked population of adult masou salmon (*O. masou*) in Hokkaido, Japan and is now widespread in the northern regions of that country. Under natural conditions, outbreaks of disease due to OMV affect only *Oncorhynchus* spp., but several other salmonid species are susceptible to OMV including fry of coho salmon, kokanee salmon (*O. nerka*), chum salmon (*Oncorhynchus keta*), and rainbow trout. Furthermore, the viral agent has limited survival outside the host. The most

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significant losses are recorded in coho salmon (Kimura and Yoshimizu, 1989). The disease is more common in the freshwater phase of the salmonid life cycle and alevins of 1 month old are particularly susceptible to infection. However, larger fish (up to 1 kg in weight) may also be affected by the disease. Horizontal and vertical infection may play a role in the transmission of OMV (Kimura and Yoshimizu, 1989).

Clinical signs

Infection with OMV is a systemic and frequently lethal condition that is associated with oedema and multiple haemorrhage. The virus multiplies in endothelial cells, haematopoietic tissue and hepatocytes. Affected fish are dark and often have severe exophthalmos with petechial haemorrhage under the lower jaw and along the ventral surface. A white mottled appearance of the liver is recorded, progressing to a pearly white colour of the whole organ. A pale kidney and a multifocal, severe necrosis of the liver is also common (Tanaka *et al.*, 1984). Gill epithelial cells become swollen and slough. There is a marked splenomegaly with associated necrosis of the ellipsoids, and the digestive tract is generally devoid of food.

Infected juvenile coho salmon develop ulcers on the skin, lesions in the liver and tumours on the mouth, caudal fin, operculum and body surface. Infected rainbow trout show few signs, mainly limited to ulcerative lesions of the skin, intestinal haemorrhage and lesions in the liver (Kimura *et al.*, 1981).

Studies involving experimental infection with OMV have shown that there is some variation in histopathological findings between species of juvenile salmon. The kidney is the apparent target organ in chum salmon, with necrosis of the haematopoietic tissue, hyaline droplet degeneration and pyknosis. Partial necrosis occurs in the spleen, liver, pancreas and stomach, whereas in masou salmon, haematopoietic necrosis has been reported (Tanaka *et al.*, 1984).

OMV has oncogenic potential and induces a mandibular epithelial neoplasm and other tumours of the fins, body surface and cornea in surviving fish (Ishigaki *et al.*, 1987; Yoshimizu *et al.*, 1988). These tumours are characterized as papillomatous and consist of several layers of proliferating epithelial cells supported by thin connective tissue. Multiple mitotic figures confirm the proliferative nature of the tumour. Post-infection, tumours may be found for up to a year (Yoshimizu *et al.*, 1988). Fish surviving the septicaemia frequently become carriers of infection and may shed the virus in the faeces, urine, sexual products and, probably, in the skin mucus. In clinically infected fish, the highest titre of virus occurs in the kidney, liver, spleen and in tumour tissue (Ishigaki *et al.*, 1987). OMV may be transmitted horizontally, via exposure to a significant titre of virus in the freshwater environment. Exposure to a higher titre of virus would be required to initiate infection in adult fish.

Diagnostic techniques

The diagnosis of OMV involves virus isolation from diseased fish, using cell lines such as CHSE-214 or RTG-2 and a serum neutralization test with a specific OMV antiserum. Optimal temperature for virus isolation is 15°C (Kimura and Yoshimizu, 1989). Work by Gou *et al.* (1991) reported a DNA probe able to detect 10 copies of viral DNA per cell.

Prevention

The spread and prevalence of infection of OMV may be managed by disinfecting eyed ova and treating hatchery water with UV radiation. Fish-to-fish transmission of OMV is effected by holding 5-month-old fry with fry infected by immersion. The resulting rate of mortality was similar to that observed as a result of infection by immersion (Kimura and Yoshimizu, 1989).

Direkbusarakom *et al.* (1996) showed that some traditional Thai herb extracts reduced the CPE due to OMV in CHSE cells by 50%. The extracts demonstrated low toxicity to the CHSE cells; however, considerably more research is warranted before the

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efficacy of herb treatments for salmon is known.

Erythrocytic inclusion body syndrome

Introduction

Erythrocytic inclusion body syndrome (EIBS) and other inclusion syndromes, including those caused by viral erythrocytic necrosis virus (VENV) (Evelyn and Traxler, 1978) and intraerythrocytic virus (Landolt *et al.*, 1977), are cytoplasmic viral infections of the erythrocytes of salmonids and other fish groups. In Japan, EIBS is characterized by a severe anaemia in cultured coho salmon, whereas in farmed Atlantic salmon from Scotland and Ireland there is no significant association between the EIBS virus and clinical disease (Rodger and Richards, 1998).

Characterization

Natural infections of EIBS virus have been described in pink (*Oncorhynchus gorbuscha*), chum and chinook salmon in the Pacific Northwest of the USA (Piacentini *et al.*, 1989). EIBS has been reported in farmed Atlantic salmon in Norway (Lunder *et al.*, 1990) and Scotland (Rodger and Richards, 1998), coho salmon in Japan (Okamoto *et al.*, 1992) and rainbow trout in Ireland (Rodger *et al.*, 1991). The virus particles that have been examined are icosahedral, enveloped and in salmonids measure 70–80 nm in diameter (Arakawa *et al.*, 1989; Thorud *et al.*, 1990; Rodger *et al.*, 1991). Tentatively, the viruses causing EIBS and EIBS-like conditions are placed in the *Iridoviridae* (Reno *et al.*, 1978).

Impact

Natural disease outbreaks have been reported in Atlantic salmon, coho salmon, chinook salmon and rainbow trout (Lunder *et al.*, 1990; Michak *et al.*, 1992). The virus can be transmitted in fresh water (MacMillan and Mulcahy, 1979; Leek, 1987) and its presence in young yolk-sac fry and alevins could suggest vertical transmission (Rohovec and Amandi, 1981). Fish reared from eggs in borehole water have been confirmed positive for EIBS virus, supporting the likelihood that vertical transmission of this virus occurs. EIBS has been associated with mortality exceeding 25% (Piacentini *et al.*, 1989); however, virus inclusions are also noted in apparently healthy fish (Rodger *et al.*, 1991; Rodger and Richards, 1998). Mortality directly attributed to EIBS virus is difficult to establish as other pathogens including *Flavobacterium psychrophilum* and IPNV often occur concurrently (Thorud *et al.*, 1990; Evensen and Lorenzen, 1997). Field observations suggest that EIBS affects the susceptibility of salmonids to secondary pathogens (Arakawa *et al.*, 1989; Piacentini *et al.*, 1989).

Clinical signs

Lethargy, pale livers and internal haemorrhage have been reported in chinook and coho salmon (Leek, 1987; Takahashi *et al.*, 1994). The initial stages of EIBS begin with an 11 day incubation period (at 12°C) but with no pathological changes, followed by the appearance of inclusions in increasing numbers, leading eventually to cell lysis and anaemia (Piacentini *et al.*, 1989). Although cell lysis may be virus-directed, the fish immune system may also contribute to the lysis of infected cells by sensitized lymphocytes and lymphokines being released in response to viral antigens. Fish with EIBS may appear healthy, but in North America and Japan anaemia has been consistently reported with an increased incidence of other infections (Piacentini *et al.*, 1989; Takahashi *et al.*, 1992; Maita *et al.*, 1998). Lunder *et al.* (1990) reported no liver lesions in Atlantic salmon or other specific clinical signs associated with this infection. Increased haemosiderin has been reported in experimentally and naturally infected chinook salmon in Idaho, USA (Foott *et al.*, 1992), but this may also result from other infections including vibriosis. Lipid peroxidation (Sakai *et al.*, 1994) and fatty acid changes in hepatic phospholipid

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(Maita *et al.*, 1996) occur in sea-cultured coho salmon naturally infected with EIBS virus. However, Maita *et al.* (1998) suggested that diet could also influence these factors.

Two types of inclusions in red blood cells are described, type A and B. The former is characterized by large single inclusions and type B by small multiple inclusions (Michak *et al.*, 1992; Rodger and Richards, 1998). Salmon infected in fresh water mainly show type A inclusions, whereas type B inclusions are dominant in seawater (Rodger and Richards, 1998). This may reflect a level of maturation of the virus or increased infection levels resulting in more type B inclusions. Although many post-smolts carried EIBS-like virus prior to transfer to seawater, there was no correlation between type B inclusions, clinical disease and mortality (Rodger and Richards, 1998). Similarly, Jarp *et al.* (1996) found no correlation between EIBS and plasma chloride levels following transfer to seawater.

Electron microscopy of erythrocyte inclusions in naturally infected chinook salmon showed viral particles 75 nm in diameter, randomly scattered throughout the cytoplasm (Leek, 1987). In Atlantic salmon, particles measure 80 nm, are hexagonal in shape with an electron-dense outer margin and are located in packages within an adjacent membrane (Thorud *et al.*, 1990). Viral particles occurred between the inner and outer layer of the nuclear membrane.

Diagnostic techniques

The examination of methanol-fixed and Leishman–Giemsa-stained blood smears for cytoplasmic inclusion bodies in red cells is a diagnosis for EIBS (Thorud *et al.*, 1990). Staining with pinacyanol chloride may increase staining consistency (Leek, 1987; Yasutake, 1987). Acridine orange-stained blood films examined by fluorescent microscopy provide an additional method for displaying intraerythrocytic inclusions (Piacentini *et al.*, 1989). To date, the virus has not been grown in tissue culture, nor is it known if other cell types are infected.

Prevention

There are insufficient data on EIBS and EIBS-like conditions to formulate adequate preventive measures. Coho salmon infected in fresh water are able to recover and are apparently resistant to reinfection for at least 242 days without any evidence of anaemia. Acquired resistance is maintained after transfer to seawater (Okamoto *et al.*, 1992).

Recommendations

In some cases, or in certain groups of fish, severe anaemia, pale liver and internal haemorrhage are associated with erythrocytic inclusions, whereas in other groups inclusions may be present but with no apparent effect (Rodger and Richards, 1998). The reason for this is unclear and further study is necessary. Similarly, gaps in our knowledge concerning the possible correlation between a focal cardiomyopathy, infectious dose and the possibility that other cell types might be infected should be examined. Long-lasting immunity has been described in coho salmon (Okamoto *et al.*, 1992) and such studies could be extended to other susceptible groups.

Diseases Caused by Bacterial Pathogens

Furunculosis

Introduction

Aeromonas salmonicida, the causative agent of furunculosis, is one of the most studied bacterial pathogens of fish. It is widespread, having been documented in Europe, North America, Japan, Korea, Australia and South Africa. Furunculosis has been an important disease in wild and cultured stocks of fish since the 1890s (Emmerich and Weibel, 1894). In fact, losses due to furunculosis were of such a magnitude in wild salmon in Scotland that the Furunculosis Committee was created. The reports of that committee were the basis

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of the Diseases of Fish Act in 1937, the earliest fish disease legislation in the world.

Although *A. salmonicida* is primarily a freshwater pathogen, outbreaks of furunculosis are often seen in seawater. The pathogen is not limited to salmonids and many species of fish are affected. Several excellent reviews of *A. salmonicida* and furunculosis are available (Bernoth, 1997; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999).

Characterization

A. salmonicida is a non-motile bacterium and readily isolated from the kidney and other organs. Most isolates produce a characteristic dark brown diffusing pigment in culture. It is traditionally described as Gram-negative, non-motile, fermentative, producing catalase and oxidase with no growth at 37°C. Classification of the subspecies of *A. salmonicida* has been the subject of much discussion. McCarthy and Roberts (1980) proposed a division of *A. salmonicida* into three subspecies. This view was supported by subsequent studies (Belland and Trust, 1988; Munro and Hastings, 1993). The following is the classification scheme as summarized by Munro and Hastings (1993).

- Group 1 strains. *A. salmonicida salmonicida*. Arbitrarily described typical strains derived from salmonid fishes.
- Group 2 strains. *A. salmonicida achromogenes*. Atypical strains derived from salmonids that represent aberrant strains that show variation in some biochemical properties and include the former species *achromogenes* and *masoucida*.
- Group 3 strains. *A. salmonicida nova*. Atypical strains associated with disease in non-salmonid fishes.

The definition of typical or atypical refers to biochemical and morphological characteristics, such as the production of pigment and extracellular proteases (McCarthy and Roberts, 1980; Böhm *et al.*, 1986; Wiklund and Dalsgaard, 1998).

Phage typing is used extensively to distinguish different isolates of *A. salmonicida*. Popoff (1984) showed 14 phage types of *A. salmonicida* using eight phages.

The physical map of a pathogenic strain A449 was recently determined by members of the Canadian Bacterial Diseases Network, and was shown to be representative of eight other furunculosis-causing strains isolated from salmonids in Canada, the USA, Europe and Japan (Umelo and Trust, 1998). These eight strains belong to a larger group of more than 40 strains that have been shown to be homogeneous by a variety of classical and molecular methods (Vaughan, 1997). These strains are considered to belong to the subspecies *A. salmonicida salmonicida*.

More than 63 unique genes have been sequenced from *A. salmonicida* (Table 4.2), and some have been subjected to functional analysis including overexpression, directed mutagenesis or allelic replacement (Vaughan, 1997). The best-characterized strain of *A. salmonicida* is A449 (Umelo and Trust, 1998). Its 4658 ± 30 kbp genome has a G + C composition of 55%. Approximate map positions have been determined for 26 protein-coding genes (including some associated with virulence), large and small subunit rDNAs, and four insertion elements. Although some associations were noted, overall their distribution around the circular genome appears random (Umelo and Trust, 1998).

Extensive research has been conducted on the pathogenicity of *A. salmonicida*. The regular surface protein layer (the A-layer) is an important factor in pathogenicity. So too are the lipopolysaccharide (LPS) layer and the possession of cytotoxic activity, as measured in *in vitro* studies (Garduño *et al.*, 1993, 1997; Daly *et al.*, 1996).

Researchers have examined the mechanisms of innate resistance in salmonids to *A. salmonicida*. Hoover *et al.* (1998) identified pentraxins and other lectin-like proteins that bound to the LPS of *A. salmonicida*. Ewart *et al.* (1999) isolated and characterized a mannose-binding lectin from Atlantic salmon sera, and then

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Table 4.2. Published gene sequences from *Aeromonas salmonicida*. Data from the National Centre for Biotechnology Information (NCBI) website (www.ncbi.nih.gov).

16 S rRNA gene
5 S rRNA
abcA protein (ATP-binding cassette membrane transporter)
Acetyl-CoA carboxylase subunit
asoA protein
asoB protein
Autoinducer synthesis protein asaI
Chaperonins groEL, groES
2′,3′-cyclic phosphodiesterase
3-Dehydroquinase (*aro*D gene product)
Dihydrofolate reductase
DNA gyrase
DNA gyrase subunit A (*gyr*A gene product)
5-Enolpyruvylshikimate-3-phosphate synthase
exeC protein
exeD protein
exeE protein
exeF protein
exeG protein
exeN protein
Ferric siderophore receptor
Flagellins flaA, flaB, flaG
General secretion pathway proteins (or precursors thereof) C, D, N
Glycerophospholipid–cholesterol acyltransferase
Haemolysins (or precursors thereof) 1, 2, 3, 4 and β
Hypothetical 21.5 kDa protein in *asa*R–*cdp*D intergenic region
Hypothetical protein in *exe*N 3′ region (not transcriptional regulator)
Hypothetical proteins, other
Hypothetical transcriptional regulator in *exe*N 3′ region
Major cold-shock protein (*csp*A gene product)
Major outer membrane proteins ompAI, ompAII
N-acyl homoserine lactone synthase (*asa*I gene product)
oriC replication inhibitor (*ici*A gene product)
3-Phosphoshikimate 1-carboxyvinyltransferase (*aro*A gene product)
Pilin proteins pilC, pilD
Pilus assembly proteins tapB, tapD
Polytopic cytoplasmic membrane protein
Porin, maltose-inducible (*lam*B gene product)
Prepilin peptidase tapD
Prepilins sfpA, tapA
Proteolysis tag (coded portion)
recA protein
Ribosomal protein S20
satA protein
Secretion proteins, including *aps*E gene product
Serine proteases and protease precursors, including *asp*A gene product
Transcriptional activator (*asa*R gene product)
vapA (S-layer protein precursor; tetragonal surface virulence array protein, or A-protein)
yggA protein

Ottinger *et al.* (1999) demonstrated *in vitro* activity against *A. salmonicida* by this lectin. They postulated that this and possibly other lectins are important as part of the innate defence system against *A. salmonicida*.

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Impact

All life stages of salmonids are susceptible to furunculosis, although young fry are less frequently infected (Munro and Hastings, 1993). Serious losses have been seen in farmed and wild salmonid stocks.

Infections by *A. salmonicida* have been documented in non-salmonids. The source of these infections is unknown. Infection by *A. salmonicida* subsp. *nova* is indigenous in cyprinids and other non-salmonids (Cornick *et al.*, 1984; Morrison *et al.*, 1984; Antychowicz and Rogulska, 1986; Evenberg *et al.*, 1986).

MacKinnon has undertaken an extensive study to determine the degree of species specificity in virulence of atypical *A. salmonicida* isolates from non-salmonids. She found that the isolates were the most virulent within the species from which they were derived, and that cross-challenges with other species showed varying degrees of virulence (A.-M. MacKinnon, Department of Fisheries and Oceans, Moncton, New Brunswick, personal communication).

Clinical signs

External signs of furunculosis depend upon the time course of the disease (Munro and Hastings, 1993). Fish may die from an acute infection without any signs of disease; chronically infected fish may present signs such as darkening, lethargy and petechial haemorrhage at the base of the fins. Fish may also exhibit furuncles from which the disease derives its name (Fig. 4.1). These furuncles may ulcerate to release necrotic tissue debris and bacteria. Internally, ascites may be seen. Frequently the intestine is empty of food, but may contain mucus and bloody cellular debris. Blood vessels around the pyloric caeca and intestine may be inflamed and the kidney swollen and liquefied. The haematocrit is often depressed and frequently the leucocyte cell layer is significantly reduced or absent. A widespread acute or subacute haemorrhaging occurs in the viscera. The kidney is soft, the spleen swollen and the liver pale. Toxins released by the bacteria liquefy the tissue causing an intense inflammatory reaction, with associated swelling causing ulceration with an exudate of blood stained tissue rich in bacteria. Bacterial invasion of the compact myocardium results in necrosis and an increase in monocytes within the subepicardial space. Foci of bacteria occur in many organs and become the centre of a generalized tissue necrosis.

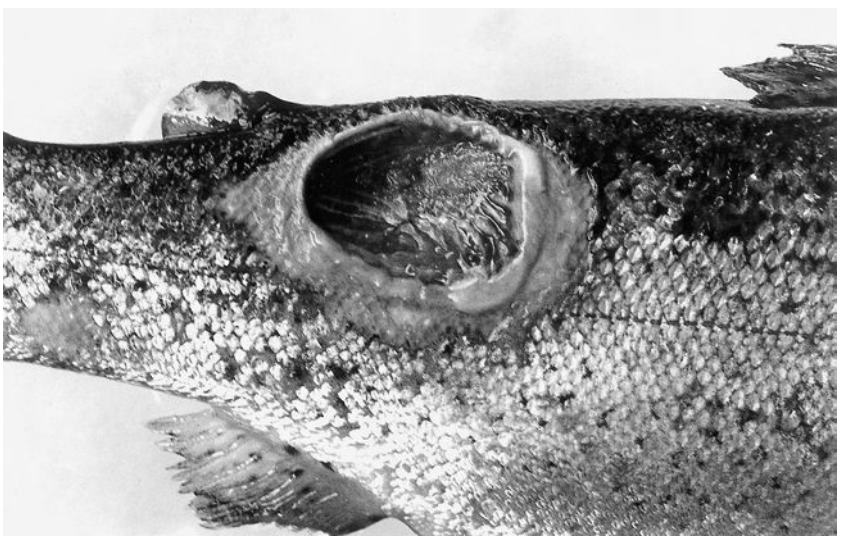


Fig. 4.1. Dorsal lesion on farmed rainbow trout attributed to infection by *Aeromonas salmonicida*.

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Diagnosis

Diagnosis is based on clinical signs, and isolation of *A. salmonicida* from fish tissues. The bacterium is grown on tryptic soy agar (TSA), or brain heart infusion agar (BHI) at temperatures below 20°C. The presence of brown, diffusable pigment is a useful tool for identification of *A. salmonicida salmonicida*; however, the pigment may be absent from some strains. On Coomassie blue agar, colonies of *A. salmonicida* are a characteristic blue colour. Upon initial isolation, colonies of *A. salmonicida* may be easily pushed across the plate by means of a bacteriological loop. Atypical colonies appear shinier and are viscous. The appearance of the bacteria in haemotoxylin and eosin (H&E)-stained sections is characteristic (Bruno and Poppe, 1996).

Within a population some fish may be infected with *A. salmonicida* but without showing clinical signs of furunculosis. In order to identify these carriers, fish can be subjected to stress tests. Elevated temperatures and other stresses are applied to induce an infectious state within a population (Bullock and Stuckey, 1975). PCR and nested-PCR techniques have also been developed to identify carriers (Hiney *et al.*, 1992; Oakey *et al.*, 1998).

Treatment

A. salmonicida is susceptible to a number of antibiotics, including oxytetracycline, oxolinic acid, trimethoprimsulphadiazine and amoxyxillin. However, antibiotic-resistant isolates have been identified and multiple resistance is common (Richards *et al.*, 1992). Both plasmid-mediated and mutational drug resistance have been detected (Aoki *et al.*, 1983; Barnes *et al.*, 1992). Oxytetracycline (OT) is one of the most extensively used antibiotics in aquaculture (Austin and Austin, 1993) and OT resistance in *A. salmonicida* has increased significantly in recent years (Smith *et al.*, 1994). Adams *et al.* (1998) have identified two plasmids of the bacterium that confer resistance to OT. In Scotland, the total number of distinct *A. salmonicida* strains isolated from cage-cultured fish by the Marine Laboratory, Aberdeen, declined from 164 in 1989 to five in 2001. The number of strains that were OT-resistant in 1990 was 52/164 isolates and 1/6 were OT-resistant in 2000.

Prevention

Duff (1942) was the first to attempt to develop a vaccine. Since then work to identify antigens of the pathogen that would be potential vaccine candidates, including those in activated whole cells, soluble extracts, immune serum and attenuated live cells with and without a modified A-layer, has continued. At present the majority of commercial bacterins are emulsified cells in an oil-based adjuvant. The side effects of this form of vaccination are discussed in Chapter 9.

There has been growing interest in the immunostimulatory properties of polysaccharides associated with the cell wall of yeasts. Some β-1,3 glucans have been tested as adjuvants in injectable furunculosis vaccines (Rorstad *et al.*, 1993). It was found that the glucan enhanced antibody formation and specific protection, and Midtlyng *et al.* (1996) found that glucan-adjuvanted furunculosis vaccines confer protection that is similar or even slightly higher than an aluminium-salt adjuvant. Culture of *A. salmonicida* in iron-depleted media results in the expression of highly protective antigens and is a technique currently used in commercial vaccines.

Work has been done on passive immunization of salmonid fry via pre-spawning vaccination of the broodstock. Kawahara *et al.* (1993) reported that when white-spotted char (*Salvelinus leucomaenis*) were vaccinated with *A. salmonicida* extracellular proteins before spawning, the eggs from these fish had increased levels of total antibodies. Furthermore, there was an increased survival upon challenge with *A. salmonicida* among fry from the broodstock. Other work has demonstrated the feasibility of depositing specific antibodies within salmonid eggs via broodstock injection (Brown *et al.*, 1997a). This may

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represent an alternative approach for immunization.

Future studies

A. salmonicida is one of the most extensively studied bacterial fish pathogens. Despite significant advances in knowledge regarding pathology, virulence and epizootiology, there are still outbreaks of the disease, and *A. salmonicida* continues to be an important pathogen in freshwater aquaculture. It is unlikely that *A. salmonicida* will be eliminated, but recent advances in understanding the molecular and biochemical basis of the host–pathogen interaction may help to develop techniques to ensure consistent and reliable control of this bacterium.

Motile aeromonad septicaemia (*Aeromonas hydrophila*)

Introduction

Motile aeromonad infections are ubiquitous and loosely encompass several species and subspecies including *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria*, although the classification of some of the group remains contentious. The bacteria are opportunistic pathogens and cause a loosely described haemorrhagic septicaemia of cultured and wild freshwater fish throughout the world and, to a lesser extent, in marine fish species. Reference here is made to the most widely described, *A. hydrophila*, a common inhabitant of aquatic systems and the causative agent of a motile aeromonad septicaemia (Roberts, 1993).

Characterization

A. hydrophila is Gram-negative, measures 0.4–1.0 μm in length and is motile by a monotrichous flagellum (Roberts, 1993). It is aerobic, catalase, oxidase and aesculin positive, produces indole, reduces nitrate and hydrolyses starch. Newman (1982) and Popoff (1984) reported biochemical profiles. Colonies are round with entire margins, and pale white to cream on TSA culture at 15°C.

Impact

Aeromonas spp. induce the following conditions in fish: a motile *Aeromonas* septicaemia, bacterial haemorrhagic septicaemia, tail and fin rot, and redsore disease. These bacteria are in soil and most natural freshwater environments. Most freshwater fish are susceptible, including rainbow trout, brown trout, tilapia (*Oreochromis* spp.), channel catfish (*Ictalurus punctatus*), striped bass (*Morone saxatilis*) and carp (Fijan, 1972; Thune *et al.*, 1982). Outbreaks are associated with rising water temperatures, crowding, handling or transfer, low oxygen levels and poor nutritional status of the fish. These stress-related conditions can lead to a rapid rise in mortality in susceptible fish (Fijan, 1972).

A. hydrophila multiplies in the intestinal epithelium and large numbers are shed in the faeces. The bacterium occurs widely in Europe, the Americas and Asia. Acute losses are reported at 20–22°C and mortality of 80% can occur in 2–3 weeks. At low temperature and in older fish, the mortality is reduced and the infection is often chronic in nature.

Toxins and extracellular enzymes, together with some structural features of the bacteria, are considered important virulence factors for *A. hydrophila* and related species (Nieto *et al.*, 1991; Mateos *et al.*, 1993). Their production is influenced by specific environmental conditions (Mateos and Paniagua, 1996). Virulent strains display cell-surface characteristics, including a paracrystalline protein array (S-layer) assembled from the monomeric S-protein (Thomas and Trust, 1994). Intracellular location of the bacterium, in tissue culture, may help to maintain a systemic infection (Low *et al.*, 1998).

Clinical signs

Motile aeromonad infections occur throughout the year, but epizootics are most

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common in the spring and early summer as the water temperature rises. These outbreaks may be a generalized septicaemia with superficial to deep grey-red ulcers, local haemorrhage, particularly from the gills and vent, abscesses, exophthalmia and abdominal distension. Fish may appear sluggish and dark in colour with tail rot. Internally, clear or blood-stained ascitic fluid, anaemia, haemorrhage around the caeca and intestine, and swollen kidneys are reported (Roberts, 1993).

Diagnostic techniques

A provisional diagnosis is based on non-specific signs. Definite diagnosis requires biochemical identification of the bacterium (Popoff, 1984). A range of commercially available media will support growth of *A. hydrophila*, including TSA and BHI after 24–48 h incubation at 20–22°C. An ELISA for detection of highly virulent strains of *A. hydrophila* and *A. sobria* serotype 0:11 has been developed by Merino *et al.* (1993).

Prevention

Infection by *A. hydrophila* often results from poor husbandry, and improvements in management practices decrease the likelihood of disease outbreaks. Antibiotic therapy has been effective, but widespread and incorrect use has resulted in antibiotic-resistant strains that have restricted the usefulness of antibiotic treatment (Mitchell and Plumb, 1980). Overall improvements in general fish health practices linked to reducing stress factors have contributed to a decline in outbreaks and consequently a reduction in use of antibiotics.

Vaccination is an alternative strategy to antibiotic use, but the antigenic diversity of *Aeromonas* challenges vaccine development. Several experimental vaccines for *A. hydrophila* have been tested, with varying degrees of success (Ascencio and Wadstrom, 1994; Ramadan *et al.*, 1994; Yin *et al.*, 1996). A marked immunopotentiating effect on both humoral and cell-mediated immune responses was noted by Ramadan *et al.* (1994), who fed ascogen (5 g kg^{-1} feed) to tilapia after vaccination.

Summary

Outbreaks involving *Aeromonas* spp. are frequently the result of poor husbandry, as the bacteria are often secondary or opportunistic pathogens (Roberts, 1993). Given the widespread occurrence of this group and the heterogeneity of strains, the development of commercial vaccines remains problematic. Extracellular products and proteins from the S-layer require further examination with respect to determining their role in pathogenesis and potential as vaccine candidates.

Enteric redmouth disease

Introduction

Enteric redmouth (ERM) disease or yersinosis is an economically important disease in freshwater aquaculture worldwide. The causative bacterium, *Yersinia ruckeri*, generally affects farmed rainbow trout fry reared in fresh water. Outbreaks are characterized by escalating losses, with congestion and haemorrhage in the kidney, liver, pancreas, musculature and intestinal tract. The severity of the disease is dependent upon the biotype of the pathogen and the salmonid host. Fish as carriers are important in the epizootiology of ERM disease, and outbreaks are associated with stressed populations. Commercial vaccines are successful.

Characterization

Y. ruckeri is a short, motile, Gram-negative, rod-shaped bacterium placed in the Enterobacteriaceae (Ewing *et al.*, 1978). Five major serovars have been recognized of which Type I (Hagerman strain) is commonly isolated and the most virulent. Stevenson *et al.* (1993) expanded this to six whole-cell serovars (I–VI) using LPS profiles. Later, Romalde *et al.* (1993) proposed four serogroups, using antigenic determinants,

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that incorporated the serovars suggested by Stevenson *et al.* (1993). A recent study to characterize Portuguese strains found that ribotyping using three restriction endonucleases was more discriminatory than outer membrane protein (OMP) or plasmid profiling (Sousa *et al.*, 2001).

Impact

ERM disease was first recorded in Idaho, USA in the 1950s among hatchery-reared rainbow trout (Ross *et al.*, 1966). Shortly afterwards the bacterium was reported from Alaska and then Canada (Wobeser, 1973). During the mid-1980s ERM disease was introduced into Europe and is now present in the UK, Norway, Denmark, France, Germany, Italy and South Africa (Bragg and Henton, 1986). Llewellyn (1980) described a bacterium similar to *Y. ruckeri* among salmonids in Australia.

The most susceptible group is young rainbow trout. Losses may be from 30 to 70% of the population (Wobeser, 1973). In the USA during 1998, of the 34.3 million total fish mortalities, 84% were attributed to ERM disease (Hinshaw, 1999). Outbreaks of ERM disease are common between 15 and 18°C, and linked with stress-related conditions such as low oxygen, handling and high stocking densities.

The movement of carrier fish is implicated as a principal cause for the spread of yersinosis and likely reservoirs of infection are therefore farmed salmonids (McDaniel, 1971). However, *Y. ruckeri* has also been isolated from wild salmonids (Petrie *et al.*, 1996) and other fish, including sturgeon (*Acipenser baeri*), walleye (*Stizostedion vitreum vitreum*), carp, goldfish (*Carassius auratus*) and minnow (*Pimiphales promelas*) (McArdle and Dooley-Martin, 1985; Michel *et al.*, 1986; Enriquez and Zamora, 1987; Vuillaume *et al.*, 1987).

Clinical signs

ERM disease is typical of other Gram-negative septicaemias and varies in severity from subclinical to subacute to acute infection with deaths in small fry (Kawula *et al.*, 1996). Predominant gross signs include darkening of the skin pigmentation and lethargy. The gills may show haemorrhage with petechiae on the liver surface, pancreas and musculature. Inflammation of the vent region, splenomegaly and a yellowish mucoid fluid in the intestine are common. Progressively blood-tinged ascites, pale gills, exophthalmos, haemorrhage of the ocular cavity and eventual rupture of the eye may also occur. At necropsy characteristic observations include profound venous and capillary congestion, particularly of the brain and blood vessels, intestinal haemorrhage, diffuse petechial haemorrhage of the musculature, splenomegaly and yellowish discharge from the vent. Necrosis of the haematopoietic tissue is the principal histological sign. Bacteria spreading to the gills, musculature and liver result in capillary dilation and haemorrhage, tissue oedema and focal necrosis. A spreading necrosis and associated pyknosis occur within the haematopoietic tissue and the splenic ellipsoids following infection by *Y. ruckeri*.

Diagnostic techniques

A provisional diagnosis made on clinical signs can be confirmed by culture of the bacterium from infected tissues. Regular cyclical shedding of *Y. ruckeri* from the intestinal tract may delay isolation (Bruno and Munro, 1989). On TSA, colonies of *Y. ruckeri* are circular with entire edges, non-pigmented and with a butyrous type of growth. The bacterium is fermentative, oxidase and cytochrome oxidase negative, catalase and citrate positive. No indole is produced in tryptone broth. Biochemical properties have been described by Hastings and Bruno (1985), Davies and Frerichs (1989) and Austin and Austin (1993). The development of selective media (Waltman and Shotts, 1984) to aid culture has not been proposed, but some biochemical tests are variable between isolates and may yield false positives (Hastings and Bruno, 1985). Immunodiagnostic approaches include the development of ELISA for specific antibodies and latex-agglutination tests (Romalde

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et al., 1995). More recently, sensitive, specific DNA primers and PCR protocols for the detection of *Y. ruckeri* in the kidney of infected fish have been reported (Argenton *et al.*, 1996; Gibello *et al.*, 1999; Temprano *et al.*, 2001).

Prevention

Commercial immersion vaccines based on a suspension of killed bacterial cells are now used. Healthy fish, generally weighing more than 4 g, are immersed or sprayed within an appropriately diluted vaccine. A booster vaccination may be included at the fingerling stage (Larsen and Pedersen, 1997). The efficacy of ERM vaccines is dependent upon species of fish, weight and water temperature, although vaccination does not eliminate mortality. Disease prevention involves adequate sanitary measures combined with regular removal of dead or moribund fish, disinfection of nets and equipment and reduction of stress to a minimum. Ozonation has been shown to be effective against *Y. ruckeri* in laboratory trials (Liltved *et al.*, 1995). Oxytetracycline, ciprofloxacin (oxolinic acid) and amoxicillin are antibiotics effective against *Y. ruckeri*. These are incorporated into the diet. Prolonged or incorrect use may have selected for strains resistant to these antibiotics (Busch, 1983). The trend may, however, be decreasing, as in Scotland (isolated by the Marine Laboratory, Aberdeen) the number of oxolinic acid-resistant *Y. ruckeri* isolates from farmed fish has declined from 67 to 14% since 1990. Similarly, no oxytetracycline-resistant strains have been found since 1992 and this parallels the decline in the amount of antibiotics prescribed.

Recommendations

Different approaches to serotyping *Y. ruckeri* have led to difficulties in comparing results between laboratories. Standardization is therefore required (as is the case with all pathogens). Development of programmes in vaccine research that identify the mechanisms of cross-protection and the antigens involved are also warranted.

Coldwater disease/rainbow trout fry anaemia

Introduction

Rainbow trout fry anaemia syndrome (RTFS) or bacterial coldwater disease (BCWD) is caused by *F. psychrophilum* (Bernardet *et al.*, 1996) and has been implicated as one of the most serious infectious diseases within the early freshwater stages of salmonids (Holt *et al.*, 1993). The first description of bacterial coldwater disease was made in West Virginia, USA, from farmed rainbow trout (Davis, 1946). Losses of more than 50% have been reported in alevins (Holt *et al.*, 1993). The disease can be seen as external lesions involving the epidermis and muscle tissue, or as a systemic infection. *F. psychrophilum* has been isolated from hatchery water, non-salmonid freshwater fish and vertebrates. Recently, the bacterium has been isolated from within newly spawned eggs of infected steelhead trout broodstock suggesting vertical transmission occurs from adult fish via eggs to alevins (Brown *et al.*, 1997b).

Characterization

The pathogen *F. psychrophilum* was initially named *Flexibacter psychrophilus* and *Cytophaga psychrophila* (Bernardet *et al.*, 1996). It was first isolated and described by Borg (1960). The bacterium is a slender Gram-negative rod, with gliding motility. Cells from young broth cultures are 0.3–0.75 μm × 2–7 μm, with some long filamentous rods of 10–40 μm (Fig. 4.2). *F. psychrophilum* does not form microcysts or fruiting bodies. The bacterium grows on Shieh's medium (Shieh, 1980), modified *Cytophaga* agar (Wakabayashi and Egusa, 1974), tryptone yeast extract (TYE) agar (Holt *et al.*, 1993) and modified Anackers medium (Lorenzen, 1993).

The salt tolerance of *F. psychrophilum* varies with the strain; however, 1.0–2.0% NaCl appears to be the maximum concentration for bacterial viability (Pacha, 1968; Bernardet and Kerouault, 1989; Holt *et al.*, 1993). *F. psychrophilum* produces a protease that is an important virulence

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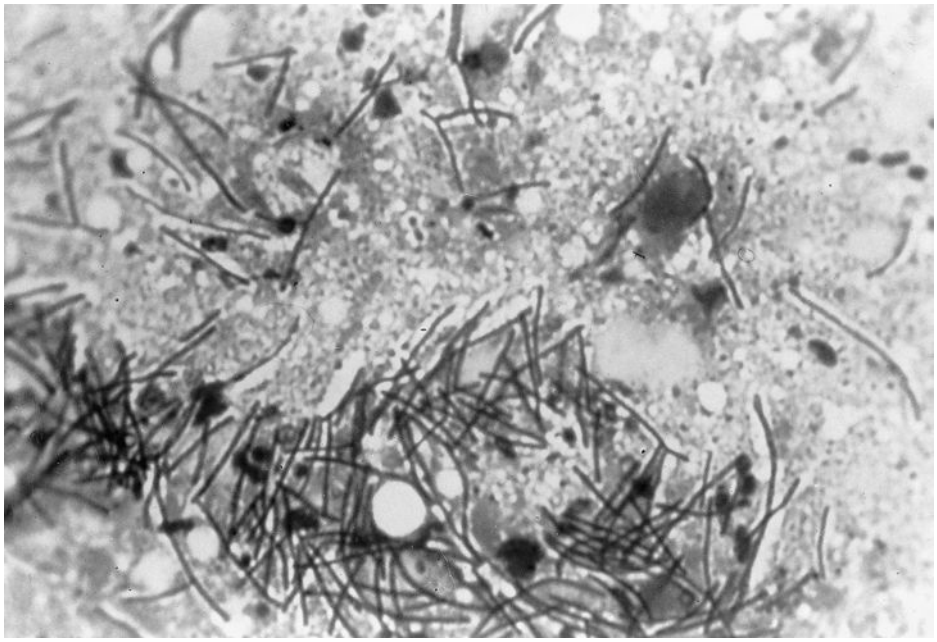


Fig. 4.2. Long filamentous rods of *Flavobacterium psychrophilum*. Fresh preparation.

factor. The optimal temperature for protease production corresponds to the environmental temperature recorded for many outbreaks of coldwater disease.

Lorenzen *et al.* (1997) applied DNA hybridization, plasmid profiling and examination of biochemical, physiological and morphological characteristics. They found similarities between Danish isolates and the type strain (NCIMB 1947) and with previously described French and American strains (Pacha and Porter, 1968; Holt, 1987; Bernardet and Kerouault, 1989). Lorenzen and Olesen (1997) further characterized *F. psychrophilum* isolates using serological methods. They found one major serotype (serotype Th), which could be further differentiated into one major (Th-1) and one minor (Th-2) subtype, and two minor serotypes (serotypes Fd and Fp^T). Serotype Fp^T included isolates from subclinical asymptomatic fish or from fish species other than rainbow trout. Chakroun *et al.* (1997) used random amplified polymorphic DNA (RAPD) analysis to differentiate strains of *F. psychrophilum* from different fish species and different geographical areas. They concluded that this method was efficient for typing *F. psychrophilum* strains and differentiation of *F. psychrophilum* from phylogenetically related species coexisting in the same environment.

Impact

The major fish affected by *F. psychrophilum* are rainbow and steelhead trout in cage culture, although outbreaks in cage-cultured coho salmon have been recorded (Borg, 1960). *F. psychrophilum* has also been isolated from the European eel (*Anguilla anguilla*), carp, tench (*Tinca tinca)* and crucian carp (Lehmann *et al.*, 1991). Fry and fingerlings are generally affected particularly if the skin is damaged (Madetoja *et al.*, 2000). Mortality can range from 20 to 90% (Bruno, 1992a). An increasing number of reports of coldwater disease in salmonids greater than 50 g have been reported; however, mortality is frequently lower (Brown *et al.*, 1997b).

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Clinical signs

Disease signs are variable and include necrotic lesions in the dorsal and caudal areas, dark pigmentation, anaemia and exophthalmos (Bruno, 1992a; Holt *et al.*, 1993). In fingerlings, later stages show skin ulceration, most commonly on the peduncle and anterior portion of the body (Fig. 4.3). Internally, the fish show signs of general septicaemia, anaemia, severe splenomegaly, and haemorrhage in the body cavity and internal organs. During chronic infection the fish may exhibit spiral swimming behaviour (Kent *et al.*, 1989) or physical deformities such as lordosis or scoliosis (Conrad and Decew, 1967).

Clumps of weakly stained Gram-negative bacteria occur loosely on and around the gill arch and secondary lamellae, which show some hypertrophy (Bruno, 1992a). The lateral skin lesions display necrosis, pyknosis and lymphocyte infiltration of the dermis and underlying muscle blocks. These signs are apparently associated with relatively low numbers of *F. psychrophilum*. Filamentous rods may be located within the spleen, liver and kidney with evidence of focal necrosis. The liver also shows increased vacuolar degeneration, pyknotic nuclei and scattered necrotic hepatocytes. In the kidney an enhanced eosinophilia of the tubules, also with some pyknotic nuclei, is noted. Colonies of *F. psychrophilum* can occur within the lumen of the olfactory capsule and in association with the epithelial lining (Bruno, 1992a).

Diagnosis

F. psychrophilum is readily cultured and a presumptive identification is based on yellow colonies, growth at 17°C but not at 30°C, and confirmation by agglutination with polyclonal antisera against *F. psychrophilum*, or by a fluorescent antibody test (FAT) using anti-*F. psychrophilum* sera. Recently, Wakabayashi and Izumi (1997) have developed a PCR to detect the pathogen within tissues. Evensen and Lorenzen (1997) have also shown that immunohistochemistry can be a sensitive and specific technique for detecting *F. psychrophilum* in fish tissues, and have suggested that it may be used as a supplementary diagnostic tool. A sensitive nested-PCR assay for the detection of *F. psychrophilum* in water samples taken from a rainbow trout farm has recently been reported (Wiklund *et al.*, 2000).

Prevention and treatment

At present there is no vaccine for RTFS and various antibiotics are used to control the bacterium.

Conclusions

One major difficulty in the management of BCWD or RTFS is that the pathogen can survive in a wide range of environmental conditions. For example, *F. psychrophilum* can be isolated from hatchery water, from the surface of eggs and from within salmonid eggs (Brown *et al.*, 1997b), possibly having entered the egg during



Fig. 4.3. Gross lesion on farmed rainbow trout attributed to *Flavobacterium psychrophilum*.

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water-hardening (Kumagai *et al.*, 2000). The possibility of vertical transmission of *F. psychrophilum* adds another dimension to the control of this disease. In addition, the bacterium is resistant to low concentrations of iodophore (Brown *et al.*, 1997b). The range of *F. psychrophilum*-like isolates has made classification confusing (Chakroun *et al.*, 1997; Lorenzen and Olesen, 1997; Lorenzen *et al.*, 1997) and further work is necessary before effective vaccines and rapid and sensitive diagnostic techniques can be formulated.

Bacterial kidney disease

Introduction

Bacterial kidney disease (BKD) is a systemic, chronic, bacterial infection of salmonids in both the fresh- and saltwater stages of their life cycle and a major bacterial problem of cage-cultured salmonids. Although known since the 1930s, the first major description of BKD was as Dee Disease following outbreaks in wild Atlantic salmon in the River Dee in Scotland (Smith, 1964). Since then, BKD has been reported throughout the world wherever salmonids are cultured or occur naturally, except Australia, New Zealand and the former USSR (Evelyn, 1988; Evenden *et al.*, 1993). The importance of vertical transmission of the causative agent, *R. salmoninarum*, will be discussed here and was mentioned briefly in Chapter 3.

Characterization

R. salmoninarum, the causative agent of BKD, was first cultured by Ordal and Earp (1956) and characterized by Sanders and Fryer (1980). The bacterium is a small (1.0 μm in length), Gram-positive, non-acid-fast, periodic acid Schiffs (PAS)-positive, non-sporulating, non-motile rod that is fastidious in its nutrient requirements (Bruno and Munro, 1986a). *R. salmoninarum* is slow-growing and a primary isolate often requires 8–12 weeks to establish growth at 15°C. Some refinements have been made in the techniques used to culture this bacterium, most notably the development of the nurse-culture technique by Evelyn *et al.* (1990). McIntosh *et al.* (1997) described a method of propagating *R. salmoninarum* in cell culture, specifically in EPC cells, thereby providing an alternative system for the study of *R. salmoninarum*.

Clinical signs

Clinical signs of BKD vary greatly and external signs include lethargy and darkening of the fish, exophthalmos, petechial haemorrhage (principally along the lateral line) and haemorrhage at the base of the fins (particularly notable on spawning salmon upon their return to fresh water). Internally, bloody ascites and enlargement of the kidney and spleen are often noted. White-grey granulomatous lesions can be seen in the internal organs, frequently in the kidney (Fig. 4.4). A pale membrane often encapsulates the spleen, kidney, heart and other organs (Bruno, 1986a). However, *R. salmoninarum* has been isolated from outbreaks where the fish have exhibited few or none of these signs.

Light microscopy

Histological lesions are reported elsewhere but notes on changes in the kidney are included here. During infection, tissue necrosis extends to large areas between the kidney tubules. Spherical granulomatous lesions containing *R. salmoninarum*, leucocytes and cellular debris can be distinguished. An opaque membrane may develop externally to the kidney capsule, appearing as thin layers of fibrin and collagen, which trap macrophages containing the bacterium, and are similar to the splenic membrane. Histologically, the glomerulus appears oedematous and *R. salmoninarum* can be detected intracellularly in endothelial cells lining the glomerular blood vessels and the lumen of the collecting ducts, although it is generally not within the proximal tubules. The nuclei of endothelial cells

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containing *R. salmoninarum* are diffusely stained and slightly cloudy (Bruno, 1986a).

Transmission

The route of invasion, attachment mechanisms and intracellular survival of the pathogen are not fully understood. Young and Chapman (1978) were the first to describe the ability of *R. salmoninarum* to survive within macrophages. Viable bacteria are within the cytoplasm of host macrophages (Fig. 4.5). Bruno and Munro (1986b) located *R. salmoninarum* in developing oocytes of experimentally infected juvenile rainbow trout, and Gutenberger *et al.* (1997) provided additional evidence for an intracellular survival mechanism. This is thought to be important in survival of the bacterium, and a number of studies have investigated the interactions between *R. salmoninarum* and salmonid macrophages. Campos-Pérez *et al.* (1997) determined that live *R. salmoninarum* elicited respiratory burst activity in rainbow trout macrophages, and that this response was enhanced when heat-killed from naive or recovered fish, but not UV-killed bacteria were used. The exact mechanism that enables *R. salmoninarum* to survive in activated macrophages is not yet known. Evenden *et al.* (1993) suggested that this intracellular survival indicates that cellular transport is involved in spreading the pathogen throughout the host; however, Flaño *et al.* (1996) speculated that *R. salmoninarum* merely incubates within phagocytes and is disseminated via the blood.

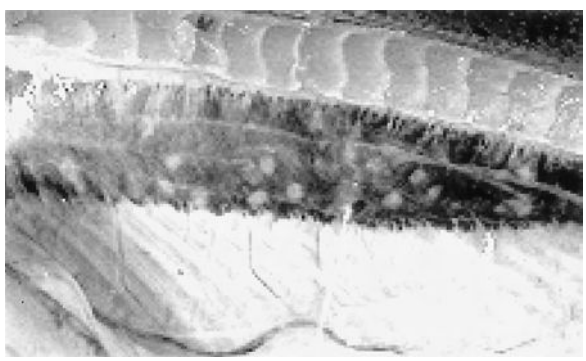


Fig. 4.4. White granulomatous lesions in kidney of farmed rainbow trout attributed to *Renibacterium salmoninarum*.

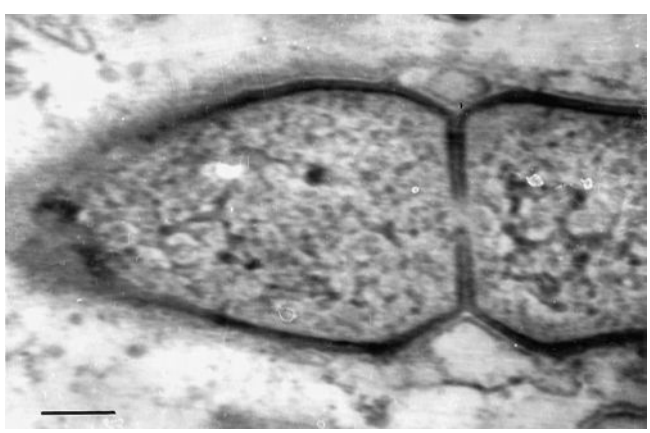


Fig. 4.5. Viable *Renibacterium salmoninarum* undergoing cell division within host macrophage. Bar, 0.1 μm.

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Virulence mechanisms

Although the pathogenic mechanisms are poorly understood, progress has been made in recent years. Characterization of virulence factors or other compounds (e.g. immunosuppressive factors) produced by *R. salmoninarum* is of interest to researchers. The main virulence factor is the 57 kDa protein, known as antigen F, or more commonly known as p57 (Getchell *et al.*, 1985; Turaga *et al.*, 1987; Bruno, 1990). This protein is the major secretory antigen produced by *R. salmoninarum* (Kaattari *et al.*, 1987) and has been associated with virulence of the bacterium (Bruno, 1988). Furthermore, it has been shown *in vitro* to be haemagglutinating (Daly and Stevenson, 1987), leucoagglutinating (Wiens and Kaattari, 1991), able to adhere to salmonid erythrocytes (Kaattari *et al.*, 1986) and to agglutinate salmon spermatozoa (Daly and Stevenson, 1989). Additional properties for p57 include contribution to the hydrophobicity of the bacterial cell surface (Bruno, 1988; Daly and Stevenson, 1990). An attenuated non-hydrophobic strain of low virulence displaying little or no surface-extractable p57 has been described (Bruno, 1988). In this case virulence may be related to the reduced levels of putative protein p57 (O'Farrell *et al.*, 2000). Cell multiplication of all strains, but particularly non-hydrophobic strains, of high-affinity chelators is reduced, and Grayson *et al.* (1995) suggested that iron reductase is an important component of its iron acquisition mechanism. Rose and Levine (1992) demonstrated that p57 is involved in the attachment of *R. salmoninarum* via opsonization by complement component C3b and subsequent binding to the CR1 receptor. Brown *et al.* (1996) have shown that vertical transmission of p57 can result in partial immunotolerance to the protein, and that fry exposed as eggs to p57 have an increased susceptibility to subsequent horizontal transmission of the pathogen. Brook trout splenic cells incubated with 0.1 or 1.0 mg extracellular protein ml^{-1} showed significantly decreased respiratory burst activity, but not phagocytic activity, as compared with untreated controls (Densmore *et al.*, 1998). Barton *et al.* (1997) further showed that p57 and some of its derivatives undergo autolytic cleavage, releasing proteolytically active polypeptides, and that these peptides may have a common mechanism characteristic of a thiol proteinase. Two copies of the gene encoding p57 (*msa1* and *msa2*) have been found. Sequence data for these were identical in the attenuated and virulent strains. While p57 may be important for *R. salmoninarum* virulence, these data suggest that differences in localization and total p57 expression are not due to differences in *msa* sequence or differences in steady-state transcript levels (O'Farrell and Strom, 1999).

Barton *et al.* (1997) also detected a soluble polysaccharide-like material in extracellular products and tissues from infected fish. This polysaccharide may have structural similarity to the O-polysaccharide of *A. salmonicida*. Sørum *et al.* (1998) showed that a galactose-rich polysaccharide and a peptidoglycan are quantitatively the most important carbohydrate constituents of the *R. salmoninarum* cell wall. Antibodies against these components are readily detectable in the sera of Atlantic salmon immunized with cell wall preparations. Maulén *et al.* (1996) have suggested that invasin molecules are involved in adhesion and attachment to host cells.

Impact

BKD is responsible annually for direct losses due to direct mortalities and losses from poor growth rates in chronically infected fish (Bruno, 1986b; Evelyn, 1988). In addition to losses within cage-cultured salmon, *R. salmoninarum* has been responsible for significant losses within valuable stocks of wild fish (Elliott *et al.*, 1989; Jónsdóttir *et al.*, 1998; Kent *et al.*, 1998). Researchers are now attempting to investigate the effect of chronic BKD infections on salmonid populations (Mesa *et al.*, 1998, 1999).

R. salmoninarum has been isolated from salmonids worldwide. All species of salmonids can be affected, although Pacific salmon species are the most susceptible. The

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pathogen has not been found in other fishes, or aquatic invertebrates (Evelyn, 1988; Sakai and Kobayashi, 1992). Although Evelyn (1988) has shown that *R. salmoninarum* can survive for a limited time in fresh water and saltwater, the bacterium can certainly survive outside the host for sufficient time to ensure transmission to other salmonids, possibly within sediments and/or faecal matter (Austin and Rayment, 1985; Balfry *et al.*, 1996). The occurrence of *R. salmoninarum* within tissue adhesions attributed to vaccine adjuvant in the body cavity has been highlighted (Bruno and Brown, 1999) and it has been suggested that subclinical infections may not be detected in these fish.

Diagnostic techniques and prevention

Culture is a sensitive method for detecting *R. salmoninarum*; however, the slow growth of the bacterium often makes this method impracticable. Immunoassays, e.g. FATs, are routinely performed on tissues taken from salmonids, using polyclonal antisera against *R. salmoninarum* (Evelyn *et al.*, 1981). ELISAs are generally sensitive and are used for screening wild and farmed populations (Olea *et al.*, 1993). Commercially available kits have been shown to vary in their efficacy (Bandin *et al.*, 1996). In North America most broodstock are screened for *R. salmoninarum* with ELISA or FATs (Elliott *et al.*, 1989), on kidney or ovarian fluid. These are carried out using polyclonal or monoclonal antisera against p57. However, there are limitations to the efficacy of these tests. Several researchers have reported cross-reactions with other bacterial species when using the antisera against *R. salmoninarum* or against p57 (Bullock *et al.*, 1980; Austin and Rayment, 1985; Brown *et al.*, 1995). To avoid false-positive reactions, monoclonal antibodies directed against p57 have been used (Wiens and Kaattari, 1989; Hsu *et al.*, 1991). Others have used Western blot analysis (Olivier *et al.*, 1992) to demonstrate the serologically reactive antigen migrating in an SDS–polyacrylamide gel to a position identical to that of p57. However, Olivier *et al.* (1992) concluded that the technique was not sufficiently sensitive to detect small amounts of p57 and thus would be useful only in diagnosing active *R. salmoninarum* infections. Other limitations of the ELISA screening method include the difficulties associated with establishing a reliable and consistent negative–positive threshold optical density value (Meyers *et al.*, 1993).

Molecular techniques hold promise for sensitive assays. PCR has been used to detect nucleic acid amplified from a variety of pathogens of aquatic vertebrates and invertebrates (Gustafson *et al.*, 1992; Kellner-Cousin *et al.*, 1993). It has been applied to detect *R. salmoninarum* within salmonid tissues (Brown *et al.*, 1994; Léon *et al.*, 1994; Magnússon *et al.*, 1994), even within individual salmonid eggs (Brown *et al.*, 1994), and the technique is sensitive and specific (Brown *et al.*, 1995). 16S RT–PCR has also been shown to be very effective for screening tissues, including blood and ovarian fluid (Rhodes *et al.*, 1998). Pascho *et al.* (1998) showed nested-PCR to be a significantly more sensitive method than membrane FAT and ELISA for detecting *R. salmoninarum* in ovarian fluid of broodstock salmon. Recently, Cook and Lynch (1999) have used a nested-RT–PCR assay to detect mRNA from between one and ten *R. salmoninarum* cells seeded into kidney homogenate. They suggested this approach was a better indicator of viable bacteria than PCR.

At present, the only preventive measure is avoidance of infection, as an effective vaccine has yet to be developed for widespread use. Recently, there have been a number of reports suggesting progress in this area. The work by Sørum *et al.* (1998) on surface polysaccharides and peptidoglycans suggests a promising area for vaccine research. Griffiths *et al.* (1998) reported promising results when they vaccinated Atlantic salmon with an avirulent strain of *R. salmoninarum* developed by Bruno (1988). Piganelli *et al.* (1999a) decreased the amount of cell surface p57 by incubating at 37°C for > 4 h. They subsequently showed that coho salmon immunized (intraperitoneal or intramuscular injection) with these p57 reduced cells showed a significantly increased mean

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time to death when challenged with live *R. salmoninarum*. Fish orally immunized with the p57 reduced cells demonstrated a significantly lower concentration of the protein 150 days after bath-challenge with *R. salmoninarum* (Piganelli *et al.*, 1999b). These findings suggest that the p57 *R. salmoninarum* may be effective as an oral BKD vaccine.

Future studies

There are gaps in our knowledge of the host–pathogen interactions, the mechanisms of *R. salmoninarum* pathogenicity and intracellular survival. We suggest that this area of research could prove fruitful. Similarly, the most sensitive diagnostic assay is not effective unless the epizootiology of the pathogen is known. For example, what is the minimum number of eggs required within a population that must be positive for *R. salmoninarum* before horizontal transmission will occur within that population? Under what conditions of density, temperature change, handling and other stressors is an outbreak most likely to occur? What is the significance of vertical transmission of antigens of *R. salmoninarum* to disease resistance?

Salmonid rickettsial septicaemia (piscirickettsiosis)

Introduction

Piscirickettsiosis caused by *Piscirickettsia salmonis* is primarily a disease of fish reared in the marine environment (see Chapter 3); however, reports from freshwater hatcheries are discussed briefly in this chapter. Furthermore, this disease is considered as an emerging problem for cage culture (Chapter 9).

Transmission

Natural outbreaks of piscirickettsiosis occur a few weeks after smolts are transferred to the sea, suggesting that the oral route is not the normal method of infection, particularly as Smith *et al.* (1999) have demonstrated that intact skin and gills are penetrated by *P. salmonis*, the causative organism. Sea lice have also been implicated in transmission, but at the present time this area is unclear.

Vertical transmission is suspected in coho salmon, and consequently salmon farmers in Chile now routinely screen their broodstock to limit spread of infection. In the laboratory (L.L. Brown, unpublished data), culturable *P. salmonis* were recovered from Atlantic salmon eggs and embryos that had been microinjected with *P. salmonis* cells just before fertilization. The eggs were fertilized after injection, incubated in flowing water and examined at the eyed and hatch stage of development for *P. salmonis*. The presence of the pathogen within each egg or embryo was determined by culture on CHSE cells, by IFAT and by Giemsa stain. These results suggest that *P. salmonis* survives within the salmonid egg at least until after hatch, and further, that the bacterium may be able to enter eggs via infected ovarian fluid and/or milt.

Natural outbreaks in fresh water

Piscirickettsiosis was initially described from fish in the marine environment, but natural outbreaks among rainbow trout and coho salmon in fresh water have also been reported in Chile (Bravo, 1994). The lesions in moribund trout were similar to those observed in marine outbreaks and *P. salmonis* was confirmed using a fluorescent antibody test.

Summary

P. salmonis is a Gram-negative obligate intracellular bacterium that causes a serious, systemic infection of salmonids in seawater in Chile and to a lesser extent in other countries. *In vitro* isolation requires culture on fish cells without antibiotics. Horizontal and vertical transmission of *P. salmonis* has been implicated. Similar pathogenic rickettsial organisms have been reported affecting salmonids cultured in

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saltwater sites in Canada, Ireland, Norway and South Africa.

Diseases Caused by Pseudofungi

Saprolegnia

Introduction

The *Oomycetes* are widespread in fresh water and are the most important group of water moulds or pseudofungi affecting wild and cultured fish. The most significant genera, particularly with respect to their impact on sexually mature broodstock and eggs, is *Saprolegnia* (Hatai and Hoshiai, 1993). Mycelial growth may occur anywhere, but normally appears as conspicuous cottony tufts of non-septate filaments around the head, caudal and anal fin (Noga, 1993). For a recent review on *Saprolegnia* and other *Oomycetes* see Bruno and Wood (1999).

Characterization

A current accurate taxonomic status of *Saprolegnia* and other *Oomycetes* is uncertain, because it relies upon the morphology of reproductive structures (Dick *et al.*, 1984; Beakes *et al.*, 1994). Consequently, identification of asexual pathogenic *Saprolegnia* isolates using classical taxonomic criteria is impractical. Willoughby (1978) subdivided *Saprolegnia diclina* into three subspecific groups based upon oogonial morphology. *S. diclina* Type 1 infects salmonids and is synonymous with *Saprolegnia parasitica* and *Saprolegnia* sp. Type 1 (Kanouse, 1932; Pickering and Willoughby, 1977). Type 2 occurs as a parasite of coarse fish and Type 3 is entirely saprophytic, with the zoospores showing direct germination (Hatai and Hoshiai, 1992). The *Oomycetes* isolated from fish are generally assigned to a single major cluster forming a coherent, separate taxon, *S. parasitica* Coker (synonym *S. diclina* Humphrey Type 1) (Willoughby, 1978). Several characteristics including cell wall composition and rDNA sequences differentiate the water moulds from the true fungi. Molecular sequences show their phylogenetic roots with the *Chromista*, the chromophyte algae and other *Protista*, rather than the true fungi (Dick, 1990; Kwon-Chung and Bennett, 1992).

Impact

Infection of eggs, fry and larger fish by water moulds is a widespread problem in cultured fish. Overcrowding, handling, temperature changes, increased organic loading, parasitism and sexual maturation increase the possibility of *Saprolegnia* infection (Pickering, 1994). Losses up to 50% of farmed catfish occur during severe winters in the USA, with an annual economic cost of US$40 million (Bly *et al.*, 1994). Among commercial catches of Atlantic menhaden (*Brevoortia tyrannus*), up to 80% of the stock may have an ulcerative mycosis (Lilley and Roberts, 1997). The economic loss is estimated at US$27 million per annum. In Japan annual losses may exceed 50% of farmed coho salmon (Hatai and Hoshiai, 1993).

Species affected

Saprolegnia sp. has been isolated from Atlantic salmon, rainbow trout, brown trout, Arctic char (*Salvelinus alpinus*) and coho salmon (Pickering and Christie, 1980; Wood and Willoughby, 1986; Hatai and Hoshiai, 1993). *S. parasitica* and *S. diclina* have been implicated in mortality of cultured rainbow trout, coho salmon and ayu (*Plecoglossus altivelis*) in Japan (Yuasa and Hatai, 1995) and infections of *S. diclina* have been reported in spawning rainbow trout in Taiwan (Chien, 1980).

Predisposing factors

The *Oomycetes* are ubiquitous, therefore fish are continually exposed to potentially pathogenic zoospores. However, infection normally only results from a change in environmental factors, predisposition or immunosuppressive components (Bly *et al.*, 1994). In wild Atlantic salmon concurrent infestations with *Saprolegnia* sp. and

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Gyrodactylus salaris (Johnsen, 1978) have been reported following sustained damage to the skin by the parasite. The association of sexual maturity with the elevated occurrence of infection is attributable to skin damage resulting from spawning activities (Richards and Pickering, 1978). Susceptibility to saprolegniasis may also be associated with an increase in cortisol and certain reproductive hormones (Pickering, 1977). Quiniou *et al.* (1998) proposed that a reduction in water temperature reduced mucus cell numbers allowing the attachment and germination of *Saprolegnia* cysts.

Clinical signs

Cotton wool-like circular tufts have been recorded on the integument and gills of host fish or eggs (Willoughby, 1989). Infection normally occurs in the epidermis and dermis where more than one species may be present (Pickering and Willoughby, 1982). Pathogenic members of the *Saprolegniaceae* may also be seen to surround and penetrate gill tissue (Fig. 4.6) (Hatai and Egusa, 1977; Bruno and Stamps, 1987).

Diagnostic techniques

Identification of oomycete fungi has relied on morphology and sporulation characteristics (Seymour, 1970; Willoughby, 1978). Sexual reproductive stages are required to enable accurate identification (Wood and Willoughby, 1986). However, some isolates take long periods to produce oogonia and this makes identification difficult (Pickering and Willoughby, 1982). Features such as size, shape and nature of the oogonial surface and wall have been used to identify *S. parasitica* (Hatai *et al.*, 1990).

Low-nutrient culture media with or without antibiotics are used for the isolation and culture of fungi. Agar plates are inoculated and incubated between 5 and 37°C and observed for newly emerging hyphae tips (Noga and Dykstra, 1986).

A PCR to examine rDNA from wide-ranging *Saprolegnia* isolates was developed with the use of the endonuclease *Bst*UI, which produced identical fingerprints from all strains of *S. parasitica* (Molina *et al.*, 1995). The authors suggested this could be used as a diagnostic test in the absence of antheridia and oogonia.



Fig. 4.6. Widespread necrosis and sloughing of gill lamellae following infection by *Saprolegnia* sp.

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RAPD using PCR with DNA from *S. diclina–parasitica* isolates was developed by Diéguez-Uribeondo *et al.* (1996). Spanish isolates have a genetic similarity of 85–100%, compared with a 20–45% similarity with other strains of the complex. Yuasa *et al.* (1977) highlighted a simple method to distinguish between *S. parasitica* and *S. diclina* based upon spore-germination type. Observations of a zoospore suspension in diluted Griffin's (GY) broth revealed a distinction between *S. diclina* (i.e. direct germination) and *S. parasitica* (i.e. indirect germination) (Yuasa *et al.*, 1977).

Prevention

The control of fungi on eggs requires the removal (by hand) of dead or infected eggs at regular intervals, and/or chemical bath treatments. Malachite green has been the traditional chemical used in fish aquaculture facilities to control or prevent external fungal outbreaks (Foster and Woodbury, 1936).

Treatment is successful in fish culture (Bailey, 1984) at concentrations of 3–5 mg l^{-1} for a 60 min exposure (Marking *et al.*, 1994). However, because of toxicological and possible mutagenic properties, the use of malachite green has been curtailed or prohibited in many countries (Schnick and Meyer, 1978).

Buffered bicarbonate iodophors are generally used to disinfect eyed ova (Marking *et al.*, 1994). Eggs are flushed using 100 mg available iodine l^{-1} in the disinfectant bath for 5–15 min and then rinsed thoroughly. For small numbers of fish, a dip treatment in the iodophors of around 30 s is adequate.

Formalin has been effective in controlling fungal infections on rainbow trout and on eggs at 250 mg l^{-1} for a 60 min exposure (Bailey and Jeffrey, 1989; Marking *et al.*, 1994). Schreier *et al.* (1996) concluded that *S. parasitica* was controlled on rainbow trout eggs that received prophylactic formalin treatments on alternate days.

Hydrogen peroxide has been successfully tested as fungicide against *Saprolegnia* on developing rainbow trout and chinook salmon (Marking *et al.*, 1994; Schreier *et al.*, 1996). Dawson *et al.* (1994) noted that exposure of eggs to a prophylactic treatment of 250–500 ml hydrogen peroxide l^{-1} (based on 100% active ingredient) for 15 min, on alternate days, inhibited fungal infection on healthy eggs.

The fungistatic effect of sodium chloride has been examined and improvements in hatching rates recorded (Phelps and Walser, 1993). Using a continuous sea-salt bath treatment, Phelps and Walser (1993) reported an improvement in the hatching success of channel catfish eggs.

Pottinger and Day (1999) used a biocide, bronopol (2-bromo-2-nitropropane-1,3-diol), formulated as Pyceze, to prevent or reduce infection with *S. parasitica* in rainbow trout and to protect eggs from infection, and suggest this is a safer alternative to malachite green. The effects of the biocide on embryonic stages of fish and the growth and survival of developmental stages need to be determined.

An alternative approach using elevated water flow for egg incubation was presented by Rach *et al.* (1995). They reported that eggs maintained at a flow rate of between 300 and 600 ml min^{-1} did not roll in the water and had a reduced rate of infection and hatching success. At 1200 ml min^{-1} the eggs were lifted into the water column and rolled moderately, resulting in an improved hatch with an absence of fungal growth.

Future studies

Oomycetes have their phylogenetic origins with the chromophyte algae, rather than the true fungi, and PCR techniques continue to enhance our taxonomic knowledge of this group. Further development of these and other techniques, such as RAPD–PCR, may help to resolve taxonomic difficulties, particularly as new species are being described (Willoughby, 1998; Bangyeekhun *et al.*, 2001). Research is also required into the lack of immune response to infection (e.g. leucocytic infiltration into pseudofungal-infected tissue as seen in channel catfish during the winter months and the inactivity of the complement system).

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Diseases Caused by Parasites

Whirling disease

Introduction

Whirling disease (WD) is a widespread, chronic parasitic infection of salmonids caused by *Myxobolus cerebralis* (Protozoa: Myxosporea). This parasite infects the head cartilage and is responsible for major economic losses to some wild and cultured trout in Europe and North America. WD was first reported in 1893 in central Europe and named for the erratic, tail-chasing, whirling motion in young fish (Hofer, 1903). Lesions in skeletal tissues and spores, particularly in the head cartilage, are used for diagnosis. In advanced stages of the disease, spores are in the spine and infected fish appear black and may be twisted posterior to the dorsal fin. Alevins often suffer 100% mortality, although there is a reduced mortality and infection in older trout.

Characterization

WD is caused by the sporogonic stage of *M. cerebralis* and is the only myxosporean in the cartilage of salmonids. A two-host life cycle involves a fish and the aquatic oligochaete worm, *Tubifex tubifex* (Markiw and Wolf, 1983; Wolf *et al.*, 1986). A re-examination of the ultrastructural features and ribosomal subunit gene sequences of myxozoans was made by Siddall *et al.* (1995) and they concluded the myxozoans were a class of highly derived parasitic cnidarians.

Impact

A heavy infection of *M. cerebralis* in young fish can result in high mortality or unmarketable, deformed individuals. Mortality among newly hatched fish may approach 90% following exposure to the infective agent as sac fry. However, 1-day-old rainbow trout appear refractory (Markiw, 1991). In many areas of the USA, WD has been implicated in the severe decline or elimination of some year classes (Vincent, 1996).

Susceptibility and host range

Young rainbow trout are most susceptible, although lake trout are apparently refractory (O'Grodnick, 1979). Other salmonids can be infected, but clinical signs of the disease may not develop. Susceptibility varies with age, species, strains and even among fish within a population (Markiw, 1992). The following list ranks species in descending order of apparent susceptibility: rainbow trout, sockeye salmon, golden trout (*Oncorhynchus aguabonita*), cutthroat trout (*Oncorhynchus clarki*), brook trout, chinook salmon, Atlantic salmon, brown trout, coho salmon, lake trout and splake (hybrids between brook trout and lake trout).

Reservoir of infectivity

The source of the infective agent for fish is usually the water supply or earthen ponds inhabited by the tubificid worm, *T. tubifex*. Predators and scavengers such as birds, which consume infected fish, can release viable spores into the environment and therefore distribute the parasite (El-Matbouli and Hoffmann, 1991a). Trout and salmon infected with WD may harbour spores throughout their life. El-Matbouli and Hoffman (1991b) found *M. cerebralis* spores can survive freezing for at least 3 months.

Geographic range

WD originated in rainbow trout in Germany, but trade in the movement of fish has helped spread the disease to many countries (Halliday, 1976), including New Zealand (Hewitt and Little, 1972) and the USA (Modin, 1998; Hoffman, 1990).

Clinical signs

In infected fish, abnormal whirling behaviour and dark or black caudal peduncle and tail fin can be used as a presumptive diagnosis. The whirling behaviour is attributed to impaired coordination caused by neural damage. Deformities, including shortening of the snout, axial skeleton or head, and

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cranial depressions, may occur and persist through the life cycle; however, injury or deficiency in dietary ascorbic acid can result in similar signs (Wolf *et al.*, 1981). Clinical signs are also influenced by the intensity of infection and water temperature. During gross examination internal organs appear normal. The pathogen is difficult to eradicate, and the use of earthen ponds for rearing juveniles is an ideal habitat for tubifex. Fish should be cultured in spore-free water, using smooth concrete raceways, plastic-lined raceways or in ponds that are regularly disinfected.

Histological sections of cartilage, particularly the skull, gill and vertebrae, stained with methylene blue, Giemsa or May–Grünwald Giemsa, show areas of lysis, inflammation and digestion of the cranial cartilage. The presence of *M. cerebralis* spores in the cartilage is considered pathognomonic for WD. However, infection in older fish is usually asymptomatic.

Life cycle

The WD protozoan has a two-host life cycle involving a fish and the aquatic oligochaete *T. tubifex* (Wolf *et al.*, 1986). Two separate stages of sporogony occur, one in each host. Spores of *M. cerebralis* are released into the environment when infected fish die or are consumed by predators. The myxosporean-type spores are ingested by these oligochaetes where they develop in the gut epithelium to the actinosporean triactinomyxon, after which infected worms release many mature forms into the water. The triactinomyxon stage enters susceptible fish through the epithelium and buccal cavity, particularly at the base of the gills and the oesophagus. The parasite reaches the cartilage via peripheral nerves and the central nervous system (El-Matbouli *et al.*, 1995). Scanning electron microscopy studies on the triactinomyxon of *M. cerebralis* have shown that attachment and complete penetration of their sporoplasm germs occur as early as 1 min post-exposure (El-Matbouli *et al.*, 1999). This was correlated with increased convulsive movements and increased mucus from the trout host (El-Matbouli *et al.*, 1999). Development time for the myxosporean in fish and the actinosporean in tubificids is directly related to temperature. Trout fry fed infected worms or exposed to water-borne triactinomyxon show black tails after 35–45 days at a water temperature of 12.5°C. Whirling behaviour frequently appears at the same time. Fully mature spores can be detected after 2.6–3.5 months at 12.5°C.

Identification

During hatchery inspections young, susceptible fish from earthen ponds instead of concrete raceways should be examined for *M. cerebralis*. The head of an anaesthetized fish is cut sagittally and a smear examined at ×20 or ×40 magnification.

Fresh or frozen gill arches should be removed, ground in several volumes of water, allowed to settle and then drops of the supernatant examined microscopically. Negative samples or lightly infected carriers (about 100 spores per head) should be subjected to a spore-concentrating procedure (O'Grodnick, 1975) or the pepsin–trypsin–dextrose (PTD) digestion method (Markiw and Wolf, 1974). Purified DNA has been used in a nested-PCR for detecting *M. cerebralis* from oligochaetes (Rognlie and Knapp, 1998) and all stages of infection in fish (Andree *et al.*, 1998).

Mature spores are lenticular in side view and nearly circular when viewed from the front. The spores are 8–10 μm in diameter and have two prominent ovate polar capsules with coiled filaments, which may be extruded in certain situations (Lom and Hoffman, 1971). The identification of *M. cerebralis* in sections from lesions of skeletal tissue is recommended for diagnosis. However, this may not be reliable with lightly infected fish that have only a few spores present. FAT examination of resin-embedded sections of infected rainbow trout has been used to detect *M. cerebralis* spores and pre-spore stages (Hamilton and Canning, 1988). The specific fluorescence of older specimens stored in formalin for a week or more is reduced and in older specimens is insignificant or non-existent.

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Control

Rainbow trout produce antibodies against *M. cerebralis*, although protection against infection has not been shown (Halliday, 1974). However, host-tissue reaction against the pathogen can decrease or even eliminate myxosporean infections in lightly infected rainbow trout (Markiw 1992), showing that immunization might be practical. Hoffman (1990) has presented a review of management practices for infected fish in hatcheries.

There is a decrease in spore development in young trout fed furazolidone or fumagillin (Taylor *et al.*, 1973; O'Grodnick and Gustafson, 1975). However, El-Matbouli and Hoffmann (1991a) reported that fumagillin, fed to experimentally infected rainbow trout, caused defects in the morphology of *M. cerebralis* spores and prevented a clinical outbreak of WD.

Future studies

WD is determined by the intensity of infection and not simply by the presence of spores. Therefore, control measures do not need to eradicate the parasite completely. Measures such as culturing resistant species, filtering the water supply, chemotherapy and periodical disinfection of the facility help in WD control. Although control of *M. cerebralis* is difficult, preventive measures can decrease the intensity of the disease and perhaps eliminate the spread to non-infected areas. Research is also required to link known species of actinosporidia to species of myxosporidia. Further research into the possible mechanical and chemical stimulation of the triactinmyxon spores of *M. cerebralis* in locating and attaching to the host is also suggested.

Proliferative kidney disease

Introduction

Proliferative kidney disease (PKD) is an economically important myxozoan of cultured salmonids in fresh water that primarily affects the kidney and spleen. It is caused by the extrasporogonic stage of an enigmatic myxozoan, referred to as PKX (Kent and Hedrick, 1985). PKX is presumed to be in the family Sphaerosporidae, genus *Sphaerospora* (Hedrick *et al.*, 1993). This disease causes severe losses in hatchery-reared salmonids in Europe and western North America (Clifton-Hadley *et al.*, 1984; Smith *et al.*, 1984, Hedrick *et al.*, 1993). Mortalities are highest during the summer months and fry are the most severely affected. Recently, our understanding of the parasite has improved with confirmation that some bryozoans harbour the mature myxospore stages (Anderson *et al.*, 1999; Longshaw *et al.*, 1999; Canning *et al.*, 1999; Feist *et al.*, 2001). Reviews on PKD and a description of the disease and its causative agent have been published (Clifton-Hadley *et al.*, 1984; Hedrick *et al.*, 1986, 1993; Morris *et al.*, 2000).

Characterization

There are two developmental stages in the salmonid host kidney: the extrasporogonic and the sporogonic stages. The extrasporogonic stage proliferates in the kidney interstitium and is often seen in tissue sections or in stained kidney imprints. PKX typically appears as large cells (≥ 20 µm) with a distinct plasmalemma (Ferguson and Needham, 1978). Sporogenesis occurs in the renal tubules. Also characteristic of PKX is the frequent appearance of cells within a cell, or endogeny (Seagrave *et al.*, 1980). This can be seen in both extrasporogonic and sporogonic stages; internal secondary cells are within primary cells. The secondary cells frequently contain internal tertiary cells and form the sporogonic stages in the kidney tubule lumen (Kent and Hedrick, 1986).

Anderson *et al.* (1999) have shown that there is a significant similarity between the reference 18S rDNA sequence of PKX in the databank and sequences obtained from myxozoan parasites in North American bryozoans and European and North American PKX. They suggested that Bryozoa (*Pectinatella magnifica*, *Cristatella mucedo*

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and *Plumatella rugosa*) are natural hosts for PKX, and that transmission and ultrastructural studies were warranted. Recently, Longshaw *et al.* (1999) reported the occurrence of PKX in a further two species of Bryozoa, *Plumatella emarginata* and *Fredericella sultana*, in the UK. Canning *et al.* (1999) identified a new species of the myxozoan genus *Tetracapsula* as the PKX organism, and proposed the name *Tetracapsula bryosalmonae*. They also successfully experimentally transmitted *T. bryosalmonae* from infected bryozoans (*F. sultana*) to naive rainbow trout

Impact

PKX is thought to be restricted to the family Salmonidae, although there is some evidence that similar infections, possibly related to other bryozoan myxozoans (*Tetracapsula* spp.), may be involved in pike and carp infections with PKX-like organisms (Bucke *et al.*, 1991; Voronin and Chernysheva, 1993). PKD affects Arctic char and rainbow trout and to a lesser extent brown trout, Atlantic salmon and Pacific salmon species. During summer months, morbidity can reach 60–100%.

Clinical signs

Clinical signs associated with PKD outbreaks include exophthalmos, pale gills, abdominal swelling and darkening. Internal gross signs include renal swelling and discoloration, ascites production, often with associated blood, splenomegaly and yellowish liver (Hedrick *et al.*, 1993).

Diagnostic techniques

Light microscopy examination is used for a presumptive diagnosis. The PKX extrasporogonic stages can be visualized directly in tissue squashes or stained imprints. Also, the characteristic 'swirls' can be seen within the kidney interstitium. These are caused by the progressive replacement of stem cells by a mixed cell infiltrate of macrophages and lymphocytes (MacConnell *et al.*, 1989).

Histopathological signs range from a mild haematopoietic hyperplasia early in the infection to a severe granulomatous response (Ferguson and Needham, 1978). This corresponds the proliferation of the extrasporogonic stages (Clifton-Hadley *et al.*, 1987). The first PKX cells can be seen 2–3 weeks post-exposure, at water temperatures greater than 15°C. These cells can most often be seen in the blood sinuses (Kent and Hedrick, 1986).

Discovery of a lectin that binds to PKX and the development of an mAb against the parasite has greatly aided diagnosis of PKD (Castagnaro *et al.*, 1991; Adams *et al.*, 1992). Saulnier and de Kinkelin (1996) produced 11 mAbs specific for PKX to identify several proteins of PKX, one of which is thought to have important antigenic properties. The study resulted in the establishment of a set of immunological probes that can be useful for diagnosis, expression and antigenicitiy studies. Saulnier *et al.* (1996) cloned, sequenced, and expressed a cDNA encoding a PKX antigenic protein of *c.* 13 kDa. This protein can be used in diagnostic applications as well as for research on the antigenicity, pathology and epizootiology of the parasite.

Saulnier and de Kinkelin (1997) have developed primers for PCR-based detection of PKX. These primers were deduced from the sequence of the small subunit rRNA gene (SSUrDNA). They suggested that the primers could be used for PCR detection of PKD in subclinical infections, for screening populations in fisheries management, and for further studies on the intermediate or definitive hosts of PKX.

Prevention and treatment

There is no vaccine to prevent PKX, therefore screening is the only available preventive measure. Molecular studies described point to some candidates for vaccine research; however, to date this has not been accomplished.

Higgins and Kent (1996) found that oral treatment with fumagillin (3.0 mg kg^{-1} fish) for 14 days after the first detection of PKX within kidney tissue resulted in a

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significant reduction in the prevalence of infection.

Ceratomyxa shasta

Introduction

Ceratomyxa shasta is a freshwater myxosporean and the causative agent of ceratomyxosis. The parasite causes high mortality in wild and captive-reared juvenile and adult salmonids in the western USA, particularly in the Columbia River basin. A massive infiltration, occlusion and destruction of several tissues by the infective stage are a direct cause of mortality.

Characterization

The aetiological agent, *Ceratomyxa shasta* (Protozoa: Myxosporea), was first described by Noble (1950) from hatchery-reared rainbow trout. This work established *C. shasta* as a new species and the first of this genus to parasitize freshwater fish.

Impact

Ceratomyxosis is recognized as an economically important condition and significant losses can occur in hatchery-reared and wild juvenile salmonids, with prespawning mortality in adult salmon. In the latter, a disease incidence as high as 94% has been reported (Chapman, 1986). In young fish, up to 100% mortality may occur. Epizootics have been reported in chinook, chum and coho salmon, and rainbow and cutthroat trout, but with variability between strains of fish (Ibarra *et al.*, 1994). Infected anadromous salmonids retain the parasite and continue to die during their marine migration. Prevalence data varies considerably with species, season and specific sampling site in a river (Margolis *et al.*, 1992).

The life cycle of *C. shasta* has not been fully described, although it is known that susceptible fish become infected while residing in or migrating through waters that contain the infective stage of the parasite (Johnston, 1980). At low temperatures (4–6°C), the progress of the disease is slow and accounts for the seasonal nature of ceratomyxosis that is generally reported between May and November (Ching and Munday, 1984).

The parasite occurs within a well defined geographic area including areas of the states of Oregon, Washington and Idaho; California north of a line due east from the southern tip of San Francisco Bay; British Columbia, south of a line east from the northern tip of Vancouver Island; and the Yukon River in Canada and Alaska (Hoffmaster *et al.*, 1988; Follett *et al.*, 1994). Distribution of *C. shasta* in these regions has apparently expanded, although this could be related to increased spread of the disease, although conversely may have occurred through improved monitoring and detection methods.

Clinical signs

The parasite manifests itself in the gut, liver, spleen and muscle, causing haemorrhage. In rainbow trout, anorexia, lethargy, dark appearance and exophthalmia are recorded, with abdominal distension due to production of a spore-containing fluid (Schafer, 1968). The intestinal tract of juvenile fish becomes swollen and haemorrhagic and the content becomes mucoid, with caseous material present in the intestine and pyloric caeca. The entire digestive tract including the liver, gall bladder and spleen, and the kidney, heart, gills and skeletal muscle may also become haemorrhagic and necrotic (Wales and Wolf, 1955). In infected juvenile chinook salmon, the fish become emaciated and then later develop large fluid-filled blebs and kidney pustules (Conrad and Decew, 1966). Infected adult salmon can develop nodular lesions in the intestine that perforate and contribute to fish mortality. These nodules may be accompanied by gross lesions in the liver, spleen, kidney and musculature.

Trophozoites in the mucosa cause a strong inflammatory response in the lamina propria. As the infection progresses, the parasite multiplies in all layers of the intestine and causes severe inflammation and desquamation of the mucosal epithelium.

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Trophozoites penetrate the intestinal tract, spread into the surrounding adipose tissues and enter the bloodstream, where they are carried to other tissues.

Histologically there is enlargement of the connective tissue within the intestinal caeca and massive infiltration by developing trophozoites and other developmental stages. Granulomatous lesions may develop in the viscera, causing peritonitis. The occlusion and destruction of the intestinal lumen is considered to be the cause of the rapid rise in mortality among infected fish.

Life cycle

The actinosporean stage of the cycle of *C. shasta* is completed through a freshwater polychaete annelid worm, *Manayunkia speciosa* (Bartholomew *et al.*, 1997).

Characterization

Spores are evident only at the terminal stages of infection and are identified by their size, shape and location. Several techniques have been suggested for spore preparation (Amos, 1985), and include wet mounts from the lower intestinal wall, ascitic fluid or gall bladder. Alternatively, air-dried smears stained using Ziehl Neelsen (ZN) without heat fixation, or smears fixed with Schaudin's fixative and then stained with Heidenhain's iron haematoxylin can be used. In stained smears the polar capsules stain red against a bluish sporoplasm and background. The trophozoites are rounded but variable in shape, and mature to form a sporoblast that usually contains 12 nucleated cells producing two groups of six cells (sporonts) and the formation of two spores in each mother cell (Yamamoto and Sanders, 1979).

Diagnosis

A presumptive clinical diagnosis, based on inflammation of the intestine, is confirmed by microscopical examination of spores in intestinal scrapings (Zinn *et al.*, 1977). The spores are evident at the terminal stages of infection and are identified by their size, shape and location. The ends of the spores are rounded, reflected posteriorly with a distinct suture line (Noble, 1950) and measure 14–23 m by 6–8 m.

The variability in size and shape of the trophozoites and their similarity to this stage in other myxosporea makes diagnosis using light microscopy difficult. Consequently, serological techniques have been developed using mAbs. The antibodies produced react specifically with the pre-spore stages and do not cross-react with trophozoite or spore stages of other myxosporeans. Hence, the use of mAbs and fluorescein or enzyme-conjugated secondary antibodies has enabled the reliable detection of early infections (Bartholomew *et al.*, 1989). A primed PCR designed to generate a specific marker for *C. shasta* DNA has been developed by Bartholomew *et al.* (1995) and is useful for detecting low levels of the parasite. The sequencing of the single-stranded rDNA (Bartholomew *et al.*, 1997) allowed the development of a sensitive PCR assay for *C. shasta* based on this sequence (Palenzuela *et al.*, 1999).

Prevention

There is no current chemotherapy for ceratomyxosis, although a selection factor on fish migrating through enzootic areas may confer some resistance. Sanders *et al.* (1972) suggested that contaminated water supplies may be filtered and treated using UV sterilization or chlorine, and that controlling the movement of eggs or live fish from *C. shasta*-endemic areas to those free of this disease can be used to contain the pathogen. The latter measure is now being used with significant effect, and recently it has been possible to introduce fish to parasite-free areas using PCR as an assay technique (Palenzuela *et al.*, 1999).

Future studies

The effect of salt water on the progress of this disease is largely unknown although mortality is believed to continue during the marine phase of the host's life cycle. Furthermore, it is unclear if anadromous

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fish are infected before they enter salt water or on returning to fresh water. Research in these areas would increase our understanding of ceratomyxosis. The increased sensitivity afforded using PCR is valuable for monitoring early and subclinical infections; however, it is unknown if the increased incidence of the disease signifies an increase or an improvement in detection methods.

Henneguya salminicola

Introduction

Henneguya salminicola (synonym *Henneguya zschokkei*) is a common internal myxosporean parasite of salmonids and coregonids. The cysts in the somatic musculature ruin the aesthetic appearance of the flesh and make it unsuitable for commercial purposes, with a consequent economic impact on salmon fisheries (Kent *et al.*, 1994).

Characterization

Several species of *Henneguya* (Protozoa, Myxozoa: Myxobolidae) are described in cultured freshwater fish. These include *H. salminicola* in salmonids, *Henneguya waltairensis* in green snakehead (*Channa punctatus*) (Kalavati and Narasimhamurti, 1985), *Henneguya exilis* and *Henneguya* sp. in channel catfish (Current, 1979; Smith and Inslee, 1980), and *Henneguya laterocapsulata* n.sp. and *Henneguya suprabranchiae* n.sp. in the catfish (*Clarias lazera*) (Landsberg, 1987). However, only *H. salminicola* is significant in cage culture (Boyce *et al.*, 1985).

Impact

H. salminicola infections are contracted in fresh water and are not usually lethal. The cysts are unsightly in the flesh and the parasite contributes to poor growth and organ displacement. The production of a heat-labile protease from the spores during the host's sea migration causes hydrolysis of the muscle resulting in liquefaction (Bilinski *et al.*, 1984; Boyce *et al.*, 1985). The marketability of some fresh, frozen or smoked products is affected, although the cysts are not readily evident in canned products. Five species of salmon along the Pacific coast of North America and Asia are susceptible to *H. salminicola* (Boyce *et al.*, 1985).

Clinical signs

Grossly visible, white, subspherical cysts up to 15 mm in diameter, with a creamy content, occur within the musculature and other tissues (Boyce *et al.*, 1985). As the cysts mature and grow, they eventually rupture through the integument, releasing many infective spores into the water. These open ulcers provide an excellent port of entry for secondary pathogens. Boyce *et al.* (1985) reported that the prevalence of infection was highest in coho and sockeye salmon and was correlated with the length of time the juvenile fish spent in fresh water.

Diagnostic techniques

Microscopic examination is required for confirmation of this infection, and diagnosis of *H. salminicola* requires the demonstration of the characteristic spores, which contain two polar capsules and two caudal projections. In addition, ultrasonic equipment has been used to detect *Henneguya* spores in whole fish. Clouthier *et al.* (1997) used molecular and antigenic characterization of spores from skeletal muscle of chum salmon to provide a potential peptide and ORF2 fusion protein that may be suitable for candidate vaccine trials.

Prevention

There are no treatments available against *Henneguya*, and the current approach is through the selection of fish from uninfected stocks.

Future studies

Continued development work on fusion proteins (Clouthier *et al.*, 1997) with the

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aim of developing a vaccine suitable for testing will be beneficial.

Ichthyobodo necator

Introduction

Ichthyobodo necator is an ectoparasite of freshwater salmonids. Mortalities of infected salmonid fry or ornamental fish can occur with moderate to severe infections; however, chronic outbreaks can result in loss of growth, and secondary gill and skin infections.

Impact

Many fish species are susceptible to *I. necator*, but infestation is particularly important among salmonid fry. Morbidity can be moderate to severe and can lead to serious secondary infections by opportunistic pathogens.

Characterization

I. necator is a small, bean-shaped flagellate, approximately 5–18 μm (Fish, 1940) in length. This protozoan belongs to the order Kinetoplastida, Family Bodonidae, and its taxonomic status was determined using electron microscopy (Joyon and Lom, 1969). Although *I. necator* is suggested as acquiring salinity tolerance and surviving and reproducing in seawater, it should be noted that the morphologically similar bodonid, *Ichthyobodo* sp., from marine Japanese flounder (*Paralichthys olivaceus*) and others from the marine environment probably represent separate species (Urawa and Kusakari, 1990).

Clinical signs

Externally, there is a white-blue haze on the skin of infected fish. Gill tissues show lamellar fusion and hyperplasia. In Atlantic salmon and rainbow trout the parasite causes hyperplasia of the malpighian cells and exhaustion of the goblet cells below infested surfaces (Robertson *et al.*, 1981). The epidermal plaque is sloughed off, leaving just a single layer of basement membrane cells. Robertson *et al.* (1981) also showed that *I. necator* caused a significant increase in cell division below parasite-infested surfaces, a pattern that is markedly different from normal cell proliferation. Pickering and Fletcher (1987) showed that *I. necator* effected the production of sacciform cells in salmonids. These cells, located in the epidermis of brown trout and Arctic char, contained an acidophilic, proteinaceous secretion. The cell number increases during chronic infections in immature trout and decreases during sexual maturation of trout and char.

Prevention

In a study of parasites on wild and cultured fishes in two lakes and a fish farm in central Finland, Valtonen and Koskivaara (1994) determined that *I. necator* was the most prevalent parasite. It was found on a wide range of fish species, including salmonids, whitefish, roach and perch. In a study examining the epizootiology of protozoans in farmed salmonids at northern latitudes, Rintamaki-Kinnunen and Valtonen (1997) showed that, unlike other protozoan parasites, *I. necator* infections had a higher prevalence within fingerling sea trout than yearlings. *I. necator* can also be transferred to seawater sites (Urawa and Kusakari, 1990) and can cause morbidity and losses within sea cages, although in some reports a separate species is also likely to be involved (Urawa and Kusakari, 1990; Bruno, 1992b; Lamas and Bruno, 1992). Under some circumstances it may be practical to move rainbow trout yearlings to a lower water temperature to prevent parasite development (Dyková, 1995).

Future studies

I. necator is a ubiquitous parasite of teleost fish, with a wide host and geographic range. There has been surprisingly little work done on the pathology and epizootiology of this flagellate. Current molecular techniques could be applied to gain valuable

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information about antigenic proteins and carbohydrates produced by the parasite, aiming towards a goal of vaccine production.

Microsporidium takedai

Introduction

Microsporidium takedai is a highly pathogenic Microspora parasite and specific to the Salmonidae (Awakura, 1974). All reports of microsporidiosis by this parasite occur in freshwater salmonids from Japan, with other species occurring in seawater fish.

Characterization

M. takedai is diagnosed by finding ovoid spores with subapically attached polar tubes that measure 2×3.4 μm in length. The target tissues are the heart and skeletal muscle, where whitish, spindle-shaped cyst-like lesions form, which are generally visible through the skin.

Impact

Eight species of salmonids are known to be susceptible and they include sockeye, pink, chum and masou salmon, rainbow and brown trout and Japanese char. Prevalence may be 100% in rainbow trout, but is usually lower in other species. A seasonal prevalence of this parasite is recognized, with the initial outbreak during the summer at water temperatures around 15°C (Urawa, 1989). The life cycle is direct and *M. takedai* is transmitted directly by ingestion of the spores present in food, or in the water.

Clinical signs

In chronic cases, the heart shows an extreme hypertrophy and deformation of the tissue with inflammatory oedema. Acute cases frequently result in high mortalities and are characterized by massive numbers of cysts within the musculature. The tissue response in skeletal muscle is similar to that observed in the heart muscle. Within each cyst numerous ovoid, proliferating microsporidia occur and measure 2.5–4.0 μm when mature (Dyková, 1995). No xenoma formation occurs. Fish surviving this infection appear to acquire protective immunity for up to 1 year.

Diagnostic techniques

Provisional identification is based on gross examination of the musculature, and confirmed by microscopical examination of the cysts.

Prevention

There is no commercially available treatment for *M. takedai*, and avoidance is the most effective means of control.

Diagnosis

Diagnosis is by gross external signs, and by identification of the flagellate using light microscopy. Skin or gill scrapings can be prepared and examined at a magnification of greater than ×25. The parasite is approximately the same size as salmonid skin cells and swims with a staggering movement.

Prevention and treatment

There is no vaccine, and prevention is largely reliant on good animal husbandry measures. Tojo and Santamarina (1998) found metronidazole, secnidazole and triclabendazole were 100% effective against the parasite in experimentally infected trout; however, the effective doses were too high to be economically viable, although there was no observed toxicity associated with the drug.

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5 Non-infectious Disorders of Coldwater Fish

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Introduction

The use of cage culture technology for the commercial on-growing of finfish has proven to be economically efficient and it continues to expand. Part of what separates this production approach from land-based technologies is the range of non-infectious diseases that confront producers and health professionals. The principal Achilles' heel of cage culture is the minimal degree of control, beyond that afforded by cage site selection, over environmental phenomena.

Coldwater cage culture is dominated by the production of salmonid species such as Atlantic salmon (*Salmo salar*), chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) in marine environments. The objectives of this chapter are to focus on non-infectious diseases that interact with this segment of the finfish aquaculture industry. Commercial cage culture of flatfish species, cod and other marine coldwater species is developing in importance, as is cage culture of rainbow trout and Arctic charr (*Salvelinus alpinus*) in freshwater lakes or brackish water bays. Some of the conditions discussed are also appropriate to the developing culture of these coldwater species.

Failure of Juvenile Salmon to Adapt to Marine Culture

The phase in which salmon smolts are moved from their freshwater rearing sites to the marine cage culture on-growing site is a period of high risk. This is also the case for transfer of steelhead rainbow trout juveniles for on-growing in marine cages (Oorschot and Boon, 1993). The Atlantic salmon hatchery industry has largely geared its activity towards production of a seawater-ready smolt in 1 year from egg hatching. This S1 smolt comes from the upper-modal growth population of juveniles in a hatchery, and is judged for seawater-readiness based on anatomic, behavioural and physiological characteristics. Variations on the theme exist. For example, entry of smolts to seawater can take place during their first autumn (S0.5), second autumn (S1.5) or second spring (S2). As yet there is no consensus on mortality rates to be expected with each regime; however, autumn entry success is usually less than for spring entry. Chinook salmon, depending on the strain being used, can be put to seacages as S0 smolt (i.e. entry in the spring of the year the eggs hatched). However, this practice has been anecdotally implicated as the cause of higher mortality rates, particularly from infections with *Renibacterium*

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salmoninarum (the causative organism of bacterial kidney disease) during the remainder of the marine production cycle.

A number of factors can lead to smolt mortalities soon after fish are transferred to seawater. With spring transfer of S1 Atlantic salmon smolts, a mortality rate during the first month post-transfer of up to 3% may not be regarded as unusual. This derives from an inability of some juveniles to adapt properly to sea water. Mortality patterns after seawater entry can vary dramatically, and their analysis can be used to point to potential aetiologies. For instance, when mortalities occur shortly after seawater introduction, this usually points to problems stemming from fish handling and transport methods. Acute patterns of mortality shortly after shipment frequently stem from anoxic conditions developing during transportation. The window of opportunity just prior to smolt movement has been viewed as a period in which health checks, treatment, vaccination, grading and inventory assessment can take place. Handling of fish is a stress that results in elevated oxygen consumption in the periods following handling (Davis and Schreck, 1997). Transporting fish to cage sites after handling may compound the oxygen debt that fish experience, especially since high loading rates (to reduce the weight of transported water) of fish are frequently used during shipment. Acute post-transfer mortality is also linked to the physiological and osmotic demands that develop if smolts are handled so roughly that scale loss occurs. Smolts are particularly prone to losing scales, and this in turn creates significant osmoregulatory problems on introduction to seawater. Smolt loss can also occur if newly introduced juveniles encounter strong currents. In reviewing diagnostic case material from Atlantic Canada (Aquatic Diagnostic Services case archives 1990–1997, University of Prince Edward Island, Canada), there have been several submissions of smolts with severe skin lesions from net abrasions and exertional muscle necrosis (Fig. 5.1) following their introduction to marine cages where water current was excessive. The type and pattern of muscle damage are interesting in that 'fingerprint lesions', which can be misdiagnosed as nutritional deficiency, can persist in survivors. Early muscle lesions include various forms of degeneration, such

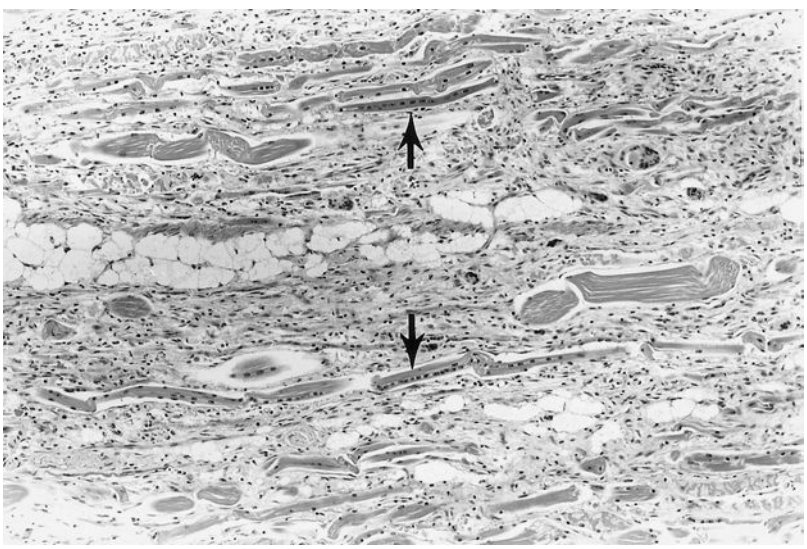


Fig. 5.1. Section of epaxial musculature from an Atlantic salmon post-transfer smolt several weeks after introduction to a cage site with excessive water current speed. Extensive satellite cell, myoblast and fibroblast proliferation have replaced areas vacated by muscle fibre necrosis and dissolution. Regenerative fibres, with characteristic nuclear rowing (arrows indicate several examples), are abundant. H&E stained.

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as segmental and discoid myofibrillar changes. Fibre necrosis is followed by mineralization. Survivors show a range of muscle changes including presence of macrophages removing cellular debris, proliferation of satellite cells, myoblast elongation and fibre regeneration (Fig. 5.1).

The scale and scope of post-transfer mortalities are of concern to the cage culture industry. In vertically integrated operations there is considerable opportunity to coordinate protocols aimed at reducing transfer stress. This can involve more precise timing of pre-transfer activities (vaccinating, grading), the timing of transfer itself and avoiding delays during transfer. Protocols for safe smolt transfer, taking into account pre-shipment, shipment and post-introduction activities, have been developed (see Pennell, 1991, for specific information).

In contrast to the acute mortality peaks, which can reflect transportation problems, a more gradual onset of post-transfer mortality is widely (but anecdotally) attributed to the problem of partial adaptation of non-smolts to the marine environment. These fish do not die at the time of transfer, but fail to regulate their blood electrolyte levels effectively. These fish are highly stressed, they darken and hang listlessly at the water surface and towards the edges of cages. Over an extended period, they undergo advanced body condition deterioration and often succumb to infectious diseases including those not typically problematic in marine environments, such as costiasis (Figs 5.2 and 5.3). The role of these stressed fish as bioamplifiers of disease such as sea lice infestations, furunculosis and vibriosis is felt to be an important factor in the epizootiology of disease outbreaks. The term 'lice-magnets', to describe the effect of these fish prior to sea lice epizootics, has been coined in reference to this effect.

The rapid changes in environmental indices experienced by smolt at seawater entry have been linked with the development of epithelial hyperplastic plaques (Nowak and Munday, 1994) and increased numbers of mucous cells (Franklin, 1990) on the gill filaments of Atlantic salmon. Environmentally induced precursor lesions are often cited as predisposing causes for infectious gill conditions. With relevance to marine culture of salmonids, these post-entry lesions are proposed to create a favourable environment for the establishment of *Paramoeba* sp. infections responsible for amoebic gill disease (AGD) in Tasmania (Nowak and Munday, 1994). An idiopathic gill condition known as clubbing and necrosis gill syndrome (CNG), which may also act as a predisposing factor for AGD, has recently been described for Atlantic salmon smolt in brackish water cage sites in

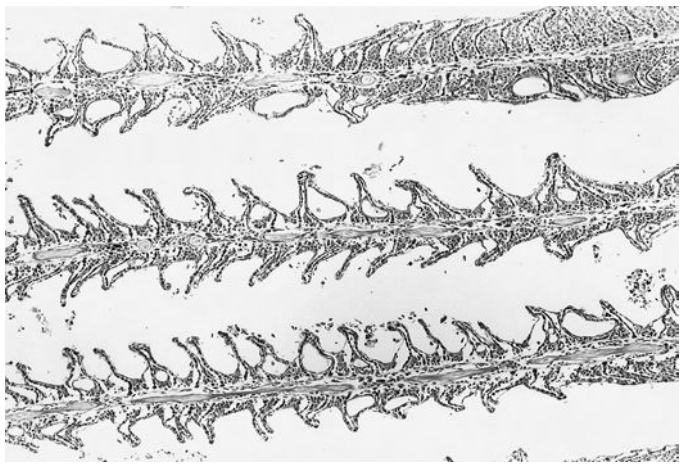


Fig. 5.2. Section of gill taken from a 'pinhead' Atlantic salmon smolt several weeks after seawater transfer. Virtually all lamellae are fused to adjacent lamellae in a pattern typical for costiasis. H&E stained.

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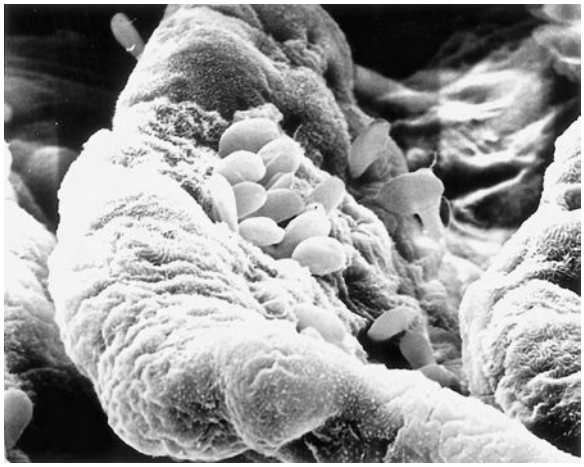


Fig. 5.3. Scanning electron micrograph of a gill lamellar surface, which is colonized by flagellated protozoans with a morphology typical of *Ichthyobodo necator*.

Tasmania (Clark *et al.*, 1997). During CNG, fish have diminished feed response and affected fish have pale gills due to excessive branchial mucus production. As yet, the specific environmental conditions responsible for the genesis of these branchial hyperplastic responses have not been determined. Similar changes have not yet been described in other parts of the world where Atlantic salmon production takes place.

Pathophysiological Effect of Routine Aquaculture Practices

During on-growing in cages, routine aquaculture practices can contribute to fish stress and physical damage. Grading and transferring fish between cages is a necessary but risky task. Dip nets and mechanical pescolators can damage fish. Farmers are generally hesitant to move or grade fish during warm water periods because of the incidence of infectious disease problems that occur post-handling. It is not known whether this link between handling and outbreaks of infectious diseases stems from the effects of physical trauma or physiological stress or both.

Grading and population splitting is a necessary event. Infrequent grading and the resultant increased variability of fish size will accentuate differences in specific growth rate during the remainder of the production cycle. Harvesting a cage that has a spread in size and condition affects marketing (Huguenin, 1997). A general solution is to complete grading procedures during spring and autumn, such that fish are relatively undisturbed during periods of warmer water.

Performing net changes to remove biofoulants is another situation in which stock are either moved or stressed. Organic growth on netting increases during warmer temperatures. Net cleaning or net changing during warm weather is considered to be stressful to stock. However, failure to clean nets predisposes cages to reduced water flow through them, but conversely increases the amount of current-induced net deformation due to heightened resistance to current flow. The latter can reduce the habitable volume of a cage.

An interesting problem reported by Williams *et al.* (1995) is an ocular degeneration that develops in cage-cultured halibut after handling. The ocular changes include gas- and fluid-filled cysts in the ocular choroid (= posterior uvea). This lesion is usually combined with damage to the ocular chambers, uveal structures and lens. The lesions closely resemble those of ocular gas bubble disease, and Williams *et al.* (1995)

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have suggested that the trauma of handling may cause gases in blood to come out of solution. They proposed that some marine species may be uniquely predisposed to this, due to relatively high levels of carbonic anhydrase within the ocular choroid. This may generate excess local oxygen production within the body of the choroid itself. Conditions favourable for the production of typical gas bubble disease as seen on land-based facilities supplied with ground water are unlikely to occur for sustained periods at sea cage sites. As such, typical gas bubble disease is not reported for cage-cultured fish.

Oil adjuvants in fish vaccines have become widely used for cage-cultured salmon (Poppe and Breck, 1997). In eastern Canada, their use has been standard practice since the occurrence of coldwater vibriosis (Hitra disease) in the early 1990s. Reduced growth rates after the use of an oil-adjuvanted vaccine have been noted and may be attributed to chronic active peritonitis and production of fibrotic visceral adhesions (Lillehaug *et al.*, 1992; Poppe and Breck, 1997) (Fig. 5.4). Poppe and Breck (1997) also noted that the degree of post-vaccination side effects varied widely between farms and within the same fish population. Additionally, where the vaccine is inadvertently administered into the gut wall, severe granulomatous enteritis extending from the serosal surface through to the mucosa can develop, and in some cases lead to leakage of gut contents into the peritoneum and death from acute peritonitis.

Behaviour-related Problems

Damage to fins, skin and eyes can result from the hierarchial activities typical of salmonids. These problems are less frequent in Atlantic salmon compared with chinook salmon. Determining whether this correlates to different degrees of domestication is complicated since evidence can be generated for both increased and decreased aggression resulting from domestication (Ruzzante, 1994). Selecting broodstock based on growth rate may be inadvertantly selecting for feeding-related agonistic behaviour if access to feed is determined by aggressive interaction and competition

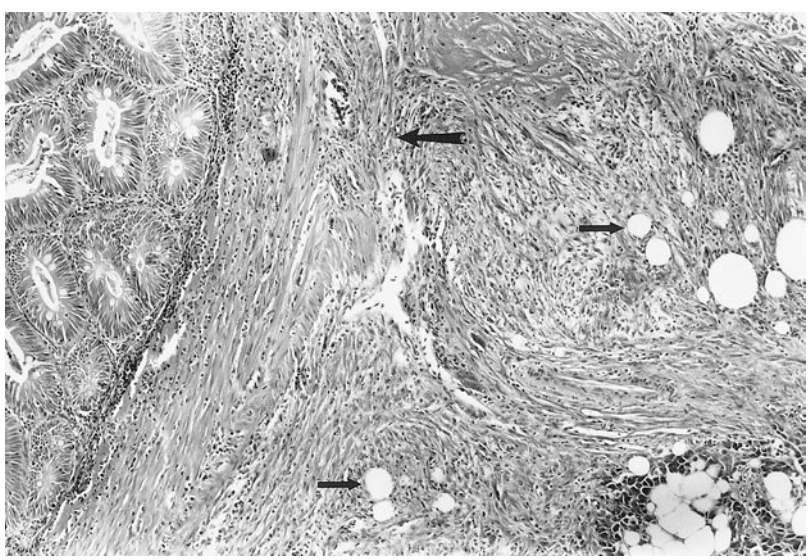


Fig. 5.4. Severe granulomatous reaction affecting the peritoneum and stomach wall of an Atlantic salmon several months after vaccination with a preparation containing an oil-based adjuvant. Persistent droplets of oil (small arrows) and the site of invasion of the granulomatous response into the muscle wall of the stomach (large arrow) are shown. H&E stained.

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(Ruzzante, 1994). Extending from this it is perhaps curious to note that aggression between salmon is often found to be inversely dependent on stocking density. This may be related to the suppression of hierarchial establishment at high densities.

Territorial behaviour of Atlantic salmon can result in bite wounds on the tail fin, peduncle region, anal fin and vent, presumably as one fish is pursued by another. Wounding, and ulcers that develop from secondary infections, have an economic effect through downgrading of the final product if healing is not complete or if scarring and/or melanization occurs. Abrasions can also develop from surface leaps, in which fish contact and become abraded from surface netting. Furevik *et al.* (1993) noted that 6% of surface leaps caused fish to contact pen netting. Culture techniques being developed for Atlantic halibut may also lead to abrasion problems. Martinez Cordero *et al.* (1994) have found that halibut will congregate on the cage bottom rather than occupy the water column. During rough weather, when sea cage bottoms heave, this could result in net abrasions although observations show at least some halibut leave the cage bottom during rough weather.

Conditions Relating to Oxyradical Production

Modern methods of feed manufacturing have drastically reduced the problems associated with nutrient imbalance and stability of ingredients. However, the imprecise knowledge of fatty acid requirements of fish, combined with the use of high fat diets to deliver protein-sparing metabolic energy, and the demands of marine coldwater fish for polyunsaturated fatty acids (PUFAs) as membrane lipid components (Winston and Giulio, 1991) create problems stemming from feed storage (Hertrampf, 1992). Rancidity, resulting in the production of malonaldehyde, develops during the exposure of PUFAs to oxygen.

In a pattern similar to the multiple presentations of fat oxidation in other domestic species, there are many clinical and pathological disease presentations that link back to the problem of antioxidant defences becoming exhausted by oxyradical production. There are quantitative differences between species for the major antioxidant enzymes – superoxide dismutate, catalase and glutathione peroxidase (Winston, 1991) – which may contribute to the differing clinical patterns relating to feed rancidity. Contributing to the overall oxyradical production/free radical quenching balance sheet are dietary factors such as the amount and saturation profile of fat components, storage factors contributing to rancidity and relative abundance of feed-additive stabilizing compounds such as tocopherol, carotene, ascorbic acid and glutathione (Winston, 1991). Variable sparing or contributing effects within this mix of components means that studies on isolated components vary considerably. As an excellent example of this, Frischknecht *et al.* (1994) compared the lesions attributed to deficiencies in vitamin C, vitamin E and also the two in combination. Rainbow trout deficient in either had suppressed growth, anaemia, muscle dystrophy and haemosiderin deposition in the spleen. With the addition of vitamin E only, similar signs developed along with deformations of the vertebral column and spontaneous haemolysis. Also, fish of different ages showed differences in these lesions.

A significant rancidity-related problem in farmed fish is lipoid liver disease. This is typically linked to auto-oxidation of fats in fish feeds (Roald *et al.*, 1981; Saraiva *et al.*, 1986). In addition to appetite suppression, clinically affected fish manifest signs stemming from red blood cell destruction and, on further examination, have large friable pale livers due to macrovesicular lipidosis and ceroid deposition (Fig. 5.5). Cell-membrane degradation products can be found in other organs, and are especially abundant in those areas with a large resident phagocyte population such as peritubular networks of the kidney (Fig. 5.6). Hepatocellular necrosis can occur when fish with lipoid liver disease are exposed to sudden heightened oxidative stress.

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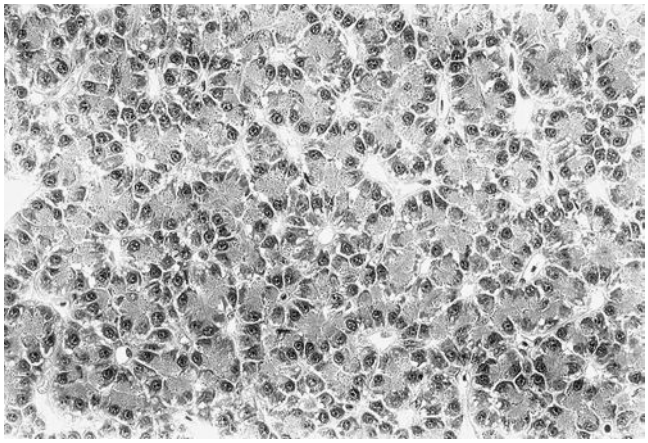


Fig. 5.5. Section of liver from a fish with hepatic ceroidosis. Hepatocytes are uniformly enlarged due to intracytoplasmic accumulations of ceroid and lipid. H&E stained.

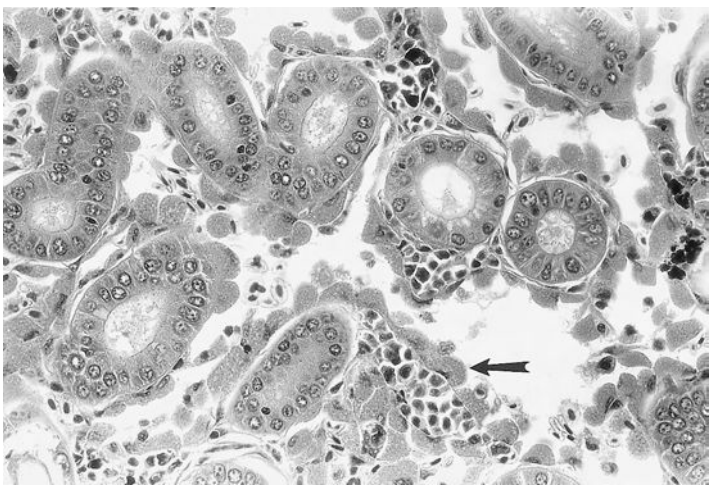


Fig. 5.6. Section of a kidney from a fish with lipoid liver disease. Macrophages of the peritubular capillary network are enlarged (arrow points to an example) due to accumulations of ceroid and other cell-membrane breakdown products. H&E stained.

Pansteatitis, a condition noted in rainbow trout in which inflammatory cells are present in any fat storage area, occurs in trout reared in brackish water and fed rancid feeds. A condition of fat cell necrosis in Atlantic halibut, which affects subdermal adipose tissue, has also been linked to an imbalance of dietary oxidants and antioxidants (Bricknell *et al.*, 1996). This disease is curious since a specific region of fat deposition is targeted, and multinucleated macrophages within the inflamed degenerate fat stores are characteristic. In a less direct manner than for the conditions cited above, increased oxidant stress arising from inadequate vitamin E levels has been shown to increase the clinical effects of salmon pancreas disease (SPD) as it occurs in Atlantic salmon (Ferguson *et al.*, 1986a,b; Raynard *et al.*, 1991).

In contrast to the epizootiological presentation of SPD, acute heart failure or

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cardiomyopathy syndrome (CMS) typically occurs in fat fast-growing salmon that have spent at least one sea winter in cages (Amin and Trasti, 1988; Ferguson *et al.*, 1990; Grotmol *et al.*, 1997). Fish often die during feeding (Grotmol *et al.*, 1997) and on necropsy an enlarged and sometimes ruptured atrium with haemopericardium is detected. Death may be from cardiac tamponade (Ferguson *et al.*, 1990). The pathology includes development of thrombi in the atria, acute myocardial lysis, the presence of debris-laden subendocardial macrophages and endothelial damage (Ferguson *et al.*, 1990). Because the disease affects rapidly growing fish and because there is myocardial damage with some similarity to that noted in SPD, nutritional and specifically oxidant factors have been speculated as playing a role. However, Grotmol *et al.* (1997) have recently identified an endotheliotropic nodavirus that reacts with antibodies raised to the striped jack nervous necrosis virus. Therefore, it may be that nutritional factors have a modulating effect on CMS presentation, as they do for SPD. It is interesting to note that Atlantic salmon, during periods of stress, accumulate adrenaline in atrial tissue, perhaps due to potent local uptake mechanisms (Fløysand *et al.*, 1992). Endogenous adrenaline in excessive amounts is known to be cardiotoxic (Carlsten *et al.*, 1983), and poikilotherms manifest this by necrosis of spongy myocardium.

Myointimal hyperplasia leading to coronary arteriosclerosis is a common condition in wild and cultured salmon, and has been shown by Saunders *et al.* (1992) to be linked to rapid growth rates of salmon as opposed to being an effect of sexual maturation. Whether this lesion is influenced by composition of dietary fat is still unknown but this remains an active hypothesis.

Intertwined with the effects of oxidative stresses contributed by high fat diets are those scenarios that can arise when dietary antioxidants are deficient in the diet. The clinical repercussions of vitamin E, vitamin C or selenium deficiencies are rarely classical in clinical presentations, and, as detailed by Frischknecht *et al.* (1994), experimental data suggest that manifestations are dictated by factors such as water temperature and fat content of diet and interactions between antioxidant components themselves.

Gastrointestinal Impaction

Water-belly syndrome (WBS) is a problem for several species of cage-reared salmonids, but especially for marine-reared rainbow trout (Staurnes *et al.*, 1990) during periods when salinity is high and water temperature is low. Changes to feeding programmes, and in particular the use of extruded pellets with a higher fat and carbohydrate content, have been cited as risk factors (Staurnes *et al.*, 1990). Affected fish are easily detected based on gross appearance. On dissection they have a massively distended stomach filled with water (Fig. 5.7). Reductions in the thickness of the abdominal wall led Staurnes *et al.* (1990) to conclude that the condition generally develops over a considerable period of time and that recovery is uncommon.

A differential diagnosis for WBS includes the gastric impaction and mural gastritis that develop in salmon due to the consumption of wood chip debris. Miller and Black (1992) noted that in areas where wood chips are hauled in open barges, considerable amounts are spilled during loading and transport. Fish that have been trained to eat pellets will also respond to floating or slowly sinking wood chips. The indigestibility of wood chips causes them to accumulate in the stomach where they act in the typical fashion of a foreign body, blocking the transit of other ingested particles from the stomach. At one farm, Miller and Black (1992) noted that 80% of salmon had woody debris (mostly bark) in their gut, and attributed 90% of the farm's mortality rates to the effects this was having on the fish. Curiously, in an attempt to deflect the ingress of floating wood chips into the cages by the use of a skirting net, they found an effect opposite to that expected. The skirting net restricted the velocity of surface water currents moving through the cages so that

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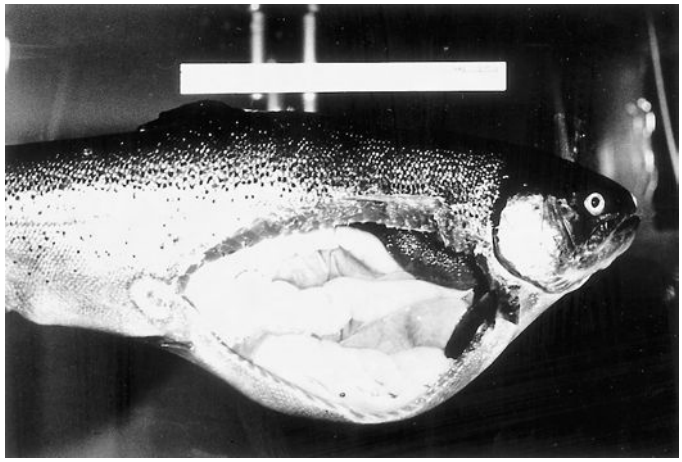


Fig. 5.7. Marine cage-cultured rainbow trout with massively distended gastrointestinal tract due to water-belly syndrome.

contact time between fish and debris was increased.

Conditions Relating to Cage Site Location

Cage-cultured fish are a physically constrained population within an environment that is partially changeable due to currents and tides. Avoidance of detrimental environmental factors is therefore restricted to the limited space and depth within the confines of the cage. Accordingly, site selection is a critical process and should, ideally, examine meteorological, locational and biological characteristics (Huguenin, 1997).

One of the more significant obstacles facing expansion and sustainability of marine cage culture is the availability of suitable sites (Huguenin, 1997). For example, in eastern Canada, there are limited numbers of sites that are sufficiently protected from severe weather while simultaneously having enough current flow to avoid summertime heating and slack-tide anoxia, or winter superchill. In other areas, problems of multiple use of water resources have brought aquaculture into conflict with other stakeholders. As an example of the detrimental effects for fish farming, aquaculture siting is frequently in areas that are near commercial marine traffic. There have been several recent incidents of oil spills from tankers (an example being the Shetland Island 'Braer' incident in the early 1990s), which have raised alarm bells about what should be expected when an oil slick comes into contact with farms. The outcome from a crude oil spill is difficult to predict, since, as reviewed by Alkindi *et al.* (1996), a number of processes occur after the spill, which affect the fate of the hydrocarbon mix and therefore the ultimate mix of toxins. As a result, the aftermath of an oil spill (and/or any other commercially shipped aquatic toxin) will largely be unpredictable.

Autointoxication is also a concern at lease sites where fallowing has not occurred, where currents are minimal and where the distance between the bottom of cages and the sea bottom is minimal. Benthic degradation and, specifically, deposition of protein-rich organic debris from salmon farm sediments is of concern to aquaculturists and environmentalists. As anoxic conditions develop within accumulating debris, conditions become favourable for the production of hydrogen sulphide. Off-gassing of hydrogen sulphide and/or release of anoxic water and hydrogen sulphide due to current-induced disturbance of the deposits are believed to be responsible for the condition known as site souring (Munro, 1990; Papoutsoglou *et al.*, 1996). Population signs related to site souring include reduced growth rate and

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increased disease problems of stock (Kiemer *et al.*, 1995). In studies on the effects of chronic exposure of Atlantic salmon smolts to sublethal concentrations of hydrogen sulphide, Kiemer *et al.* (1995) found that it caused progressive gill hyperplasia, coupled with degeneration and necrosis of hepatocytes. Hydrogen sulphide is also capable of binding to haemoglobin and inhibiting the oxygen uptake in a manner similar to that induced by exposure to nitrite. The combination of gill pathology and reduced oxygen loading kinetics of haemoglobin may perhaps act synergistically on growth performance.

Cage sites in well-protected shallow sites, such as partially enclosed bays, run the risk of periodic high temperatures and marginal to lethal dissolved oxygen levels occurring during slack tides. Additionally, Oorschot and Boon (1993) implicated higher metabolic requirements for osmoregulation at elevated water temperatures as a contributing cause to summer mortality in marine-cultured rainbow trout. Conversely, in eastern Canada, dramatic mortalities have occurred because of superchilling (Fletcher *et al.*, 1988). This problem generally occurs in mid- to late winter, on cloudless nights when air temperature plummets to below –20°C. As high tide begins to recede, water, which has superchilled (having cooled to just below its freezing temperature but not yet undergone ice crystal formation), draws back towards lease sites. As fish contact this unstable superchilled water, their body temperature drops to a level where blood and body fluids will freeze. Superchill phenomena can lead to mortality of all stock on a farm.

In general, site selection is an important process that should consider temperature profiles during summer and winter, the likelihood of declines in dissolved oxygen at slack tide, plankton bloom history, current speed at slack and peak tides, the abundance and type of predators, shelter from wind and storms, and the likelihood of exposure to upwelling currents (see Pennell, 1992, for practical review and specific guidelines).

Algal Blooms

Toxic algal blooms are a cause of catastrophic losses to marine cage culture operations and globally their incidence or recognition is on the rise. The mechanisms of interaction between algae and finfish that contact algal blooms are highly variable. Additionally, the inter-relationship of environmental factors that promote blooms are complex and challenging to model (Perry *et al.*, 1989), as is predicting the timing of diatom aggregation and subsequent mass sedimentation phenomena characteristic of bloom declines (Passow, 1991). Environmental variables control not only the growth habits of the algae, but also the motility, morphology (for example, transformation from unicellular to colonial habit) and rates of secretion of carbohydrate complexes (Tomas, 1978; Luttke, 1979). The upwelling of ocean currents, which brings about nutrient enrichment and rapid changes to water temperatures, is a common factor promoting algal blooms. Historical occurrences of coastal upwelling phenomena and algal blooms are important criteria for cage operation site selection.

Morphological characteristics of some diatoms appear to aid their ability to cause disease. For example, the chain-forming diatoms *Chaetoceros concavicornis* and *Chaetoceros convolutus* (Albright *et al.*, 1993) and some non-chain-forming *Corethron* spp. (Speare *et al.*, 1989) diatoms possess setae and spinules (barbs). These brittle spear-like projections emerge from the diatom and form a silica-rich web-like structure around the body of the frustule, which assists the buoyancy of the diatom (Fig. 5.8). Inadvertently, however, the setae are important morphological features, which aid pathogenicity. The setae projections cause the diatoms to become retained in the sieve-like arrangement of interfilamental and interlamellar spaces of the gill (Speare *et al.*, 1989) (Fig. 5.9). The colonial nature of the *Chaetoceros* diatoms, which may be an advantage to the diatom with respect to sedimentation rates (Fryxell, 1978), probably further enhances their

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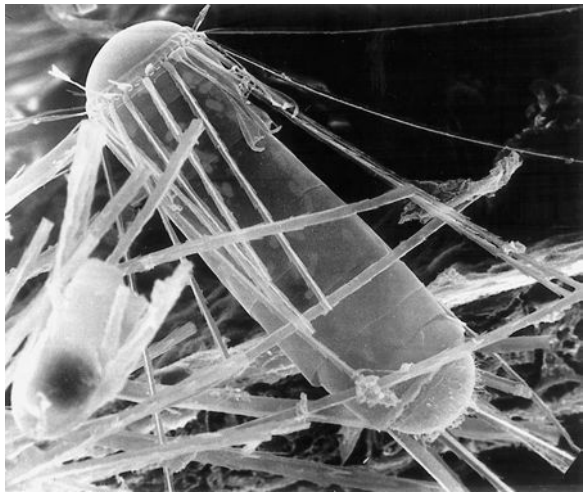


Fig. 5.8. Scanning electron micrograph showing the arrangement of the spine-like setae emerging from the corona of a *Corethron* sp. diatom. Material was collected from the gill of a marine cage-cultured coho salmon during an algal bloom.

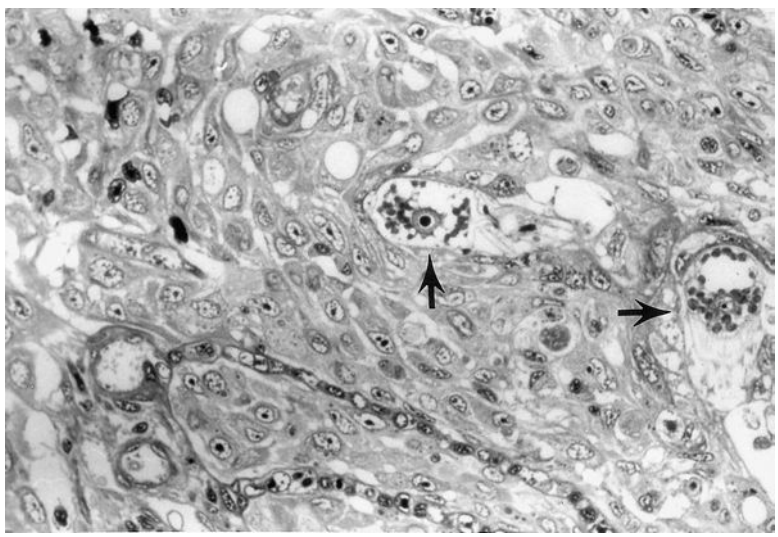


Fig. 5.9. Section of gill with epithelial hyperplasia surrounding entrapped diatoms (arrows). H&E stained.

pathogenicity, since a flexible chain of setae-bearing diatoms is more likely to become entrapped or snared during passage between gill lamellae.

There has been considerable interest in determining the pathophysiological effect of diatom blooms on fish, in order that therapeutic and supportive measures can be put in place once a bloom overruns a cage site. The clinical signs of salmon during exposure to a *Chaetoceros* sp. bloom are largely those of anoxia brought about by abundant mucous discharge on to gill lamellae (Albright *et al.*, 1993). A similar phenomenon was reported by Hishida *et al.* (1997) to explain the cause of death of marine

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cage-cultured yellowtails during a bloom of the marine alga *Chattonella marina*. Additionally it has also been shown that *C. marina* produces oxygen radicals especially during exponential growth phases (Ishimatsu *et al.*, 1996a). Whether there is a causative relationship between oxygen radical production and hypersecretion of mucus is as yet not precisely defined (Ishimatsu *et al.*, 1996b).

In clinical cases relating to the effects of exposure to *Chaetoceros* spp., there is a protracted time course of mortality and poor growth performance. This appears unrelated to the acute branchial reactions to the algae and more probably reflects the progressive pathology evoked by entrapment of the silica-rich frustules and setae within gill tissue in a manner similar to that proposed for *Corethron* spp. (Speare *et al.*, 1989). The specific lesions include a random multifocal pattern of branchial lamellar and filament fusion, with layers of squamous-transformed epithelial cells encircling captured diatom elements. Migration of leucocytes, originating from lamellar pillar channels and the gill filament's central venous sinusoid, towards the pockets of trapped diatoms is common (Speare *et al.*, 1989) and mechanistically understandable considering the antigenic nature of many forms of silica (Lugano *et al.*, 1982). Growth factors released by the leucocytes presumably have a role in evoking the epithelial hyperplasia that accompanies diatom entrapment.

Recognition of the chronic physiological disturbances, which persist in fish after exposure to an algal bloom, is critical in managing the health and performance of recovering populations. Feeding and cage management decisions need to take this recovery time-lag into consideration. For example, these fish may have little respiratory-function reserve. Therefore, unexpected mortalities may occur after routine practices such as grading or net changes. Furthermore, Albright *et al.* (1993) noted that cage-cultured Pacific salmon had reduced resistance to common indigenous bacterial pathogens following exposure to sublethal concentration of *Chaetoceros* spp. Whether this is a manifestation of stress, or a more specific mechanistic pathway is unknown.

The possible role that other setae-bearing diatoms, not typically recognized as problematic, may have on the health of cage-cultured salmon has been raised. For example, Bruno *et al.* (1989) noted the potential role that *Distephanus speculum* and *Chaetoceros debile* may have. Additionally Kent *et al.* (1995) demonstrated severe gill lesions in fish exposed to a bloom dominated by the chain-forming *Skeletonema costatum* and two species of *Thalassiosira*, which possess barbless setae. This suggests that diatom monitoring programmes that are put in place to alert fish farmers of diatom bloom status need to track a diverse range of organisms since many of them may prove to have negative effects on fish health.

Heterosigma carterae (*Heterosigma akashiwo*, *Olisthodiscus luteus*) has been reported as a significant cause of catastrophic fish mortality in many parts of the world including the Pacific Northwest. Unlike the setae-bearing diatoms whose action on the gills appears to be mechanical, *H. carterae* appears to affect fish by releasing a toxin. Supplemental oxygenation does not improve fish survival and the diatom does not provoke gill lesions (Black *et al.*, 1991). Fish experimentally exposed to this alga appear anaesthetized (Black *et al.*, 1991) and this may reflect the action of a brevetoxin (neurotoxin), which has been identified from *H. akashiwo* samples from a red tide in Japan (Khan *et al.*, 1997).

A toxin affecting permeability of gill epithelium is also believed to be the mechanistic link between algal blooms of the phytoflagellate *Chrysochromulina polylepsis* (Prymnesiophyceae) and massive mortalities of cage-cultured Atlantic salmon on the Norwegian coast (Underdal *et al.*, 1989). These authors reported that a combination of low salinity, high temperatures and high nutrient content created conditions believed to be conducive to the bloom. Additionally, the pathophysiological effect of the algal cells on the fish was affected by salinity (Underdal *et al.*, 1989), suggesting

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that the elaboration of toxin was under tight environmental control.

Exposure to microcystin hepatotoxins released into the environment from blue-green algae has been implicated as the cause of gill and liver damage in feral brown trout in Scotland (Rodger *et al.*, 1994). It is also suspected to be the cause of net pen liver disease (NPLD), a syndrome that yields a unique pattern of liver damage in cage-cultured Atlantic salmon along the Pacific Northwest (Kent *et al.*, 1988; Kent, 1990). NPLD lesions include marked hepatocellular pleomorphism with abundant megalocytes (Fig. 5.10), some of which are multinucleated. Megalocytosis persists to some degree even in livers of fish that are in a recovery/hepatocellular regenerative phase (Kent, 1990). The syndrome is most typical when it affects Atlantic salmon during their first year in seawater, beginning in summer and leading to persistent mortality rates. In some situations, cumulative mortality has reached 90% (Kent, 1990). The effect of this condition on growth rates and production performance of fish has not yet been defined, although recovery from NPLD can occur if fish are moved to clean seawater (Kent, 1990).

Sunburn

Effects of solar ultraviolet (UV) exposure have been recognized as the cause of classic sunburn lesions on the dorsum of cage-cultured fish. Additionally, the so-called 'summer syndrome' of cage-cultured Atlantic salmon, which begins as a discrete focus of skin necrosis on the dorsum and caudal peduncle (Rodger, 1991), but which can further develop into severe ulceration accompanied by secondary infections, is now believed to be linked to solar UV exposure (McArdle and Bullock, 1987; Rodger, 1991). Summer syndrome is generally a problem for salmon during their first summer at sea, and Rodger (1991) advanced the hypothesis that rapidly growing post-smolts would have many epidermal cells undergoing DNA synthesis or mitosis making them vulnerable to UV. This is supported in part in findings by Bullock and Roberts (1992) that epidermal cells migrating to cover sites of

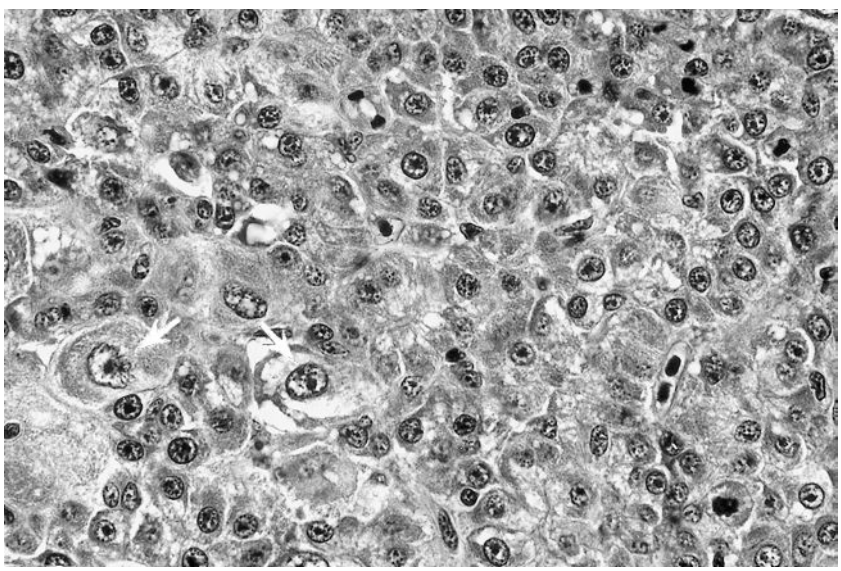


Fig. 5.10. Section of a liver from an Atlantic salmon with net pen liver disease. Hepatic megalocytosis is typical. Note extremely hypertrophied nuclei (arrows). H&E stained. (Photo courtesy of M.L. Kent, Pacific Biological Station, Department of Fisheries and Oceans.)

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repair were most vulnerable to UV. In general, this may explain why fish suffering from epidermal parasitism (particularly sea lice, which frequently target the epithelium covering the dorsal cranium) are also more vulnerable to sunburn.

Epidermal changes induced by excessive UV exposure begin as a pattern of single-cell epithelial necrosis, with necrotic cells scattered throughout the epidermis. This leads to epithelial oedema and separation of the basal layer of epithelial cells from the subjacent basal lamina. Dermal changes include degeneration of melanocytes (McArdle and Bullock, 1987). Whether or not the initial cellular injury will lead to tissue changes such as hyperplasia, ulceration or secondary infection is dependent on the degree of injury and the types and number of opportunistic pathogens.

Predators

Direct and indirect losses due to actions of predators are perhaps the most significant causes of fish mortalities in cage culture (Moring, 1989) and are often anecdotally cited by aquaculturists as a significant constraint to production. This is not surprising considering the attractiveness of a cage culture site to predators, and the often minimally effective defence afforded by predator netting.

Avoidance of predators needs to be one of the major criteria used during site selection. Historical biological data and observations are often useful in determining the local abundance of predators – for example, locations of seal colonies. Unfortunately, because of the rich biota that develops around cage culture lease sites, attraction of feral animals is unavoidable. The interaction of wildlife and farmed fish is a major point of criticism used by detractors of fish farming. Common predators include aquatic mammals such as sea lions, seals and otters, in addition to predatory or opportunistic species such as ospreys, eagles, herons, kingfishers and gulls (Moring, 1989).

Methods used to reduce predator losses have involved cage nettings (over cage surfaces to deter birds and as a skirting to deter marine mammals), visual and acoustic scaring devices, dogs, nightwatchmen, trapping and, in some cases, killing. The economic consequences of predation are numerous. The costs of fish being killed and eaten, or escaping through predator-caused pen tears, would be relatively straightforward to cost-analyse. However, it is usually impossible to gather the data to determine the extent of losses. These losses then become part of the unaccounted-for 'shrinkage' phenomenon of cage inventory (Moring, 1989). Wounds inflicted upon fish are also a concern. Seals will often swim rapidly into the netting and grab hold of and chew off parts of fish that they encounter. Carss (1993) noted that herons commonly dropped fish while trying to pull them through cage nets. The presence of these injuries provides access for primary pathogens. Seal attacks are particularly problematic, since their occurrence increases during periods of colder water temperature. Cold water temperature generally acts to delay wound healing and these persistent wounds become sites for infection. In addition, fish with wounds are downgraded at the time of marketing. As part of an environmental assessment of aquaculture, an excellent synthesis of the impact of predation and methods to limit predation, as practised in British Columbia, has been produced by Iwama *et al.* (1997)

In addition to the costs associated with fish being wounded or killed, the presence of predators frequently stresses the fish to the point where they cease feeding. Farmers cite the presence of predators as a common precursor to outbreaks of infectious diseases such as vibriosis and furunculosis, suggesting that the fish are stressed when they sense predators nearby.

Neoplastic Conditions

Historically, the development of tumours in animals has been considered a non-infectious disease event, which arises due

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to either random or provoked cellular genetic defects. However, within this category, the role of infectious agents is increasingly becoming recognized, and drawing the line between environmental and microbial contributions to oncogenesis is becoming more difficult.

The epizootiology of several types of common tumours supports the idea of random genetic or developmental events as the underlying cause. At the processing line, such disorders as multiple hepatic cysts (Bruno and Ellis, 1986) and polycystic kidneys are sometimes noted. These cysts are generally fluid filled and, in fish as in mammals, may have their origins in failure of component cells (such as biliary duct cells and renal tubular epithelium) to properly maintain cellular polarity. Vectorial processes involved in normal secretion are therefore disturbed (Molitoris and Nelson, 1990) and cysts may form due to the resulting secretions. Nephroblastomas, tumours emerging from the surface of the posterior kidney, in which poorly developed sections of the nephron are formed, are also noted with regularity in market-ready salmon. These tumours seem to have little or no effect on growth performance, although the tumour can grow to massive size.

Thyroid hyperplasia and neoplasia also occur in farmed fish (AVC case archives). Differentiating responsive hyperplasia of the thyroid gland from a benign neoplastic process can be difficult. This differentiation is often critical since many causes of thyroid hyperplasia stem from nutritional deficiencies, which must be corrected. Epizootiological considerations are useful in this regard, as well as monitoring the response (such as regression of follicular hyperplasia) to dietary management. Spontaneous thyroid carcinoma also can occur in cage-cultured salmon. The histological appearance can include bizarre cellular formations in which the diagnosis of their cellular origins may be impossible without the use of immunohistochemical markers. Hepatic tumours may be single animal disease phenomena, but when they occur in groups of farmed fish, other factors such as feed contamination with aflatoxins (Nunez *et al.*, 1991) should be investigated.

Haemic tumours are well represented in cage-cultured salmon. Spontaneous lymphosarcoma has been intermittently diagnosed in Atlantic and chinook salmon cultured in Canada (AVC case archives), as elsewhere in the world, and is characterized by infiltration of many organs, and particularly the renal interstitium, by small uniform-sized lymphocytes (Fig. 5.11). Whether these tumours reflect spontaneous genetic mutations, or develop due to the

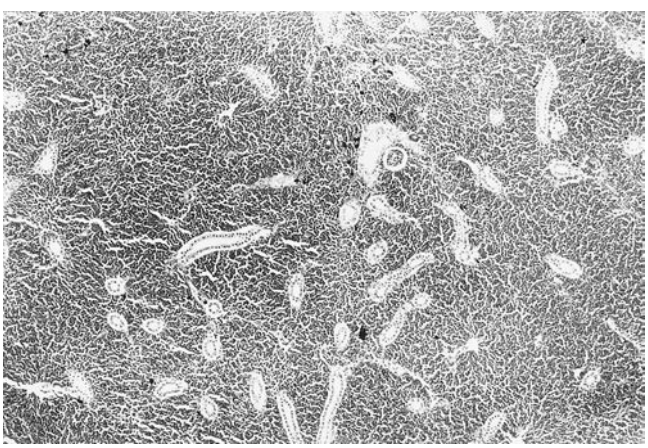


Fig. 5.11. Section of a kidney from an Atlantic salmon in which the inter-tubular portions have been overrun by a population of monomorphic lymphocytes. This pattern is typical for lymphosarcoma. H&E stained.

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interaction of the host genome with an infectious agent such as a novel or endogenous retrovirus, is as yet unknown. Based on the epizootiology, however, it would appear that typical salmon lymphosarcoma is not infectious. In contrast to this, Kieser *et al.* (1991) have reported an outbreak of an epitheliotropic lymphoblastic lymphoma in coho salmon. Its histological picture contrasts with spontaneous lymphosarcoma by the relative immaturity of many of the neoplastic cells. At a population level, it contrasts remarkably in that progression of the disease within the population occurred.

Plasmacytoid leukaemia (PL), also known as 'marine anaemia', is a haemic neoplasm, which principally affects cage-cultured chinook salmon. Fish with PL have massive numbers of plasma cells and plasma cell precursors, including many plasmablasts in mitosis. These cells are widely distributed in the fish and, in particular, infiltrate organs such as the kidney, spleen, liver, retro-orbital areas and the lamina propria of the gut (Kent *et al.*, 1990). PL contrasts with typical lymphosarcoma and the epitheliotropic lymphoblastic lymphoma in that its infectious nature has been repeatedly demonstrated (Kent and Dawe, 1990; Newbound *et al.*, 1993) although the exact nature of the infectious agent remains controversial. Nevertheless, its waxing and waning nature within farmed populations suggests that its expression as a clinical disease entity may be tied to environmental conditions and/or the presence of other disease conditions. Further details on PL are presented elsewhere in this volume.

Disease Conditions Relating to the Use of Medications

There are relatively few scientific reports dealing with the adverse effects of medications applied to fish in cage culture. However, this lack of reporting does not reflect the observations that are frequently made by aquaculturists and fish health professionals. Indeed, several of the bath treatments directed at sea lice are applied in such a manner that behavioural signs of toxicity are routinely noted and used to signal that the treatment period should be terminated. Treatment-induced fish kills, especially those attributed to the effects of organophosphate-based sea lice treatments, can be quite extensive (Roth *et al.*, 1993). This has prompted a search for organophosphates with wider therapeutic margins, in addition to other methods of sea lice control.

Bath treatments in cage-culture situations are problematic because of difficulties in calculating the volumes of water in cages fitted with tarpaulins. Additionally, the underlying disease conditions and pre-treatment stress levels of target fish can lead to results that are unexpected based on comparisons with scientific data, the latter being typically generated from exposure of healthy fish under optimum environmental conditions. Reduction of dissolved oxygen concentrations, during field treatments, is particularly important with respect to organophosphate treatments, since, although brain acetylcholinesterase (AChE) levels recover after organophosphate exposure (Morgan *et al.*, 1990), low oxygen levels at the time of treatment cause a greater and more persistent suppression of AChE (Høy *et al.*, 1991). The latter problem may make fish vulnerable to the toxic effects of a regime of repeated organophosphate baths (Høy *et al.*, 1991).

Bath treatment of fish with high concentrations of hydrogen peroxide has recently been used as an alternative to organophosphates for treatment of salmon with sea lice (Bruno and Raynard, 1994). However, depending on the concentration used, the water temperature and the exposure time, hydrogen peroxide has the potential to cause mortalities (Bruno and Raynard, 1994) stemming from extensive branchial epithelial necrosis and consequent branchial oedema (Johnson *et al.*, 1993a; Kiemer and Black, 1997) (Fig. 5.12). As an alternative to bath treatments for lice, ivermectin (an avermectin compound) has been used successfully when incorporated into the feed (Johnson and Margolis, 1993).

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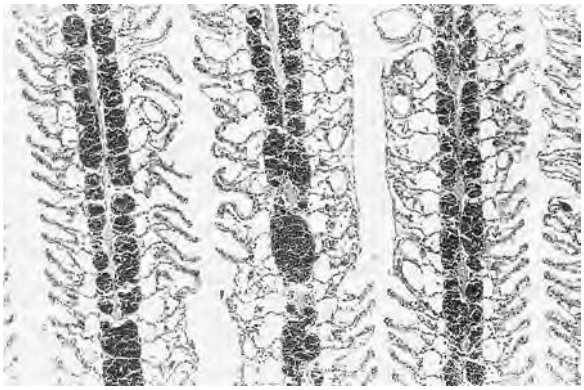


Fig. 5.12. Section of a gill from an Atlantic salmon collected 20 min after the commencement of a bath treatment with hydrogen peroxide (1250 ppm). The gill structure is altered by the presence of widespread lamellar fusion, severe lamellar oedema and marked congestion of the central venous sinusoids of the filament. H&E stained.

Although Johnson *et al.* (1993b) noted a difference in toxic effects between species of salmon, they were unable to correlate histopathological changes with the toxicity.

The incorporation of antibiotics into feed can lead to feed refusal or reduced feeding rates (Hustvedt *et al.*, 1991), particularly if the feeding rate is low and, consequently, the concentration of antibiotic in the feed is relatively high on a percentage basis. Whether antibiotics can directly be associated with fish deaths is difficult to determine; however, Hiney *et al.* (1994) noted an increased mortality rate in Atlantic salmon during a treatment with the fluoroquinolone antibiotic 'flumequin'. Intuitively it should also be expected that diseases may alter the rates of antibiotic metabolism and clearance. This phenomenon has been largely unexplored; however, it was suspected by Bruno (1989) to explain the development of patterns of liver pathology in fish suffering from furunculosis and being treated with oxytetracycline. Bruno (1989) suggested that the antibiotic may have had an anomalous effect on the livers due to compromised antibiotic excretion.

Summary

Non-infectious disorders of cage-cultured fish can stem from many sources. They may lead to distinct clinical entities or play a role in the pathogenesis of infectious conditions. In general, because of the fixed nature of cage culture operations, environmental factors that may cause disease or stress will continue to be major considerations when cage sites are being selected. Current research into non-infectious disorders, despite the importance of these problems to aquaculture bioeconomics, is relatively fragmented and typically does not attract research money in a manner comparable with funding for infectious disorders

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6 Infectious Diseases of Warmwater Fish in Marine and Brackish Waters

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Introduction

The culture of marine finfish in cages that hang from floating rafts was successfully initiated in Japan in the 1950s and in Southeast Asia in the 1970s. In those early years, fish for culture were obtained from the wild. While in many regions this is still the way culture is started, some species of fish are today successfully hatchery-produced. The cage culture system is basically similar throughout the world wherever intensive mariculture is practised. However, disease types and severity are greatly influenced by the species of fish, the conditions in which the animals are cultured and the husbandry management.

Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following rough, although often unavoidable, handling operations. Once conditions suitable for pathological changes develop, progress to disease in the warmwater environment is rapid. Early detection of behavioural changes and clinical signs in the cultured animals are critical for proper diagnosis of the disease.

The warmwater culture of marine finfish in floating cages is concentrated in two main geographic regions, namely West Asia and Southeast Asia. The West Asia regions present wider fluctuations of environmental conditions, particularly water temperature, whereas in Southeast Asia they are generally more stable. The species of fish cultured in the various regions reflect these environmental differences. Other warmwater areas where cage culture is practised on a commercial scale are the tropical islands of the Pacific Ocean.

The most common species of marine fish cultured in floating cages are summarized in Table 6.1.

Diseases Caused by Viruses

Viral diseases in cage-cultured fish have been on the increase since the 1980s in East Asia and the 1990s in Southeast Asia (Nakai *et al.*, 1995; Arthur and Ogawa, 1996; Muroga, 1997; Bondad-Reantaso, 2001; Roongkamnertwongse *et al.*, 2001; Zhang, 2001). Virological research received a new impetus following the high mortality in hatchery-bred juvenile fish soon after being placed in sea cages (Fukuda *et al.*, 1996; Park and Sohn, 2001). With the increasing awareness of virus-related diseases and with new species of fish being selected for culture, more reports of known and new

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Table 6.1. Species (common name) of marine fish cultured in cages in Asia.

Species	East Asia	Southeast Asia	West Asia
Serranidae (seabass and groupers)			
Dicentrarchus labrax (European seabass)	–	–	+
Epinephelus aeneus (white grouper)	–	–	+
E. coioides (greasy grouper)	+	+	+
E. malabaricus (black dot grouper)	+	+	–
E. bleekeri (brown grouper)	+	+	–
E. fuscoguttatus (tiger grouper)	+	+	–
Lutjanidae (snappers)			
Lutjanus argentimaculatus (mangrove snapper)	+	+	–
L. johni (golden snapper)	–	+	–
L. russellii (Russell's snapper)	+	–	–
Chanidae (milkfish)			
Chanos chanos (milkfish)	+	+	–
Centropomidae (snooks)			
Lates calcarifer (Asian seabass)	–	+	–
Mugilidae (mullets)			
Mugil cephalus (mullet)	–	–	+
Scorpaenidae (scorpion fishes)			
Sebastes schlegeli (black rockfish)	–	–	+
Carangidae (jacks)			
Seriola quinqueradiata (yellowtail)	+	–	–
S. dumerili (yellowtail)	+	–	–
Caranx (*Pseudocaranx*) *dentex* (striped jack)	+	–	–
Trachurus japonicus (horse mackerel)	+	–	–
Tetraodontidae (puffers)			
Takifugu rubripes (tiger puffer)	+	–	-
Sparidae (seabream)			
Sparus aurata (silver seabream)	–	–	+
Rhabdosargus sarba (goldlined seabream)	+	–	–
Pagrus major (red seabream)	+	–	–
P. schlegeli (black seabream)	–	–	+
Acanthopagrus bifasciatusi (black seabream)	–	–	+
Sciaenidae (drumfish)			
Sciaenops ocellatus (red drum)	–	–	+
Pleuronectidae (flounders)			
Paralichthys olivaceus (Japanese flounder)	+	–	-

viral diseases are to be expected. The viruses reported in cultured marine fish are summarized in Table 6.2.

Lymphocystis

Lymphocystis is a highly contagious infection caused by a cytoplasmic DNA iridovirus. The disease follows a chronic course and, in general, mortalities are limited. The infected fish recover within a few weeks of the onset of the outbreak, displaying little or no scar tissue (Paperna *et al.*, 1982). Although the unsightly appearance of the typical lesions renders the infected fish unmarketable, juveniles are considerably more susceptible to the infection than larger, market-sized stages.

Host range. Although known to infect 30 families of marine fish (Wolf, 1988), lymphocystis is a host-specific disease (Overstreet and Howse, 1977; Chao, 1984), therefore the disease is most probably caused by a group of different viral strains.

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Table 6.2. Viral diseases of warmwater maricultured finfish.

Disease	Causative agent	Species affected
	DNA virus	
Lymphocystis (LCDV)	Iridovirus	Japanese flounder (*Paralichthys olivaceus*)
		Japanese seabass (*Lateolabrax japonicus*)
		Seabream (*Sparus aurata*)
		Red drum (*Sciaenops ocellatus*)
Red seabream iridoviral disease	RSIV	Red seabream (*Pagrus major*)
		Grouper (*Epinephelus malabaricus*)
	RNA virus	
Viral nervous necrosis (SJNNV and VNN)	Nodavirus	Striped jack (*Pseudocaranx dentex*)
		Black spotted grouper (*Epinephelus bleekeri*)
		Greasy grouper (*Epinephelus coioides*)
		Black spotted grouper (*Epinephelus malabaricus*)
		Grouper (*Epinephelus tauvina*)
		Marbled leopard grouper (*Plectropomus maculates*)
		European seabass (*Dicentrarchus labrax*)
		Asian seabass (*Lates calcarifer*)

In Southeast Asia, only seabass (*Lates calcarifer*) has been reported to be affected by this disease (Limsuwan *et al.*, 1983; Chao, 1984). In Israel, it was reported in seabream (*Sparus aurata*), a species reared in the Red Sea but originally imported from the Mediterranean Sea (Paperna *et al.*, 1982), and in the red drum (*Sciaenops ocellatus*), originally imported from the USA (Colorni and Diamant, 1995). Lymphocystis has been listed as a major viral disease of maricultured fish in Japan (Muroga, 1995). In East Asia, outbreaks of this disease have been reported for seabass (*Lateolabrax japonicus*) (Miyazaki and Egusa, 1972; Chen, 1996; Park and Sohn, 1996), yellowtail (*Seriola quinqueradiata*) (Matsusato, 1975), Japanese flounder (*Paralichthys olivaceus*) (Tanaka *et al.*, 1984; Park and Sohn, 1996), red seabream (*Pagrus major*) (Chen, 1996; Park and Sohn, 1996; Muroga, 1997) and rockfish (*Sebastes schlegeli*) (Chun, 1998). Matsuoka (1995) reported that the incidence of this disease has increased since the early 1990s, particularly in Japanese flounder.

Geographic distribution. Lymphocystis disease is not restricted to warm seas, but is widespread throughout the world in both marine and freshwater fish (Wolf, 1988).

Diagnosis. The disease is characterized by tumour-like masses of tissue on the body surface (Fig. 6.1). These growths are clusters of extremely hypertrophic fibroblastic dermal cells (Fig. 6.2). In yellowtail, the infected cells are dispersed, covered by a layer of epithelium and surrounded by black pigment cells, thus appearing as small black dots (Matsusato, 1975). Occasionally internal organs can become infected (Colorni and Diamant, 1995).

Lymphocystis-infected cells are mainly spherical in shape with a thick elastic membrane, but may be distorted when in clusters, due to pressure from adjacent cells. The infected cells apparently stimulate proliferation of the adjacent healthy tissue. After 2 weeks of infection, the cells enlarge significantly. Both nucleus and nucleolus present large basophilic cytoplasmic inclusion bodies that react positively for DNA.

Diagnosis of lymphocystis disease is confirmed through histological sections and appropriate staining of the tissue lesions. In fact, this is one of the few viral diseases that can be identified histologically. The observation of the typical icosahedral virions by electron microscopy offers further confirmation. Horizontal transmission is the most probable route, facilitated by high stocking density and unfavourable environmental conditions. In Southeast Asia, trash fish

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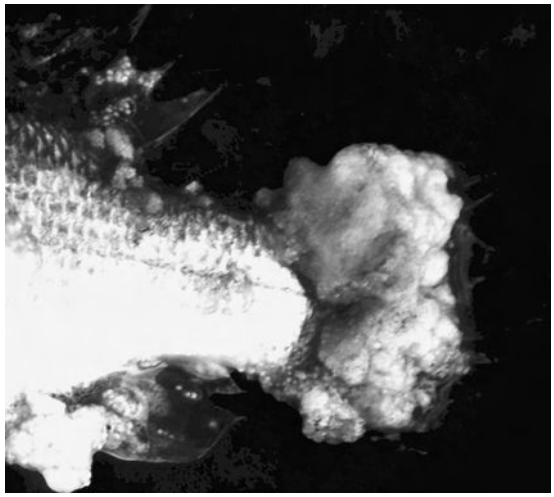


Fig. 6.1. Lymphocystis in Asian seabass, *Lates calcarifer*.

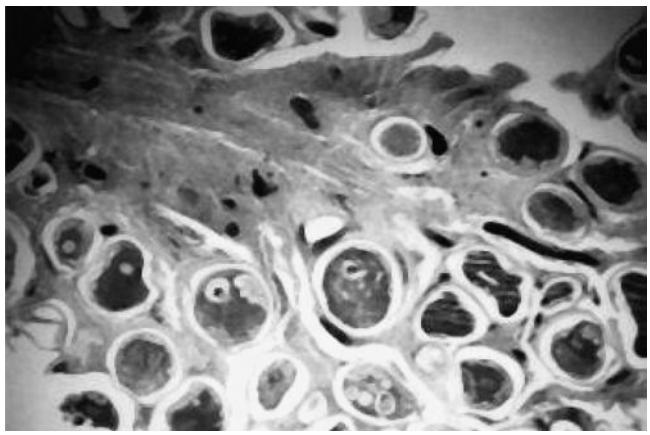


Fig. 6.2. Hypertropic fibroblastic cells in caudal fin of Asian seabass, *Lates calcarifer*.

used as feed may be another source of infection (T.S. Leong, personal observation).

Prevention and control. There is presently no effective therapy for this disease. A decrease in stocking density and culling of visibly infected individuals are the only known measures that can be adopted to reduce the impact of the disease.

Red seabream and brown-spotted grouper iridovirus

The virus belongs to the family *Iridoviridae*. In electron microscopy, the particles appear hexagonal in shape, with a diameter of 200–240 nm (in red seabream) and 140–160 nm (in brown-spotted grouper) (Danayadol *et al.*, 1997; Kasornchandra and Khongpradit, 1997).

Host range. Red seabream iridovirus (RSIV) was first diagnosed in Japan where it caused a systemic infection in farmed red seabream (Inouye *et al.*, 1992). This serious disease, however, affects other cultured marine fish species (Nakajima *et al.*, 1995). In 1993/94, an iridovirus disease similar to RSIV was reported in cultured grouper (*Epinephelus malabaricus*) in Thailand (Danayadol *et al.*, 1997). So far, RSIV has

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been found in more than 20 marine species (Matsuoka *et al.*, 1996; Nakajima, 1997). Juvenile and young red seabream cultured in cages are highly susceptible to the disease.

Geographic distribution. Red seabream iridovirus disease and similar iridoviral diseases have been reported in Southeast and East Asia.

Diagnosis. Affected fish become lethargic and severely anaemic. The gills are haemorrhagic. The spleen is hypertrophic and the iridovirus appears in a crystalline array in the enlarged, basophilic splenic cells (Inouye *et al.*, 1992). Presumptive diagnosis based on Giemsa staining of histological sections can be confirmed by immunofluorescence with a monoclonal antibody or a PCR assay (Inouye *et al.*, 1992; Nakajima and Sorimachi, 1994; Nakajima *et al.*, 1995, 1997). RSIV can be isolated in several cell lines including RTG-2, CHSE-214 and FHM (Inouye *et al.*, 1992). Sensitivity was particularly high in KRE-3 and BF-2 (Nakajima and Sorimachi, 1994). However, viral infectivity decreased progressively with cell subcultures. The grouper iridovirus grew well in epithelioma papulosum cyprini (EPC) and grouper fin (GF) cell lines (Kasornchandra and Khongpradit, 1997).

Prevention and control. An experimental vaccine prepared by Nakajima *et al.* (1997) produced a higher survival in treated red seabream than in the control group, suggesting the possibility of controlling the disease through vaccination.

Viral nervous necrosis (VNN)

The terms fish viral encephalitis and encephalopathy have been used to describe a number of infections with a similar syndrome. VNN was first reported in Japanese parrotfish (*Oplegnathus fasciatus*) in a 1985–1987 disease outbreak (Yoshikoshi and Inoue, 1990) and the causative agent has since been identified as a member of the *Nodaviridae* and named striped jack nervous necrosis virus (SJNNV) (Mori *et al.*, 1992).

Electron microscopy demonstrates that the virus particles are packed in the cytoplasm of affected retinal and brain cells and are non-enveloped, icosahedral in shape, about 20 nm in grouper and 25–34 nm in striped jack (Danayadol *et al.*, 1993; Nguyen, 1996).

Host range. Since it was first reported in Japanese parrotfish, VNN has been diagnosed in more than ten fish species in Japan (Mori *et al.*, 1991; Arimoto *et al.*, 1993; Muroga, 1995; Nakai *et al.*, 1995). Similar viral diseases have been reported in Asian seabass (see Glazebrook *et al.*, 1990; Munday *et al.*, 1992; Comps *et al.*, 1994), grouper (*Epinephelus* spp.) (Chong and Chao, 1986; Danayadol *et al.*, 1993, 1995; Chua *et al.*, 1995; Boonyaratpalin *et al.*, 1996; Tanaka *et al.*, 1998; Bondad-Reantaso *et al.*, 2001), European seabass (*Dicentrarchus labrax*) (Breuil *et al.*, 1991; Comps *et al.*, 1994), turbot (*Scophtalmus maximus*) (see Bloch *et al.*, 1991) and halibut (*Hippoglossus hippoglossus*) (see Grotmol *et al.*, 1995). A similar syndrome was reported in the European seabass cultured in Martinique, French Caribbean Islands (Bellance and Gallet de Saint Aurin, 1988; Gallet de Saint Aurin *et al.*, 1990).

Geographic distribution. VNN disease has been found in all warmwater marine environments where marine fish have been cultured in cage environments, particularly in juvenile stages.

Diagnosis. Infected fish exhibit whirling movements, lethargy, dark body coloration, loss of balance and hyper-excitability in response to noise and light. Mortalities are usually high and occur within a week of the onset of first signs. Extensive spongiosis is typically observed in the retina, brain and central nervous system (Glazebrook *et al.*, 1990; Yoshikoshi and Inoue, 1990; Arimoto *et al.*, 1992, 1993; Munday *et al.*, 1992; Danayadol *et al.*, 1995; Boonyaratpalin *et al.*, 1996; Nguyen, 1996). SJNNV can be detected

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by ELISA and PCR (Arimoto *et al.*, 1992; Mushiake *et al.*, 1992, 1994). A PCR method based on the sequence of the virus coat protein gene (RNA2) was used to diagnose the virus in spawners, suggesting vertical transmission of the infection (Arimoto *et al.*, 1992; Mushiake *et al.*, 1992; Nishizawa *et al.*, 1994, 1995).

Prevention and control. At present there is no known method of therapy, but vaccination using recombinant coat protein of live piscine nodavirus in sevenband grouper, *Epinephelus septemfasciatus*, resulted in significantly lower mortality in the virus challenge tests, indicating great potential for protection against the virus.

Diseases Caused by Bacteria

Many clinical signs of bacterial diseases of cultured marine fish are similar. Definitive diagnosis requires the isolation and *in vitro* culture of the organisms involved. A great number of aquatic bacteria are opportunistic and under normal environmental conditions do not cause disease, becoming pathogenic only when the balance of the host/environment is changed by elevated stocking densities, inadequate nutrition, deteriorating water quality, rough handling (e.g. net changing, grading) and other stress factors. The bacteria reported in cultured marine fish are summarized in Table 6.3.

Epitheliocystis

The epitheliocystis organism is a chlamydia-like, obligate, intracellular prokaryote that has not been cultured *in vitro*. Infection primarily involves epithelial cells of the gills that become packed with a large mass of the minute coccoid organisms. Transmission is apparently horizontal and direct. Extensive infections occur in juveniles and are often lethal. Epitheliocystis is highly infective and host-specific, indicating that the disease in different species of fish is most probably caused by different strains of epitheliocystis organisms (Lannan *et al.*, 1999). Two distinct developmental life cycles have been recently hypothesized for a highly pleomorphic chlamydia-like organism that causes epitheliocystis infection in seabream (*S. aurata*) (Crespo *et al.*, 1999).

Host range. Epitheliocystis infections have been reported from over 25 species of fish, including Carangidae, Centrarchidae, Centropomidae, Mugilidae, Pleuronectidae, Serranidae and Sparidae (see Crespo *et al.*, 1999).

Geographic distribution. Epitheliocystis is not limited to a warm marine environment and has been reported worldwide (see Noga, 1996; Crespo *et al.*, 1999; Lannan *et al.*, 1999).

Diagnosis. Affected fish typically display flared opercula and fast, shallow respiration. In histological sections, epithelial hyperplasia and fusion of adjacent gill lamellae are apparent. Infected cells (up to 220 × 100 μm in size, depending on developmental stage) are basophilic and appear either amorphous or uniformly granular.

Prevention and control. At present, no effective therapy for epitheliocystis is known.

Gram-negative bacteria

Vibriosis

Vibriosis is the disease caused by a group of bacteria belonging to the family Vibrionaceae. The infectious disease they cause is one of the most significant in mariculture. Age and sex of fish are not relevant factors in the disease (Sano and Fukuda, 1987; Arthur and Ogawa, 1996; Leong, 1996; Sako, 1996; Shariff and Arulampalam, 1996). In Southeast Asia, 4–6-week-old caged grouper weighing approximately 200 g often die overnight without any apparent signs of disease except that the body darkens. This condition is referred to

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Table 6.3. Bacterial diseases of warmwater maricultured finfish.

Causative agent	Disease	Species affected
Gram-negative pathogens		
Vibrionaceae		
Listonella anguillarum	Vibriosis	Yellowtail (*Seriola quinqueradiata*)
		Amberjack (*Seriola dumerili*)
		Horse mackerel (*Trachurus japonicus*)
		Red seabream (*Pagrus major*)
Vibrio alginolyticus	Vibriosis	Greasy grouper (*Epinephelus coioides*)
		European seabass (*Dicentrarchus labrax*)
		Seabream (*Sparus aurata*)
Vibrio parahaemolyticus	Vibriosis	Golden snapper (*Lutjanus johni*)
		Seabream (*S. aurata*)
Photobacterium damsela	'Pasteurellosis'	Yellowtail (*S. quinqueradiata*)
		Amberjack (*S. dumerili*)
		European seabass (*D. labrax*)
		Seabream (*S. aurata*)
		Red drum (*Sciaenops ocellatus*)
Enterobacteriaceae		
Edwardsiella tarda	Edwardsiellosis	Japanese flounder (*Paralichthys olivaceus*)
Cytophagaceae		
Flexibacter maritimus	Saltwater myxobacteriosis	Red seabream (*P. major*)
		Greasy grouper (*E. coioides*)
		Asian seabass (*Lates calcarifer*)
		Mangrove snapper (*Lutjanus argentimaculatus*)
		Japanese flounder (*P. olivaceus*)
Gram-positive pathogens		
Streptococcus spp.	Streptococcosis	Greasy grouper (*E. coioides*)
		Yellowtail (*S. quinqueradiata*)
		Amberjack (*S. dumerili*)
		European seabass (*D. labrax*)
		Red drum (*S. ocellatus*)
		Tilapia (*O. mossambicus*) (adapted to seawater)
Acid-fast pathogens		
Nocardiaceae		
Nocardia seriolae	Nocardiosis	Yellowtail (*S. quinqueradiata*)
Mycobacteriaceae		Amberjack (*S. dumerili*)
Mycobacterium marinum	Mycobacteriosis	Seabream (*S. aurata*)
		European seabass (*D. labrax*)

as 'sleepy-grouper syndrome', but its aetiology is still controversial. In Singapore and Indonesia, cases of sleepy-grouper syndrome were originally attributed to a virus (Chua *et al.*, 1994; Arthur and Ogawa, 1996), with vibrios considered as secondary invaders. In Malaysia, groupers with sleepy-grouper syndrome were found with high numbers of monogeneans as well as with gastroenteritis vibriosis (however, fish were not examined for virus) (Leong and Wong, 1993).

Host range. The majority of marine fish cultured in cages are susceptible to vibriosis, with some fish species more sensitive to the infection than others. In East Asia, yellowtail, red seabream, horse mackerel and flounder are particularly susceptible. In Southeast Asia, grouper, seabass and snapper have been frequently reported as affected by vibriosis. The greasy grouper (*Epinephelus coioides*) (Fig. 6.3) is more susceptible than black-spotted grouper (*E. malabaricus*) and brown-spotted grouper

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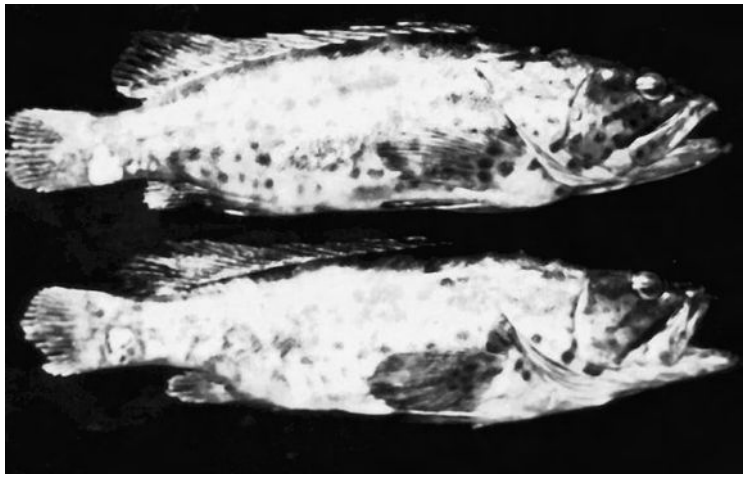


Fig. 6.3. Vibriosis in greasy grouper, *Epinephelus coioides*.

(*Epinephelus bleekeri*), even when all three species are cultured in the same cage. It occurs frequently during periods of fluctuations in salinity, increased organic load, or stress brought on by net changing and grading of fish. The period following initial stocking is particularly critical. Horizontal transmission is the most probable route, with bacteria being shed from open lesions.

In Israel, mortalities of seabream cultured in the Red Sea were associated with isolation from the blood stream of *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio anguillarum* or *anguillarum*-like. Infection by these microorganisms, however, was low (Colorni *et al.*, 1981).

Geographical distribution. Vibrios are ubiquitous in all marine environments and most are facultative pathogens. The species of vibrios involved in diseases reflect regional differences. In East and West Asia, the most commonly isolated species are *Vibrio ordalii*, *Vibrio ichithyoenteri*, *Vibrio trachuri*, *Vibrio damsela* and *Listonella* (*Vibrio*) *anguillarum* (Kusuda and Kawai, 1997). In Southeast Asia, *V. parahaemolyticus* and *V. alginolyticus* are the main species involved (Wong and Leong, 1986, 1990).

Diagnosis. Vibriosis is characterized by haemorrhagic septicaemia. The clinical signs are capillary congestion and 'red boils' appearing on the body surface and gradual darkening of the body. Initially, the haemorrhage usually enlarges into irregular and deep lesions, which disintegrate the skin, exposing the underlying muscle, which becomes necrotic. Vibrios produce a wide variety of proteases and extracellular enzymes that are responsible for the extensive tissue damage (Thune *et al.*, 1993).

Two forms of vibriosis are recognized. The first form produces external haemorrhage and is referred to as the dermatitis form of vibriosis. The second form is less common and is referred to as gastroenteritis vibriosis. The latter does not have external signs (Muroga *et al.*, 1990; Egusa, 1992). In the dermatitis form of vibriosis, internal pathology occurs as the disease progresses, with congestion and haemorrhage of the liver and enlargement and liquefaction of spleen, liver and kidney. The histopathological changes are associated with intestinal haemorrhage and destruction of the tunica mucosa. Groupers with sleepy-grouper syndrome tend to give out a characteristic strong, foul smell from the abdomen when examined. Biochemical and immunological methods are used for identification, but most require culture and isolation of these pathogens. The organisms are Gram-negative rods with motile polar flagella, non-capsulated and non-spore producing. They are positive for oxidase and catalase,

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and generally ferment a large number of carbohydrates.

Prevention and control. Leong *et al.* (1997) reported that groupers vaccinated against vibriosis do not show any sign of sleepy-grouper syndrome. The vaccinated groupers were healthier and grew faster, suggesting that the sleepy-grouper syndrome in grouper could in fact be gastroenteric vibriosis. Good husbandry practice and adequate nutrition are essential to prevent the development of vibriosis. The initial stage of the disease can be treated with a number of sulphur drugs in feed with good results. The dosage varies between 50 and 200 mg kg^{-1} fish weight day^{-1} for 10–20 days (Sano and Fukuda, 1987). Intraperitoneal injection has been most effective for the treatment of vibriosis in adult grouper.

'Pasteurellosis'

'Pasteurellosis' is the second most important infectious disease in cultured yellowtail in Japan. It causes the loss of thousands of tonnes of cultured yellowtail (Sano and Fukuda 1987; Sako, 1996). The route of infection is probably oral. Stress is an important predisposing factor to infection.

Although the aetiological agent was first described by Janssen and Surgalla (1968) as a member of the *Pasteurella* genus, its taxonomic position was later questioned by Gauthier *et al.* (1995) who placed it in the genus *Photobacterium* and renamed it *Photobacterium damsela* subsp. *piscicida*. However, while confirming that the pathogen should be included in the genus *Photobacterium*, Thyssen *et al.* (1998) found no evidence, morphological or biochemical, to justify its classification as a subspecies of *Photobacterium damselae*.

Host range. The seabream in Israel (Colorni, 1998) and yellowtail, black seabream, horse mackerel and Japanese flounder in Japan and Korea have been reported to be seriously affected by the bacterium (Sano and Fukuda, 1987; Kusuda and Salati, 1993; Park and Sohn, 1996; Sako, 1996).

Geographical distribution. The majority of disease outbreaks involving this bacterium have been reported in both Mediterranean and Red Sea Israeli fish farms (Colorni, 1998), as well as in Japan, America and Mediterranean countries. This bacterium is affected by water temperature and the disease tends to occur in the summer months with water temperature between 20 and 25°C.

Diagnosis. Pasteurellosis is a septicaemic disease with no external signs except occasional darkened spots on the body surface in yellowtail (Kubota *et al.*, 1970a; Fukuda and Kusuda, 1981). A large number of white spots of 0.5–3.5 mm corresponding to foci of bacterial colonization engulfed by phagocytes is found in the spleen and kidney, and to a lesser extent in the liver (Kubota *et al.*, 1970a,b, 1972; Egusa, 1992). The numbers of macrophages increase in the spleen, kidney, gill and liver, which often appear necrotic and enlarged (Figs 6.4 and 6.5). Many bacteria are able to survive in the macrophage (Nelson *et al.*, 1989). The diseased fish rapidly lose their vigour, sink to the bottom of the cage and die.

P. damsela is Gram-negative, non-motile, usually short (0.5–0.7 × 0.7–2.6 μm), bipolar and pleomorphic (from coccoidal to rod-like, depending on the culture and environmental conditions). A variety of media, including yeast peptone agar, brain and heart infusion agar and blood agar containing 1.5–2.0% NaCl can be used to isolate the bacterium. Colonies are small (1–2 mm in diameter) and translucent.

Prevention and control. Ampicillin (Aoki and Kitao, 1985) and florfenicol (Yasunaga and Yasumoto, 1988) have been reported to be effective when administered in feed. This bacterium is known, however, readily to become resistant to antibiotics. Vaccine preparations also gave satisfactory results (Fukuda and Kusuda, 1985; Kusuda and Hamaguchi, 1988; Kusuda *et al.*, 1988).

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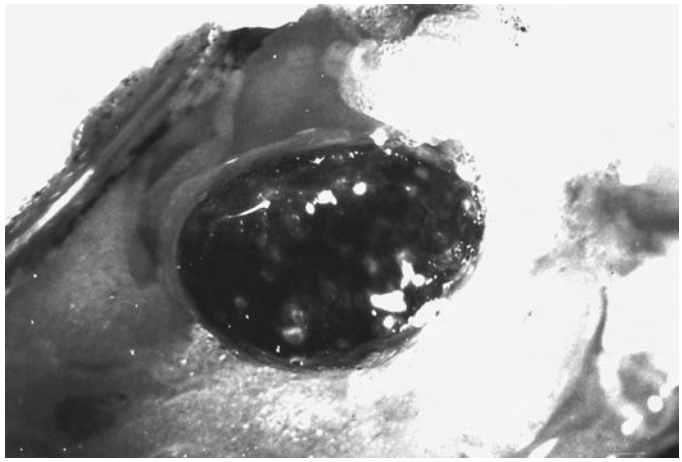


Fig. 6.4. Enlarged spleen with white spots in seabream, *Sparus aurata*, typical of 'pasteurellosis' by *Photobacterium damsela*.

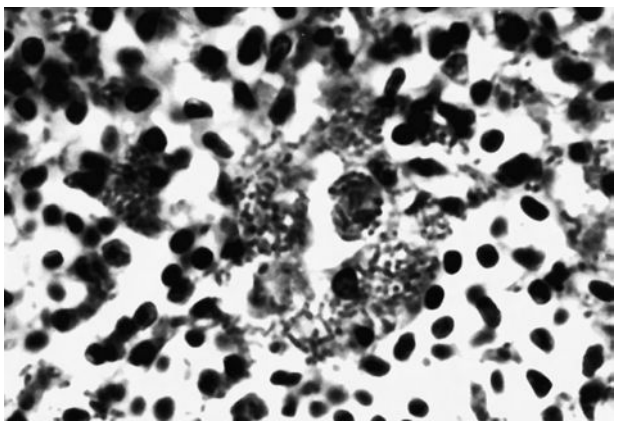


Fig. 6.5. Splenic foci of *Photobacterium damsela*, typical of 'pasteurellosis' in seabream, *Sparus aurata*.

Edwardsiellosis

Edwardsiellosis, caused by bacteria of the genus *Edwardsiella* (family Enterobacteriaceae), is a systemic bacterial disease, reported in warm freshwater and marine fish. The pathogenesis in cultured marine fish has not been well documented. Two species are involved, *Edwardsiella tarda* (infecting a variety of both freshwater and marine fish) and *Edwardsiella ictaluri* (infecting mainly cultured catfish of the genus *Ictalurus*) (see Chapter 7).

Host range. This bacterial disease has been reported from a large variety of cultured marine fish including mullet (*Mugil cephalus*) (Kusuda *et al.*, 1976a), crimson seabream (*Evynnis japonica*) (Kusuda *et al.*, 1977), yellowtail (*S. quinqueradiata*), red seabream (*Chrysophrys major*) (Yasunaga *et al.*, 1982) and Japanese flounder (*P. olivaceus*) (Nakatsugawa, 1983).

Geographic distribution. E. tarda has a worldwide distribution, occurring in both freshwater and marine environments. It causes severe disease problems in a variety of cultured marine fish, mainly in Southeast Asia (see Plumb, 1999).

Diagnosis. Edwardsiellosis is characterized by cutaneous haemorrhagic ulcers, which

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gradually deepen into the muscle forming large necrotic abscesses. Internally, greyish-white spots develop in the spleen and kidney (Kusuda *et al.*, 1977). *E. tarda* is an enteric, Gram-negative motile rod with peritrichous flagella. It grows on media with 0.5–4% NaCl, a temperature range of 15–41°C and a pH level of 5.5–9.0, with small circular transparent colonies (Wakabayashi and Egusa, 1973; Kusuda *et al.*, 1976a, 1977; Amandi *et al.*, 1982; Yasunaga *et al.*, 1982; Nakatsugawa, 1983; Farmer and McWhorter, 1984; Waltman *et al.*, 1986). Little variation in the biochemical and biophysical characteristics existed in 116 isolates from Taiwan and the USA (Waltman *et al.*, 1986). Traditional diagnosis of edwardsiellosis involves isolation of the bacterium and identification by biochemical tests. A fluorescent antibody (FA) method and an ELISA have been developed (Kusuda and Salati, 1993).

Prevention and control. Infection of *E. tarda* can be treated by application of antibiotic medicated feed. Salati (1988) reported that the most effective drug is oxolinic acid, followed by trimethoprim, oxytetracycline, furazolidone and piromidic acid. Although the bacterium is sensitive to a wide variety of antibiotics, strains resistant to chloramphenicol, furazolidone and sodium nifurstyrenate have been detected (Aoki *et al.*, 1977, 1989; Waltman and Shotts, 1986).

Most studies on vaccination against *E. tarda* have been carried out on eel in Taiwan and Japan (Song and Kou, 1979; Song *et al.*, 1982; Salati *et al.*, 1983; Salati, 1985; Salati and Kusuda, 1985a,b), but overall little research has been carried out on the vaccination of cultured marine fish. Salati *et al.* (1987) showed that vaccination of red seabream with formalin-killed cells and crude lipopolysaccharide (LPS) preparation of *E. tarda* enhanced phagocytosis and increased antibody titres.

Gliding bacterial disease/tail rot disease

A columnaris disease in Asian seabass was reported in Thailand in 1983 (Danayadol *et al.*, 1984) and the bacterium involved was identified as a *Flexibacter* sp. (Ruangpan, 1985; Ruangpan *et al.*, 1987). Since 1988, disease epizootics have been observed whenever the seabass fingerlings have been introduced for culture in netcages throughout Southeast Asia (Chong and Chao, 1986; Perngmark, 1992; Leong, 1994).

A disease associated with gliding bacteria was described in red seabream and black seabream in Japan by Masumura and Wakabayashi (1977). This disease is similar in appearance to columnaris disease of freshwater fish and the aetiological agent also belongs to the genus *Flexibacter*. Wakabayashi *et al.* (1986) proposed the name *Flexibacter maritimus* for the organism, which has an obligate requirement for seawater irreplaceable by NaCl alone for growth (Hikida *et al.*, 1979). In Southeast Asia, gliding bacteria were reported as *Flexibacter* sp. (Danayadol *et al.*, 1984; Ruangpan, 1985; Baxa *et al.*, 1986; Chong and Chao, 1986: Wakabayashi *et al.*, 1986; Ruangpan *et al.*, 1987; Leong, 1994).

Two species of *Flexibacter* have been described, *F. maritimus* from seabream (*P. major*) (see Wakabayashi *et al.*, 1986) and *Flexibacter ovolyticus* from Atlantic halibut (*H. hippoglossus* L.) (see Hansen *et al.*, 1992). The flexibacter-like bacteria that cause tail rot syndrome in cultured marine fish, particularly Asian seabass, have not been characterized.

Host range. A variety of marine fish cultured in cages has been reported to be affected by gliding bacteria. In East Asia, yellowtail, red seabream, black seabream, Japanese flounder, tiger puffer, grouper and grey mullet are susceptible to this gliding bacterial disease (Masumura and Wakabayashi, 1977; Baxa *et al.*, 1986, 1987a,b; Arthur and Ogawa, 1996; Lavilla-Pitogo *et al.*, 1996; Liao *et al.*, 1996; Park and Sohn, 1996; Sako, 1996; Kusuda and Kawai, 1997).

In Southeast Asia, caged Asian seabass (Fig. 6.6) are most susceptible to tail rot, followed by the mangrove snapper, golden snapper and grouper, though to a lesser extent (Danayadol *et al.*, 1984; Ruangpan, 1985; Chong and Chao, 1986; Ruangpan

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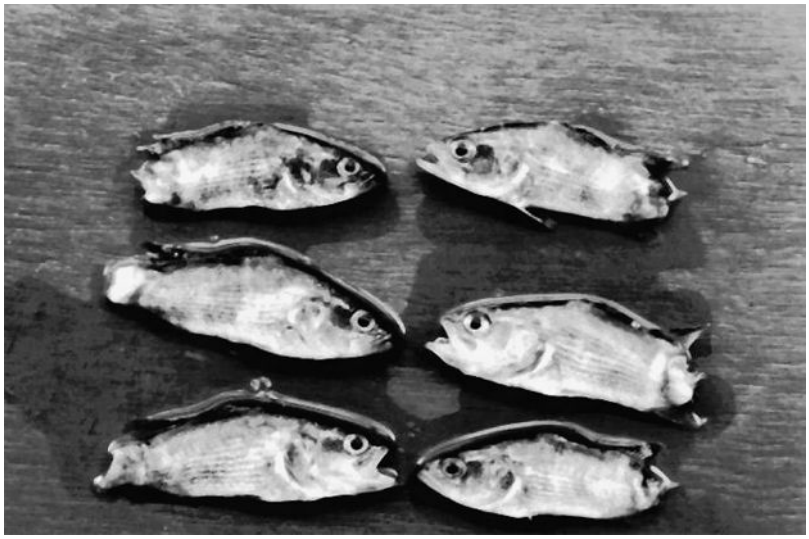


Fig. 6.6. Trail rot syndrome in juveniles of Asian seabass, *Lates calcarifer*.

et al., 1987; Leong *et al.*, 1992; Perngmark, 1992). Histopathological study of the tail rot syndrome in Asian seabass has indicated that the onset of the disease is through the pathogen infection in the tail region and proliferation in the epidermis and dermis (Perngmark, 1992).

Geographic distribution. Gliding bacteria of the genus *Flexibacter* appear to have a worldwide distribution. Only *F. maritimus* has been reported in East Asia.

Diagnosis. In seabream and yellowtail in East Asia, the gliding bacteria first gain entry through the damaged caudal fin, where the tissues are gradually eroded away by the action of the bacteria. The bacteria then invade the muscular region, the muscles disintegrate and typical tail rot occurs. No pathological changes are normally observed in the internal organs. The disease usually affects seabream and Asian seabass fry, 2–3 weeks after their introduction into sea cages.

F. maritimus is a long slender Gram-negative rod, which exhibits gliding movements on a wet surface. Culture (on cytophaga medium) requires at least 30% seawater, which cannot be replaced by NaCl. Colonies are pale yellow.

Prevention and control. It is difficult to prevent and control the disease in the cage environment. The standard treatment is feed medicated with oxytetracycline or a bath in sodium nifurstyrenate. However, the results are usually unsatisfactory. A combination of freshwater treatment and reduction of stocking density helps to reduce mortality in affected seabass (T.S. Leong, unpublished data).

Gram-positive bacteria

Two Gram-positive bacteria are of major importance in maricultured fish: *Enterococcus seriolicida* and *Streptococcus iniae* (Kusuda and Salati, 1999).

Streptococcosis

The taxonomy of fish streptococci is still controversial, but more than one species causing a similar syndrome is involved. The disease is most severe when farmed fish are stressed and water temperature is high. The onset of the disease is related to the rapid growth of the bacterium in the intestine where both extracellular and intracellular toxins are produced (Kusuda *et al.*, 1978; Kimura and Kusuda, 1979, 1982).

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Kusuda and Hamaguchi (1989) showed that the former have haemolytic activity and the latter leucocidal activity. The disease is transmitted by contact (Robinson and Meyer, 1966), but feed may also be a source of infection (Taniguchi, 1983).

Host range. A large variety of freshwater and marine fish species has been reported to be susceptible to *Streptococcus* spp. The farmed species affected by streptococci are grouper and rabbitfish in Southeast Asia (Chong and Chao, 1986; Leong, 1994), and yellowtail, red seabream, Japanese flounder, Japanese seaperch, rockfish and horse mackerel in East Asia.

In Israel, *Streptococcus* spp. have been isolated from European seabass, tilapia (*Oreochromis mossambicus*) adapted to seawater and red drum. This last isolate was identified as *S. iniae* (Eldar *et al.*, 1999).

Geographic distribution. Streptococcosis is not confined to warm water, and both freshwater and marine species can be affected. Heavy losses have been reported in yellowtail, horse mackerel and Japanese flounder in Japan (Kusuda *et al.*, 1976b; Kitao *et al.*, 1979; Sako, 1996) but this disease has been known to occur in a variety of fish in Australia, Italy, Israel, South Africa and the USA (see Austin and Austin, 1993). In Israel, severe mortalities were recorded among the red drum cultured in cages on the Mediterranean coast (Eldar *et al.*, 1999).

Diagnosis. The clinical signs vary depending on the fish species affected. In tilapia, *S. iniae* infection produces panophthalmitis and meningitis with only minor pathological changes in other organs (Eldar *et al.*, 1995). In red drum, clinical signs include lethargy, loss of orientation, protrusion of the eye with clouding of the cornea and erosion of the skin (Eldar *et al.*, 1999). Other common signs are darkening of the body, erratic swimming, haemorrhage in the intestine, liver, spleen and kidney, and abdominal distention. Necrosis in the heart, gill, skin, spleen and eye have also been reported (Egusa, 1992).

Confirmation of the diagnosis requires culturing the pathogen, preferably on a blood-enriched medium. Pathogen presence can also be confirmed through direct or indirect fluorescent antibody methods (Kusuda and Kawahara, 1987; Kawahara *et al.*, 1989). Recent studies have placed some isolates in the genus *Enterococcus* (Kusuda *et al.*, 1991; Kusuda and Salati, 1999). *Streptococcus* spp. (Fig. 6.7) are non-motile, Gram-positive, spherical to ovoid-shape cells, less than 2 μm in diameter. When grown in liquid media, they occur in pairs or form short chains (Kusuda and Kawai, 1982; Kusuda *et al.*, 1991). Most are facultative anaerobes, without endospores, while some form capsules. Streptococci can be isolated from diseased fish using brain heart infusion agar with or without 1.5–2% NaCl.

Prevention and control. Control is mainly by chemotherapy. Antibiotic treatment with

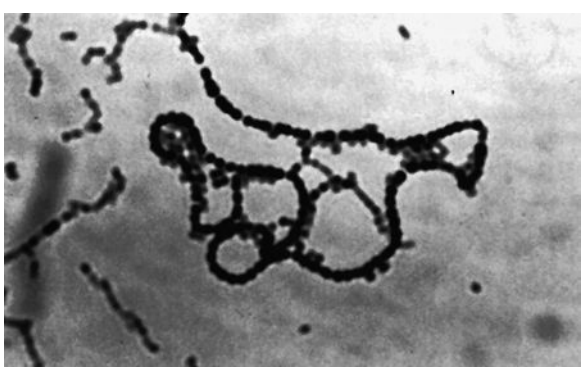


Fig. 6.7. *Streptococcus iniae* from seabass, *Dicentrarchus labrax*.

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erythromycin, spiramycin and josamycin has proved effective (Kashiwagi *et al.*, 1977a,b; Shiomitsu *et al.*, 1980; Kusuda and Onizaki, 1985; Kusuda and Takemaru, 1987; Takemaru and Kusuda, 1988a,b,c, 1990).

Acid-fast bacteria

Mycobacteriosis

The aetiological agents of mycobacteriosis, *Mycobacterium marinum* and other *Mycobacterium* spp., cause systemic, chronic infections in fish and other aquatic animals, and can occasionally cause skin ulcers in humans.

Host range. Since its first isolation from the European seabass in 1990 in Eilat (Red Sea, Israel) (Colorni, 1992), *M. marinum* has been detected in at least 18 other species of local fish and may have spread from sea cages to other farmed and native species in the Gulf of Eilat (Diamant and Colorni, 1995). The commercial species found to be infected are seabream (*S. aurata*), striped bass (*Morone saxatilis*), sheepshead (sharpsnout) (*Puntazzo puntazzo*), red drum (*S. ocellatus*), rabbitfish (*Siganus rivulatus*), mullet (*M. cephalus*), red seabream (*P. major*), hybrid red seabream (*P. major* × *S. aurata*), grouper (*Epinephelus aeneus*) and tilapia (*O. mossambicus*). Of these, only *S. rivulatus* and *M. cephalus* are native to the Red Sea.

Geographic distribution. Fish mycobacteriosis is not restricted to warm seas, but is widespread throughout the world in both marine and freshwater environments. Different endemic strains of *M. marinum* exist, specific to geographic regions (Colorni *et al.*, 1996).

Diagnosis. The disease follows a chronic course and remains asymptomatic for a long time. Superficial ulcers and exophthalmia are often the only external signs. Spleen and kidney, however, are severely affected and are enlarged with granulomatous lesions that appear macroscopically as whitish nodules (Fig. 6.8). In advanced cases these lesions spread to liver, heart, mesentery, etc. Special media (such as Löwenstein–Jensen or Middlebrook) are required for the culture of these mycobacteria, whose growth is usually slow (2–3 weeks for the first colonies to become visible). A Ziehl–Nielsen stain reveals the typical slender acid-fast rods (Fig. 6.9).

Prevention and control. There is no effective control.

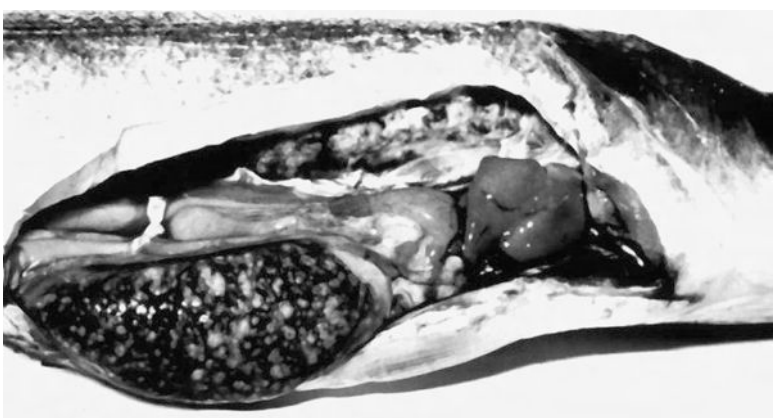


Fig. 6.8. Extremely enlarged granulomatous spleen of seabass, *Dicentrachus labrax,* infected with *Mycrobacterium maritimus*.

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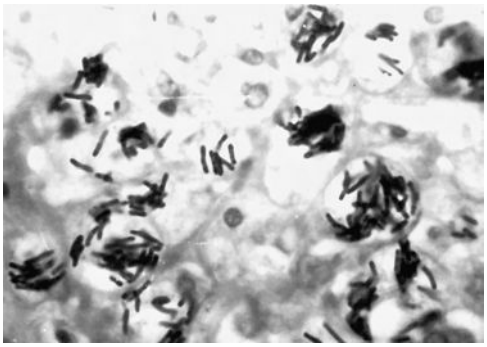


Fig. 6.9. *Mycobacterium marinum* in seabass, *Dicentrachus labrax* (Ziehl–Neelsen stain).

Nocardiosis

Nocardiosis is a chronic bacterial disease that affects both freshwater and marine fish. In Japanese yellowtail, amberjack and striped jack it is caused by *Nocardia kampachi* (Kariya *et al.*, 1968; Kawatsu *et al.*, 1976; Sako, 1996).

Host range. *N. kampachi* appears to be restricted to yellowtail, amberjack and striped jack (Kariya *et al.*, 1968; Kubota *et al.*, 1968).

Geographic distribution. Norcadiosis caused by *N. kampachi* is restricted to East Asia, primarily where yellowtails are cultured.

Diagnosis. Many clinical characteristics of nocardiosis are similar to mycobacteriosis. The disease occurs sporadically during autumn but outbreaks can extend from July to February. Early signs of infection include anorexia, inactivity, skin discoloration and emaciation. In the late stages, nodular skin lesions may ulcerate or extend to skeletal muscle and visceral organs, causing abdominal distension.

The morphology of *Nocardia* varies, but cells are generally filamentous, branched or beaded. The bacterium is acid-fast and can grow on a variety of media containing carbon and nitrogen sources. It can be isolated on brain heart infusion agar (BHIA), tryptone soya agar (TSA) and nutrient agar (NA), with optimum growth temperature at 20–30°C (Kusuda and Taki, 1973; Kusuda *et al.*, 1974). It produces flat, wrinkled colonies after 10 days at 25°C. *N. kampachi* does not grow at 37°C.

Prevention and control. There is no effective therapy for this disease. The route of infection in fish is not known, but is probably through direct contact or contaminated food. A clean environment is an important factor in preventing the occurrence of the disease. Kusuda and Nakagawa (1978) showed that *N. kampachi* can survive for more than 90 days in the presence of 100 mg l^{-1} fish extracts, but only 2 days in open seawater.

Diseases Caused by Protistans

A large, heterogeneous group of pathogenic one-cell organisms are associated with fish. Some are ectoparasites while others are endoparasites. Many of these organisms are not selective in their host preferences and can cause severe damage to any marine fish in intensive culture. Others may coexist with their host as epicommensals or as facultative parasites. The obligate parasitic species are host-specific, thus better adapted to coexist with their host causing limited harm (Lom, 1984). The endoparasites may considerably alter the appearance, taste and odour in the affected fish.

Myxosporean infections

Myxosporeans are endoparasites that can reside either in visceral cavities such as the gallbladder, swimbladder and urinary tract (coelozoic species), or settle as inter- or intracellular parasites in blood, muscle or connective tissue (histozoic species). Spores with four polar bodies in the stellate arrangement typical of the genus *Kudoa* (6.4–13.6 µm in length) have been found in the viscera of seabream cultured in the Red Sea (Paperna, 1982). This histozoic myxosporean may have originated in the Mediterranean Sea and been introduced into the Red Sea with infected seabream.

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The parasite causes relatively benign infections, usually limited to a few individuals.

A debilitating myxosporean disease was described in *S. aurata* by Diamant (1992) and Diamant *et al.* (1994). The aetiological agent, *Myxidium leei*, is a histozoic species that settles in the intestinal mucosa. In heavy infections, fish present an enlarged abdomen, with the intestinal tract filled with purulent, foul-smelling liquid. Histologically, small plasmodia (22 µm average size), which each give rise to two spores, are detected between the epithelial cells of the mucosa along the entire intestinal tract. As the same parasite was later discovered in other Mediterranean sparids and grey mullets (Lom and Bouix, 1995), *M. leei* too was probably imported with its host into the Red Sea. Recently, Diamant (1997) demonstrated that transmission of this organism does not require an intermediate host.

Another histozoic myxosporean, *S. epinepheli*, was described in the urinary system of adult groupers (*E. malabaricus*) from Southeast Asia (Supamattaya *et al.*, 1990, 1993). Presporogonic stages, round to oval in shape (1.98–10.75 µm), carried in the blood stream, settle in the kidney tubules where sporogenesis occurs. Mature spores, subspherical to spherical in shape (7.8–10 µm in length, 12.3–14.5 µm in thickness, 7–9.5 µm in width) present two round polar capsules. The epithelium of the renal tubules harbouring the parasites appears highly vacuolated. The life cycle of myxosporean parasites from marine hosts is unknown, but as Diamant (1997) demonstrated for *M. leei*, the notion that an intermediate host is indispensable for the completion of the myxosporeans' life cycle needs to be revised.

A *Sphaerospora*-like myxosporidean was reported to have caused a high cumulative mortality (90%) in cultured cobia, *Rachycentron canadian* (L.), in Taiwan (Chen *et al.*, 2001). The extrasporogonic and/or sporogonic stages appeared in the blood, glomerulus, renal tubules and renal interstitium. Matured spores with polar filaments were elongated or spherical, with numerous refractile granules in the cytoplasm.

Infections by ciliates

Brooklynellosis

Brooklynella hostilis is a ciliate protozoan that was first described in aquarium fish by Lom and Nigrelli (1970) as a gill pathogen. However, *B. hostilis* can also cause serious skin lesions (Noga, 1996). In heavy infections the ciliates destroy the host's surface tissue with their cytopharyngeal armature, feeding on tissue debris, ingesting blood cells and causing haemorrhage in the gills (Lom and Dyková, 1992).

Host and geographic distribution. European seabass (*D. labrax*) and lutjanids cultured in Martinique suffered heavy infestations of this parasite (Gallet de Saint Aurin *et al.*, 1990). *B. hostilis* has been detected repeatedly in mariculture facilities in Kuwait and Singapore (Lom and Dyková, 1992). Recently, it was diagnosed in cage-cultured seabream (*S. aurata*) in the Red Sea (Diamant, 1998).

Diagnosis. *B. hostilis* is recognizable by its oval, dorsoventrally flattened shape, notched oral area and size, measuring 36–86 × 32–50 µm (Lom and Dyková, 1992).

Prevention and control. There are no reported methods for caged fish.

Cryptocaryonosis

Cryptocaryonosis is a disease caused by the holotrich ciliate, *Cryptocaryon irritans*, a parasite belonging to the class Colpodea (order Colpodida) (Diggles and Adlard, 1995). Only one species, *C. irritans*, is reported for the genus. However, intraspecific variants exist (Diamant *et al.*, 1991; Colorni and Diamant, 1993; Diggles and Lester, 1996b; Diggles and Adlard, 1997). The ciliate invades the skin, eyes and gills of a suitable host, impairing the physiological functions of these organs. Its life cycle is quadriphasic and includes a parasitic phase on the fish (trophont), during which *Cryptocaryon* feeds and can be observed continuously revolving in the

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fish epithelia. After growing for 3–7 days to a maximum size of 300–400 μm, the parasite spontaneously leaves its host (as a protomont) and within several hours encysts and starts dividing (tomont), eventually producing up to 200 free-swimming infective stages (theronts). Sizes and numbers of theronts vary with the geographic locations, fish host species and water temperature (Colorni and Burgess, 1997). The theront life span is approximately 24 h, but its infectivity rapidly decreases after the first 6–8 h post-excystment (Yoshinaga and Dickerson, 1994; Diggles and Lester, 1996a).

Host range. This ciliate protozoan shows low host specificity and is capable of infecting most marine teleosts. Host susceptibility, however, may vary (Nigrelli and Ruggieri, 1966; Wilkie and Gordin, 1969; Colorni, 1985). Grouper cultured in marine cages in Hong Kong were susceptible to the protozoan infection; other fish species were not (ADB/NACA, 1991). Cultured grouper, snapper and Asian seabass fingerling are susceptible to this protozoan during the early stage of cage culture (Chong and Chao, 1986; Glazebrook and Campbell, 1987; Leong, 1994).

Geographic distribution. Although typical of tropical seas, this parasite has a worldwide distribution that extends well into temperate environments (Diamant *et al.*, 1991).

Diagnosis. The parasite burrows into the fish epithelia and appears macroscopically as pinhead-size whitish 'blisters', more conspicuous on coloured fish and on the translucent parts of the fins. Heavily infested fish may frequently come to the surface, gasping for oxygen. Mucus production increases. A definitive diagnosis of cryptocaryonosis can be made from the examination of a gill clip or a wet mount of fin or skin scraping for the presence of the large, revolving ciliate protozoans.

Prevention and control. The presence of *C. irritans* in cage-cultured fish means that the cages are kept in too shallow waters. If logistically feasible, the cages should be moved into an area where sufficient depth and currents prevent the theronts from reinfecting the fish (Colorni, 1987).

Diseases Caused by Metazoans

Monogenea

The monogeneans are gill and skin flukes frequently encountered in mariculture systems. Most monogeneans are host-specific, but some species have a wide host range. Monogeneans are hermaphroditic. Their direct life cycle, together with the availability of constantly stressed fish hosts in high stocking density environments, facilitates fish-to-fish infestation (Paperna *et al.*, 1984; Leong and Wong, 1987; Cone, 1995).

Capsalid monogeneans

Capsalid monogeneans are generally found on the fish skin and under the scales, while a few are found on the gills. They can move actively on the body surface, feeding on epithelial cells and mucus. The body of these parasites is relatively large and flat, with a conspicuous muscular disc haptor at the posterior end (Fig. 6.10). The haptor may be subdivided by septa. At the anterior end is a pair of large disc-like adhesive organs. The intestinal caeca are diverticular and end blindly. Three genera, *Benedenia*, *Neobenedenia* and *Megalocotyloides*, are commonly found infecting marine fish cultured in floating net cages (Table 6.4).

Host range. The capsalid monogeneans have been reported from a wide host range in the wild (Yamaguti, 1963; Paperna *et al.*, 1984), in cultured marine fish and in marine aquarium fish (Nigrelli, 1943; Paperna and Overstreet, 1981; Leong and Wong, 1987; Ogawa *et al.*, 1995a,b; Hla Bu *et al.*, 1998). High mortalities associated with heavy infestation of *Benedenia epinepheli* (Ogawa *et al.*, 1995a) were reported in Japanese flounder (*P. olivaceus*) in Shimane Prefecture and in black rockfish (*Sebastes schlegeli*) (with

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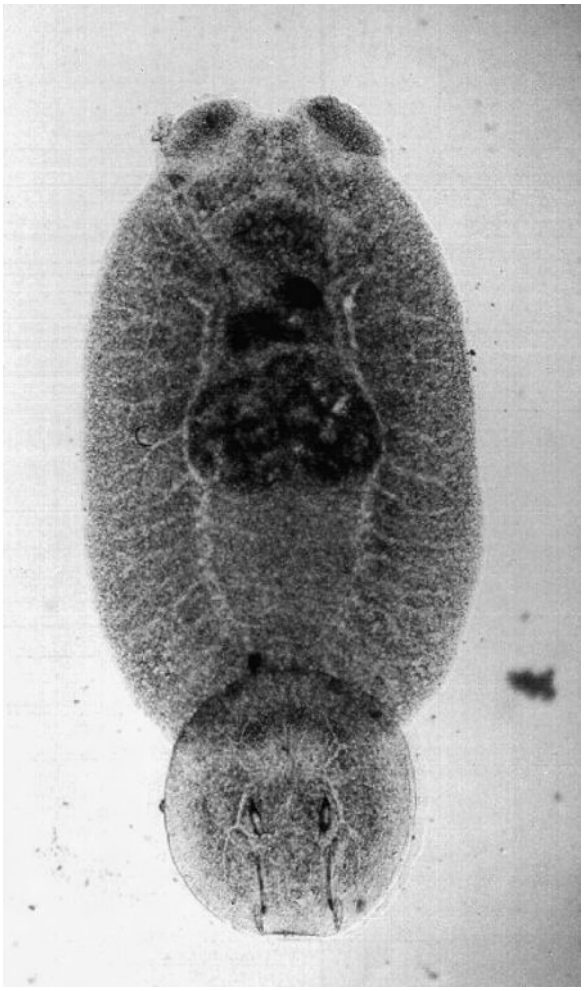


Fig. 6.10. Capsalid monogenean commonly found on cultured marine fish.

an intensity of 900–1500 worms per fish) in Yamaguchi Prefecture. Ogawa *et al.* (1995b) reported that 100% mortality of juvenile amberjack (*Seriola rivoliana*) in Okinawa was associated with *N. girellae.*

Some capsalid monogeneans appear to have low host specificity. For example, Ogawa *et al.* (1995a) reported that *B. epinepheli* was found in 25 fish host species, with tetraodontid fish being most susceptible, as up to 3000 individuals of *B. epinepheli* were recovered from a single individual in Japan. In Malaysia, 13 species of cultured marine fish were found with capsalid monogeneans (*Benedenia lutjani, B. epinpheli* and *Neobenedenia girellae*). Both *B. epinpheli* and *N. girellae* were found to be equally distributed on the greasy grouper (*E. coioides*) and the Asian seabass (*L. calcarifer*). In general, serranid, sparid and lutjanid fish appear to be more susceptible than other species. In the golden snapper (*Lutjanus johni*) up to 60 *B. lutjani* per infected host were counted. Tilapia cultured in cages in the marine environment were heavily infested by *Neobenedenia melleni* in Hawaii and in the West Indies (Kaneko *et al.*, 1988; Robinson *et al.*, 1989; Gallet de Saint Aurin *et al.*, 1990; Hall, 1992).

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Geographic distribution. The capsalid monogeneans have a wide distribution pattern (Table 6.5). More benedenid species have been reported from the East Asia region than from other regions. This is probably due not only to a greater variety of fish species cultured, but also to the availability there of more diagnostic facilities.

Cultured fish infested with capsalid monogeneans gradually withdraw from the group, quit eating and their bodies gradually darken. Heavily infected fish swim erratically and rub against the net, which results in dermal ulceration and subsequent bacterial invasion.

The active feeding of the monogeneans on mucus and epithelial cells leads to haemorrhage, inflammation and mucus hyperproduction (Paperna, 1991; Egusa, 1992). The monogeneans often settle on and around the eyes, damaging the cornea and causing blindness (Egusa, 1992; Ogawa *et al.*, 1995a; Colorni, 1998).

Prevention and control. The capsalid monogeneans are found on a large variety of wild fish and it is difficult to prevent and control them in the cage environment. Despite their size (up to a few mm) the monogeneans may go unnoticed. Whenever logistically

Table 6.4. Capsalid monogeneans found in various cultured marine finfish.*

	Fish species										
Capsalid species	1	2	3	4	5	6	7	8	9	10	11
Benedenia epinepheli	+	+	+	+	+	+	+	+	+	–	–
B. hoslinai	–	–	–	–	–	–	–	–	–	–	+
B. lutjani	+	+	+	+	+	+	+	–	–	–	–
B. monticelli	–	–	–	–	–	–	–	–	+	–	–
B. seriolae	–	–	–	–	–	–	–	–	–	–	+
Benedenia spp.	+	+	–	+	+	+	+	–	–	–	–
Neobenedenia girellae	+	+	+	+	–	–	–	–	–	+	+
N. melleni	–	–	–	–	–	–	–	–	+	+	–
Neobenedenia sp.	+	+	–	+	+	+	+	–	–	–	–
Megalocotyhoides epinepheli	+	+	–	–	–	–	–	–	–	–	–
M. convoluta	+	–	–	–	–	–	–	–	–	–	–

(1) *Epinephelus coioides*; (2) *E. malabaricus*; (3) *E. bleekeri*; (4) *Lutjanus johni*; (5) *L. argentimaculatus*; (6) *Lates calcarifer*; (7) *Pinjalo-pinjalo*; (8) *Pagrus major*; (9) *Liza* and *Mugil* spp.; (10) *Prepchromis* spp.; (11) *Oplegnathus fasciatus*.
*Many more cultured marine finfish susceptible to capsalids are not listed here.

Table 6.5. Geographical distribution of various benedenid species in cultured marine fish.

Benedenid species	East Asia	Southeast Asia	West Asia
Benedenia epinepheli	+	+	–
B. monticelli	–	–	+
B. seriolae	+	–	–
B. lutjani	–	+	–
B. hoshinai	+	–	–
Benedenia spp.	–	+	–
Neobenedenia girellae	+	–	–
N. melleni	+	–	+
Neobenedenia sp.	–	+	–
Megalocotyloides epinepheli	+	+	+
M. convoluta	+	+	–

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feasible, a freshwater dip of 3–5 min should be made to dislodge the parasites from the host (Leong, 1997; Zafran *et al.*, 2000). The treatment kills the parasites, which turn white and become more visible. Ellis and Watanabe (1993) reported that the egg, juvenile and adult stage of *N. melleni* in cultured tilapia could be eliminated in a 5 day hyposalinity (15 g l^{-1}) treatment. Tropical cleaner-fish, such as the wrasse (*Thalassoma bifasciatum*) and gobies (*Gobiosonia genie*, *G. ocenops* have been used to control *N. melleni* infecting seawater-cultured tilapia (Cowell *et al.*, 1993).

Diplectanid monogeneans

Diplectanid monogeneans are found only on the gills of fish hosts, feeding on mucus. Two genera of diplectanids are found to infest cultured marine finfish: *Diplectanum* and *Pseudorhabdosynochus*.

Host range. The diplectanid monogeneans have been reported from a wide variety of fish hosts (Beverly-Burton and Suraino, 1981; Kritksy and Beverly-Burton, 1986; Leong and Wong, 1987; Hla Bu *et al.*, 1999). In the tropical environment, they are commonly found on cultured serranids, sparids and centropomids (Table 6.6).

Diplectanid species are very host-specific and do not infect other fish species. *Pseudorhabdosynochus epinepheli*, *Pseudorhabdosynochus lanteuensis*, *Pseudorhabdosynochus coioidesis* and *Diplectanum grouperi* are found only in serranids, whereas *Pseudorhabdosynochus lateis, Pseudorhabdosynochus monosquamodisci* and *Diplectanum penangi* (Fig. 6.11) are found only in centropomids. Diplectanids are not known to infect cultured lutjanid fish.

Geographic distribution. The geographic distribution of diplectanid monogeneans in cultured marine fish is shown in Table 6.7.

Table 6.6. Diplectanid monogeneans found in various cultured marine finfish.

	Fish species				
Diplectanid species	*Dicentrarchus labrax*	*Epinephelus coioides*	*E. malabaricus*	*E. bleekeri*	*Lates calcarifer*
Pseudorhabdosynochus epinepheli	–	+	+	+	–
P. lanteuensis	–	+	+	–	–
P. lateis	–	–	–	–	+
P. monosquamodisci	–	–	–	–	+
P. coioidesis	–	+	+	+	–
Diplectanum penangi	–	–	–	–	+
D. grouperi	–	+	+	+	–
D. aequans	+	–	–	–	–
D. laubieri	+	–	–	–	–

Table 6.7. Diplectanid monogeneans found in various culture locations.

Diplectanid species	East Asia	Southeast Asia	West Asia
Pseudorhabdosynochus epinepheli	+	+	–
P. lanteuensis	+	+	–
P. lateis	–	+	–
P. monosquamodisci	–	+	–
P. coioidesis	+	+	–
Diplectanum penangi	–	+	–
D. grouperi	–	+	–
D. aequans	–	–	+
D. laubieri	–	–	+

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More diplectanids are reported in cultured marine fish from Southeast Asia but due to the numerous transfers of live fish from one region to another, similar reports are to be expected from East Asian regions, where many exotic marine finfish were recently imported by fish farmers.

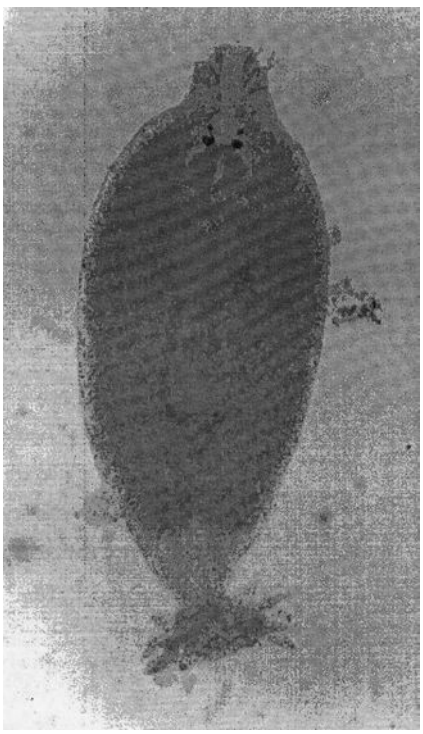


Fig. 6.11. *Diplectanum penangi*, a monogenean worm infecting the Asian seabass, *Lates calcarifer*.

Diagnosis. Fish infected with diplectanid monogeneans do not show conspicuous signs; haemorrhage, hyperplasia and fusion of lamellae at the points of attachment are commonly observed (Oliver, 1977; Giavenni, 1983; Hla Bu and Leong, 1997). The body of diplectanid monogeneans is elongated and characterized by a large flat haptor with squamodiscs at the posterior end.

Prevention and control. It is difficult to prevent the introduction of diplectanids into the culture system, especially when fingerling and juvenile fish are obtained from the wild. Freshwater treatment, which works effectively on benedenids, is not effective against diplectanids. The population size of diplectanids may be controlled in culture systems through appropriate stocking density of the fish in each cage.

Dactylogyrid monogeneans

Dactylogyrid monogeneans are parasites on the gills of cultured snapper. They are mucus feeders. One genus, *Haliotrema*, has been reported from cultured lutjanids (Leong and Wong, 1987, 1989; Liang and Leong, 1992; Leong, 1994).

Host range. The monogeneans found in various cultured marine finfish are shown in Table 6.8. They show restricted infectivity of fish host and are found mainly on lutjanid fish, with the exception of *Haliotrema*

Table 6.8. Dactylogyrid monogeneans found in various cultured marine finfish.

	Fish species				
Dactylogyrid species	1	2	3	4	5
Haliotrema johni	+	+	+	–	–
H. noncalcaris	–	+	–	–	–
Haliotrema sp. A	+	+	–	–	–
Haliotrema sp. B	+	+	–	–	–
Haliotrema sp. C	–	+	–	–	–
Haliotrema sp. E	–	+	–	–	–
Haliotrema sp. F	+	–	–	–	–
Haliotrema sp.	–	–	+	–	–
Haliotrema epinepheli	–	–	–	–	+

(1) *Lutjanus johni*; (2) *L. argentimaculatus*; (3) *L. russelli*; (4) *Pinjalo-pinjalo*; (5) *Epinephelus coioides*.

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epinepheli, which is found on serranids as well.

There are nine known species of dactylogyrids found in various species of cultured marine snappers, all belonging to the genus *Haliotrema* (Table 6.8). The mean intensity of infection of *Haliotrema johni* (Fig. 6.12) in cultured golden snapper (*L. johni*) (mean range of 162–409 per fish) was found to be an order of magnitude higher than that in wild snapper (33 per infected fish) (Leong and Wong, 1989). Furthermore, the prevalence and mean intensity of infection of *H. johni* in cultured disease fingerling golden snapper (90–97%; 107–314 per infected fish) were much higher than those in wild golden snapper (8–60%; 13–29 per infected fish) (Leong and Wong, 1987). In Penang, Malaysia, *Haliotrema noncalcaris* was found in all cultured mangrove snapper (*Lutjanus argentimaculatus*), with a mean range intensity of 45–58 per infected fish. Both *Haliotrema* sp. A and *Haliotrema* sp. B were also dominant numerically in both golden and mangrove snappers (Liang and Leong, 1992).



Fig. 6.12. *Haliotrema johni,* a monogenean infecting cultured golden snapper (*Lutjanus johni*).

Geographic distribution. The dactylogyrids are commonly found in golden and mangrove snapper cultured in Southeast Asia and in East Asia (Hong Kong).

Disease signs and pathology. Moderate infection of dactylogyrids in cultured finfish does not show significant clinical signs. In heavy infection, however, the forehead of the golden snapper is often devoid of scales and with epidermal lesions from the repeated rubbing against the net in response to the irritative action of the parasite (Chong and Chao, 1986). The histopathology of dactylogyrids on cultured marine finfish has not been reported.

Prevention and control. It is not possible to prevent fish from being infected by these monogeneans. Reducing stocking density of juveniles in the cage would probably reduce the build-up of population size of monogeneans in the fish. In heavily infected golden snapper, a formalin treatment of 300 ppm for 30 min significantly reduces *H. johni* (48%) and *Haliotrema* sp. (78%), while treatment with fresh water reduced *Haliotrema* sp. by 91% (Liang and Leong, 1992). Dipterex and malachite green were found to be ineffective in reducing the population size of these monogeneans (Liang and Leong, 1992).

Microcotylid monogeneans

Microcotylid monogeneans are gill parasites in which the haptor has numerous clamps that are important for taxonomic identification. Some clamps are situated on long stalks, while others are found on the body surface at the posterior end. The mouth of microcotylids is adapted for blood sucking.

Host range. The microcotylid monogeneans have been reported on a wide range of hosts in the wild (Yamaguti, 1963) and on cultured marine fish (Paperna, 1991; Egusa, 1992). The microcotylids reported in cultured marine fish are shown in Table 6.9. Only a few of the many species of cultured

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Table 6.9. Microcotylid monogeneans found in various cultured marine finfish.

Microcotylid species	Fish host	Culture sites
Bivagina tai	*Pagrus major*	East Asia
Choricotyle elongata	*Pagrus major*	East Asia
Heterobothrium okamotoi	*Pagrus major*	East Asia
H. tetradonis	*Takifugu rubripes*	East Asia
Heteraxine heterocerca	*Seriola quiqueradiata*	East Asia
Metamicrocotyle cephalus	*Mugil* spp.	West Asia
Microcotyle mugilis	*Mugil* spp.	West Asia
Microcotyle sp.	*Lutjanus russelli*	East Asia
M. chrysophryei	*Sparus aurata*	West Asia
M. labrachis	*Dicentrarchus labrax*	West Asia
Polylabris sp.	*Siganus* spp.	West Asia

fish have been reported to be infected by the microcotylids, which display a certain degree of host specificity.

Geographic distribution. The geographic distribution of microcotylid monogeneans is shown in Table 6.9. None of them has been found in Southeast Asia.

Disease signs and pathology. Cultured marine finfish infected by microcotylids do not always show clinical signs. There were few or no pathological changes in grey mullets, European seabass and gilthead seabream infected with microcotylids (Paperna, 1991). Conversely, in other cultured fish, such as yellowtail and red seabream, mortality in the cages has been directly attributed to the heavy infestation of these flukes. An average of 85 *Heteraxine heterocerca* were counted per infected yellowtail (Egusa, 1992). A large amount of mucus is secreted when the parasite is present in high numbers, gill filaments are destroyed, and haemorrhage due to parasite feeding activity can cause severe anaemia (Egusa, 1992). Parasitized fish often suffer from a concurrent bacterial infection.

The microcotylids have a flat body and are relatively large, reaching 17 mm in *H. heterocerca* on yellowtail and 20 mm in *Heterobothrium tetradonis* on puffer fish (Egusa, 1992). Very little is known about their development. The optimum temperature for eggs of *H. heterocerca* to hatch is 18–25°C. At 25°C, approximately 4 days are necessary but it was found possible to induce hatching between 10 and 28°C (Matsusato, 1968). The spawning period of *Bivagina tai* extends from November to January and eggs hatch in approximately 8.5 days at 18.5–19.5°C (Fujita *et al.*, 1969).

Prevention and control. Preventing the introduction of microcotylid monogeneans into cage culture systems is difficult whenever wild juvenile marine finfish are used for stocking. Finfish infected with microcotylids have been successfully removed with immersion in hyposaline water (Okamoto, 1963; Akazaki *et al.*, 1965; Fujita *et al.*, 1969), sodium pyrophosphate–hydrogen peroxide but not by oral administration of bithionol (Okamoto, 1963; Akazaki *et al.*, 1965; Fujita *et al.*, 1969). In addition, freshwater immersion was successful in removing *Choricotyle* sp. in seabream (Egusa, 1992).

Sanguinicolid digeneans

Digenean species are endoparasitic and require one or more intermediate hosts (mostly snails) for the completion of their life cycle. The first larval stage emerging from an egg (miracidium) is ciliated and free-swimming and develops into the sporocyst and redia stages, eventually producing cercariae and metacercariae. The parasite is most lethal, particularly to juveniles, when the metacercariae migrate within the fish body. The adult digeneans live in piscivore birds.

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Only digenean species belonging to the family Sanguinicolidae have been reported to cause mortality in cultured marine finfish (Ogawa and Egusa, 1986; Herbert *et al.*, 1994; Ogawa and Fukudome, 1994; Harisson, 1995). Heterophyiid metacercariae have also been reported in the muscles of marine fish, particularly in the mullets (*Liza* spp. and *Mugil* spp.) (Egusa, 1992).

The infective stage of sanguinicolids penetrates the fish host eventually gaining entry and settling into the vascular system. The flukes as well as their eggs are normally found obstructing the blood flow in the gill arteries, ventral aorta and the heart.

Host range. The sanguinicolids reported in various cultured marine finfish are shown in Table 6.10. So far, only cultured carangid (*Seriola* sp.), seabass, grouper and snapper have been reported to be infected with four genera of sanguinicolids, comprising five species. Surveys by Ogawa *et al.* (1989, 1993) and Ogawa and Fukudome (1994) revealed that nearly all the cultured *Seriola* spp. examined were infected by *Paradeontacylix* spp. All seabass from Pulau Ketam, Selangor, Malaysia, larger than 10 g were found to be infected by *Cruoricola lates* (Herbert *et al.*, 1994), while 63% of 19 examined seabass weighing between 18 and 1073 g in Penang were infected (Harisson, 1995). According to Herbert *et al.* (1994), the majority of *C. lates* are found in the venules around the stomach, pyloric caeca, intestine and excretory bladder. In Penang, 70% of cultured greasy grouper (*E. coioides*), with a weight range between 52 and 364 g, were infected by the sanguinicolid, *Pearsonellum corventum*, with a mean intensity of eight per fish (Harisson, 1995).

Geographic distribution. The few sanguinicolids are reported to show a limited geographical distribution, in accordance with that of their host (Table 6.10). *Paradeontacylix* spp. are found in East Asia, whereas *Cruoricola* sp., *Pearsonellum* sp. and *Cardicola* sp. are found in Southeast Asia. Without any doubt, however, increased awareness will widen the geographic distribution as well as the variety of hosts in which these worms have been detected.

Diagnosis. No particular signs are observed in fish infected with sanguinicolids, except that the fish die with open mouth and flared opercula (Ogawa and Fukudome, 1994). Field observations indicate that affected fish gasp for oxygen and die soon after being fed, suggesting that they require more oxygen when actively competing for food (Ogawa *et al.*, 1989). Heart and gills are the main organs affected by the sanguinicolids. In the gills, hyperplasia is extensive, especially in the area around encapsulated eggs, resulting in lamellar fusion. Eggs lodged in the heart tissue may form nodules, most of them in the ventricle, where muscle cells atrophied, but did not become necrotic (Ogawa *et al.*, 1989).

Prevention and control. There are no known methods for controlling sanguinicolids in fish cultured in cages. Fish caught in the wild become infected in their natural habitat. As all seabass fingerlings cultured in Southeast Asia are hatchery-produced,

Table 6.10. Sanguinicolid blood flukes in cultured marine fish according to geographical location.

	Fish host				
Parasite	*Seriola quinqeradia*	*Lates calcarifer*	*Epinephelus coioides*	*Lutjanus* spp.	Cultured site
Paradeontacylix grandispinnis	+	–	–	–	East Asia
P. kampachi	+	–	–	–	East Asia
Cruoricola lates	–	+	–	–	Southeast Asia
Pearsonellum corventum	–	–	+	–	Southeast Asia
Cardicola sp.	–	–	–	+	Southeast Asia

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C. lates infects the fish once they are moved into the cages.

Diseases Caused by Crustaceans

Crustaceans belonging to the Branchiura, Copepoda, Isopoda and Amphipoda are frequently found on the body surface and/or gills of caged marine fish. Some, like *Argulus* spp., glide freely on the body surface, while others anchor themselves to the host.

They do not require an intermediate host for their transmission. The mature female lays eggs, which develop into free-living nauplii and copepodid larval stages. All larval stages undergo several moultings from one stage to another before metamorphosing into adults in a suitable fish host.

Host range. The number of parasitic crustaceans reported from cultured marine finfish are relatively few and are shown in Table 6.11. The only branchiuran reported is *Argulus* sp. infecting grey mullet (*M. cephalus*), milkfish (*C. chanos*) and Asian seabass (*L. calcarifer*) (Paperna and Overstreet, 1981; Chong and Chao, 1986; Arthur and Ogawa, 1996). *A. scutiformis* was reported from *Takifugu rubripes* (Egusa, 1992). The life history of marine argulids has not been reported.

Most of the copepods reported are caligids, which could cause epizootics in the farms. A large population of yellowtail was infected with *Caligus spinosus*, which caused serious injuries to the fish host in Japan (Fujita *et al.*, 1968), and *Caligus patulus* in milkfish cultured in the Philippines (Lavina, 1977; Jones, 1980; Lin, 1989). In Malaysia, cultured groupers (*E. coioides* and *E. malabaricus*) are often infested by *Caligus* spp., which are also found in cultured snapper and seabass, and by *Ergasilus borneoensis* (see Leong and Wong, 1988; T.S. Leong, unpublished data). Izawa (1969) reported that the developmental stages of *C. spinosus* in cultured yellowtail included

Table 6.11. Parasitic crustaceans found in cultured marine finfish.

Parasite crustacean	Fish host	Cultured site
Alcirona insularis	*Epinephelus* sp.	Caribbean
Aega sp.	*Lates calcarifer*	Southeast Asia
	L. calcarifer	Southeast Asia
	Chanos chanos	Southeast Asia
Argulus scutiformis	*Mugil cephalus*	Caribbean
	Takifugu rubripes	East Asia
Caligus spinosus	*Seriola quinqueradiata*	East Asia
C. patulus	*Chanos chanos*	Southeast Asia
Caligus sp.	*Epinephelus coioides*, *L. calcarifer*, *Lutjanus johni*	Southeast Asia
Ergasilus borneoensis	*E. coioides*	Southeast Asia
Elaphognathia sp.	*M. cephalus*	West Asia
Gnathia sp.	*L. calcarifer*	Southeast Asia
	C. chanos	Southeast Asia
	L. johni	Southeast Asia
Gnathia piscivora	*M. cephalus*	West Asia
Lemathropus latis	*L. calcarifer*	Southeast Asia
Lernaea cyprinacea	*C. chanos*	Southeast Asia
Nerocila sp.	*E. coioides*, *L. calcarifer*, *L. johni*	Southeast Asia
Pseudocaligus apodus	*M. cephalus*	West Asia
P. fugus	*T. rubripes*	East Asia
Jassa sp.	*E. coioides*, *L. calcarifer*, *L. johni*	Southeast Asia
Microjassa sp.	*E. coioides*, *L. calcarifer*, *L. johni*	Southeast Asia
Lembos sp.	*E. coioides*, *L. calcarifer*, *L.johni*	Southeast Asia
Stenothole sp.	*E. coioides*, *L. calcarifer*, *L. johni*	Southeast Asia

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two nauplius stages, one copepodid stage, three chalimus stages and two juvenile stages before the copepod metamorphosed into an adult.

The isopod, *Nerocila* sp., has been found in grouper, seabass and snapper in Singapore and Malaysia, whereas *Aega* sp. has been detected in seabass in Thailand (Chong and Chao, 1986; Ruangpan, 1988) Larval stages of gnathiid isopods were also frequently encountered in grouper, seabass, milkfish and mullets (Paperna and Por, 1977; Leong and Wong, 1988).

The amphipods, *Lembos* sp., *Microjassa* sp., *Jassa* sp. and *Stenothol* sp., are found in grouper, seabass and snapper cultured in Malaysia (Leong *et al.*, 1998).

Geographic distribution. As indicated in Table 6.11, parasitic crustaceans, particularly the caligids and isopods, are widely distributed especially in Southeast Asia. Fish mortality has been attributed to the presence of large numbers of these crustaceans.

Diagnosis. The nature and severity of pathogenic effects depend on the interaction between the host and the parasite. Some of these parasites are mobile and cause less damage than those that are stationary, as the latter firmly anchor themselves to the host.

Argulids, however, though motile, do cause considerable injury to the host because they tend to remain in one spot for a long period of time. The pointed styles of argulids pierce the skin while feeding on mucus. Furthermore, acting as a cephalothoracic suction cup, they exert a great pressure with their body. The presence of large numbers of argulids results in haemorrhage as well as thinning of the epithelial cells.

Many parasitic crustaceans on the gills attach themselves by grasping or anchoring. The epithelial cells are generally reduced or lost, resulting in the inflammation and thickening of the local epithelial layer, haemorrhage, haemolysis, hyperaemia and hyperplasia. These signs are evident in the gills of grouper infested with *E. borneoensis* and Asian seabass infested with *Lernathropus latis*.

Mortalities caused by copepods have been reported for *Caligus spineus* on yellowtail in Japan (Fujita *et al.*, 1968), *Pseudocaligus apodus* on grey mullets in Israel (Paperna and Lahav, 1974), *Caligus patulus* in milkfish in the Philippines (Lavina, 1977) and *Pseudocaligus fugus* in puffer fish in Japan (Arthur and Ogawa, 1996).

The isopod, *Aega* sp., caused mortality in juvenile Asian seabass *L. calcarifer* in Thailand (Ruangpan, 1988) and an unidentified isopod on yellowtail in Japan (Kubota and Takakuwa, 1963). The larval stages of gnathiid isopods have been found in Asian seabass, snapper and milkfish in Malaysia and Thailand (Leong and Wong, 1988) and *Gnathia piscivora* in mullets in Israel (Paperna and Por, 1977). The gnathiids feed on fish blood and can cause severe anaemia when present in large numbers.

Prevention and control. Parasitic crustaceans are generally introduced along with fish caught in the wild for culture, but several of them are transmitted by wild fish around the cages. Prevention, therefore, is difficult. Organophosphate insecticides are commonly used for treatment. Fujita *et al.*, (1968) successfully treated yellowtail infected with *Caligus elongatus* by means of 50 s immersion in 100 ppm solution of Dipterex. Freshwater dip proved to be effective for *Caligus* sp. in cultured grouper and snappers in Malaysia, and *C. elongata* in red drum (Landsberg *et al.*, 1991). Other chemicals that have been used include formaldehyde, hydrogen peroxide and ivermectin.

Concluding Remarks and Current Perspectives

Like other aquaculture systems, cage aquaculture uses resources and produces wastes. Certain types of site habitats are particularly sensitive to cage aquaculture development and the impacts of cage fish farming on the aquatic environment can exert with time a

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negative feedback effect on cage operations (Beveridge, 1996).

Many species of wild fish are attracted by the cage structures, for shelter and abundant supply of food. They often gain access into the cages through the mesh, while farmed species manage to escape into the surrounding environment. The mingling of fish populations creates new opportunities for disease transfer (Diamant and Colorni, 1995; Colorni, 1998) and the interactions between cultured and feral fish may have important ecological implications. Translocated species may carry exotic diseases that could spread and devastate indigenous wild populations or may be themselves extremely sensitive to a local pathogen. In any case, the introduction of large numbers of caged fish to a system tends to have dramatic effects on disease agents. It has become increasingly apparent that high stocking densities of caged fish cause 'pathogen loading' in the surrounding area, where patterns of occurrence, prevalence and pathogenicity change greatly.

Only a limited number of therapeutic drugs are licensed for use in fish. When drugs are used, costs are usually high and residues may remain in the fish flesh after treatment, which means a long withdrawal period before the fish can be marketed. Also, accumulation of therapeutics in waste products can compromise water quality. Use of antibiotics, in particular, may not only enhance the development of resistant strains of bacterial populations but may also suppress the immune system of the fish (Adams *et al.*, 1997).

Considerable effort has been made in recent years to develop effective, safe and economical vaccines for numerous bacterial and viral diseases. Since *in vitro* culture of the causative agent or its inactivation is not always feasible, subunit vaccines have been prepared using recombinant technology. Here, the pathogens' antigenic determinants that elicit a protective host response have been identified and isolated, molecularly cloned and expressed in the bacterium *Escherichia coli* or the yeast *Saccharomyces cerevisiae*. Using biotechnology for the growth of these organisms, expressed 'foreign' antigenic proteins can be produced in bulk. The food is the most practical delivery system of these products to caged fish, requiring no extra labour and no handling stress. However, oral vaccines have so far proved disappointing, providing protection that is generally weak and of short duration, presumably because protective antigenic determinants are destroyed in the fish foregut. Encapsulation of vaccines is needed to ensure that the essential antigenic determinants reach the second gut segment in a non-degraded and immuno-stimulatory form (van Muiswinkel, 1995).

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7 Infectious Diseases of Warmwater Fish in Fresh Water

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Introduction

Cage culture of freshwater fish, which began in Cambodia in the late 1800s, is now commonly practised in Southeast Asia and gaining popularity in India (Chapter 1). In developing tropical countries, this type of fish culture is still either at the subsistence or semi-intensive level or is at the experimental stage, as for *Chrysichthys* spp. in Africa (Aqua Farm News, 1993).

Fish cultured in cages in Southeast Asia include tilapia, carp, catfish, snakeheads and eleotridids (Table 7.1). The tilapias, one of the common species in freshwater cages, are also cultured in cages in warm marine waters (Chapter 6) (Aqua Farm News, 1993). The catfish cultured include the Ictaluridae (*Ictalurus* spp.), Claridae (*Clarias* spp.), Pangasiidae (*Pangasius* spp.), Siluridae (*Silurus glanis*) and Bagridae (*Hemibagrus* spp.) (Aqua Farm News, 1993). Most catfish are of Southeast Asian origin, the exception being channel catfish cultured in the USA, which have been introduced into cages in Indonesia (Rabegnatar *et al.*, 1990). The most common catfish species cultured in cages in Southeast Asia is *Pangasius hypophthalmus*. Exotic Chinese carp, common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*) together with *Puntius gonionotus* and *Leptobarbus hoevenii* dominate the cyprinids (Table 7.1). Due to the variety of common names available for a particular fish species in Southeast Asia, the scientific names will be used as much as possible.

Publications and reports are available on diseases of feral and cultured fish in warm fresh water (Lio-Po, 1984; Kabata, 1985; ADB/NACA, 1991; Lim 1991d, 1992; Paperna, 1991, 1996; Arthur, 1992; Thune *et al.* 1993; Arthur and Lumalan-Mayo, 1997; Fijan, 1999). However, there is a paucity of information on diseases of fish in freshwater cage culture, even though cage culture began in Southeast Asia (Chapter 1) (Christensen, 1989; Aqua Farm News, 1993). Diseases are normally either mentioned in passing or are not included, particularly in those publications dealing with cage culture (Christensen, 1989; ADB/NACA, 1991; Dharma *et al.*, 1992; Nasution *et al.*, 1992; Alawi and Rusliadi, 1993; Aqua Farm News, 1993). In addition, publications on diseases in fish culture do not distinguish between diseases found in cage culture and pond culture (Davy and Chouinard, 1982; Arthur, 1987; ADB/NACA, 1991; Aqua Farm News, 1993). This is further exacerbated by the lack of comprehensive investigation into

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Table 7.1. Freshwater fish species cultured in cages in some tropical countries.

Country	Fish species	References
Bangladesh	*Catla catla*	Karim and Harun-al-Rashid Khan (1982)
	Cirrhina mrigala	
	Cyprinus carpio	
	Hypophthalmichthys molitrix	
	Oreochromis niloticus	
Cambodia	*Channa micropeltes*	Thana (2000)
	Cirrhinus microlepis	
	Labeo sp.	
	Clarias sp.	Guerrero (1979)
	Leptobarbus hoevenii	
	Oxyeleotris sp.	
	Pangasius sp.	
India	*C. catla*	Natarajan *et al.* (1983)
	C. mrigala	
	Labeo bata	
	Labeo rohita	
	Channa striata	Sukumaran and Sanjeeviraj (1983)
	Oreochromis mossambicus	Jameson (1983)
Indonesia	*C. striata*	Indra (1982)
	Oxyeleotris marmoratus	
	Tilapia	
	C. carpio	Jangkaru and Rustami (1979)
Malaysia	*Aristichthys nobilis*	Annual Fisheries Statistics (1998)
	C. striata	
	Ctenopharyngodon idellus	
	C. carpio	
	Hemibagrus nemurus (also known as *Mystus nemurus*)	
	H. molitrix	
	L. hoevenii	
	O. marmoratus	
	Puntius gonionotus	
	Tilapia	
Philippines	*A. nobilis*	Palisoc (1988)
	Chanos chanos	
	C. carpio	
	H. molitrix	
	O. niloticus	
Sri Lanka	*O. niloticus*	Siriwardena (1982)
Thailand	*Clarias* spp.	Tugsin (1982)
	C. carpio	
	Goby sp.	
	O. niloticus	
Vietnam	*Barbus* spp.	Pantulu (1979)
	Leptobarbus sp.	
	C. striata	T.T. Dung (personal communication)
	O. marmoratus	
	Pangasius bocourti	
	Pangasius conchophilus	

diseases encountered in cage culture systems in warm waters.

Disease outbreaks in cage culture have a greater impact because of high stocking

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densities and close proximity of cultured fish with each other as well as with feral fish. For example, there were 64 reported disease outbreaks in cage-cultured channel catfish in the USA during 1990 with mortality in 91% of these cases (Masser *et al.*, 1991). Also, diseases appear to occur more frequently in cages than in ponds (Collins, 1988). Cage culture exposes fish to pathogens of feral fish and perhaps to a greater number of intermediate hosts in parasitic diseases. Fish reared in cages may also present a potential health threat to man, especially when they are reared in unsanitary waters in areas where fish-borne zoonotic diseases are prevalent (see Ko, 1995) or when located in polluted areas. Diseases afflicting pond-reared and cage-cultured fish are in most cases similar, hence those that are important in pond aquaculture will be treated as potential problems for the cage culture. For example, *Piscinoodinium pillulare*, the causative agent of velvet disease of cyprinids, was first reported on pond-reared fish but is now found on cage-cultured fish (F. Shaharom, personal communication). The paucity of information on diseases in cage-cultured fish is partly due to the lack of studies on identification of pathogens/disease mechanisms and/or the absence of mandatory reports on disease outbreaks in many countries. Hence, we expect diseases to become more prevalent in the future as we move into more intensive fish culture, find out more about infectious agents, and adopt a system where it is mandatory to report disease outbreaks. In the current review, we have also included unpublished information from colleagues as well as from personal observations, and wherever possible we have provided the correct identification of pathogens and supplementary information on them.

Viral Infections

Viral infections can cause mass mortality, especially in fry or fingerlings, while older fish may develop resistance or are hardly affected. Most viral infections occur in fish at low water temperatures. This may explain the paucity of viral infections recorded in warm freshwater fish. Stress from handling, poor water quality, water temperature, age of fish, high stocking density and poor nutrition are factors that facilitate the development of viral diseases.

Among viral infections in fish, the channel catfish virus disease has the most impact on cage culture while the grass carp haemorrhagic virus and the spinning tilapia syndrome are also potential viral problems. In addition, other viral epizootics have been reported in common carp and tilapia that may have implications in fresh warmwater cage culture systems (Sano *et al.*, 1993; Oyamatsu *et al.*, 1997; Fijan, 1999). The epizootic ulcerative syndrome (EUS), a disease associated with a rhabdovirus, bacteria and the pseudofungi, *Aphanomyces invadans*, is discussed in the section on Diseases of Complex Infectious Aetiology.

An insufficient number of susceptible fish cell lines hampers isolation and diagnosis of viral pathogens. Cell lines currently used for isolation of warm freshwater virus are from: bluegill fry (BF-2) (Wolf and Quimby, 1966), brown bullhead (BB), channel catfish ovary (CCO) (Bowser and Plumb, 1980), *Epithelioma papulosum cyprini* (EPC) (Fijan *et al.*, 1983), grass carp kidney (GCK-84), grass carp gonad (GCG), grass carp fin (GCF) (Wolf, 1988), rainbow trout gonad (RTG-2), snakehead fry (SSN-1) (Frerichs *et al.*, 1993), catfish spleen (CFS) and snakehead spleen (SHS) (Lio-Po *et al.*, 1999).

Electron microscopy for the diagnosis of viral infections is not commonly used due to inaccessibility to this equipment in most tropical countries. As an alternative, serological tests are applied such as neutralization index determination, Western blot, ELISA, fluorescent antibody technique (FAT) and indirect fluorescent antibody test (IFAT). Recent molecular biology techniques such as PCR, RT–PCR and gene probes are becoming popular for the diagnosis of fish viral infections.

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Channel catfish virus disease (CCVD)

Channel catfish (*Ictalurus punctatus*) is the principal host of channel catfish virus (CCV). Outbreaks occur in most southern states in the USA, while low-grade mortality can be induced in blue catfish (*Ictalurus furcatus*) and channel catfish × blue catfish hybrids by experimental injection (Plumb *et al.*, 1975).

Pathology. CCV causes acute infection in cultured channel catfish fry and fingerlings less than 10 cm in length. It can also infect channel catfish juveniles and adults following waterborne exposure to CCV (Plumb, 1971; Hedrick *et al.*, 1987). Clinical signs are abdominal distension, exophthalmia, pale or haemorrhagic gills and petechial haemorrhage at the base of the fins and throughout the skin (Fig. 7.1). Infected fish swim erratically at the surface in head-high or hanging position. Mortality approaching 100% in channel catfish younger than 4 months old occurs at water temperatures above 25°C within 7–10 days. The virus does not induce mortality below 15°C. Secondary external lesions caused by bacteria, e.g. *Flavobacterium columnare* or *Aeromonas hydrophila*, or by aquatic stramenopiles may develop.

CCVD develops into a haemorrhagic viraemia after replicating in the kidney and in the spleen. Thereafter, the virus is transported via the blood to the intestine, liver, heart and brain (Plumb and Gaines, 1975). Thus, hyperaemia of the visceral cavity, enlarged spleen, and empty stomach and intestine have been observed (Plumb, 1994). Necrosis of the renal haematopoietic tissue and tubules, oedema, necrosis and congestion of the liver, intestinal oedema and congestion and haemorrhage in the spleen are characteristic histopathological findings. Skeletal muscle haemorrhage is seen in experimentally infected fish. The virus can be isolated from the kidney, intestine, liver, spleen, brain and muscle tissues (Plumb, 1971; Plumb and Gaines, 1975). The portal of entry for CCV from water is through the gills and the gut (Nusbaum and Grizzle, 1987). Channel catfish surviving a CCV infection grow slowly; e.g. experimentally induced CCVD survivors ranged from 11 to 15 g compared with 73–93 g in unexposed channel catfish 6 months after a standardized feeding regime (McGlamery and Gratzek, 1974).

The virus remains viable in dead fish kept on ice for 14 days and at –20°C for 100 days (Plumb *et al.*, 1973). It remains infective for 2 days in pond water at 25°C and for 11 days in dechlorinated tap water. However, it is rapidly inactivated in pond mud and by drying (Plumb, 1994).

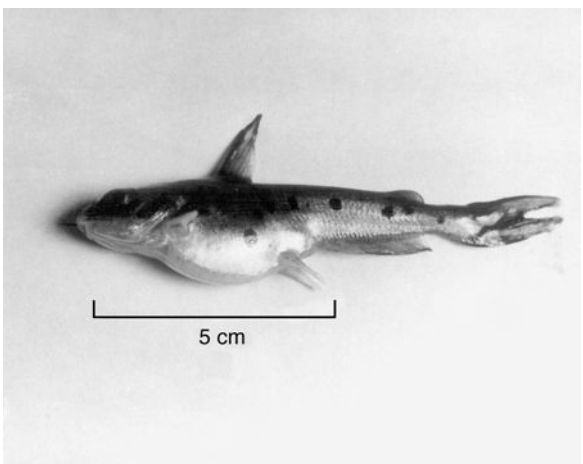


Fig. 7.1. Channel catfish (*Ictalurus punctatus*) infected with the channel catfish virus (CCV) (courtesy of Dr John Plumb).

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Transmission of CCV occurs both horizontally and vertically. The virus is readily transmitted from fish to fish. The exact mode of transmission is unknown but is most likely through the branchial and intestinal epithelium. Upon intraperitoneal injection, the virus is detected in the kidney after 24–48 h, in the intestine and liver after 72–96 h and in the brain after 96–120 h post-injection (Plumb, 1971). In experimental infections, fry die within 3 days of exposure (Wolf, 1988). The virus also occasionally persists in apparently healthy adult channel catfish broodfish but in most cases CCV cannot be isolated from adult fish, and has been isolated from fingerlings in only two of seven farms with positive broodfish (Bowser *et al.*, 1985).

Diagnosis. CCV, designated as *Herpesvirus ictaluri*, is a herpesvirus of the family *Herpesviridae* (Wolf and Darlington, 1971). It is enveloped, with icosahedral symmetry and measures 90–100 nm in diameter. The virus can be isolated from the kidney of fish with active infections using CCO or BB cells. Inoculated cells develop cytopathic effects (CPE) 24–48 h post-exposure, with optimal viral replication at 25–30°C. Identification is confirmed using electron microscopy, serum neutralization tests, IFAT, ELISA using monoclonal antibodies, CCV DNA probes and PCR (Wise *et al.*, 1985; Office International des Epizootie (OIE), 1995; Baek and Boyle, 1996).

Prevention and control. Detection of CCV in catfish broodstock will help prevent its spread to young catfish. The use of virus-free stock is the best preventive method. Alternatively, the use of resistant fish stocks or hybrids of channel catfish is recommended. Quarantine and killing of CCV-infected stock including surveillance for feral fish carriers should be practised. This should be of utmost consideration before introduction of channel catfish into tropical countries. Vaccination is still at the experimental stage and there is no chemotherapy.

Grass carp haemorrhagic disease (GCHD)

The disease was first observed in China in the 1980s. It commonly affects grass carp but can also infect black carp (*Mylopharyngodon piceus*), topmouth gudgeon (*Pseudorasbora parva*) and rare minnow (*Gobiocypris rarus*). It can also replicate in silver carp and in Chinese minnow (*Hemiculter bleekeri*) without any clinical signs. Outbreaks occur in Southern China during the summer when water temperatures range from 24 to 30°C (Nie and Pan, 1985; Wolf, 1988; Jiang, 1995; Fijan, 1999).

Pathology. Acute infections cause significant mortality of more that 80% in fingerlings and up to 70% in yearlings. Clinical signs include exophthalmia and severe haemorrhage of the gills and fin bases. Internally, haemorrhage occurs in the musculature, oral cavity, intestinal tract, liver, spleen and kidney. Naturally and experimentally infected fish have reduced erythrocytes, plasma protein, calcium and urea nitrogen but serum potassium is elevated. Experimental infection by bath and by injection induced typical signs of the infection. Disease and mortality are observed within 1–2 weeks exposure of fish in water at temperatures of 25°C or higher (Wolf, 1988; Fijan, 1999).

Diagnosis. The grass carp haemorrhagic virus (GCHV) is a non-enveloped, doubly encapsidated icosahedron with 5:3:2 symmetry, 92 capsomeres, with an overall diameter of 60–80 nm and a 40 nm inner capsid (Wolf, 1988). It is resistant to ether and chloroform. It is presently classified under the genus *Aquareovirus* (Family Reoviridae) (Li *et al.*, 1997). The virus can be propagated in GCK-84, GCG and GCF cells yielding titres of 10^8–10^9 $TCID_{50}$ ml^{-1}. *In vitro* viral replication is optimum at 28–30°C inducing CPE in 3–4 days post-inoculation (Wolf, 1988). The virus in fish with clinical signs and in carrier fish can be confirmed using RT–PCR (Li *et al.*, 1997).

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Prevention and control. Experimental vaccination using inactivated virus induced 80% protection by day 4 at temperatures above 20°C, by day 20 at 15°C, and by day 30 at 10°C, and this protection lasts for up to 14 months (Wolf, 1988). Zhu *et al.* (1993) as cited by Fijan (1999) reported that the 'Kelieao–Yufukang', a combination of two drugs, has *in vitro* and *in vivo* anti-GCHV activity.

Spinning tilapia (ST) syndrome

This virus was recently detected in Mozambique tilapia, blue tilapia (*Oreochromis aureus*), Nile tilapia (*Oreochromis niloticus*), and mango tilapia (*Sarotherodon galilaeus*) in Australia. The disease is caused by an iridovirus (Ariel and Owens, 1997).

Pathology. Affected tilapia fry swim in a spiral pattern, sink to the bottom then rise and hang at a 45° angle just under the water surface, gasping for air. They do not feed, are darker in colour and exhibit 'fin clamping'. Tilapia fry manifesting the spinning syndrome die within 24 h and a 100% mortality often occurs within 60 days. Naive tilapia fry experimentally exposed to diseased fry via cannibalism developed similar signs after 12 days. Histopathologically, the renal tubules are shrunken, haemorrhaging and infiltrated with eosinophilic granular cells. In addition, focal myolysis occurs in muscles. These histopathological lesions and the size range (110–140 nm) of the virus are similar to those caused by the Bohle iridovirus (BIV) in tilapia fingerlings (Ariel and Owens, 1997). The Bohle iridovirus also infects amphibians (Cullen *et al.*, 1995).

Diagnosis. So far, the virus has not been isolated in cell culture from diseased tilapia but the disease is usually diagnosed based on clinical signs and is confirmed by electron microscopy.

Prevention and control. No treatment is available but prevention through quarantine and restriction of transfer of stocks from endemic to non-endemic areas is recommended.

Bacterial Diseases

High stocking density of fish leads to increased feed rations and waste. This also results in bacterial problems with concomitant increases in ammonia and nitrite toxicity (Mitchell, 1997). Stress and trauma from handling are also predisposing factors. Most bacterial pathogens produce enzymes that facilitate their entry/invasion into the fish host tissues. Although they may cause primary infection, they may also act as secondary disease agents to a primary virus or parasite. The major bacterial infections among warm freshwater fish are motile *Aeromonas* septicaemia, *Pseudomonas* septicaemia, edwardsiellosis, enteric septicaemia, columnaris disease and streptococcal septicaemia/meningoencephalitis.

Motile *Aeromonas* septicaemia (MAS)

This disease was formerly known as haemorrhagic septicaemia, infectious dropsy, infectious abdominal dropsy, red pest, red disease, red sore or rubella. The syndrome is caused by the motile *A. hydrophila* (previously named *Aeromonas punctata* or *Aeromonas liquefaciens*). *Aeromonas sobria* and *Aeromonas caviae* are rarely associated with fish epizooties.

MAS affects freshwater and occasionally brackishwater and marine warmwater fish worldwide. It is the most frequently diagnosed bacterial fish disease and was the most severe disease problem among cage-cultured channel catfish in the USA between 1972 and 1980 (Plumb, 1994). Subsequently, it became the third most common bacterial infection (1987–1991) among cage-cultured channel catfish in the USA, accounting for 13–22% of disease outbreaks (Duarte *et al.*, 1993). The infection occurs mostly from February to July, with some outbreaks occurring in September and November. In the tropics,

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MAS infections are often reported in pond- and pen-cultured milkfish (*Chanos chanos*), common carp, grass carp, Nile tilapia and giant gourami (*Osphronemus goramy*) (Kou, 1972; Ruangpan *et al.*, 1985; Karunasagar *et al.*, 1986; Lio-Po *et al.*, 1986; Saitanu *et al.*, 1986; Supriyadi, 1986; Areerat, 1987; Angka *et al.*, 1988; Okaeme *et al.*, 1989; Yambot, 1997). Moreover, *A. hydrophila* has been associated with epizootic ulcerative syndrome (EUS)-affected striped snakeheads (*Channa striata*, also known as *Ophicephalus striatus*) and walking catfish (*Clarias batrachus*) in the wild, as well as in ponds and cages (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Subasinghe *et al.*, 1990; Torres, 1990; Lio-Po *et al.*, 1992; Pathiratne *et al.*, 1994; Angka *et al.*, 1995; Karunasagar *et al.*, 1995; Thanpuran *et al.*, 1995; Rahman *et al.*, 1999).

Pathology. *A. hydrophila* is a free-living, mesophilic bacterium found in soil, freshwater lakes, ponds, streams, bottom mud, domestic tap water and sewage. It is often associated with the normal flora of fish. Thus, the bacterium has been isolated from both healthy and diseased fish (Lio-Po and Duremdez-Fernandez, 1986; Lio-Po *et al.*, 1986, 1992; Torres, 1990). It causes infections not only in aquatic animals but also in avian hosts, cows and humans.

Infected fish lose their appetite, become lethargic and swim near the surface. External signs may vary according to fish species but are generally similar to clinical signs of other bacterial septicaemia infections in fish, i.e. exophthalmia and distended abdomen. However, septicaemia in acute MAS can be fatal with no clinical signs. Among milkfish reared in pens in a freshwater lake in the Philippines, acute signs of petechial haemorrhage of the skin and fin bases including dermal and caudal fin rot were observed (Lio-Po *et al.*, 1986). Yambot (1997) also isolated *A. hydrophila* from cage-cultured tilapia in the Philippines with haemorrhagic skin, ulceration, loss of scales, mouth sores, eye abnormalities, fungal growth and/or tail and fin rot. Fingerlings to adult Nile tilapia can be infected by *A. hydrophila* (Yambot, 1997). A case of natural infection in tilapia caused by *A. hydrophila* was associated with epidermal lesions so severe that the vertebrae were exposed (Lightner *et al.*, 1988). This severe condition is not uncommon among EUS-affected fish (Roberts *et al.*, 1994b). Hence, it is not surprising that the bacterium has been consistently isolated from EUS-affected fish (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Subasinghe *et al.*, 1990; Torres, 1990; Lio-Po *et al.*, 1992; Pathiratne *et al.*, 1994; Angka *et al.*, 1995; Karunasagar *et al.*, 1995; Thanpuran *et al.*, 1995; Rahman *et al.*, 1999).

In channel catfish, *A. hydrophila* infection has three categories: (i) motile aeromonad septicaemia with external signs; (ii) cutaneous, manifesting lesions that are limited to the skin and underlying muscle; and (iii) latent septicaemia with no external signs (Grizzle and Kiryu, 1993). Internal clinical signs include oedema, haemorrhage and necrosis. The disease is acute in very young fish while adults generally develop chronic infections (Plumb, 1994).

Motile aeromonad infections are predisposed by stress from temperature shock, low dissolved oxygen, high ammonia, handling or hauling, and an ongoing primary infection (Plumb *et al.*, 1978; Lio-Po *et al.*, 1986). These predisposing conditions are possibly immunodepressive, and the virulence of the *Aeromonas* strain is an important factor in the development of MAS epizootics (Thune *et al.*, 1993). Moreover, *A. hydrophila* is often reported in mixed infections with *Edwardsiella tarda*, *E. ictaluri*, *Flavobacterium columnare*, *Streptococcus* spp. or with parasites (Kanai *et al.*, 1977; Liu *et al.*, 1990; Duarte *et al.*, 1993). In carp dropsy, *A. hydrophila* was a component in the pathology of the disease, which is attributed to a virus as prime aetiological agent (Roberts, 1993).

Experimental *A. hydrophila* infections may be induced in milkfish with up to 100% mortality in 2 days after immersion exposure of scarified fingerlings, but not in fish with intact skin (Lio-Po and Duremdez-Fernandez, 1986). In addition, intraperitoneal injection with the bacterium causes

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mortalities within 12 h of injection of the pathogen (Lio-Po and Duremdez-Fernandez, 1986). In walking catfish and snakeheads, *A. hydrophila* induced dermal lesions after intramuscular injection of at least 10^5 cells per fish, which eventually ulcerated (Fig. 7.2) (Lio-Po *et al.*, 1992). Compared with other bacteria associated with EUS lesions, such as *Pseudomonas* sp., *Aquaspirillum* sp. and *Streptococcus* sp., *A. hydrophila* induced the most severe lesions upon intramuscular injection of snakeheads (Lio-Po *et al.*, 1998). Experimental infection of Nile tilapia fingerlings by immersion yielded an LD_{50} of 1.5×10^6 colony-forming units (cfu) ml^{-1} with 100% mortality at 10^8 cfu ml^{-1} and no mortality at 10^3 cfu ml^{-1} (Yambot, 1997). Attempts to induce external gross lesions in walking catfish by dermal cut, dermal scraping, fish bite, oral feeding, gastric lavage and cohabitation with a golden snail carrier (*Ampullarius* sp.) were unsuccessful (Lio-Po *et al.*, 1996). In contrast, in channel catfish with mechanically abraded skin, *A. hydrophila* experimentally induced systemic infection in 80% of exposed fish while cutaneous lesions developed in the remaining fish (Matsche and Grizzle, 1999).

Histopathologically, marked necrosis of the muscle fibrils occurred in snakeheads intramuscularly injected with *A. hydrophila* (Lio-Po, 1998). Walking catfish injected intraperitoneally with *A. hydrophila* developed focal necrosis in the liver, kidney, intestine and dorsal musculature (Angka, 1990). The infection elicits an intense inflammatory response, with massive infiltration of monocytic and granulocytic cells into infected tissues (Huizinga *et al.*, 1979; Ventura and Grizzle, 1988). Infected goldfish are anaemic, e.g. low red blood cell, haematocrit and haemoglobin counts (Brenden and Huizinga, 1986). In addition, there is a shift in the differential counts of lymphocytes to a predominance of neutrophils.

Motile aeromonads secrete extracellular products (ECPs) and these include toxins, protease, cytotoxin, haemolysin, leucocidin, gelatinase, elastase, staphylolysin, caseinase, enterotoxin and a dermonecrotic factor (Hsu *et al.*, 1981; Olivier *et al.*, 1981; Kanai and Wakabayashi, 1984; Lallier *et al.*, 1984; Krovacek, 1989; Yadav *et al.*, 1992). Moreover, cytotoxin-producing strains were associated with EUS-affected fish (Yadav *et al.*, 1992). Dermonecrotic strains of this bacterium secrete haemolysin at 10 and 30°C (Olivier *et al.*, 1981). However, correlation between virulence and ECP production was not consistent (Leaño *et al.*, 1996). Recently, Cascon *et al.* (2000) described the molecular characteristic of an elastase secreted by *A. hydrophila* that is important in its

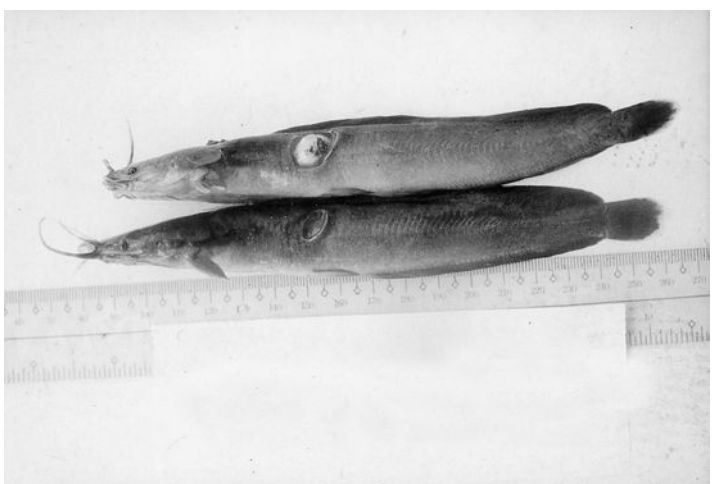


Fig. 7.2. Catfish (*Clarias batrachus*) showing ulcerative lesions 4 days post-intramuscular injection with *Aeromonas hydrophila*.

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pathogenicity. This protease has a high amino acid sequence similarity to proteases secreted by *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Vibrio* spp. An investigation of 12 strains of *A. hydrophila* isolated from fish showed that protease production varied among strains, with peak protease production optimum at 27.6 ± 4.9°C. (Uddin *et al.*, 1997). The protease levels increased during the late log phase to early stationary phase.

Siderophore production is also described but this is not related to virulence of the bacterium (Santos *et al.*, 1988; Leaño *et al.*, 1995). Virulence of *A. hydrophila* varies, even among isolates from the same epizootic (Lio-Po *et al.*, 1992). Also, Rahman *et al.* (1997) reported that *A. hydrophila* stored in 0.60 and 0.85% NaCl solutions had higher virulence than the cultured bacterium when injected intraperitoneally into carp and goldfish. Subsequent studies showed that a significantly higher number of starved *A. hydrophila* adhered to the skin of crucian carp (*Carassius carassius*) than the cultured bacterium (Rahman and Kawai, 1999). Attachment of *A. hydrophila* to carp epithelial cells is attributed to a 43 kDa outer membrane protein adhesin because of an abundance of this particular receptor on the cell surface (Lee *et al.*, 1997). Dooley *et al.* (1986) earlier described a crystalline surface layer, or S-layer, of 52 kDa protein, and further correlated this to strain virulence (Murray *et al.*, 1988; Ford and Thune, 1991).

Diagnosis. Motile *Aeromonas* spp. are flagellated, Gram-negative, short rods. They do not produce pigments and are resistant to vibriostat 0/129 (2,4-diamino-6,7-diisopropylpteridine phosphate). The bacteria grow at a temperature range of 18–39°C (Uddin *et al.*, 1997). In tryptic soy agar (TSA) or in brain heart infusion agar (BHIA) at 25–30°C incubation for 24–48 h, *Aeromonas* spp. produce white to creamy, convex, moist colonies. In Rimler–Shotts medium, the bacteria form orange–yellow colonies at 35°C (Shotts and Rimler, 1973). The three important *Aeromonas* spp. in fish can be differentiated using biochemical tests (Lio-Po *et al.*, 1992; Plumb, 1994). Joseph and Carnahan (1994) have further classified these into seven species (Table 7.2). Molecular identification can be applied by ribotyping of restriction genomic DNAs of aeromonads using different fragments of the 16S rDNA gene of *Escherichia coli* as a probe (Lucchini and Altwegg, 1992). Also, amplified fragment length polymorphism (AFLP) as a high-resolution genotype tool for classification of *Aeromonas* spp. and pulse-field gel electrophoresis as a rapid technique for typing of *A. hydrophila* have been developed (Huys *et al.*, 1996; Talon *et al.*, 1996). Igbal *et al.* (1998) furthur recommend the application of genetic identification using DNA–DNA hybridization.

Both virulent and non-virulent strains have been isolated from diseased fish (Torres, 1990; Lio-Po *et al.*, 1992; Leaño *et al.*, 1996). Definitive identification of either strain is a major difficulty and has been the subject of a number of research efforts. Cartwright *et al.* (1994) developed monoclonal antibodies for detection of virulent strains using either ELISA or fluorescein isothiocyanate (FITC) immunofluorescence. Virulent strains of *A. hydrophila* require about 30 min to induce CPE in EPC cells, while avirulent strains do not induce this pathological effect (Leung *et al.*, 1996). A PCR method that is reported to be rapid, sensitive and specific for the detection of virulence factors of *Aeromonas* spp. has been developed (Bin Kingombe *et al.*, 1999). Recently, identification of the genetic differences and virulence genes among different strains of *A. hydrophila* using a suppression subtractive hybridization (SSH) technique was reportedly successful (Zhang *et al.*, 2000).

Prevention and control. MAS outbreaks are common in eutrophic lakes and ponds. Outbreaks of *A. hydrophila* infections in fish-pen-reared milkfish are usually related to transport and handling stress, adverse environmental conditions of low oxygen concentration, low pH, and increased levels of ammonia and carbon dioxide (Walters and Plumb, 1980; Lio-Po, 1984; Lio-Po *et al.*, 1986). Moreover, tilapia fingerlings during seining can get caught between nets and

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Table 7.2. Comparison of distinguishing profiles of mesophilic clinical *Aeromonas* species (reprinted from *Annual Review of Fish Diseases*, Vol. 4, Joseph and Carnahan, 1994, with permission from Elsevier Science).

	Result[a] for:						
Characteristic	*A. hydrophila* (*n* = 46)	*A. veronii* bv. *sobria* (*n* = 26)	*A. veronii* bv. *veronii* (*n* = 8)	*A. caviae* (*n* = 33)	*A. schubertii* (*n* = 6)	*A. jandaei* (*n* = 9)	*A. trota* (*n* = 13)
Esculin hydrolysis	+	–	+	+	–	–	–
Voges–Proskauer reaction	+	+	+	–	V	+	–
Pyrazinamidase activity	+	–	–	+	–	–	–
cAMP-like factor (aerobic only)	+	+	+	–	–	V	–
Fermentation							
Arabinose	V	–	–	+	–	–	–
Mannitol	+	+	+	+	–	+	+
Sucrose	+	+	+	+	–	–	–
Susceptibility							
Ampicillin	R	R	R	R	R	R	S
Carbenicillin	R	R	R	R	R	R	S
Cephalothin	R	S	S	R	S	R	R
Colistin[b]	V	S	S	S	S	R	S
Decarboxylase							
Lysine	+	+	+	–	+	+	+
Ornithine	–	–	+	–	–	–	–
Arbutin hydrolysis	+	–	+	+	–	–	V
Indole	+	+	+	+	–	+	+
H_2S[c]	+	+	+	–	–	+	+
Glucose (gas)	+	+	+	–	–	+	+
Haemolysis (TSA with 5% sheep erythrocytes)	+	+	+	V	+	+	V

[a]+, positive for > 70% of isolates; –, negative, i.e. positive for < 30% of isolates; V, variable; R, resistant; S, susceptible.
[b]MIC (single dilution), 4 μg ml^{-1}.
[c]H_2S from GCF medium.

subsequently develop MAS causing more than 25% mortality after stocking in cages (J.A. Plumb, personal communication). Therefore, prevention of these stressful conditions will minimize MAS outbreaks.

Limited success has been achieved with vaccination against MAS in milkfish (G.D. Lio-Po, unpublished data) but vaccination is protective in tilapia (Ruangpan *et al.*, 1985). Indian major carp vaccinated with *A. hydrophila* yielded increased agglutinating antibody titre (Karunasagar *et al.*, 1991). Vaccination by intraperitoneal injection of formalin-killed *A. hydrophila* to catfish (*Clarias macrocephalus*) is more effective than immersion or oral administration (Areechon *et al.*, 1992). Biofilm vaccine at 10^{13} cfu g^{-1} of *A. hydrophila* in catla (*Catla catla*), rohu (*Labeo rohita*) and common carp for 15–20 days elicited high serum antibody titre and protective response for 60 days (Azad *et al.*, 1999). Blue gourami (*Trichogaster trichopterus*), when intraperitoneally immunized with major adhesin (43 kDa) in Freund's complete adjuvant, developed protective immunity to challenge by homologous and heterologous strains of *A. hydrophila* and one virulent

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strain of *Vibrio anguillarum* (Fang *et al.*, 2000). Vaccination studies in carp immunized with crude lipopolysaccharide showed that the mechanism of immunity is attributed to a sensitized thymocyte–macrophage system (Baba *et al.*, 1988a,b).

Recently, immersion vaccination of carp with *A. hydrophila* bacterins showed a distinct increase of lysozyme level in fish mucus, with stronger bacteriolytic properties 7 and 28 days after immunization (Kozinska, 2000). Moreover, a single intraperitoneal injection of 20 mg β(1,3)-D-glucan kg^{-1} into blue gourami enhanced the immune response against *A. hydrophila* for up to 29 days (Samuel *et al.*, 1996). Subsequent studies showed that the use of four glycans, namely Bar (glycan extracted from barley), krestin, scleroglucan and zymosan, significantly increased survival rates of tilapia and grass carp after infection with *A. hydrophila* (Wang and Wang, 1997).

Prophylactic bath treatments with 1–3% NaCl will help reduce post-handling infections. Likewise, bath treatments with 2–4 mg potassium permanganate l^{-1} are also effective for external lesions. Medicated feed with 2–4 g oxytetracycline kg^{-1} feed (50–100 mg kg^{-1} fish) for 14 days is recommended (Plumb, 1994). However, drug-resistant strains of *A. hydrophila* may evolve (Aoki, 1999).

Pseudomonas septicaemia

Pseudomonas spp. are ubiquitous in water and are opportunistic pathogens. In freshwater culture systems, *Pseudomonas fluorescens* has been implicated in epizootic outbreaks in Nile tilapia, grass carp, silver carp and bighead carp (*A. nobilis*) (Miyashita, 1984; Lio-Po and Sanvictores, 1987; Thune *et al.*, 1993).

Pathology. The clinical signs in fish affected with *Pseudomonas* septicaemia are very similar to those with MAS. Gross signs include ascites, exophthalmia, septicaemia and ulcers. The infection may be acute or chronic, with the latter commonly associated with skin lesions. Histopathological findings in Nile tilapia include focal necrosis, abscess and granulomas in the eyes, gills, liver, swim-bladder, kidney and spleen (Miyashita *et al.*, 1984). The bacterium also causes mortalities in 2-week-old Nile tilapia fry (Lio-Po and Sanvictores, 1987).

P. fluorescens is part of the normal flora of tilapia gut (Sugita and Kadota, 1980). It remains viable in fresh water for up to 150 days (Duremdez and Lio-Po, 1984) and secretes an extracellular proteinase (Li and Fleming, 1967).

Diagnosis. As clinical signs of *Pseudomonas* septicaemia resemble those of MAS, isolation and identification of the bacterial pathogen is required. *P. fluorescens* is a Gram-negative rod with one to three polar flagella. It grows on nutrient agar, *Pseudomonas* F agar and blood agar (Austin and Austin, 1987). For strains pathogenic to fish, the optimum growth temperature is 20–25°C. These secrete oxidase, catalase and gelatinase but not amylase, galactosidase, urease or hydrogen sulphide. It is citrate-positive, oxidative for glucose and produces a fluorescent pigment (Plumb, 1994).

Prevention and control. Stress from low dissolved oxygen concentrations, high stocking density, physical trauma and poor nutrition are predisposing factors in the development of *Pseudomonas* septicaemia (Post, 1983). Therefore, avoidance of these conditions is necessary in the prevention of its outbreak. Suggested bath treatments during the early stage of the disease include 1–2 mg benzalkonium chloride l^{-1} for 1 h, 0.5–1 mg furanace l^{-1} for 5–10 min or 1–5 mg malachite green l^{-1} for 1 h (Austin and Austin, 1987).

Edwardsiellosis and enteric septicaemia

E. tarda is synonymous to *Paracolobactrum anguillimortiferum* and to the *E. anguillimortiferum* described by Wakabayashi and Egusa (1973) and Kuo *et al.* (1987),

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respectively. Edwardsiellosis has been reported in 25 countries worldwide (Austin and Austin, 1987). The disease affects eels (Wakabayashi and Egusa, 1973), channel catfish (Meyer and Bullock, 1973), mullet (Kusuda *et al.*, 1976), tilapia (Lio-Po *et al.*, 1982), carp (Sae-Oui *et al.*, 1984) and striped bass (Herman and Bullock, 1986). Although, there are no reports of cage-cultured fish affected by edwardsiellosis, it poses a health threat. *E. tarda* can also cause serious infections in humans.

Enteric septicaemia is attributed to *E. ictaluri* in freshwater fish. This is a major pathogen of cage-reared channel catfish and accounts for about 30% losses in the southeastern USA. The estimated annual loss attributed to this pathogen is US$20–30 million (Plumb and Vinitnantharat, 1993). The majority of cases occur in May and June and again in September and October when water temperatures are between 22 and 28°C (Plumb and Schwedler, 1982). A morbidity rate as high as 68% was observed in May 1987, and an estimated 10–32% yearly morbidity rate among the primary diagnostic cases of cage-cultured and pond-cultured channel catfish in 1987–1991 (Duarte *et al.*, 1993).

E. ictaluri has been reported in Thailand, the USA and Australia. It is pathogenic to channel catfish but only very slightly pathogenic to blue catfish. White catfish (*Ictalurus melas*) and brown bullhead (*Ictalurus nebulosus*) are occasionally infected, while natural infections of walking catfish have been reported in Thailand (Plumb, 1994). Information on the pathology, epizootiology, diagnosis, prevention and control of *Edwardsiella* is detailed in Chapter 4 and in Plumb (1999).

Columnaris disease

Columnaris disease is an acute to chronic infection of freshwater fish and a common bacterial infection in the southeastern USA (Duarte *et al.*, 1993; Mitchell, 1997). Outbreaks are from March to September with peaks in June, and usually follow outbreaks of other diseases. The disease occurs in Asia, the USA and Europe, affecting warmwater fish like channel catfish and other ictalurids, cultured eels, common carp and tilapia (Plumb, 1994). The pathogen is *Flavobacterium columnare*, formerly called *Flexibacter columnaris*, *Cytophaga columnaris*, *Chondrococcus columnaris* and *Bacillus columnaris*.

Pathology. Infection primarily begins at the mouth, fins and gills. Clinical signs include frayed fins with greyish to white margins, depigmented, necrotic skin lesions with yellowish or pale margins, which can develop into shallow ulcers, yellowish mucoid material at the mouth and light to dark brown gill discoloration. Gill lesions initiate at the distal end of the filaments, which extend to the base. Epithelial vacuolation, necrosis, congestion, oedema, fusion and degeneration of the secondary lamellae subsequently follow. Acute mortality is usually associated with gill lesions. Internal pathology or host inflammatory response may occur, and the pathogen may be isolated from internal tissues (Thune *et al.*, 1993; Plumb, 1994; Shotts and Starliper, 1999).

Farkas and Olah (1986) described the three stages of gill necrosis. The first stage is initiated and maintained by environmental stress (probably ammonia, pH, temperature or any toxins in the rearing water) but *F. columnare* is seldom detectable on gills that are pale or dark purple. The second stage consists of bacterial invasion of the damaged gill at water temperatures above 20°C, causing gill necrosis, resulting in a grey-white coating of the gills. In the third stage, the white coating of the gills disappears and the infected gills become distorted. Different stages of gill necrosis may be observed in the same fish population.

Transmission of the bacterium is via water. The disease is most commonly associated with stress from high temperatures, elevated organic loads, high stocking density, low dissolved oxygen and trauma from excessive handling. In channel catfish, it occurs more often at temperatures between 25 and 32°C with significant mortality.

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Young fish are more susceptible than older fish. It may occur as a primary infection or as a mixed infection with another bacterium, *E. ictaluri* or *A. hydrophila*, or in association with a parasite, e.g. *Henneguya* sp. or *Ichthyobodo* sp. (Hawke and Thune, 1992; Duarte *et al.*, 1993; Plumb, 1994). Columnaris disease appears to follow outbreaks of other diseases (Duarte *et al.*, 1993).

Survivors of columnaris disease release the pathogen into the water at rates of up to 5×10^3 cells ml^{-1} h^{-1} (Fujihara and Nakatani, 1971), and surviving fish may release the bacterium for up to 140 days post-infection. The severity of lesion depends on the virulence of the strain and the ability of the pathogen to produce proteolytic enzymes. *F. columnare* produces an extracellular chondroitin AC lyase that degrades chondroitin and hyaluronic acid in fish connective tissue (Griffin, 1991). Bertolini and Rohovec (1992) also reported four extracellular proteases with molecular weights of 32, 34, 40 and 47 kDa. Newton *et al.* (1997) further observed that more protease is secreted into a medium with low nutrients and salt (Ordal's medium) than into media with high concentrations of nutrients or salt (TYES, Hsu–Shotts, modified Shieh's media).

Gills or dermal/muscular capillaries of infected fish become congested and degenerate (Plumb, 1994). Kuo *et al.* (1981) showed that survival of fish given 0.35–1.4 mg iron $100\ g^{-1}$ fish prior to challenge with the pathogen was reduced from 3 days to 1 day. Furthermore, highly virulent strains of *F. columnare* adhered more readily to the gills than low virulence strains, and were enhanced in ion-rich water, in the presence of nitrite or organic matter and at 28°C temperature (Decostere *et al.*, 1999).

The bacterium can survive up to 16 days at 25°C in hard, alkaline water with a high organic load, but survival decreases at pH 7 or less and in waters with less than 50 mg $CaCO_3$ l^{-1} and with low organic matter (Fijan, 1968). In sterile mud at 25°C, the organism survives for 16 days (Becker and Fujihara, 1978).

Diagnosis. *F. columnare* is a slender, Gram-negative, non-flagellated rod (about 0.5×4–12 μm) with gliding motility and forms 'hay stacks' or columns. Primary isolation of the pathogen can be achieved on selective Cytophaga agar supplemented with 5 μg neomycin ml^{-1} and 200 IU polymyxin B ml^{-1} (Hawke and Thune, 1992). *F. columnare* colonies are yellow to orange and rhizoid. This aerobic organism cannot tolerate more than 0.5% NaCl and it grows between 4 and 36°C, producing gelatinase, caseinase, catalase, oxidase and chondroitin sulphatase (Song *et al.*, 1988).

Diagnosis of the disease is dependent on the appearance of typical lesions on the skin, fins and gills, including the detection of the filamentous bacterial cells in wet mounts made from lesions. Based on the gene sequence of the 16S rRNA of the bacteria, Bader and Shotts (1998) designed primers for its detection using PCR.

Prevention and control. Disease prevention is by maintenance of fish under optimal environment conditions, proper handling of fish, prophylactic treatment and good health management practices (Plumb, 1994). Daily oral vaccination with heat-killed *F. columnare* for 4 weeks reportedly reduced mortality of rainbow trout from 48 to 8%, with protection correlated with antibody levels (Fujihara and Nakatani, 1971). Moore *et al.* (1990), however, showed that immunization of channel catfish using formalin-inactivated *F. columnare* bacterin by immersion yielded inconsistent results.

Potassium permanganate at 5 mg l^{-1} (depending on the organic load of the rearing water) in combination with oxytetracycline added to feed at 50 mg kg^{-1} fish day^{-1} for 10 days is effective in controlling outbreaks in cages. Potassium permanganate (based on the cage volume) mixed with a few litres of water and then poured through a 7.5 cm diameter polyvinyl chloride (PVC) pipe into the cage and allowed to dissipate into the pond by diffusion is also effective (Duarte *et al.*, 1993).

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Streptococcal septicaemia/meningoencephalitis

In freshwater cage-cultured Mozambique tilapia (*Oreochromis mossambicus*), epizootics attributed to streptococcal septicaemia were reported in Taiwan (Tung *et al.*, 1985). Other outbreaks have included the disease in Nile tilapia, hybrid tilapia (*O. niloticus* × *O. aureus*), rainbow trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*) in Israel, Japan and the USA (Kitao *et al.*, 1981; Kitao, 1993; Eldar *et al.*, 1994; Perera *et al.*, 1994; Baya *et al.*, 1996; Stoffregen *et al.*, 1996). Pathogenic species are *Streptococcus iniae* (phenotypically identical to *Streptococcus shiloi*) (Eldar *et al.*, 1995b), *Streptococcus difficile* and other *Streptococcus* spp.

Most reports on streptococcal infections have occurred among wild and cultured marine chinook salmon (*Oncorhynchus tshawytscha*), rabbitfish (*Siganus canaliculatus*) and barramundi (*Lates calcarifer*) in the USA, Singapore and Japan (Moring, 1982; Foo *et al.*, 1985; Bromage *et al.*, 1999). *S. iniae* specifically causes infections in marine finfishes, as discussed in Chapter 5.

Pathology. Among tilapia (15–20 cm in length) cage-cultured in a dam, this bacterium caused cumulative mortality of 50–60% within 1 month (Tung *et al.*, 1985). Clinical signs include unilateral and bilateral exophthalmia with or without conjunctival haemorrhage and corneal opacity. Petechiae occur on the underside of the operculum, around the anus, caudal and pectoral fins and mouth, with darkening of the body and discoloration of the dorsal and lateral trunk and peduncle with nodular or abscess formation. Abdominal swelling with ascites is common. Affected fish are anorexic, swim sluggishly in a circle, turning laterally, and eventually die.

Internal signs include petechiae and haemorrhage of the intestinal tract, liver and pyloric caeca. Systemic infection has been observed with evidence of bacterial dissemination in the heart, liver, kidney, stomach, small intestine, brain, eyes and musculature. Multiple necroses with granuloma occur in the hepatic parenchyma. The spleen develops hyperplasia of the reticuloendothelial cells with necrotic foci. Degenerative changes in the renal tubules, catarrhal enteritis in the small intestine and stomach, bacterial meningitis and abscess formation in the muscles have been noted.

The disease was experimentally reproduced in trout and tilapia using 10^7 and 10^8 cfu of *S. shiloi* and *S. difficile*, respectively, with virulence increased to 10^2 and 10^5 cfu after *in vivo* passage (Eldar *et al.*, 1995a). *Streptococcus* is also more pathogenic to Nile tilapia than to channel catfish (Chang and Plumb, 1996). In a mixed infection experiment with *Streptococcus* sp. and *A. hydrophila* as inocula, mortality was higher among experimental fish inoculated with both bacterial pathogens compared with those inoculated with either *Streptococcus* or *A. hydrophila* (Liu *et al.*, 1990). Infection via the nares is a potential route in Nile tilapia and hybrid striped bass (Evans *et al.*, 2000). Experimental transmission occurs by immersion, injection, orally or by cohabitation and is enhanced by injury to the skin or stressful environment. Sources of infection are water, mud, contaminated feed or carrier fish (Plumb, 1994).

Environmental factors influenced the development of streptococcal disease in Nile tilapia. Shoemaker *et al.* (2000) showed that significantly higher mortality (about 28.4%) developed in medium (11.2 g l^{-1}), compared with 4.8% in low (5.6 g l^{-1}) fish density treatments exposed to 2.5×10^7 cfu ml^{-1} *S. iniae* by immersion. Moreover, the infection could be transmitted by cohabitation with *S. iniae*-infected Nile tilapia for 48 h. In another study, Bunch and Bejerano (1997) demonstrated that low oxygen and high nitrite levels increased mortality in hybrid tilapia exposed to *Streptococcus* sp. However, these factors had no additive effect. Furthermore, streptococcal infection in Nile tilapia fingerlings may occur in association with *Trichodina* infestation (J.A. Plumb, personal communication).

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Diagnosis. Streptococcal organisms can be isolated in culture from the brain, kidney, heart, spleen and exophthalmia in Todd–Hewitt (TH) broth (DIFCO), nutrient agar supplemented with sheep or goat's blood, brain heart infusion agar or TSA for 24–48 h at 20–30°C (Kitao *et al.*, 1981). Modified Hucker's Gram-staining showing small, Gram-positive cocci, approximately 0.3–0.5 μm in diameter, most often occurring in chains, is a presumptive diagnosis. These organisms are non-motile and encapsulated. Plumb (1994) divided *Streptococci* associated with fish epizootics into four major groups: (i) group B, which is non-haemolytic; (ii) group D alpha- and group D beta-haemolytic; (iii) alpha-haemolytic strains that do not react with Lancefield antisera; and (iv) other *Streptococci* from freshwater and marine fish. The pathogen does not grow in 40% bile, 6.5% saline, 0.1% methylene blue milk or at 10 or 45°C (Kusuda and Salati, 1999). Details on the classification of *Streptococci* spp. based on biochemical and serological tests are in Kitao (1993) and Plumb (1994). All isolates from freshwater fish are beta-haemolytic (Kitao *et al.*, 1981; Tung *et al.*, 1985).

Prevention and control. Avoidance of stress due to adverse or poor water quality, rough handling, high stocking density, non-removal of infected or dead fish and overfeeding should be followed. Formalin-killed *S. difficile* vaccine injected intraperitoneally protects tilapia (Eldar *et al.*, 1995c). Recently, Klesius *et al.* (2000) showed that intramuscular injection of a combined vaccine prepared from two strains of *S. iniae* obtained from Nile tilapia provided relative percentage survivals of 63.1 and 87.3% when challenged with its homologous pathogens. Medicated feed with enteroflaxin at 5 mg kg^{-1} body weight for 10 days (Stoffregen *et al.*, 1996) or with erythromycin–doxycycline mixture at 100 mg and 70 mg kg^{-1} body weight for 6 days are also effective (Tung *et al.*, 1985). Formalin treatment was used for the associated *Trichodina* (J.A. Plumb, personal communication).

Pseudofungal Diseases

Stramenopiles are pseudofungal organisms previously classified as mycotic microbes (Alexopoulos *et al.*, 1996). Infections induced by the stramenopiles (Family Saprolegniaceae, Class *Oomycetes*) are commonly called 'water mould infections', cotton tuft disease or saprolegniasis. Branchiomycosis and mycotic granulomatosis also occur in cultured fish in fresh waters. The EUS is associated with a rhabdovirus, the bacterium *Aeromonas hydrophila* and/or the stramenopile, *Aphanomyces invadans* (see section on Diseases of Complex Infectious Aetiology, p. 246).

Saprolegniasis

The *Oomycetes* are distributed worldwide and affect warmwater fish in ponds, lakes, dams and rivers. In India, *Achlya* spp., *Aphanomyces*, *Dictyuchus*, *Saprolegnia* and *Pythium* were isolated from rohu, grass carp, common carp, catla, banded gourami (*Colisa fasciatus*), *Labeo bata*, climbing perch (*Anabas testudineus*) and giant snakehead (*Channa micropeltes*); *Aphanomyces* spp. from rohu and *Puntius ticto*; and *Saprolegnia* spp. from dwarf gourami (*Colisa lalia*), banded gourami, *Nandus nandus*, *Heteropneustis fossilis* and *Notopterus notopterus* (Srivastava, 1980; Bisht *et al.*, 1996). Saprolegniasis was also reported in Nile tilapia, mango tilapia and common carp in Taiwan, Egypt, Nigeria and Hungary (Chien, 1981; Okaeme *et al.*, 1989; ElSharouny and Badran, 1995; Jeney and Jeney, 1995).

Pathology. *Aphanomyces piscicida* causes mycotic granulomatosis in ayu (*Plecoglossus altivelis*) and dwarf gourami. External clinical signs include red spots on the body surface due to fungal growth, swelling, erosion and ulcers. Histologically, fungal-like hyphae and granulomas are seen in the internal organs and musculature. It is also highly pathogenic to goldfish (*Carassius auratus*),

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Rhodeus ocellatus, bluegill (*Lepomis macrochirus*) and crucian carp (Hatai and Egusa, 1977; Hatai *et al*., 1994). In experimentally infected ayu, typical mycotic granulomatosis occurred, while in common carp no inflammatory response was observed (Wada *et al*., 1996).

Fungal-like *Aphanomyces* spp., *Achlya*, *Allomyces* and *Saprolegnia* are also associated with EUS in snakeheads (Roberts *et al*., 1993; Paclibare *et al*., 1994; Willoughby *et al*., 1995). However, only *Aphanomyces* has been experimentally shown to induce lesions in naive snakeheads (Chinabut *et al*., 1995; Lilley and Roberts, 1997). Bruno and Wood (1999) provided a recent review on saprolegniasis, which is also discussed in detail in Chapter 4.

Branchiomycosis

Another fungal-like pathogen, *Branchiomyces*, has also been implicated as a cause of loss of 85% of juvenile red tilapia hybrid (*O. niloticus* × *O. mossambicus*) and green tilapia hybrid (*O. niloticus* × *O. aureus*) in Israel (Paperna and Smirnova, 1997). Carp are also susceptible (Post, 1983).

Pathology. Affected fish are lethargic with ragged or corroded gills, which are either bright red or white to brown depending on the degree of necrosis. Histological examination of the gill filaments of infected fish demonstrates the proliferation of hyphae of up to 11 μm in diameter. At the onset of sporulation, the hyphae contain multinucleated plasmodia, which develop into daughter plasmodia. The final stage of cell division yields a sporont filled with spores. Spores are released from the necrotic gills and remain suspended in the water or fall to the bottom.

In severe infection, some filaments undergo complete degeneration with necrotic residues of the pseudofungus. As a result, the pseudofungi reduce the blood supply to the gills, causing necrosis and sloughing away of the gill tissue. Hence, the disease is commonly named 'gill rot' (Post, 1983). Secondary bacterial invasion of the filament edges follows.

The presence of organic matter, algal blooms, dissolved fertilizer, low dissolved oxygen, pH between 5.8 and 6.5, high stocking density and temperatures between 25 and 32°C are predisposing factors. Under favourable conditions, the disease may develop in 2–4 days although *in vitro* culture of the pathogen produced spores on day 14 of culture (Post, 1983).

Diagnosis. Two species have been described: *Branchiomyces sanguinis* and *B. demigrans*. Squash preparations of the gills examined using light microscopy can be used to differentiate the two species. *B. sanguinis* has a thin hyphal wall (0.2 μm), spores of 5–9 μm diameter and affects the gill filaments and gill lamellar capillaries. *B. demigrans* has a thicker hyphal wall (0.5–0.7 μm), spores of 12–17 μm diameter and infects the parenchyma of the gills (Post, 1983).

Prevention and control. Affected fish should be burned and/or buried. Survivors of the epizootic are carriers of the pathogen and should not be cultured with naive fish or transported into *Branchiomyces*-free geographical areas.

Diseases of Complex Infectious Aetiology

Epizootic ulcerative syndrome (EUS)

EUS affects wild and cultured snakeheads, catfish (*Clarias* spp.), *Mastacembelus armatus*, *Puntius* spp., giant snakehead, *Oxyeleotris marmoratus*, *Glossogobius giurus*, blue gourami, snakeskin gourami (*Trichogaster pectoralis*), *Trichopsis vittata*, Siamese fighting fish (*Betta splendens*), swamp eels (*Monopterus albus*) and several wild fish species (Lilley *et al*., 1998). Major outbreaks occurred in Malaysia in 1979, in Indonesia in late 1980, in Thailand in 1981, in Kampuchea, Myanmar and Lao

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PDR in 1984, in the Philippines in 1985, in Sri Lanka in 1987, in Bangladesh and India in 1988, and in Bhutan and Nepal in 1989 (Tonguthai, 1985; Lilley *et al.*, 1998; Lio-Po, 1998). In addition, EUS was observed in Vietnam, Singapore and Pakistan. EUS-like lesions on fish were also reported in 1972 in Australia (Rodgers and Burke, 1977) where infected fish included mullet (*Liza* spp., *Mugil* sp.), sand whiting (*Sillago ciliata*), *Acanthopagrus australis* and *Arrhamphus sclerolepis*. The disease was then called red spot disease (RSD). Similarly, in Papua New Guinea, *Toxotes chatareus*, *Kurtus gulliveri*, *Bunaka* spp., goby, freshwater anchovy and spotted scat (*Scatophagus argus*) were severely affected in 1975 (Haines, 1983). Fish with EUS were found in all types of freshwater systems, including lakes, rivers, streams, culture ponds, rice paddies, irrigation canals and reservoirs. Cage-cultured snakeheads in the Philippines are very susceptible to the disease (Lio-Po *et al.*, 1992). Similarly, it was reported among cage-cultured *P. gonionotus* and *L. hoevenii* (Christensen, 1989).

Pathology. Lesions associated with EUS are characterized by severe, ulcerative, dermal necrosis with extensive erosion/sloughing of the underlying musculature (Fig. 7.3). The necrotic muscular tissue emits a foul odour. Fish have frank ulcers that consist of eroded dermal layer, exposing the underlying musculature, which may be haemorrhagic. In less severe infections, there is scale loss with erosion of the skin surface with or without haemorrhagic signs. To date, EUS is defined as a seasonal epizootic condition of freshwater and estuarine warmwater fish of complex infectious aetiology characterized by the presence of invasive *Aphanomyces* and necrotizing ulcerative lesions typically leading to a granulomatous response (Roberts *et al.*, 1994a).

In general, EUS outbreaks show a seasonal pattern (Phillips and Keddie, 1990). In Laguna de Bay, the Philippines, the EUS morbidity rate among snakeheads was estimated to be 59% in January, 1986 (Mines and Baluyot, 1986). Outbreaks are more common from September to March, which correlates with the period when the water temperature in the region is at its lowest range of below 25°C. Such low temperatures reduce the immune response of fish (Catap and Munday, 1998).

The spreading pattern of outbreaks of EUS in Southeast and East Asia strongly indicates the infectious nature of the aetiological agent. The actual pathogen of this disease has been in dispute for years. A rhabdovirus, *Aeromonas hydrophila* and *Aphanomyces invadans* have been associated with EUS-affected fish (Frerichs *et al.*, 1986; Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Lio-Po *et al.*, 1992, 2000; Pathiratne *et al.*, 1994; Chinabut *et al.*, 1995; Karunasagar *et al.*, 1995; Thanpuran *et al.*,



Fig. 7.3. Snakehead (*Channa striata*) affected with epizootic ulcerative syndrome.

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1995; Kanchanakhan, 1996; Lilley and Roberts, 1997; Lilley *et al*., 1998). Saitanu *et al*. (1986) also detected a virus associated with EUS.

The association of a rhabdovirus with EUS in Thailand and in the Philippines was first reported by Frerichs *et al*. (1986) and by Lio-Po *et al*. (2000). The virus is bullet-shaped, typical of the rhabdovirus genus (Family: *Rhabdoviridae*) and induces a CPE in BF-2, SSN-1, CFS, CCO and SHS cells, producing virus titres in the latter cells of 10^6 $TCID_{50}$ ml^{-1} at 25°C in 2–3 days (Lilley and Frerichs, 1994; Lio-Po *et al*., 2000). Optimum replication in SHS cells is at 15–25°C. Characterization and serological comparison of the virus with other fish rhabdoviruses associated with EUS-affected fish in Thailand showed that the Philippine virus isolate is morphologically similar and slightly antigenically related to the ulcerative dermal rhabdovirus (UDRV) (Lio-Po *et al*., 2000). Earlier experiments on the pathogenicity of rhabdovirus from EUS fish were not demonstrated (Frerichs *et al*., 1993). However, subsequent studies experimentally induced lesion development and mortality in virus-injected snakeheads reared at 20–22.5°C but not at 28–32°C (Lio-Po *et al*., 2001). Similarly, Kanchanakhan (1996) reported that rhabdoviruses can experimentally cause skin damage in juvenile snakeheads at ~20°C. This lower temperature range corresponds to the water temperature during the cooler months of December through to February when outbreaks of EUS among freshwater fish occur in the Philippines and in other EUS-affected countries.

A. hydrophila has been consistently isolated from lesions of EUS-affected fish (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Subasinghe *et al*., 1990; Torres, 1990; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Rahman *et al*., 1999). Pure cultures of the bacterium inoculated intramuscularly induced dermonecrotic lesions in healthy catfish and snakeheads (Lio-Po *et al*., 1992, 1996, 1998; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995). This bacterium grows at a temperature range of 18–39°C and secretes a dermonecrotic factor at temperatures of 10 and 30°C (Olivier *et al*., 1981; Uddin *et al*., 1997). Moreover, cytotoxin-producing strains were associated with EUS-affected fish and hypothesized to play an important role in the pathogenesis of the disease (Yadav *et al*., 1992).

The pseudofungi *Aphanomyces* spp., *Achlya*, *Allomyces* and *Saprolegnia* have also been reported in EUS-affected snakeheads (Roberts *et al*., 1993; Paclibare *et al*., 1994; Willoughby *et al*., 1995). Isolates of *A. invadans* were experimentally shown to induce lesions in most test snakeheads or sand whiting (Roberts *et al*., 1993; Chinabut *et al*., 1995; Catap and Munday, 1998). Other studies have reported that the pseudofungi grow invasively through the fish muscle causing severe myonecrosis (Callinan *et al*., 1995; Chinabut *et al*., 1995; Lilley and Roberts, 1997). Granuloma development was observed at 26°C or above, while fish at lower temperatures showed acute inflammation (Chinabut *et al*., 1995). In addition, Catap and Munday (1998) observed that sand whiting injected with zoospores of *Aphanomyces* sp. at 26°C developed highly inflamed, haemorrhagic external lesions, while similarly treated fish held at 17°C had slightly inflamed injection sites. The temperature-related growth rate of this pathogen appears to correlate with the findings that *Aphanomyces* isolates from EUS-affected fish generally thrive better at 26–30°C than at lower temperatures (Lilley and Roberts, 1997).

Diagnosis. The virus is typical of the bullet-shaped rhabdoviruses with an estimated size of 65 × 175 nm (Lio-Po *et al*., 2000). Filtrates derived from the visceral organs of EUS-affected fish can induce a CPE when inoculated into susceptible cells. The bacteria, *A. hydrophila*, and the pseudofungus, *Aphanomyces* sp., can be isolated from ulcers and muscles of EUS-affected fish by methods described in the section on motile *Aeromonas* septicaemia (Chapter 4) and in Lilley *et al*. (1998). Histopathology of muscular lesions of affected fish shows the development of a necrotic granulomatous

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mycosis, which may eventually invade the abdominal viscera (Lilley *et al.*, 1998). Bacterial colonies are also histologically demonstrated in EUS-affected snakeheads (Lacierda, 1995).

Prevention and control. Quarantine and restricted movement of EUS-susceptible fish from endemic areas to non-endemic sites should be practised. Prophylactic treatment with 5 ppm Coptrol (a chelated copper compound) was reported to prevent induction of EUS lesions while a proprietary mixture, CIFAX, may be curative (Lilley *et al.*, 1998). Moreover, recent studies showed that fish fed with the immunostimulant Salar-bec survived better when challenged with *A. invadans* (Miles *et al.*, 2001).

Parasitic Diseases

Although there is information on parasitic diseases of fish in tropical aquaculture (Kabata, 1985; Lim, 1991d, 1992; Paperna, 1991, 1996; Arthur, 1992; Arthur and Lumalan-Mayo, 1997), there is little or no information dealing specifically with parasitic diseases in cage culture systems. This paucity of information on disease pathogens and control measures and the lack of regulations concerning movement of diseased fish and mandatory reporting of diseases and mortalities in developing countries, coupled with the diverse species cultured, have made management of parasitic diseases in warmwater cage culture a difficult task. The diseases and specific identity of the parasites infecting warm freshwater cultured fish (in particular cage cultured fish) are seldom known and at best only the genera are recorded (Paperna, 1991). Overall, there is also a lack of knowledge about the actual disease patterns, the pathology and prevailing factors predisposing fish to the disease (Christensen, 1989; Dharma *et al.*, 1992; Nasution *et al.*, 1992; Alawi and Rusliadi, 1993). The lack of comprehensive investigations into the diseases encountered in cage culture systems has resulted in the abandonment of some lucrative projects such as the culture of *Oxyeleotris marmorata* in cages in Thailand (ADB/NACA, 1991). Thus, subsequent details of the diseases encountered in cage culture systems are discussed under generic and other taxonomic groupings, rather under the specific pathogens in question.

Generally, wild/feral fish have greater parasite species diversity but lower population abundance and the converse is true for cultured fish but further studies are required (L.H.S. Lim, personal observation; Lerssutthichawal, 1999). Personal observations and discussions with tropical fishery scientists and the current literature indicate that not all parasites known from other forms of culture systems have a similar impact on cage-cultured fish.

Diseases caused by protistans

The protozoan or protistan parasites that cause disease in fish belong to several phyla and these include the Ciliophora, Myxozoa, Microspora, Sarcomastigophora and Apicomplexa (Dickerson and Dawe, 1995; Dykova, 1995; Lom, 1995; Lom and Dykova, 1995; Molnar, 1995; Noga and Levy, 1995; Woo and Poynton, 1995). The commonly reported pathogenic protistans in or on fish reared in cages in warm waters include the myxosporeans, trichodinids and the dinoflagellates (Christensen, 1989; T.T. Dung, personal communication; F. Shaharom, personal communication). *Leptobarbus hoevenii* cultured in cages in Indonesia are infected with myxosporeans (Christensen, 1989). In Vietnam, fish in cage culture are plagued by *Trichodina*, *Balantidium* (in the intestines of catfish) and *Glossatella* (T.T. Dung, personal communication). The oodinid dinoflagellate *Piscinoodinum* sp. infects grass carp, bighead carp and *P. gonionotus* in pond culture, as well as catfish and tilapia in cage culture (Shaharom-Harrison *et al.*, 1991; F. Shaharom, personal communication). Various other protistan parasites have also been recorded but their prevalence is not known. For example, *Ichthyobodo* (*Costia*) and *Oodinium* are known to affect hybrids

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of *Clarias* in tropical warm freshwaters (Paperna, 1991), resulting in pale gills and excessive mucus secretions, causing the fish to gasp for air. The lack of reports on protistan diseases in warmwater cage culture systems could be due to lack of expertise in diagnosing the disease and/or the absence of reporting procedures, rather than the absence of the disease agents.

Movement of fish for culture has contributed to the worldwide distribution of many of their parasites, especially parasitic protistans. For example, *Eimeria cheni* and *Eimeria sinensis*, originally found in farmed carp in China, are now found in Europe (Molnar, 1976). Nile tilapia imported into Thailand from Egypt were also infected with *Eimeria vanasi* (Paperna, 1991), while cichlid fish farmed in Israel (Landsberg and Paperna, 1985) were infected by *E. vanasi* and *Gousia cichlidarum*. A few protozoan diseases found in cold waters could be regarded as emerging disease problems in cage culture in warm waters since these could be carried with their host species.

Myxosporean diseases

Myxosporeans are observed as cysts, infecting the skin and subcutaneous layer, muscle, gills, central nervous system as well as visceral organs. These cause extensive lesions as cysts break, and mortality occurs in cultured as well as feral fish (Lom and Dykova, 1995). In most cases in Southeast Asia, the specific myxosporidean pathogens are not known and at best the identification is at generic level. *Thelohanellus* (Myxobolidae), *Myxobolus* (Myxobolidae) and *Myxosoma* (Myxidiidae) have been reported from exotic carp and indigenous cyprinids in the Indian continent, Southeast Asia and China (ADB/NACA, 1991; Paperna, 1991). *Thelohanellus* has been reported on *P. gonionotus*, common carp and *Clarias* spp. in Peninsular Malaysia (Paperna, 1991; ADB/NACA, 1991). Myxosporeans are a major problem in Central Java (Indonesia), infecting *L. hoevenii* and *P. gonionotus* reared in ponds. However, in cages, the parasite was only found on *L. hoevenii* (Christensen, 1989). *Myxobolus koi* has been found on the gills of common carp and goldfish in Japan (Egusa, 1992) and on fish farms in Israel, Indonesia and the Indian continent, causing high mortality among the younger fish (ADB/NACA, 1991; Paperna, 1991), while *Myxobolus artus* is found on common carp in East and Southeast Asian countries (Lom and Dykova, 1995).

Pathology. *M. koi* infections on the gills of common carp and goldfish result in many small white to large pinkish to red cysts in the gill tissue (Paperna, 1991; Egusa, 1992). Large cysts are enclosed in the host connective tissues, which turn dark red due to haemorrhaging, leading to congestion and degeneration of the gill capillaries. The movement of the opercula and respiratory processes are further affected by increased mucus production and epithelial proliferation. Spores of *M. koi* were also observed in the heart, liver, kidney and intestine (Hoshina, 1952). According to Lom and Dykova (1995), *Thelohanellus pyriformis* forms large plasmodia in the subcutaneous tissue and muscle of cyprinids causing fatal epizootics in Indonesia. Little is known about the pathology caused by the other myxosporeans.

Diagnosis. One characteristic sign of myxosporean infections is small white and/or large cysts on the gills. For example, *M. koi* are observed as small white cysts and large pinkish to reddish cysts in the gill tissues of common carp and goldfish (Paperna, 1991; Egusa, 1992). Opercular movements of infected fish are hampered and respiration is affected by the increased mucus secretion and epithelial proliferation (Hoshina, 1952). Some myxosporeans are confined to the body and these occur as white cysts under the scales, often near the tail or fins, resulting in sores or ulcers on the skin (Christensen, 1989). Identification of the myxosporeans is based on the morphological characteristics of the spores. Cysts on the skin or gills are removed and gently broken to release the spores (preferably) on

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glass slides. The multicellular spores are unique in possessing nematocyst-like polar capsules (Lom and Dykova, 1995). They are usually oval–pear to round shaped, anterior end pointed, posterior end rounded, 1–2 polar capsules with polar filaments, sporoplasm with or without iodophilic vacuole and with or without posterior processes (Shulman and Shtein, 1962; Lom and Dykova, 1995).

The spores of *Myxobolus* are oval to pear-shaped with two polar capsules at their pointed anterior; the posterior end is rounded and lacks processes. The spores of *Henneguya* are round, oval or fusiform with two anterior polar capsules and valves with two caudal processes from the posterior end. The oval to round spores of *Myxosoma* are different in having two polar capsules at one end and lack processes and iodophilic vacuoles, while *Thelohanellus* has oval to round spores with smooth valves without processes and one medially displaced polar capsule.

Prevention and control. There is no effective treatment and the best method is to remove and destroy heavily infected fish from cages (Christensen, 1989). In light (early) infections, the cysts should be carefully removed and destroyed. Treatment with saline (0.23–5.0%), copper sulphate (0.025–0.05%), potassium permanganate, formalin, methylene blue, glacial acetic acid or phenol is not effective (Hoshina, 1952) as myxosporean spores are highly resistant to chemicals. The inclusion of certain drugs (such as Proguanil and furazolidone) in the fish feed has been shown to reduce spore production and alleviate lesions (Lom and Dykova, 1995). Although the life cycles of some species of myxosporeans are known to involve intermediate hosts such as oligochaetes (Lom and Dykova, 1995), for the majority of cases, the life cycles have not been elucidated and the actual intermediate hosts not identified. Hence, control of myxosporeans via eradication of intermediate hosts (oligochaetes) is not a viable option at the present time. Eradication of heavily infected hosts appears to be the most viable option for the moment.

Diseases caused by ciliates

The ciliates (Phylum Ciliophora) are common ectoparasites of fish, especially in hatcheries and on young fish in grow-out ponds. *Ichthyophthirius multifiliis* is the most well known pathogenic ciliate and is related to the marine pathogen, *Cryptocaryon irritans*. Others include the trichodinids and *Chilodonella*. However, in the majority of reported cases in tropical aquaculture, the specific identities of these ciliates are not known. Besides the known obligatory parasitic (pathogenic) ciliates, there are also facultative parasites (*Tetrahymena*, for example), which are opportunistic organisms.

Trichodinid diseases

Pathogenic trichodinids include *Chilodonella*, *Trichodina*, *Tripartiella* and *Trichodenella*. A large number of trichodinids are associated with the goldfish, common carp, grass carp, silver carp and bighead carp and these were introduced into Israel and Southeast Asia from China (Chen, 1955; Paperna, 1991). The trichodinids (*Trichodina acuta*, *Trichodina centrostrigeata* and *Trichodina heterodentata*) from African cichlids have also been introduced into Southeast Asia (Albaladejo and Arthur, 1989; Bondad-Reantaso and Arthur, 1989).

Trichodinids can be found on snakeheads and *Pangasius conchophilus* cultured in cages in Vietnam (T.T. Dung, personal communication). Although chilodonellosis occurs mainly in cold waters, *Chilodonella hexasticha* has also been found on the bighead carp in Malaysia (Shariff, 1984).

Trichodinids commonly cause mortality in hatcheries and these may continue to be a problem after fish are transferred to cage culture systems. Trichodinids are prevalent on young clariid hybrids of African catfish (*Clarias gariepinus*) and *Clarias* sp. in cages. These are also found on silver carp, bighead carp and grass carp in hatcheries in China and Vietnam, and are also on pangasiids and *Catla* sp. in cage culture. In Nepal, trichodinids cause mortality among the fry during spring and autumn. Although there

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are many species of trichodinids, only a few are known to be pathogenic (Lom, 1995).

Pathology. Pathological effects are dependent on the host's response, the intensity of infections and environmental conditions, since stressful conditions can compromise the host's ability to counteract infections (Paperna, 1996). Some trichodinids live specifically on the body surface or on the gills, while others are found both on the skin and the gills (Paperna, 1996). In skin infections, the preferred sites are the bases of fins. These parasites damage epithelial tissue through adhesion and crawling actions (Paperna, 1996). They feed on the epithelial cells causing abrasion and some trichodinids may suck out cellular contents, damaging cells, which degenerate and disintegrate resulting in erosion and desquamation of the epidermis (Paperna, 1996). The host responds to the infection by increased mucus secretion and epithelial hyperplasia, cellular destruction and inflammation. The damaged gills and epidermal tissues are targets for bacterial invasion. The infected epidermis thickens, becomes turbid with mucus and sloughed epithelial cells, and the fish becomes emaciated. When the gills are infected, excessive mucus is produced, with massive destruction of the gills, and proliferation of epithelial cells causing difficulty in respiration. Trichodinids are usually found in association with monogenean and other protozoan infections. Massive infections causing damage in the epidermis as described above result in mortality due to disruption in the respiratory functions of the gills (Paperna, 1996). Young fish in overcrowded and confined stressful habitats are usually heavily infected with trichodinids, while older fish have fewer but more host-specific species (Paperna, 1996).

Diagnosis. Trichodinids are easily observed microscopically from skin and gill scrapings (Paperna, 1996). Taxonomy of the trichodinids is based on the structure of the buccal ciliature, the morphology of the adhesive disc and the number and size of its components (Lom, 1995). Trichodinids are essentially flat discs, with somatic ciliature consisting of 3–4 ciliary wreaths around the aboral surface of the body, which is transformed into an adhesive disc. The disc is a proteinaceous skeleton, composed of a ring of hollow conical denticles. The denticles consist of blades (centrifugal flat projections) and horns (rod-like centripetal projections), connected to each other by radial pins (Fig. 7.4).

There are five genera of fish trichodinids (Lom, 1995). In warm freshwater cage culture systems, only *Trichodina* spp. has been identified. *Trichodina* is characterized by denticles with massive central conical parts, flat semi-circular blades, straight thorns and a diameter of 50–100 μm. For general identification, skin and gill smears containing trichodinids should be air-dried, fixed in Bouins for 20 min, washed in 70% ethanol, rehydrated and stained in a haematoxylin stain, dehydrated and mounted. For specific identification of the trichodinids, the adhesive disc is studied using a silver impregnation method (Welborn, 1967; Paperna, 1996). Air-dried smears should be fixed in 2% silver nitrate for 7–9 min in the dark, washed in distilled water and exposed to sunlight or UV light for 5–10 min.

Prevention and control. In most cases, an outbreak of trichodinid infections is the result of adverse environmental conditions, which are common in intensive culture systems. The best preventative measure is to ensure that good quality environmental conditions are maintained. To eliminate trichodinids from aquaculture systems, several chemicals have been recommended (Lom, 1995): saline solution (0.1–0.2% as a dip for 1–2 days), formalin (150–250 ppm as a dip for 30–60 min), acriflavine (indefinitely in water at 10–20 ppm) and potassium permanganate (0.1% as a dip for 30–45 min). Formalin has been used effectively to control trichodinids in warm waters. The efficacy of formalin in controlling trichodinids depends on water quality (pH, salinity and ambient temperature) and species of fish treated. Van As *et al.* (1984) showed that

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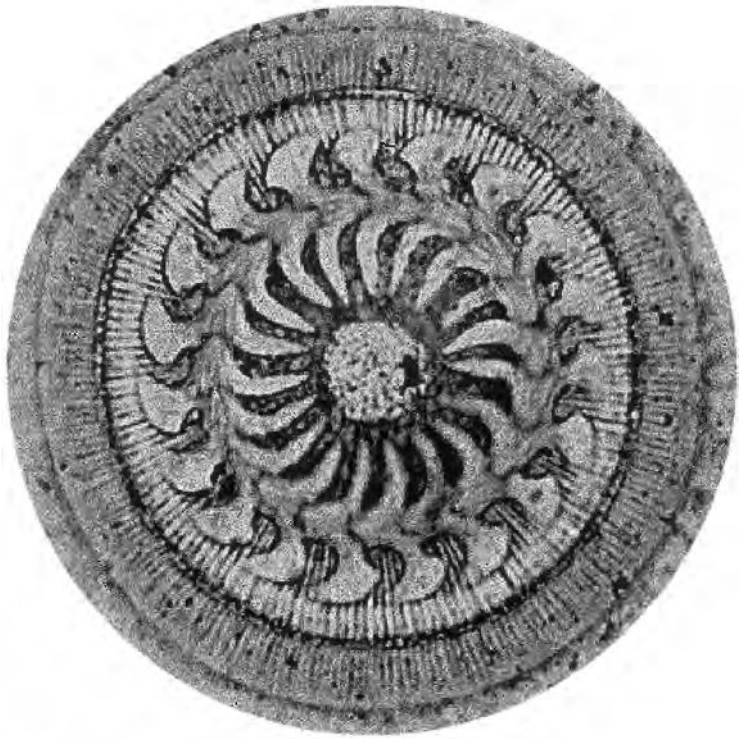


Fig. 7.4. *Trichodina acuta* from the skin of *Ctenopharyngodon idellus* (Klein's silver impregnation) (courtesy of Dr Richard Arthur, Canada).

25 ppm for 24 h was effective in cleaning infected carp, while 45 ppm for 24 h was needed to clean tilapia.

Ich or white spot disease

Ichthyophthirius multifiliis is a pathogenic ciliate infecting freshwater fish causing ichthyophthiriosis (also known as ich or white spot). This pathogen was first reported from China (Dickerson and Dawe, 1995), but is now a cosmopolitan pathogen in temperate and tropical warmwater fish (ADB/NACA, 1991). It is predicted to spread with the increase in aquaculture activities and also via the aquarium trade (Paperna, 1996). The outbreaks of ich are dependent on water temperature and, as temperature increases, the life cycle of this parasite is completed in a shorter time (Dickerson and Dawe, 1995), making them a potential danger to cage culture systems in tropical warmwaters. This parasite is maintained within the fish as a low subclinical (enzootic) infection and as encysted tomonts. It persists in the environment, becoming epizootic clinical infections when fish are stressed as a result of poor management practices (e.g. poor feed, overcrowding and poor sanitation). The pathogen is not host-specific and recovery from the disease confers resistance to reinfection (Paperna, 1996).

Pathology. The feeding or trophont stage is located within the epidermis (gills or skin) of the fish (feeding on the basal layer of the epidermis). The matured tomonts leave the fish and damage the epidermis causing detachment from its basal membrane; they secrete a gelatinous cyst wall and divide asexually to form tomites, which differentiate into infective theronts and are released into the water. The tomites develop into infective theronts, which penetrate the epidermis of the fish becoming established in the basal layer of the epithelium just

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above the basal membrane, and feed on epithelial cells. The rate of development of these stages is dependent on water temperature (see above). Intense and prolonged infections cause epithelial proliferation, haemorrhagic inflammation and subsequent disintegration of the integument.

Diagnosis. Clinical signs include anorexia and lethargy, and the disease is characterized by white spots on the skin and gills (Dickerson and Dawe, 1995). Skin and gill scrapings examined under the microscope reveal the ciliate (1 mm in diameter) with a small cytostome. *Ichthyophthirius* fixed and stained with Giemsa or haematoxylin reveals a large crescent-shaped macronucleus and small micronucleus.

Prevention and control. This pathogen is particularly difficult to control. An integrated approach incorporating appropriate culture practices (locating cages in areas where water movement is continuous and stocking of clean and healthy fish), immunization and chemotherapy in cases of heavy infestations are probably the most effective means of disease control (Dickerson and Dawe, 1995; Paperna, 1996).

The chemicals recommended for treatment include sodium chloride, malachite green, formalin and potassium permanganate (Dickerson and Dawe, 1995; Paperna, 1996). The efficacy of these chemicals is dependent on a number of factors such as environmental conditions, the fish species in question and the different developmental stages of the parasites (see below). For example, encysted tomonts in the environment are resistant to antiparasitic chemicals (Paperna, 1996). The stages of the parasite that can be destroyed are the dividing tomonts and the newly released tomites. Several chemicals have been listed for use against this pathogen, and the cost-effective chemicals suitable for large-scale farming systems are malachite green (0.05–0.15 ppm used continuously for 3–4 days) and a mixture of formalin and malachite green (50 and 0.05 ppm) (Paperna, 1996). The fish species has to be taken into consideration when chemicals are used since some species, especially catfish, do not respond well to malachite green (Paperna, 1996). Potassium permanganate has been used successfully in ponds to control ich but its effectiveness is affected by the amount of organic matter in the water (Dickerson and Dawe, 1995). Malachite green in a non-water-soluble formulation in feed has been reported to be effective against trophonts (Schmahl *et al.*, 1992). Immersion of fish infected with ich in Toltrazuril or triazinone (10 μg ml^{-1}) for 4 h (repeated daily for 3 days) has been shown to be effective against trophonts (Dickerson and Dawe, 1995). However, malachite green has been reported to be carcinogenic and its use is limited to aquarium fish, and should not be used in fish cultured for human consumption (Dickerson and Dawe, 1995).

Studies have shown that fish that have recovered from ich infections develop immunity against the parasite (Dickerson and Dawe, 1995). Immunization and vaccination offer another way to protect fish against ich. Experimental immunization using killed vaccines, intraperitoneal inoculation with live theronts and controlled exposure to infective tomites have been used (Paperna, 1996; Sin *et al.*, 1996). An experimental recombinant vaccine (from a 316 bp gene fragment of the immobilizing antigens, or i-antigens, of *I. multifiliis* and expressed in *Escherichia coli*) has been developed for ichthyophthiriosis (Woo, 1998). Goldfish inoculated with the recombinant protein vaccine in Freund's adjuvant survived a parasite challenge (He *et al.*, 1997).

Diseases caused by dinoflagellates

There are five genera of parasitic oodinid dinoflagellates: *Amyloodinium*, *Piscinoodinium*, *Crepidoodinium*, *Ochthyodinium* and *Oodinioides* on fish (Noga and Levy, 1995). The ichthyotoxins produced by dinoflagellates cause massive mortality in cultured and feral fish (Steindinger and Baden, 1984). The important freshwater pathogenic dinoflagellate in fish is *Piscinoodinium*,

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which is closely related to the marine dinoflagellate pathogen, *Amyloodinium*. *Piscinoodinium* is not host-specific and has been reported on feral, aquarium and cultured food fish species from diverse families in warm waters (Lom and Schubert, 1983; Paperna, 1991, 1996; Shaharom-Harrison *et al.*, 1991).

Velvet or rust disease

Fish with excessive mucus covering the body together with a rust-coloured appearance of the skin are infected with *Piscinoodinium pillulare*, the causative agent for velvet rust diseases, gold dust disease, pillularis disease and freshwater *Oodinium* disease (Shaharom-Harrison *et al.*, 1991). *Piscinoodinium*, like its marine relative *Amyloodinium*, is found on a wide range of host species and is known to cause mortality in warmwater fish (Paperna, 1996).

P. pillulare has been reported from 14 tropical ornamental fish species as well as cultured carp and cyprinids (Shaharom-Harrison *et al.*, 1991; Noga and Levy, 1995). In Peninsular Malaysia, *P. pillulare* occurs on aquarium fish, cultured grass carp, bighead carp, *P. gonionotus* and *L. hoevenii*, causing mortality in the latter (Shaharom-Harrison *et al.*, 1991). This pathogen also causes disease in cage-cultured *Hemibagrus nemurus* in the Trengganu River and in Tilapia cultured in Kenyir dam, Malaysia (F. Shaharom, personal communication), although not to the same extent as that found on pond cultured fish.

Pathology. Histopathological changes of gill structure occur with a massive proliferation of the gill epithelium, fusion of adjacent lamellae and separation of the gill respiratory epithelium resulting in a severe hyperplasia of the entire gill filament (Shaharom-Harrison *et al.*, 1991). The trophonts of *P. pillulare* penetrate the host cells by nail-like extensions resulting in degeneration and collapse of the cells, leading to focal erosion and proliferation of the epithelium and obliteration of the gill lamellae. The inner strata of the epithelium become spongious and may undergo complete lysis (Lom and Schubert, 1983; Paperna, 1991).

Diagnosis. Initial diagnosis can be based on clinical signs and confirmed by microscopic examination of the trophont stage. *Piscinoodinium* infects skin and gills with clinical signs similar to amyloodiniosis. Infected fish have a yellow to rust-coloured (velvety) skin, dense covering of mucus resulting in darkening of the skin, dyspnoea, anorexia and skin ulcers (Shaharom-Harrison *et al.*, 1991).

All oodinids have a parasitic trophont stage and a sessile, stalked, sac-like trophozoite stage, which feeds on the skin and gill epithelia. The trophont has a prominent stalk, which anchors the parasite to the host. It probably uses the stalk to absorb nutrients. After feeding, the trophont detaches, withdraws the stalk and forms an encysted tomont (reproductive cysts). The tomont divides asexually forming dinospores, the mobile infective stages. The trophonts and tomonts are important for definitive diagnosis, and microscopic identification of these stages is necessary. Trophonts are oval with smooth walls, usually visible to the naked eye as white spots (80–100 μm) and in Lugol's iodine turn dark blue.

Piscinoodinium is distinguished from other oodinid dinoflagellates on the basis of the morphology of the trophont, especially the type of host attachment and mode of nutrition (Lom, 1981). Fish should be examined live or immediately after death, and snips of the gills can be removed from live or recently dead fish and examined. Trophonts are removed by brushing the fish gently in a dish of water and the sediment is examined under the microscope. The trophont of *Piscinoodinium* is a yellow-green, pyriform or sac-like cell, almost round, 12×29 μm, with a rudimentary sulcus and a short stalk with an attachment disc extending from its base and thin holdfasts (rhizocysts) radiating from the stalk (Lom and Schubert, 1983). Head parts of the rhizocysts are inverted in separate compartments (rhizothecas) in the sole of the disc, while their shafts are firmly embedded in the host cell cytoplasm. The theca covers

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the entire cell except for the area of the attachment disc.

Prevention and control. Outbreaks of oodinid infections result from stress due to poor environmental conditions. Hence, environmental manipulation is probably a viable approach to control outbreaks of *Piscinoodinium*. Formalin detaches trophonts, but does not inhibit division (Paperna, 1996). A copper ion concentration of about 0.15 ppm (mixture of 5-hydrate copper sulphate with citric acid monohydrate) in water is effective in controlling *Piscinoodinium* (Paperna, 1996). A salt dip for 1–3 min dislodges the trophonts, while immersion for 3–5 days in a combination of 7 g salt l^{-1} and 40 mg potassium permanganate l^{-1} is also effective. However, freshwater fish cannot tolerate high salt concentration and potassium permanganate higher than 2 mg l^{-1} (van Dujin, 1973; Plumb, 1979).

Diseases caused by monogeneans

Monogeneans are among the most commonly reported parasitic agents of fish (ADB/NACA, 1991). They are mainly ectoparasitic on the gills, buccal cavity, body surface and fins of freshwater fish although some are endoparasitic (Gussev and Fernando, 1973; Euzet and Combes, 1998). Monogeneans are oviparous with the exception of the viviparous gyrodactylids. Although they rarely cause disease in wild fish, apart from the benedenids (Paperna, 1975), they are important pathogens in intensive fish culture (Paperna *et al*., 1984). Their direct life cycle results in rapid and continuous recruitment, especially in warm waters; this makes monogeneans especially dangerous in intensive culture. Disease caused by monogeneans is normally more debilitating than fatal, and subsequent mortality is usually attributed to viral or bacterial infection. Monogeneans stress the fish hosts by destroying the epidermal integrity of the fish, thus predisposing their hosts to other pathogens. Cone (1995) suggested that monogeneans could be the mechanical vectors of bacterial and viral diseases, but further confirmation is needed. In intensive culture systems, where intensity of infection can be high on the gills, monogeneans can cause death directly by inhibiting respiration through physical damage to the gills. Fish mortality from monogenean infections may result from damage to gill tissues and skin caused by attachment organs, and by feeding on the integument, which stimulates cell proliferation and secretion of copious amounts of mucus (Paperna, 1991). Cage culture in tropical areas is usually conducive to the perpetuation of parasitic diseases with high stocking density. The nets trap eggs, infective larvae and food debris around the cages, which attract carrier/reservoir feral fish.

Most monogenean genera are specific to a group of related host species. *Dactylogyrus* is found on cyprinids and catfish harbour *Thaparocleidus*. Although at species level most species are specific to a particular host species, some species, like *Thaparocleidus caecus*, are found on a number of pangasiids (Lerssutthichawal, 1999).

Many of the monogenean species on warm freshwater cultured fish have not been identified or are incorrectly classified. For example, *Dactylogyrus* spp. have also been incorrectly implicated as being pathogenic to snakeheads, tilapia and clariids cultured in Southeast Asia (Kabata, 1985). These fish possess their own unique and specific monogeneans (Lim and Furtado, 1983, 1986; Lim, 1986, 1991a). *Trianchoratus* is found on snakeheads other than giant snakehead, which is infected by *Sundanochus* spp., while *Cichlidogyrus* spp. infect the tilapias and *Quadriacanthus* spp. and *Bychowskyella* spp. infect the Southeast Asian clariids. The gyrodactylids, on the other hand, are ubiquitous, although at species level they might be host-specific.

The most commonly reported monogeneans on warm freshwater cultured fish are the *Dactylogyrus* spp. on carp, *Cichlidogyrus* spp. on cichlids, *Bychowslyella* spp. and *Quadriacanthus* spp. on clariids, *Trianchoratus* spp. on snakehead, *Pseudodactylogyroides* spp. on *O. marmorata*, and *Thaparocleidus* spp. on catfish other than

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clariids and *Gyrodactylus* spp. (see below). *Pseudodactylogyrus* spp. have been recorded from eels (*Anguilla* spp.) in warm waters of Indonesia (K. Buchmann, personal communication), and it should be noted that *Pseudodactylogyrus* infections caused mass mortalities of cultured eels, especially *Anguilla japonicus*, in Europe in the 1980s (Buchmann *et al.*, 1987; Buchmann, 1997).

In the majority of cases, the specific identity of the pathogenic monogeneans, signs and pathology of the infection, disease mechanism and control and preventative measures have not been specifically elucidated and documented. For instance, it is known that *Thaparocleidus siamensis* occurs in greater intensity than *Thaparocleidus caecus* on cultured *P. hypophthalmus* in Peninsular Malaysia and Thailand (Lim, 1990, 1996; Lerssutthichawal, 1999), but it is not known which of the two species is pathogenic. The translocation of monogeneans, along with their hosts, has been well documented for the various *Dactylogyrus* spp. on imported Chinese carp, *Cichlidogyrus* spp. on tilapia and recently for *Quadriacanthus clariadis* on the *C. gariepinus* imported into Thailand (Paperna, 1991; Lerssutthichawal, 1999).

There is information on the signs, pathology and control measures for some species of *Dactylogyrus*, *Gyrodactylus* and *Cichlidogyrus*, but not for other monogenean pathogens. In most cases, the information is derived from pond culture systems and not from cage culture systems. It should be also noted that habitat can affect parasitic infections, as indicated by the infestation of tilapia by *Neobenedenia* spp. instead of *Cichlidogyrus* spp. when farmed in cages in estuarine waters (see Chapter 5).

Diseases caused by Dactylogyrus *species*

Dactylogyrus species are specific to the Cyprinidae although they are also found on Hemiramphidae (L.H.S. Lim, unpublished data) and one species on a catfish (Gussev, 1976). This genus is frequently listed as a disease-causing agent since cyprinids are the most cultured fish group. Cyprinids cultured in cages and pens include the common carp, grass carp, bighead carp, silver carp and *Catla* spp., as well as other Southeast Asian carp such as *P. gonionotus* and *L. hoevenii* (see Chapter 1).

The four important species of *Dactylogyrus* that cause disease in cultured common carp in Israel are *Dactylogyrus anchoratus*, *Dactylogyrus extensus*, *Dactylogyrus minutus* and *Dactylogyrus vastator* (Paperna, 1991). These have different temperature preferences: for example, *D. extensus* flourishes at low water temperatures (optimum temperatures of 16–17°C), while *D. vastator* prefers warmer waters (20–24°C). Currently, *D. minutus* can be found on common carp in Taiwan (Paperna, 1991). The grass carp are infested with *Dactylogyrus lamellatus* and *Dactylogyrus ctenopharyngodonis*, silver carp with *Dactylogyrus hypophthalmichthys*, *Dactylogyrus suchengtaii* and *Dactylogyrus scriabini* and bighead carp with *Dactylogyrus aristichthys* and *Dactylogyrus nobilis* (Paperna, 1991). In Peninsular Malaysia, *D. nobilis* and *D. aristichthys* are found on cultured bighead carp and *D. lamellatus* on grass carp (Shaharom, 1988). *P. gonionotus* and *L. hoevenii* are infested with *Dactylogyrus leptobarbus* and *Dactylogyrus lampam* (Mizelle and Price, 1964; Lim and Furtado, 1986; Lim, 1991b), respectively, in Peninsular Malaysia. In Thailand, however, there are seven species of *Dactylogyrus* on feral *P. gonionotus* (Chinabut and Lim, 1993). *Dactylogyrus* has also been shown to cause mass mortality of fry, small fish and broodfish (Paperna, 1991).

Pathology. The pathology caused by *Dactylogyrus* spp. on exotic carp has been reported in studies done in Europe, but not for the species infecting the indigenous cyprinids of Southeast Asia. Feeding on epithelial cells and anchorage (attachment) by the monogeneans cause severe destruction of the gills resulting in haemorrhage and metaplasia of the gill tissue. Secondary bacterial infections usually occur and result in death of the fish. The pathologies caused by *D. vastator* and *D. lamellatus* are similar (Molnar, 1972; Paperna, 1991). *D. vastator* infestations cause severe hyperplasia of the epithelium of gill filaments. Extensive

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proliferation of the respiratory epithelium of the gills interferes with respiratory functions and may be a direct cause of death. The sites of proliferation are dependent on the preferred sites of the monogenean species. *D. vastator* prefers the tips of the gill filaments and causes mass mortality in young fish but seldom on fish greater than 32–35 mm since the functions of the remaining gill filaments are not affected. Massive infestations of *D. extensus* can cause mortality in 4–7 kg broodfish (Paperna, 1991).

Diagnosis. Fish infected with *Dactylogyrus* spp. are lethargic and usually found swimming on the surface of the water. Fish heavily infected with *Dactylogyrus* have pale to greyish gills, swollen at the edges, and the opercula appear to open wider than normal and secrete excessive amount of mucus (Christensen, 1989). Heavily infected fish are also anorexic and are usually found gasping for air and exhibiting abnormal behaviour such as jumping out of the water.

Dactylogyrus spp. are usually found on the gills, although in massive infections they can also be found on the buccal cavity. *Dactylogyrus* spp. can kill directly by damaging gill structures and affecting respiration. In warm eutrophic waters with low oxygen, this becomes serious. *Dactylogyrus* infections usually result in secondary bacterial infections with subsequent mortality. At present, *Dactylogyrus* infections are confirmed by examination of the gills and infected fins for presence of the monogeneans.

The signs and pathology of monogenean infections are neither generic- nor species-specific. Hence, diagnosis of monogenean infection is based on the identification of the pathogen itself. Correct diagnosis of monogeneans requires proper preparation of the parasite specimens. Gills can either be completely removed or gill clippings can be taken from the infected fish. Each parasite is removed carefully from the gills under a dissecting microscope, placed on a slide and covered with a coverslip. Excess water is removed and the corners of the coverslip sealed with nail polish to prevent it from moving (Lim, 1991c). Ammonium picrate is added underneath the coverslip to clear and fix the specimens, which are examined using a phase contrast microscope. Monogenean species are usually identified on the basis of the sclerotized reproductive and haptoral armaments on the cleared and flattened specimens. The *Dactylogyrus* are oviparous monogeneans with or without four eye-spots, 14 marginal hooks, two anchors, one to two connective bars and two needle-like structures and spindle-shaped dactylogyrid-type seminal vesicles. The descriptions for the various *Dactylogyrus* are found in Gussev (1985) for imported carp, in Lim and Furtado (1986) and Chinabut and Lim (1993) for *P. gonionotus* and in Mizelle and Price (1964) for *L. hoevenii*. Presently, other diagnostic techniques (such as immunological) are not known.

Prevention and control. The main method for control of monogeneans is the application of chemicals. Chemotherapeutic treatments include dips or baths in salt, formalin or organophosphates (Dylox, Dipterex, Neguvon, Chlorophos), Bromex-50 and potassium permanganate (Paperna, 1996; T.S. Thana, personal communication; T.T. Dung, personal communication). The recommended doses and concentrations vary according to host and parasite species, as well as physico-chemical properties of the waters. A 1 h bath with formalin at 1:4000 (< 10°C), 1:5000 (10–15°C) or 1:6000 (15°C) and a bath with 3–5 ppm potassium permanganate for 1–2 h (Hoffman and Meyer, 1974) has been recommended. Trichlorfon (Dylox) may be added in the food (50 mg kg^{-1} fish) four times at 3 day intervals each month during the critical periods. Lime and other chemicals have been recommended for pond applications: 0.4–0.5 ppm of trichlorphon (0.0-dimethyl-2,2,2 trichloro-1-hydroxyethyl phosphanate) has been used in Japan and 0.2 ppm dimethyl-1,2 dibromo-2,2 dichloroethyl phosphate (Bromex) in Israel (Egusa, 1992). These chemicals will be effective if the cages are in ponds. However, they will not be effective for large bodies of water and rivers where cages are usually located. The above chemotherapeutic formulations are for specific regions, and effective doses

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have to be tested for different waters. If chemicals prove ineffective, most farmers will simply destroy their heavily infected fish (personal communication with farmers). Eradication of feral reservoir fish from ponds is possible but not when the cages are in rivers or large lakes. The best alternative management strategy includes good husbandry based on knowledge of the reproductive biology and ecological requirements of the parasites such as temperature dependency. Using healthy fish fry from reliable hatcheries, limiting stocking density of fish, providing good quality feed and sanitation of nets will help to keep infestation at a low level. Some fish are able to acquire immunity against monogenean infections (Paperna, 1964, 1991) and more studies should be done to see if this could be used in the control of monogenean infections.

Diseases caused by Cichlidogyrus *species*

Cichlids are cultured in warm freshwater cages as well as in warm estuarine waters. Tilapias cultured in freshwater are affected by *Cichlidogyrus* spp., while in marine waters they are infected by the marine monogenean, *Neobenedenia melleni* (see Chapter 5). Tilapia (cichlids) is cultured in cages in freshwater in Indonesia, Vietnam and the Philippines as well as Malaysia. Cichlids are hosts to species of *Cichlidogyrus*, *Onchobdella* and *Enterogyrus* (an endoparasitic monogenean present on Sri Lankan cichlids). Several species of *Cichlidogyrus* and *E. cichlidarium* have been introduced with their fish hosts into the Philippines, Indonesia and Peninsular Malaysia (L.H.S. Lim, unpublished data; Shaharom, 1985). The *Cichlidogyrus* species on tilapia in Indonesia have been incorrectly identified as *Dactylogyrus* spp. (ADB/NACA, 1991). *Cichlidogyrus* spp. are also found on tilapia in cages in Vietnam (T.T. Dung, personal communication).

As noted by Paperna (1980) and Paperna *et al.* (1984), no reports of mortality due to *Cichlidogyrus* spp. have been recorded, but *Cichlidogyrus sclerosus* was found to cause severe gill damage in tilapias cultured in the Philippines (Kabata, 1985). *Neobenedenia* spp. found on tilapia in cages in estuarine waters are more pathogenic than *Cichlidogyrus* spp. (see Chapter 5).

Diagnosis. The behaviour of the fish can indicate the presence of parasites and this is similar to *Dactylogyrus* infection. However, accurate diagnosis requires removing the gills or gill clippings; the monogeneans are collected and prepared as stated above for *Dactylogyrus*. *Cichlidogyrus* can be distinguished from other monogeneans by having a haptor with four anchors, with two bars, one of which is V-shaped and the other made up of three parts. To identify the different *Cichlidogyrus* species, consult Paperna (1980).

Prevention and control. As for *Dactylogyrus* infections.

Trianchoratus *and* Sundanonchus *infections*

Monogenean species belonging to these two genera are found to infect the channids. *Sundanonchus* spp. are restricted to *C. micropeltes* while *Trianchoratus* spp. are found on the other channids. Although these monogenean species are found on and known to plague cultured snakeheads, there is no report of mortality due to these monogeneans.

Diagnosis. Methods for collecting and preparation of monogenean species for diagnosis are the same as for the *Dactylogyrus* spp. above. *Trianchoratus* spp. have four anchors, of which one pair is vestigial, no connective bars, 14 marginal hooks and a dactylogyrid-type seminal vesicle (Lim, 1986), while *Sundanonchus* spp., infecting giant snakeheads, can be differentiated from the other monogeneans in having four anchors, with two connective bars (dorsal bar may be split into two), 16 marginal hooks, a dactylogyrid-type seminal vesicle and an X-shaped vitelline duct (Lim and Furtado, 1985; Kritsky and Lim, 1995).

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Prevention and control. As for *Dactylogyrus* infections.

Diseases caused by Pseudodactylogyroides marmoratae

Pseudodactylogyroides marmoratae has been found on cage-cultured *O. marmorata*, a highly priced fish in Malaysia and Vietnam (Leong and Wong, 1998; T.T. Dung, personal communication). *O. marmorata* is cultured in cages in Peninsular Malaysia, Indo-China and Thailand. However, this fish is no longer cultured in Thailand because of disease problems (ADB/NACA, 1991). Other than the fact that this parasite causes disease, practically nothing is known about the signs, its pathology or how to control this pathogen.

Diagnosis. The monogenean species are collected and prepared as given for the *Dactylogyrus* species above. Morphologically, *Pseudodactylogyroides* spp. (Fig. 7.5) possess four anchors, of which one pair is usually underdeveloped and small and the larger pair has a patch-like inner root, two connective bars, 14 marginal hooks and a dactylogyrid-type seminal vesicle (Lim, 1995).

Prevention and control. As for *Dactylogyrus* infections.

Diseases caused by Thaparocleidus *species*

Species belonging to this monogenean genus are found on cultured pangasiids and bagrids in Southeast Asia (Lim, 1990; Lerssutthichawal, 1999; A. Pariselle, personal communication). As with *Pseudodactylogyroides*, little is known about the pathology caused by this group of monogeneans, or its level of pathogenicity.

Diagnosis. Monogenean species from pangasiids are collected and prepared as for *Dactylogyrus* infection above. *Thaparocleidus* spp. (Fig. 7.6) have four anchors, two connective bars, one of which may be whole or separated into two, 14 marginal hooks and a sac-like seminal vesicle (Lim, 1996).

Prevention and control. As for *Dactylogyrus* infections.

Diseases caused by Gyrodactylus *species*

The gyrodactylids are easily differentiated from the dactylogyrids since they are viviparous with developing embryos in the uterus. The young gyrodactylids do not need to search for a host. It has been postulated that gyrodactylids are able to disengage and reattach to new hosts, especially under intensive culture where fish are in close proximity to each other.

Some *Gyrodactylus* spp. have wide host specificity and cause fish mortality. Gyrodactylids are easily translocated via the live fish trade, for example *Gyrodactylus turnbulli* is spread via the aquarium trade to England, New England States, Nova Scotia and Peru from Singapore (Cone, 1995). Although the gyrodactylids are important pathogens in warmwater culture systems, there is a paucity of information on this group. Studies on the pathogenicity of the *Gyrodactylus* spp. are mostly from temperate countries (Paperna, 1991; Cone, 1995). *Gyrodactylus* infection is common on *Clarias* spp. such as *C. batrachus, C. macrocephalus, C. gariepinus* and the hybrid of *C. macrocephalus* and *C. gariepinus* reared in cages in Thailand (Aqua Farm News, 1993). Paperna (1991) reported *Gyrodactylus rysavyi* and *Macrogyrodactylus* on *C. gariepinus* in Africa. These parasites may have an impact on the future of cage culture of *C. gariepinus* in the Ivory Coast as well as the *Clarias* culture in Thailand, the Philippines and Indonesia. *Gyrodactylus fuscus* has been found on *Clarias fuscus* in North Vietnam. Unidentified *Gyrodactylus* spp. infect cage-cultured *L. hoevenii* and *P. gonionotus* in Indonesia (Christensen, 1989). *Gyrodactylus* is found on tilapia cultured in freshwater and brackish waters (Natividad *et al.*, 1986).

Pathology. *Gyrodactylus* spp. are usually found on the skin and fins, although there are species that can be found on the gills. They are also found in conjunction with protozoan and bacterial infections. Mucus

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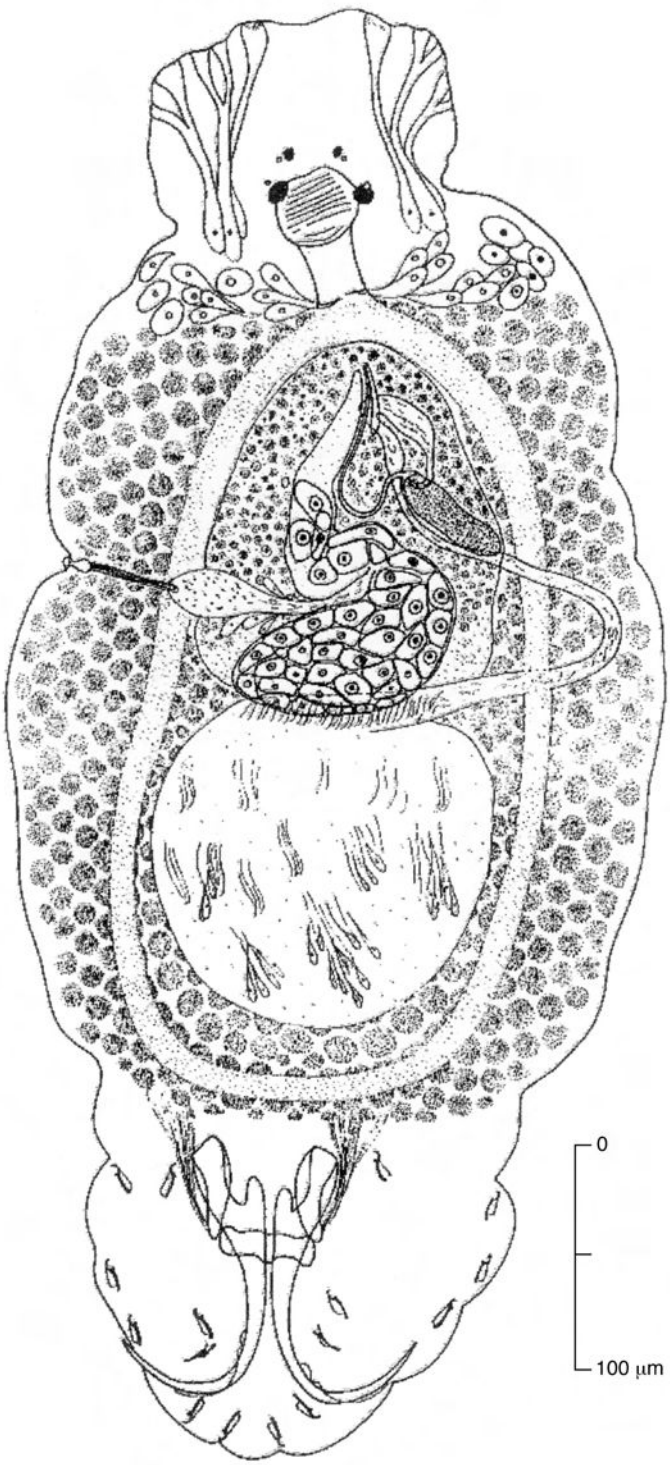


Fig. 7.5. *Pseudodactylogyroides marmoratae* from the gills of *Oxyeleotris marmorata* (reproduced with permission from *Systematic Parasitology*).

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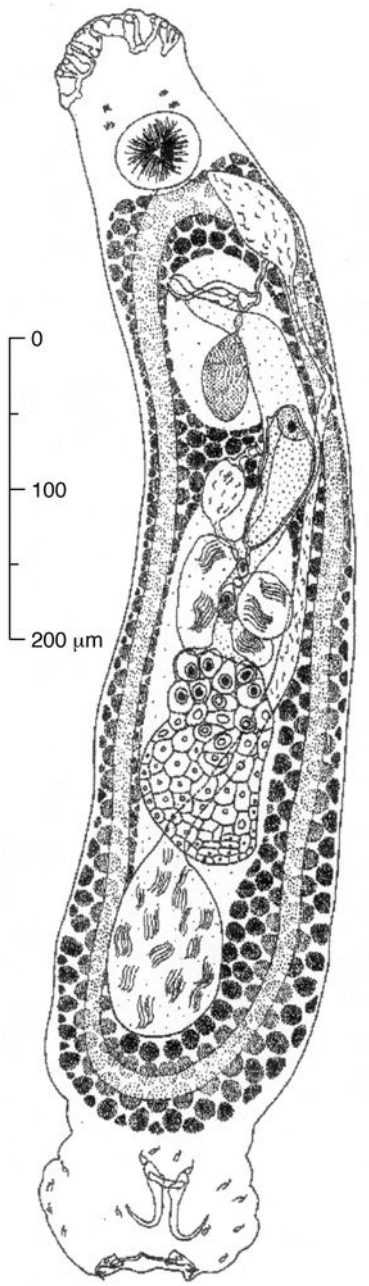


Fig. 7.6. *Thaparocleidus caecus* from the gills of *Pangasius hypophthalmus* (reproduced with permission from *The Raffles Bulletin of Zoology*).

secretion is increased during heavy infections, fins become frayed, skin ulcerated and gills damaged by the feeding and attachment processes of the worm. Fish infected with *Gyrodactylus* exhibit abnormal behaviour, i.e. rubbing against the net, anorexia, hyperproduction of skin mucus, haemorrhagic ulcers on the body sides, fin rot (mainly anal and caudal fins) and thickening and opacity of the eye cornea. At this stage, it is easy to detect parasites on the eyes, skin and fins. The skin usually appears whitish. At the later stage of infections, reddish inflamed areas develop on the skin and the eyes may become opaque and blind (Christensen, 1989).

Diagnosis. Initial diagnosis can be based on clinical signs with confirmation by examination of the parasites. The monogeneans are collected from skin and gill scrapings and prepared as for *Dactylogyrus* spp. (see above). The anterior region of the gyrodactylid is divided into two lobes with two sets of head glands. Its haptor is armed with 16 hinged, marginal hooks, two anchors and two connective bars. *Gyrodactylus* spp. are difficult to identify (Paperna, 1991). The body size, excretory systems, dimensions and morphology of the sclerotized parts (reproductive spines, anchors, marginal hooks, connective bars) are important criteria for species differentiation (Malmberg, 1970). *Gyrodactylus* spp. in the tropical regions are poorly studied and more investigations are required.

Prevention and control. A formalin bath using 20–25 ml of 40% formalin in 100 l of well-aerated, clean water for 30 min is used. Other formulations include formalin at 1:2000 for 10 min and ammonia solution at 1.5 ml ammonia 1^{-1}. The latter two methods reduce infections but do not eradicate them. Trichlorphon (0.25 ppm) is also effective (Meyer, 1968).

Diseases caused by other helminths

Although there are pathogenic trematodes, nematodes, cestodes and acanthocephalans in tropical aquaculture (Paperna, 1996), the pathogenic species causing disease in tropical cage culture systems are unknown. For instance, trematodes and cestodes have been found in cage-cultured *Pangasius*

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bocourti and in snakeheads in Vietnam but their identities are unknown (T.T. Dung, personal communication). With the introduction of more exotic species into tropical waters there will probably be more reports of helminthic parasites in aquaculture in the future. The impact of helminthic infections in aquaculture is not known: although the intestines of several feral giant snakeheads infected with the acanthocephalan species *Gorgorhynchus ophicephali* were observed with perforations (personal observation), its impact on cultured giant snakeheads is not known. Since there are no reports of massive mortalities caused by cestodes, trematodes or nematodes, the following sections will briefly deal with known helminth infections in fish species that are cultured in cages in warm waters.

Trematode infection

Sanguinicola (blood fluke) has been recorded on exotic cultured grass carp and bighead carp (Anderson and Shaharom-Harrison, 1986). Thus far, no *Sanguinicola* has been reported on clariids of Southeast Asia although *Sanguinicola dentata* is found on *Clarias lazera* (now known as *C. gariepinus*) from Africa (Paperna, 1996) and this species has been imported into Thailand for culture purposes. Metacercariae causing 'black spots' in cichlids and clariids in Africa (Paperna, 1996) could spread to other tropical waters. Kabata (1985) noted the presence of clinostomatids and heterophyids in farmed fish in the warm waters, but thus far none have been reported among cage-cultured fish.

Nematode infection

Nematodes are common on feral as well as food fish (L.H.S. Lim, unpublished data; Kabata, 1985). The nematode *Anguillicola crassa* could become important since its host, *A. japonicus*, is cultured in Taiwan and on a smaller scale in Indonesia. The other nematode of importance is *Philometroides cyprini* in common carp (Paperna, 1996). Camallanids are common on feral catfish (L.H.S. Lim, unpublished data; Kabata, 1985) but little is known about their effect in cage culture.

Cestode infection

The adult Asian tapeworm, *Bothriocephalus acheilognathii*, causes mortality in heavily infected grass carp in Europe (Paperna, 1996). The Asian tapeworm is not confined to cultured imported carp but has spread to native fish in warm waters of Asia (Peninsular Malaysia) and Israel with the imported grass carp, silver carp and bighead carp (Shaharom, 1985; ADB/NACA, 1991; Paperna, 1991, 1996). Paperna (1996) has provided a detailed account of the disease caused by this cestode species. Cestodes are also present in cultured and wild fish in warm waters: *Lytocestus* spp. are found in cultured and wild *C. batrachus*, while *Senga* spp. are found in cultured and wild snakeheads, *Channa* spp. (Furtado, 1963; Furtado and Lau, 1971; Furtado and Tan, 1973).

Cestode infections in fish and resulting mortality are sporadic. Fish infested with intestinal (adult) cestodes have retarded growth, erratic swimming behaviour and distended abdomen, become emaciated, cease to feed, develop a haemorrhagic enteritis caused by the destruction of the intestinal epithelium, and heavily infected fish have varying degrees of aseptic dropsy (Paperna, 1996). The cyclopoid copepod is the intermediate host, and the cestodes could be an important pathogen in cage culture systems since fish are in intimate contact with the environment.

Diseases caused by parasitic arthropods

Lernaea and *Ergasilus* spp. (Copepoda), *Argulus* (Branchiura) and *Alitropus* (Isopoda) have been recorded on a wide range of cultured fish species (Kabata, 1985; ADB/NACA, 1991). *Lernaea* and *Argulus* cause the most problems in warmwater aquaculture in Southeast Asia and the Indian continent. They were introduced into these countries via fish importation (ADB/NACA, 1991) and will be discussed in greater detail below. The isopod

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Alitropus is another common arthropod in aquaculture systems (Fig. 7.7) and is associated with poor fish growth and increased fish mortality (L.H.S. Lim, unpublished data; Lester and Roubal, 1995). It could be a potential pathogen in warm freshwater cage cultures. However, nothing much is known about its impact in aquaculture. Infestation by the copepods *Ergasilus*, besides causing cage-cultured fish to lose weight and appear unsightly, causes gill damage and, in heavy infestations, results in gill dysfunction (Kabata, 1985). *Ergasilus* was recorded to cause fish mortality in Indonesia, especially in young fish (ADB/NACA, 1991). *Ergasilus* is a common crustacean parasite of fish and a potential pathogen in cage culture systems; however, little is known about its ecology or pathology.



Fig. 7.7. *Alitropus* species (Isopoda) found on the skin of *Channa micropeltes* (picture by K.S. Liew).

Lernaea *infections*

Lernaea or anchor worm causes the most damage in warm freshwater fish and is usually associated with high mortality. *Lernaea* spp. seem to prefer warm waters of 26–30°C (Shields and Tidd, 1974). Although it is known that this parasite causes disease in cage-cultured fish in Southeast Asia, the extent of its impact and damage to aquaculture has not been estimated (Kabata, 1985).

Pathology. *Lernaea cyprinacea* is distributed widely with the global translocation of carp and is now recorded in 45 species of cyprinids as well as in other orders of fish, especially the siluriforms (Lester and Roubal, 1995). *Lernaea* is found in India, Nepal, Bangladesh, Thailand, Indonesia, Peninsular Malaysia, Vietnam, China and Japan (ADB/NACA, 1991). Lernaeosis occurs in China on silver carp, bighead carp, grass carp and black carp, in India and Bangladesh on all the major carp, in Vietnam on bighead carp, grass carp, silver carp, common carp, crucian carp and snakehead, and in Indonesia on common carp, *P. gonionotus*, spotted gourami, mudfish and catfish. In 1976, these parasites reached epizootic levels, destroying about 30% of fish in over 7500 ha of ponds, ricefields and open waters in West Java and North Sumatra (ADB/NACA, 1991). In Southeast Asia, *Lernaea polymorpha* is found on bighead carp and silver carp (Shariff and Sommerville, 1986).

Haemorrhaging and gross lesions occur at the site of *Lernaea* infections and are associated with bacterial and other secondary infections. There are relatively few studies on the effects of anchor worm infection on the fish hosts in warm waters. Some authors suggest that the attached females feed on host blood, while others suggest that they probably ingest host cells and absorb tissue fluids (Egusa, 1992). Lester and Roubal (1995) provided detailed information on the other signs associated with *Lernaea* infections, and these included blindness, epidermal and dermal necrosis and haemorrhage and encapsulation of the embedded horns of *Lernaea*. Copepodids of *Lernaea* may cause disruption and necrosis of the gill epithelia, and

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large numbers of larvae on the gills may cause fish mortality. Lesions caused by penetration of metamorphosing females are generally associated with punctuate haemorrhage, and muscle necrosis is evident at the point of penetration of parasites (Khalifa and Post, 1976). Penetrating female *L. polymorpha* cause punctuate haemorrhage in bighead carp resulting in mortality in heavily infected fish (Shariff and Sommerville, 1986). *L. cyprinacea* in the eyes cause blindness.

Diagnosis. There are over 40 species of pathogenic *Lernaea* (Kabata, 1983), but in most outbreaks, the specific identity of the parasite is unknown. *Lernaea* spp. are macroscopic and easily seen with the naked eye on the surface of fish. Only females of *Lernaea* are parasitic and are highly modified so they do not resemble free-living copepods. Adult *Lernaea* females have their anterior end embedded into the body musculature of their host, while their long rod-shaped body with two egg sacs protrudes outside the host tissue. The anterior head region is modified as a small hemispherical cephalothorax, which contains the mouth, with a well-developed holdfast, bifurcate dorsal process and simple ventral process (anchor). The anterior region may even penetrate into the body cavity and embed into visceral organs. *Lernaea* spp. are distinguished by the shape of the anterior anchors, which may be modified by bone or other structures encountered during development in their host tissue. *Ergasilus*, on the other hand, is recognizably a copepod with a second antenna modified for attachment and a pair of multiseriate egg sacs arising from the genital segment.

Prevention and control. Several chemicals are recommended but their efficacy requires further careful testing (Kabata, 1985; Egusa, 1992; Paperna, 1996). Kasahara (1962) effectively used Dipterex (organophosphate trichlorphon) to control and eradicate the larval stages of *L. cyprinacea* in the water column. At temperatures of 20–27°C, concentrations of 0.5 and 0.2 ppm kill the nauplii in 1 and 2 days, respectively. Copepodid stages are killed in 24–36 h at 0.2 ppm and in 12–18 h at 0.5 ppm at 20°C, but Dipterex is not effective on adult females. However, in cages located in rivers or large lake systems, the use of chemicals is ineffective and dipping fish in chemicals seem to be insufficient to get rid of all the copepodid stages (Lester and Roubal, 1995).

Argulus *infections*

The majority of the branchiurans are freshwater parasites (about 75% of the 120 species of *Argulus*), with few estuarine or marine species (Kabata, 1985). *Argulus* or fish louse (Fig. 7.8) is macroscopic and easily observable on the skin and fins and also in the oral cavity. Infected skin becomes opaque with frayed fins. This ectoparasitic crustacean feeds on the mucus layer, flesh and blood of the fish. The prolonged feeding and strong attachment of *Argulus* by its suckers on to the host result in direct mechanical damage to the skin and disruption of epithelial structure, resulting in lesions and subsequent invasions by opportunistic pathogens such as pseudofungi (Singhal *et al.*, 1986; van der Salm *et al.*, 2000). There are at least four species of argulids (Branchiura, Argullidae) that are economically important as parasites of fish in warm freshwater aquaculture, i.e. *Argulus japonica*, *Argulus foliaceus*, *Argulus indicus* and *Argulus siamensis*, and these have been introduced along with their cyprinid hosts and are now reported from both local indigenous cyprinids and



Fig. 7.8. *Argulus* species (Branchiuran) found on the skin of *Channa micropeltes* (picture by K.S. Liew).

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non-cyprinid hosts in the introduced areas (Paperna, 1991). *A. japonica* is in Israel, while *A. foliceaus* is in Thailand, Peninsular Malaysia and Sri Lanka on carp and native cyprinid species (Kabata, 1985). *A. indicus*, an Asiatic species, is on anabantids, chaniids, tilapias and native cyprinids in Indonesia, Thailand and India. *A. siamensis* is reported in Thailand from anabantids and *Cirrhina* spp. (Gopalakrishnan, 1968; Kabata, 1985) and in India on a snakehead species (*Channa gachua*) (Ramakrishna, 1951). *A. japonica* is an important parasite of warm freshwater fish, while *Argulus coregoni* parasitizes cold freshwater fish.

Pathology. This parasite is not host-specific and is found on a wide range of fish species from cyprinids to siluriforms and perciforms (see above). The life cycle of the parasite is direct and the eggs hatch into free-swimming larvae, which must find a host within 2–3 days. It is reported to cause massive mortality of fish in Bangladesh, and in the majority of cases the outbreaks were seasonal, usually in the colder months (Kabata, 1985). *Argulus* usually infects the young fish from spring until early summer. The parasite is also common in India, affecting the major Indian carp, especially *Rohu* spp. In Peninsular Malaysia, argulids have been found on wild fish such as *C. micropeltes* (L.H.S. Lim, unpublished data), the imported fry of bighead carp and grass carp (Shaharom, 1988). *Argulus* spp. are found on the sand goby and snakeheads in cages in Vietnam (T.T. Dung, personal communication). Heavily infected fish are lethargic, listless, cease to feed and rub themselves on the substrate in an attempt to dislodge the parasite.

The lesion or wound made by the feeding *Argulus* may be restricted to the epidermis or may penetrate through to the stratum spongiosum of the dermis and even the stratum compactum turning the dermis oedematous (Lester and Roubal, 1995). The area may become necrotic with secondary bacterial and fungal infections. Mortality may be associated with changes in the ionic and osmotic homeostasis, anorexia and secondary infections. Kabata (1970), Paperna and Zwener (1976) and Paperna (1980) noted that lytic and toxic substances were secreted into the dermal area, while feeding caused acute haemorrhagic, inflamed wounds. *Argulus* feeding on blood causes fish to become anaemic and its piercing proboscis stylet causes haemorrhagic spots on the epidermis. The spots are formed by epidermal hyperplasia. Bacterial infections occur around the site of infection. *Argulus* may also be a vector of viral infections. Ahne (1985) showed that spring viraemia of carp (SVC) was transmitted by *A. foliaceus*, and in Israel, carp pox (carp papilloma) occurred in conjunction with *A. japonica* infestation (Sarig, 1971).

Diagnosis. The parasite is oval to round, dorso-ventrally flattened (about 4–8 mm in diameter), with a pair of modified sucker-like first maxillae. Its proboscis or feeding organ is for inserting into the epidermis and the underlying tissue of the fish hosts to feed on blood (Fig. 7.8).

Prevention and control. Several chemicals, especially organophosphate insecticides, formalin, chlorine, sodium chloride and even antimalarial drugs, are recommended (Kabata, 1985; Egusa, 1992; Lester and Roubal, 1995; Paperna, 1996), but their efficacy in different types of water bodies is not known. Studies carried out in warm waters of Israel and Africa show that some (see below) of the insecticides are effective in killing argulids within the safety margin for fish (Paperna, 1996). Lindane has been used to clean fish of argulids prior to marketing (Paperna, 1996). The chemicals in use are gemmexane (this is toxic to fish and man), Pyrethrum (not tested on a large scale yet) (Paperna, 1996), Dipterex, trichlorphon, Neguvon, malathion, formalin and antimalarial drugs such as quinine hydrochloride (13 ppm) (Puffer and Beal, 1981; Kabata, 1985; Singhal *et al*., 1986). However, not all the chemicals are equally effective for the different developmental stages of argulids. For example, Dipterex (0.2–0.3 ppm) is effective against the adults

and larvae causing them to fall off the fish and die but has no effect on the eggs (Egusa, 1992). The water chemistry and temperature are important factors in the use of these chemicals. The occurrence of these parasites, despite the amount of chemicals used, indicates that the eggs are still in the system and that the chemicals used are not effective in destroying the eggs. The strategy of not stocking the ponds until the larval stages have died could be effective. Other methods of control include the use of substrates such as wooden slats to trap eggs, filtering incoming water to remove larval stages, stocking clean fish, quarantining incoming fish with treatment if necessary before stocking, and stocking with argulid-predatory fish (Kabata, 1985).

Conclusions and Recommendations for Future Research

Microorganisms and parasites are normal flora and fauna inhabiting the skin, fins, gills and the gastrointestinal tract of fish. Under normal conditions, many of these organisms do not induce disease in their fish host. However, man-made pollutants and/or intensification of fish culture result in increased environmental changes, which may be stressful to fish. Bacterial multiplication, for instance, is enhanced with increasing organic matter from uneaten feeds. The stress predisposes fish to invasion by opportunistic pathogens, with subsequent morbidity and mortality. Stress is also associated with handling, stocking, grading and shipping of fish.

Often fish mortality can be attributed to several factors (e.g. fish condition, pathogens and environment) and it is difficult to determine the significance of any one of these factors (Mitchell, 1997). Parasites such as the monogeneans may not have a direct effect on fish mortality but they debilitate the fish, making it more susceptible to other pathogens. Their organs of attachment usually create portals of entry for viral, bacterial and pseudofungal pathogens of fish. In addition, some parasites are reservoirs of viral pathogens.

Despite the long history of aquaculture in the tropics and the importance of disease in aquaculture, there have been few concerted efforts to document and investigate the diseases of fish cultured in cages and ponds. This may be due to lack of trained manpower and institutional support. The diversity of fish cultured in warm waters does not help to alleviate this problem. The usual approach to disease and health management is to use chemicals (usually indiscriminately) or, if this does not work, to discard the fish species and start afresh with another species. Also, there is no mandatory requirement in many of the developing and underdeveloped countries to report fish death, and until recently fish were usually imported and stocked without quarantine. It must be taken into consideration that the movement of fish, especially across international boundaries, may transfer fish pathogens as well (Hedrick, 1996). In this regard, the provisions of the Office International des Epizooties (OIE) (1995) International Aquatic Animal Health Code should be adhered to.

The lack of institutional support results in reduced research on pathogens and consequently an inability to control and prevent diseases. There is also a lack of trained personnel in disease management and little reliable information on the specific identity of pathogens. A related issue is the lack of legislation and guidelines pertaining to the use of drugs and chemicals in aquaculture. Currently, drugs and chemicals are used indiscriminately (usually the aetiological agents are not identified), and without a specific withdrawal period prior to the sale of the fish. Trained competent fish disease managers, who are able to diagnose pathogens and are capable of dispensing proper prevention and control measures, are important to sustain aquaculture.

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8 Non-infectious Disorders of Warmwater Fish

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Introduction

Compared with infectious diseases and disorders, few non-infectious diseases and disorders in cultured fish have severe biological or economic impact. Culture practices, however, often establish environments that promote infectious disease by weakening the immune response or by promoting conditions that favour infectious agents (Barton, 1997). Generally, non-infectious diseases and disorders result from adverse physical, chemical and biological factors and from poor nutrition (Southgate, 1993). Adverse factors can be changes in temperature, pH and dissolved gases, suspended solids, endogenous toxins, anthropogenic toxicants, solar radiation and physical damage such as from predation. Neoplastic diseases can be initiated by infectious agents (viruses), exposure to carcinogenic chemicals or by hereditary genetic disturbances. In this chapter, nutritional disorders are discussed from the standpoint of dietary imbalances in protein, lipid, carbohydrates, minerals and vitamins. Also included are starvation, imbalances in specific dietary constituents and dietary toxicity.

These categories are broad, with a great deal of knowledge available for some fish species and certain diseases and disorders, and little or none for others. Most of the available information for warmwater cultured species is anecdotal except that for the channel catfish (*Ictalurus punctatus*). In this chapter, we attempt to assemble the available information on non-infectious diseases and disorders in warmwater fish with an emphasis on cultured species. As aquaculture expands, more warmwater marine and freshwater species will be developed for culture. Therefore, much of this information is prospective rather than retrospective with information drawn particularly from warmwater species, but also from the more extensively studied coldwater cultured species such as the salmon and trout. We make no attempt to define the line between disease and disorder, the distinction of which is often blurred. For recent reviews of and on non-infectious disorders in fish in general, readers are referred to Ferguson (1989), Leatherland and Woo (1999), Noga (1996), Roberts (1989) and Stoskopf (1993). Reviews dealing specifically with channel catfish are also available (Johnson, 1993; Schwedler *et al.*, 1985).

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Environmental factors

Chemicals

Concern centres not only on the toxic effects of excessive exposure, which are typically dramatic, killing all adequately exposed individuals quickly, but also on the potential for bioaccumulating toxicants and contaminating the fish product. Long-term, low-level exposures can predispose the fish to infectious disease, respiratory distress, retarded growth, loss of reproductive ability and deformities. Toxic agents can be classified in several ways including mode of action, target organ, and chemical class and use (see Rand *et al.*, 1995). Based on chemical characteristics, aquatic toxicants include metals, inorganic compounds, organic compounds, pesticides and radionuclides. With regard to the metals, copper, zinc, cadmium, lead and mercury are of particular concern in aquatic systems followed by aluminium, chromium, selenium, silver, arsenic and antimony. Metal contamination can arise from mining and industrial processes, ageing water pipes and tanks. Metal toxicity varies with factors including water quality (pH, hardness and temperature), species and form of the metal and bioavailability. Inorganic compounds of concern include chloride, chlorine, nitrogen, phosphorus, ammonia, arsenic, nitrites, nitrates and sulphides. Chlorine is used to decontaminate municipal waters and can be highly toxic in closed systems such as those used for ornamental fish (Butcher, 1993). Hydrogen sulphide and methane result from the breakdown of organic material in water. As with the metals, the toxicity of inorganic compounds varies with fish species, water quality and bioavailability.

Anthropogenic toxicants. Anthropogenic organic compounds, as products or by-products of industrial processes, represent a new and ever-changing class of aquatic toxicants (Rand *et al.*, 1995). Of particular concern are the polychlorinated biphenyls, dioxin, furans, polycyclic aromatic hydrocarbons (PAHs) and organic solvents. These compounds have different mechanisms of toxic action, environmental persistence and bioavailability. Petroleum by-products, especially PAHs, represent real hazards not only for their direct toxicity but also for their potential carcinogenic properties (see Eisler, 1987).

Exposure to anthropogenic contaminants in aquaculture systems is rather uncommon, but the effects can be devastating. Many pesticides, including herbicides and molluscides, are directly toxic to fish. Routes of contamination of aquatic systems include wastes from manufacturing operations, agricultural runoff and runoff from urban sources. Depending on the chemical, toxic effects from these sources can be acute or chronic, and many of the compounds such as organochlorines have cumulative effects (Branson, 1993). Some organophosphorus pesticides are known to cause skeletal abnormalities in several species of fish. Couch *et al.* (1979) reported vertebral abnormalities in the sheepshead minnow (*Cyprinodon variegatus*) exposed to trifluralin, and similar abnormalities were reported from brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) exposed to trifluralin (Wells and Cowan, 1982). Middaugh *et al.* (1990) also reported vertebral abnormalities in juvenile inland silversides (*Menidia beryllina*) exposed to terbufos during embryogenesis. In all cases, the lesions consisted of compressed and fused vertebrae, hypertrophied bone, and proliferating fibroblasts, osteoblasts and osteocytes (Fig. 8.1).

Ammonia. Ammonia poisoning is one of the most common water quality problems occurring in aquaculture. Ammonia is the primary nitrogenous waste product of fish, but it is also formed by the decay of organic matter. Additional sources include inflowing water, especially in areas where agricultural runoff occurs, overstocking or overfeeding. Ammonia can cause mortality quickly, but often it causes a sublethal stress. Most fish health experts believe that the toxic form, unionized ammonia, primarily causes direct damage to the gill epithelium

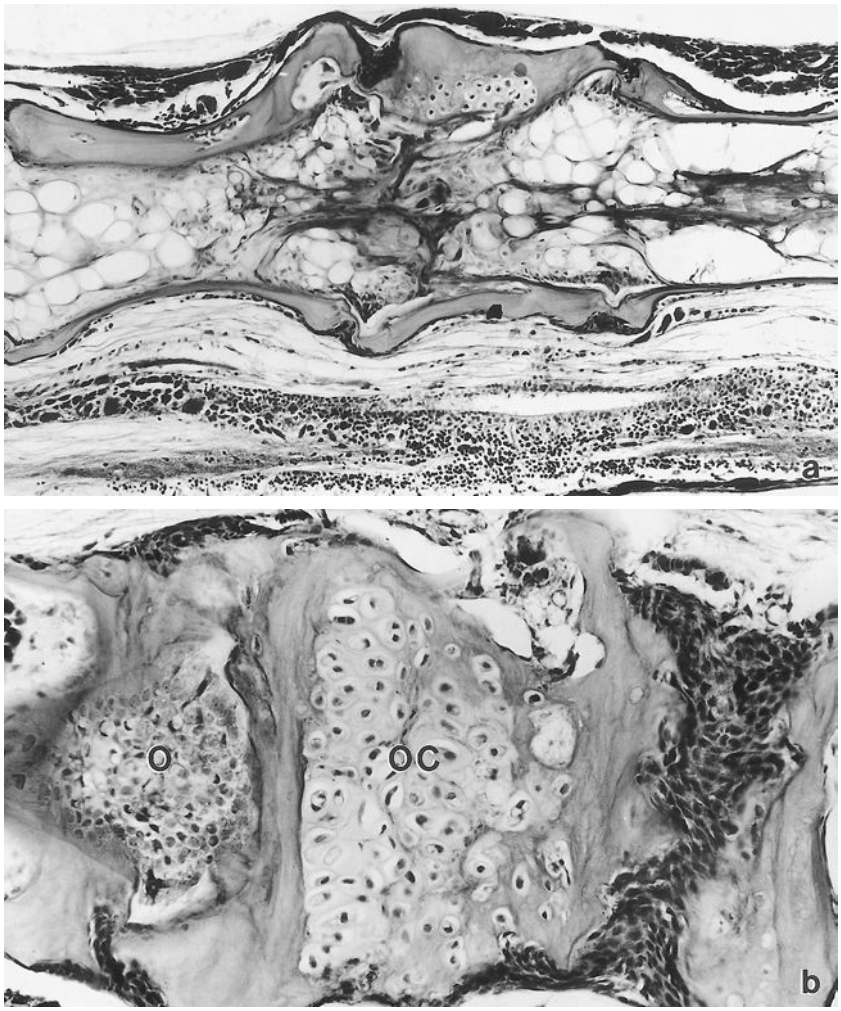


Fig. 8.1. Sections through inland silversides (*Menidia beryllina*) exposed to terbufos. (a) Vertebral compression and hypertrophy of vertebral walls. (b) Advanced lesion with fused vertebrae. Note proliferation of fibroblasts and osteoblasts in the zygapophyseal region (O) and osteocytes (OC) in the bony matrix.

with hyperplasia and hypertrophy (Branson, 1993) and reduced respiratory capability. However, Daoust and Ferguson (1984) reported that no lesions attributable to ammonia were seen in the gills of rainbow trout (*Oncorhynchus mykiss*) exposed to high concentrations of ammonia. This study raised questions about the precise role of unionized ammonia in the formation of proliferative gill lesions. Daoust and Ferguson (1984) indicated that the oxygen content of the water in their systems was optimum, whereas in crowded aquaculture situations, high levels of metabolic ammonia are usually accompanied by low oxygen levels. Under conditions of low oxygen, the susceptibility of fish to the toxicity of various pollutants increases.

Oxygen–nitrogen (gas-bubble disease). Gas-bubble disease is one of the most important disorders of cultured fish and is associated with the supersaturation of the water with nitrogen or oxygen. Usually, gas emboli are produced by excess nitrogen (Marking, 1987) because oxygen is assimilated

metabolically and is less likely to form persistent bubbles. With red snapper (*Lutjanus campechanus*) maintained in the laboratory, overaeration can result in gas-bubble disease (Vernon Minton, Alabama Department of Natural Resources, 1999, personal communication). In hatcheries, gas-bubble disease may be caused by leaks in pumps or valves or by sudden temperature gradients. Clinical features of gas-bubble disease are variable. In fish larvae, bubbles are most frequently encountered in the dermis and yolk sac (Roberts, 1989). In older specimens, bubbles are usually seen in the eyes, skin, gills and mouth. Internal gaseous accumulations can also occur in the swimbladder and visceral peritoneum and exophthalmos can result from retrobulbar gas emboli. The primary histological feature observed in chinook salmon (*Oncorhynchus tshawytscha*) with gas-bubble disease was oedema of the secondary gill lamellae with concomitant degeneration of the overlying respiratory epithelium (Pauley and Nakatani, 1967). Variables such as the size and species of fish affected, the extent and duration of the supersaturation, and the water temperature all play a role in the prognosis.

Physical parameters

pH. The pH of water is a measurement of the concentration of hydrogen ions present. It is an important water chemistry variable, which has a significant effect on fish health. The optimum pH range for most warmwater species is between 6.5 and 9.0, and direct toxic effects occur outside this range (Tomasso, 1993). Sublethal effects in alkaline waters include gill damage, damage to the skin and scales and changes to the cornea and lens. Most species will die when exposed to a pH of 10 or greater (Branson, 1993). Acute acid poisoning is characterized by tremors and hyperactivity (Schwedler *et al.*, 1985). However, chronic acid stress is more common. Direct damage to skin and gills occurs in acidic waters and results in problems with oxygen uptake and osmoregulation. Low pH stimulates increased mucus production, which in turn interferes with gas and ion exchange (Noga, 1996). Poor growth, reproductive failure and increased accumulation of heavy metals are associated with chronic low pH stress (Haines, 1981). Most species will die when the pH is below 4. The pH of the water also has an effect on the toxicity of many compounds including ammonia, cyanide, sulphides and some biocides (Sprague, 1985). Aluminium ion, for example, is solubilized and more toxic in acid pH and as a result, aluminium toxicity can occur concurrently with low pH (Noga, 1996).

Solar radiation (sunburn). In natural waters, fish avoid exposure to potentially damaging solar radiation by moving to deeper water or to shaded areas. In some aquaculture conditions in which fish are held in relatively shallow waters unshaded and at high densities, they may be exposed to excessive amounts of solar radiation. The UV-B portion of the solar spectrum is considered to cause sunburn (Roberts, 1989). In water with high particulate levels, UV-B does not penetrate more than a few millimetres. However, in clear water, UV-B can penetrate about 1 m. Sunburn has been experimentally induced in fish at depths of more than 0.5 m (Bullock and Roberts, 1979).

The integument of fish is more susceptible to damage by UV-B than the skin of mammals because of the lack of a keratinized layer and the presence of dividing cells in all layers (Roberts, 1989). Additionally, fish epidermis normally does not possess protective melanin-containing cells. Lesions typically occur on the most exposed areas, including the head, dorsal fin, pectoral fins, tail and dorsum. Histologically, early lesions are characterized by the presence of swollen Malpighian cells containing dark, shrunken nuclei surrounded by a large clear halo (Bullock and Coutts, 1985; Roberts, 1989). In some specimens, Malpighian cells exhibit karyorhexis, the lower epidermis becomes oedematous, and eventually the skin surface ulcerates. These ulcerated areas can serve as portals of entry for secondary or opportunistic pathogens.

Biotic factors

Algal blooms. Algal blooms have caused fish kills in coastal waters throughout the world in both wild and captive marine fish (Möller and Anders, 1986; White, 1988; Anderson, 1995) and many species of noxious phytoplankton are becoming an increasingly serious threat to fish culture, especially in near-coastal marine systems (Noga, 1996). These blooms can kill fish through physical damage to the gills by the spicules of diatoms, asphyxiation caused by oxygen depletion, gas-bubble trauma due to oxygen supersaturation from algal photosynthesis and direct chemical toxicity from toxins produced by the algae. At the present time, effects are more commonly seen in cultured coldwater than in warmwater fish. This is likely to change as culture technology is applied to more species of warmwater fish. Losses in salmon and yellowtail cultured in seawater have been caused by toxin-producing algae such as *Heterosigma akashiwo* (see Okaichi, 1983; Black *et al.*, 1991), *Chatonella antiqua* (see Gaines and Taylor, 1986) and a number of other species (Kent, 1992). In the Pacific Northwest, blooms of the dinoflagellate, *H. akashiwo*, and the diatoms *Chaetoceros convolutus*, *Chaetoceros concavicornis* and *Corethron* sp. have been associated with mortality in pen-reared fish (Gaines and Taylor, 1986; Speare *et al.*, 1989; Black *et al.*, 1991). In British Columbia from 1986 to 1989, approximately US$11 million of pen-reared salmon were lost to algal blooms (Kent, 1992).

In warmwater fish, important toxic algal species are the cyanobacteria, the blue-green algae. Some of these organisms (e.g. *Microcystis*, *Nodularia*) produce potent hepatotoxins and others produce neurotoxins (Carmichael, 1994). In cultured channel catfish, little is known about the occurrence or consequences of cyanobacterial toxin. Schwedler *et al.* (1985) reported only one fish kill case submitted to the Mississippi Cooperative Extension Service Fish Disease Diagnostic Laboratory in Stoneville, Mississippi, from 1980 to 1984 that was related to algal toxins. Distressed fingerling channel catfish around the pond margin were found to have ingested large quantities of *Microcystis aeruginosa*. Affected fish exhibited tremors and convulsions but only a few died. *Microcystis aeruginosa*, and other toxic species of blue-green algae occur commonly in catfish ponds and are often present in the gut of catfish (Schwedler *et al.*, 1985). Recently, Zimba *et al.* (2001) demonstrated that microcystin exposure was the probable cause of significant numbers of channel catfish mortalities in farm ponds in Mississippi. The role of those organisms, as well as prevalent toxic dinoflagellates such as *Gymnodinium breve*, the red tide organism, in disease and mortality events in warmwater fish requires further investigation.

Gross abnormalities. A range of morphological abnormalities have been reported in fish taken from contaminated waters, including jaw deformations (Pohl, 1990), skeletal deformities such as lordosis, kyphosis and scoliosis (van den Avyle *et al.*, 1989; Pohl, 1990), skull deformities (Lindesjöö and Thulin, 1992), fin erosion (Reash and Berra, 1989) and ocular disorders (Tyler and Everett, 1993). Fournie *et al.* (1996) reported an increased prevalence of gross abnormalities in fish collected from estuarine sites contaminated with municipal and industrial wastes in the mid-Atlantic and Gulf of Mexico. Skin lesions, including epidermal papillomas in the brown bullhead (*Ameiurus nebulosus*) (Fig. 8.2), were the most prevalent abnormalities seen from a total of 24,291 specimens examined. Other abnormalities included ocular, skeletal and branchial chamber abnormalities. The prevalence of gross abnormalities was three times higher for demersal (bottom-dwelling) fish than for pelagic (mid-water) fish and was about eight times higher at sites with high sediment contaminant concentrations. Morphological abnormalities in fish have been used as indicators of environmental contamination and include spinal deformities (van den Avyle *et al.*, 1989; Pohl, 1990), deformities of the jaw and skull (Pohl, 1990; Lindesjöö and Thulin, 1992) and external

lesions or tumours (Tyler and Everett, 1993). In a study of mixed species of tilapia from three sampling sites in two rivers in southern Taiwan, the fish showed a range of morphological abnormalities (Sun *et al.*, 1999). Commonly encountered morphological abnormalities in tilapia were scale disorientation, fin deformities and vertebral abnormalities (Fig. 8.3). These changes have been related to environmental contamination, including heated effluents, fish age and crowding (Sun *et al.*, 1999). Tyler and Everett (1993) found fin deformities in 19% of barbel (*Barbus barbus*) from a stream with poor water quality in England, but postulated that the fin deformities were age-related. Another morphological abnormality observed in the Taiwan river tilapia was deformity of the operculum and gill. Similar opercular deformities have been reported to occur naturally in cultured *Oreochromis mossambicus* (Handwerker and Tave, 1994), suggesting that some of the opercular deformities observed were unrelated to environmental contamination. Deformities of the skeleton appear to be the morphological deformity that is most strongly related to environmental contamination (Pohl, 1990; Lindesjöö and Thulin, 1992). Nevertheless, skeletal deformities were rare in Taiwan river tilapia. Pohl (1990) found the highest percentage of vertebral deformities in European smelt (*Osmerus eperlanus*) in the mesohaline zone of the



Fig. 8.2. Brown bullhead (*Ameiurus nebulosus*) from Black River, Maryland, showing epidermal papilloma on the lower lip.

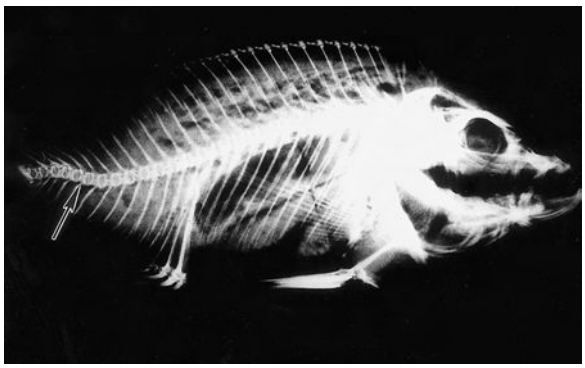


Fig. 8.3. Vertebral deformity (arrow) in the posterior portion of a tilapia (*Oreochromis mossambicus*) from a river in southern Taiwan.

Elbe estuary, although jaw deformities were more common upstream in less saline waters. He hypothesized that the combination of hydrological factors such as salinity and toxic organic and inorganic chemicals contributed to the high percentages of deformed smelt.

Neoplasia. The development of neoplasia (tumours) is generally age-related, and such lesions are rarely seen in cultured fish that are marketed at a relatively young age. Much of the research on neoplasia in fish began with the findings of aflatoxin-induced liver neoplasms in cultured rainbow trout in the 1960s (see Bailey *et al.*, 1987). Neoplasia in cultured warmwater fish appears to be rare, although there is a single report on neoplasms in sunshine bass from a commercial aquaculture pond in Mississippi (Thiyagarajah *et al.*, 2001; A. Thiyagarajah, 1999, personal communication). The neoplasms reported from this hybrid included a disseminated lymphosarcoma, branchii blastoma and hepatocellular carcinoma. All warmwater fish, however, are not necessarily refractory to the induction of neoplasia, especially by chemical carcinogens. Mummichog (*Fundulus heteroclitus*) collected from a known creosote-contaminated site in Virginia were found to have a high prevalence of hepatic neoplasia and, in advanced cases, mummichog livers had multiple large tumour masses that were grossly visible (Fig. 8.4) (Vogelbein *et al.*, 1990). Furthermore, warmwater aquarium fish such as the Japanese medaka (*Oryzias latipes*) and platyfish × swordtail (*Xiphophorus* spp.) hybrids are used in carcinogenesis studies (see Hawkins *et al.*, 1995) because, in part, they are especially prone to developing neoplastic lesions following exposure to carcinogens. With other warmwater fish, accounts of neoplasia are scattered. In ornamental fish, papillomas that are seen as external, raised lesions are often caused by viruses. For example, a herpesvirus causes carp pox (Butcher, 1993). Neurofibromas and fibromas are common in goldfish (*Carassius auratus*) and melanomas in some platyfish × swordtail hybrids. The aetiology of epidermal papillomas in brown bullhead has not been determined (Fournie *et al.*, 1996). However, sediments at the location where the papillomas occurred had high levels of heavy metals and other contaminants. Similarly, the cause of high prevalence of pigmented subcutaneous spindle cell tumours in gizzard shad (*Dorosoma cepedianum*) from lakes in Oklahoma has not been determined (Ostrander *et al.*, 1995).

There is a wide species-related susceptibility to neoplasia. This has been shown in laboratory studies and is obvious in a cursory examination of reports on neoplasia in fish. Channel catfish appear notably resistant to the development of neoplastic lesions both in culture operations and in the laboratory (Beleau, 1993). Considering the high commercial production of catfish, relatively few tumours or any other neoplastic lesions have been reported. The

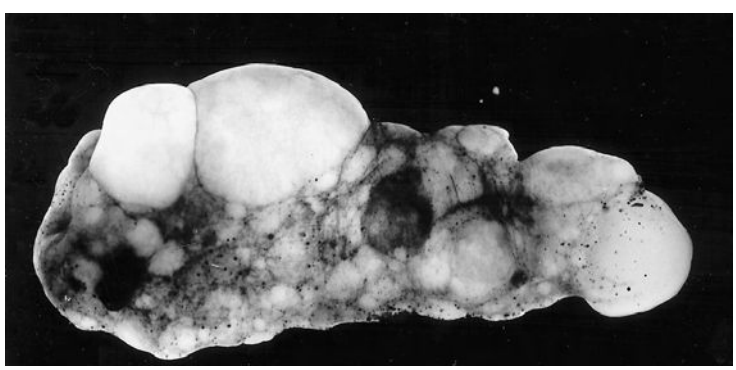


Fig. 8.4. Liver from mummichog (*Fundulus heteroclitus*) collected at a creosote-contaminated site in the Elizabeth River, Virginia, showing normal liver (upper left) and liver with multiple tumour nodules.

related brown bullhead, however, appears susceptible to environmentally induced neoplasia (Baumann *et al.*, 1990; Baumann and Okihiro, 2000).

As in other animals, some neoplastic diseases that have been reported in fish are caused by viruses such as the carp pox cited above. Virus-induced neoplasia is particularly prevalent in cultured salmonids. A plasmacytoid leukaemia of chinook salmon has caused significant mortality at numerous netpen farms in British Columbia (Kent, 1992). Plasmacytoid leukaemia is characterized by proliferation and infiltration of immature plasma cells in the visceral organs and retrobulbar tissue. Mortality rates associated with this condition have been varied, with one farm losing about 50% of its production stock in the fish's second year in seawater (Kent, 1992). Preliminary transmission studies in the laboratory indicated that this neoplastic disease was caused by an infectious agent (Kent and Dawe, 1990; Newbound and Kent, 1991). Eaton and Kent (1992) subsequently demonstrated that an oncogenic virus was the cause of the disease. Although virus-induced neoplasia has not been frequently reported from warmwater cultured fish, a virus has been implicated in schwanommas (neurofibrosarcomas) in bicolour damselfish (*Pomacentrus partitus*) from the Florida Keys (Schmale and Hensley, 1988).

Systemic non-infectious granulomatosis. Granulomatous lesions in visceral organs are commonly encountered in cultured fish, especially those subjected to non-specific or specific stress. Mycobacteria are the most common cause of granulomas, particularly those that occur in the spleen (Ferguson, 1989). Recently, granulomatous lesions occurring in the absence of an infectious agent such as mycobacteria were recognized (Herman, 1996). The lesions in brook trout (*Salvelinus fontinalis*) were related to the use of cottonseed meal in the diet (Dunbar and Herman, 1971) and in gilthead bream (*Sparus aurata*) to formulated feeds, fish meal, or frozen fish that had been stored for prolonged periods (Paperna, 1987). Similar diseases and conditions have been reported from cultured fish such as turbot (*Scophthalmus maximus*) in France (Messager *et al.*, 1986), goldfish (see Hernandez and Fernandez, 1986) and several species of African cichlids (Noga, 1986). Recently, granulomatous disease was found in cultured red snapper fed initially on copepods then exclusively on raw squid and fish (J. Ogle and R. Overstreet, 1999, personal communications). No infectious agent was seen in the lesions, which occurred mainly in the liver and kidney, and attempts to transmit the disease to cultured sheepshead minnow failed.

Systemic non-infectious granulomatous disease has rarely been reported in wild fish. However, tilapia (*Oreochromis* spp.) from rivers in southern Taiwan were widely affected by granulomatous lesions mainly in the spleen, liver, kidney and in and around the heart (Sun *et al.*, 1999). Histologically, granulomas in the river tilapia were typically organized with layered, compressed membranous structures surrounding an amorphous mass (Fig. 8.5). In infectious granulomas, the enclosed amorphous material often stained positive with Ziehl–Neelson staining, indicating the presence of mycobacteria. In cultured tilapia from Taiwan, systemic granulomatous lesions were associated with organisms resembling rickettsia (Chen *et al.*, 1994; Chern and Chao, 1994). Rickettsia-like organisms (RLOs) were also found in granulomatous lesions from cultured tilapia in Hawaii (J.A. Brock, 1997,



Fig. 8.5. Two granulomas (G) associated with macrophage aggregates in the spleen of a tilapia (*Oreochromis* spp.) from a river in southern Taiwan.

personal communication). Those lesions appeared similar to those from the Taiwan river tilapia. Extensive analysis of the Taiwan river tilapia splenic granulomas by Ziehl–Neelson staining and by electron microscopy produced no evidence of parasitic, bacterial or viral infections or any other foreign bodies as the causative agent. Further indications that the granulomatous lesions were not caused by an RLO were the lack of splenomegaly and macroscopically visible nodules reported in confirmed cases of RLO infection (Rodger and Drinan, 1993; Chen *et al.*, 1994; Chern and Chao, 1994). Also, examination of granulomatous lesions in spleen and liver tissue of an RLO-infected cultured tilapia from Taiwan showed striking differences in morphology and pigmentation when compared with the lesions from Taiwan river tilapia (P.L. Sun, unpublished observations). Granulomas in the tilapia from southern Taiwan rivers could be related to the abundance of macrophage aggregates that occurred particularly in the spleen, as the percentages of macrophage aggregates and granuloma appeared to follow the same pattern of occurrence among seasons and stations. Fournie *et al.* (2001) demonstrated that splenic macrophage aggregates that are closely related to granuloma are effective biotic indicators for discriminating fish exposed to degraded habitats.

Nutritional disorders of warmwater fish

A proper diet is important for fish, and poor nutrition can cause or contribute to many disorders and diseases. Depending on nutritional requirements, feeding habits and food preferences, fish can be classified as herbivorous, carnivorous, omnivorous, scavenging, plankton eating and parasitic (Wuthipunchai, 1993). Nutrition in fish has been widely studied and the subject of several reviews on the role of nutrition in growth, health and disease resistance (Chance *et al.*, 1964; Takeuchi and Watanabe, 1977; Mazik *et al.*, 1987). Here, we focus on the relationship of nutrition to diseases in warmwater fish, especially those being cultured or under consideration for cage culture. Although nutrition can have a profound effect on the development of disease, its role is often difficult to discern because the specific nutritional requirements involve species-specific factors, with host genetic variation, nutritional bioavailability and adaptation to food deprivation all playing a role.

The effects of nutritional deficiency and imbalances of protein, fatty acids, carbohydrates, vitamins and minerals have been studied in numerous fish species (Schaefer *et al.*, 1995; Davis and Gatlin, 1996; Rajan *et al.*, 1996). Deficiencies or excesses of particular nutrients can have profound effects on the immune system by influencing the integrity of the epithelial tissues and the composition of body tissues and fluid. Compromise of immune responses and reduced mucus secretion can predispose the fish to infections (Lall and Olivier, 1993).

Protein imbalance

Optimum growth and reproductive performance of fish depend largely on protein requirements, which vary for each species. In aquaculture, overfeeding protein promotes the excretion of nitrogenous compounds into the fish's environment, which can then lead to stress, which makes the fish vulnerable to diseases (Viola and Lahav, 1993). In carp (*Cyprinus carpio*), a minimum protein level of 30–35% has been recommended for normal growth (Takeuchi *et al.*, 1979; Watanabe *et al.*, 1987), but to achieve maximum performance in growth as well as disease resistance, 40% protein is required (Kaneko, 1968; Kiron *et al.*, 1993; Rajan *et al.*, 1996). The level of dietary protein affects not only weight gain but also feed conversion and protein conversion efficiency (Hassan *et al.*, 1995). Proper protein content in the diet ensures the formation and activity of essential enzymes and hormones involved in the metabolism and growth of the fish (Buttle *et al.*, 1995).

Protein deficiency in fish diets results in growth retardation (Fig. 8.6) and can affect oocyte growth and maturation. Tilapia (*Oreochromis niloticus*) fed a 10% protein diet failed to reach maturation (Gunasekera *et al.*, 1995). The lack of a single essential amino acid can result in a deficiency syndrome: fish may exhibit signs of food rejection, become anorexic, and growth retardation of up to 25–34% can occur in less than 10 weeks (Halver and Coates, 1957). In juvenile sunshine bass (*Morone chrysops* × *Morone saxatilis*), the highest weight gain and best feed and protein utilization values were observed among fish fed a diet containing DL-methionine and *N*-acetyl-DL-methionine as compared with L-methionine, as the former regimen satisfied their amino acid requirements (Keembiyehetty and Gatlin, 1995).

Amino acid requirements in fish are quite similar to those of other vertebrates (Dupree and Halver, 1970). Crystalline amino acid in the feed is assimilated at different rates by different fish species. If the levels of amino acid intake are not optimum, imbalance in tissue amino acids may result. Growth enhancement is difficult to predict as the addition of non-essential amino acids to essential amino acids improves both growth and feed conversion efficiency (Plakas and Katayama, 1981). A mixture of glutamate, glycine and glutamic acid gave the best results in growth performance in rainbow trout (Schuhmacher *et al.*, 1995). In larvae of red seabream (*Pagrus major*), replacing casein (5 g casein per 100 g diet) with a low level of crystalline amino acids enhanced growth and survival (Lopez-Alvarado and Kanazawa, 1995). However, replacing over 15 g casein produced a detrimental effect on larval performance.

The protein requirement of fish is influenced by both the quality of the protein and the energy content of the feed, as well as by the size of the fish and the culture conditions. Salinity may affect the growth rate and the protein conversion efficiency. In seabream, culture at 15 ppt salinity enhanced growth and reduced oxygen consumption, ammonia excretion rates, liver lipid levels and hepatic glucose-6-phosphate activities (Woo and Kelly, 1995). However, hepatic hexokinase activity was stimulated, probably due to the high protein level. The reorganization of the metabolism could allow protein sparing and favour a shift towards the preferential utilization of carbohydrate and lipid (Viola and Lahav, 1993).

A deficiency of protein in the feed and unfavourable amino acid proportions can

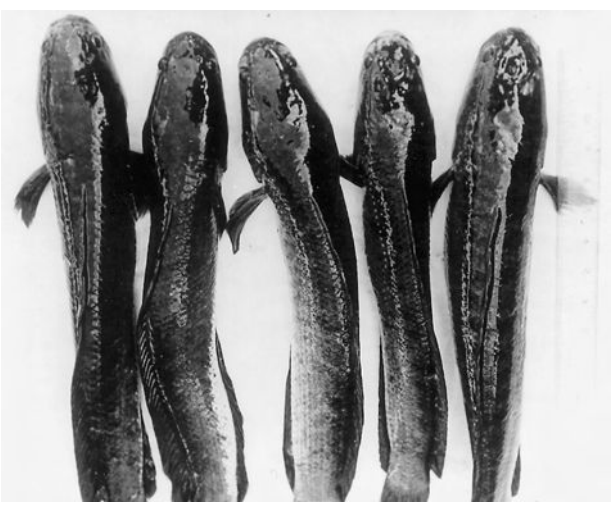


Fig. 8.6. Blade-like bodies of snakehead fish (*Chana striatus*) fed a low protein diet. Compare with a normal fish on the right. (Courtesy of Dr J. Tangtrongpiros.)

cause liver damage and lipoid degeneration in visceral organs (Amlacher, 1960). In carp, low protein and high carbohydrate concentrations can dramatically increase the fat content in the body cavity and the musculature. Lipoid degeneration can be considerable in such a situation, where the protein content decreases with increasing levels of body fat. Feeding fish with maize may also cause liver lipoid degeneration due to the amino acid imbalance of proteins in maize. Since lysine and tryptophan levels are extremely low in maize, biosynthesis of nicotinamide is unsuccessful, resulting in tryptophan and nicotinamide deficiency. Signs of protein deficiency can be recognized in the skin, which can develop petechial haemorrhage. In carp, the intestine can empty and become inflamed and thin, and kidneys become necrotic and greyish in colour (Albrecht, 1967).

The source of the protein can be important for certain situations. For example, adding the protein fraction of squid meal into fish feeds can reduce deficiency problems in marine fish egg quality because the amino acid composition in fish eggs is similar to that of squid. A balanced composition of essential amino acids is expressed in vitellogenin synthesis and endocytosis (Tandler *et al.*, 1995).

Lipid imbalance

Lipids are essential for fish growth and body composition (Anwar and Jafri, 1995; El-Zipdeh *et al.*, 1995; Jafri *et al.*, 1995). In walking catfish (*Clarias batrachus*), weight gain, growth rate, feed conversion, protein efficiency and muscle protein deposition were improved with a diet of 9%, rather than 7%, lipid (Anwar and Jafri, 1995). Increased dietary lipid also resulted in higher accumulation of fat in the liver and viscera of the sand goby (*Oxyeleotris marmoratus*) (Fig. 8.7).

Diseases caused by lipid imbalance occur when feed has spoiled or been stored under low temperatures for long periods. The effects of poor lipid nutrition are often seen in the liver. Degenerative changes and ceroid deposition of the liver occur due to the destruction of vitamin E from unsaturated fatty acid auto-oxidation. Ceroid formation can be prevented by adding vitamin E as an antioxidant to the diet (Faktorowitsch, 1960; Roald, 1976). In common carp, extensive dorsal atrophy, weight loss and death due to feeding of rancid fat have been reported (Yokote, 1970). Histopathological studies of fish fed rancid fat show myopathy of the skeletal musculature, ceroid and haemosiderin deposition

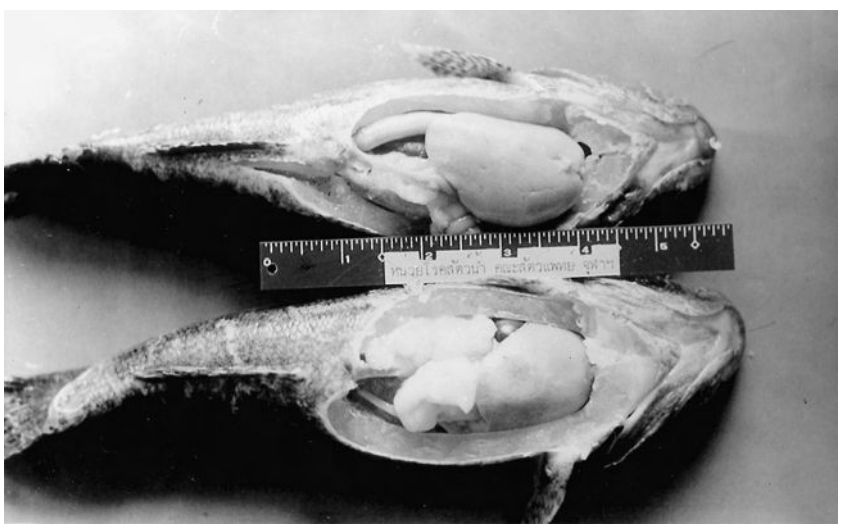


Fig. 8.7. Pale and enlarged liver with fatty infiltration in a sand goby (*Oxyeleotris marmoratus*). (Courtesy of Dr J. Tangtrongpiros.)

and kidney swelling (Miyazaki and Kubota, 1981). The cause of this disease is the formation of peroxides and secondary products of catabolism, which affect enzyme activity. The addition of α-tocopherol to the diet can prevent the condition (Hata and Kaneda, 1980). Degenerative myopathy is seen in channel catfish maintained on feed containing purified fatty acids. Data indicate that an elevated level of vegetable oil used in the feed is responsible for poor growth performance. This growth-suppressing effect may be caused by the linolenic fatty acid contained in vegetable oils (Lewis *et al.*, 1985).

For marine fish, the beneficial effects of highly unsaturated *n*-3 fatty acid (*n*-3 HUFA) have been recognized (Liu *et al.*, 996). In seabream larvae, the requirements of *n*-3 HUFA are age-dependent (Tandler *et al.*, 1995). An optimum dietary level of *n*-3 HUFA during early larval development accelerates growth by 250%. Japanese flounder (*Paralichthys olivaceus*) fed a diet with *n*-3 HUFA supplemented with L-ascorbyl-2-phosphate-Mg (APM) showed improved weight gains and feed conversion efficiencies over those fed an APM-unsupplemented diet (Kosutaruk *et al.*, 1995). The total lipid concentration in the whole body and the liver generally increased, because of the accumulation of triglyceride, as the APM level increased.

Other essential fatty acids involved in the growth and survival of marine fish include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These fatty acids are incorporated preferentially into polar lipids, particularly phosphatidylethanolamine. Choline and phosphatidylethanolamine are involved in the production of healthy biomembranes (Tandler *et al.*, 1995). The importance of unsaturated *n*-6 fatty acids in marine fish has also been suggested, specifically arachidonic acid, which is incorporated in phosphatidylinositols (Robin, 1995). For normal growth, Korean rockfish (*Sebaster schlegeli*) need 1.0% EPA or DHA in the diet. DHA is superior to EPA as an essential fatty acid for fish (Lee *et al.*, 1994). In fish, a deficiency of food lipid results in a low fat deposition, which under stress may lead to an energy-deficiency condition, with multiple physiological disturbances (Spangenberg and Schreckenbach, 1984).

Immunological studies of B-cells in the kidney and spleen of vaccinated fish showed that fish fed diets with a low ratio of (*n*-3)/(*n*-6) polyunsaturated fatty acid (PUFA) may be less resistant to infection than those fed diets with a higher ratio (Thompson *et al.*, 1996). Fish with PUFA deficiency may exhibit gill lesions that are usually diffuse and occur among the arches, sometimes with chloride cell degeneration (Ferguson, 1989). Deficiency of *n*-3 PUFA in the diet can also cause albinism as well as a cessation of larval metamorphosis (Estevez and Kanazawa, 1995)

Carbohydrate imbalance

Carbohydrates are important energy components in fish feeds. The influence of carbohydrate on digestibility, growth, carcass composition and energy retention has been investigated for various kinds of carbohydrate sources (Schwarz and Kirchgessner, 1993). Fish species show different abilities to utilize carbohydrates. Common carp can use up to 40% carbohydrates or up to 40% fats, as long as the protein content of the feed is not less than 32% (Ogino and Takeda, 1976; Takeuchi *et al.*, 1979). Optimum carbohydrate levels enhance growth, feed conversion efficiency, protein utilization and condition. In the air-breathing freshwater fish *Channa punctatus*, a diet containing 15% carbohydrate is recommended (Sakthivel and Baskaran, 1995). Fed at a 25% carbohydrate, *C. punctatus* showed lower levels of dry matter, protein and ash content but higher lipid content. Furthermore, with increasing dietary carbohydrate levels and decreasing protein levels, the activities of hepatopancreatic glucose-phosphate isomerase, glucose-6-phosphate and phosphogluconate dehydrogenases, and malic enzyme, as well as hepatopancreatic glycogen and serum triglyceride contents, increased, while those of glucose-6-phosphatase, arginase, glutamic pyruvic transaminase (GPT) and glutamic oxaloacetatic transaminase (GOT), together with

serum-free amino acid, decreased. These relationships indicate that dietary carbohydrate stimulates glycolysis and lipogenesis but depresses gluconeogenesis and amino acid degradation in the hepatopancreas. This may be part of the carbohydrate–protein sparing process (Shimeno *et al.*, 1995).

In an attempt to reduce the protein level in commercial fish feeds, higher carbohydrate levels have been added for their possible protein sparing effects. Most of the fish fed with a higher level of carbohydrate showed a reduction in feed intake, higher body fat content and a slower growth rate when compared with controls. It is suggested that high intake of carbohydrate may have an inhibitory effect on the appetite or the palatability of feed is reduced because of the progressive toughness of the feed pellets as their starch content increases. To overcome the problem of feed acceptance, flavouring agents and improved pellet texture are recommended (Garcia-Gallego *et al.*, 1991).

Mineral imbalance

Dietary minerals ensure proper metabolism and growth in fish. Most minerals such as calcium, phosphorus, potassium, cobalt, manganese, iron, chloride, iodine and zinc are taken up through both the water and the feed. Usually, calcium is abundant in water and fish can readily absorb it directly (Ichikawa and Oguri, 1961), supplementing that obtained from food (Ichii and Mugiya, 1983). However, excessive calcium in the diet suppresses growth in channel catfish and tilapia (*Oreochromis aureus*) (see Andrews *et al.*, 1973; McClain and Gatlin, 1988; Gatlin and Phillips, 1989; Scarpa, 1989) and reduces the phosphorus available to common carp (Nakamura, 1982). The level and source of dietary calcium affect weight gain and feed conversion efficiency. Reduction in growth and feed efficiency has been observed in fish fed increasing levels of calcium carbonate ($CaCO_3$) possibly due to changes in the pH of the digestive tract, which, in turn, are influenced by the pH of the diet (Gatlin and Philips, 1989; Scarpa, 1989).

Bone mineralization may also be influenced by the calcium source. A calcium carbonate-supplemented diet can reduce bone ash and bone calcium and phosphorus levels. The solubility of calcium seems to be a primary factor influencing calcium absorption and bone mineralization. Stomach pH may also affect the absorption and availability of calcium (Gatlin and Scarpa, 1993). Dietary phytic acid may form insoluble protein–phytate complexes, which can affect the bioavailability of minerals. Plasma levels of calcium, zinc and iron are affected by dietary mineral composition, especially when diets contain a high level of phytic acid, calcium and magnesium. This may result in histopathological changes in intestinal epithelium in carp (Hossain and Jauncey, 1993). Cellular hypertrophy and vacuolization of the intestinal epithelium can result from direct toxicity of phytic acid or an impaired magnesium bioavailability (Erdman, 1979).

Maintaining a balanced dietary calcium and phosphorus ratio has been considered more critical for terrestrial animals than for fish. In several fish species, increasing dietary calcium did not affect growth and bone mineralization (Ogino and Takeda, 1976; Sakamoto and Yone, 1976; Watanabe *et al.*, 1980). The recommended supplementation of both calcium and phosphorus in the form of $CaHPO_4.2H_2O$ achieved a dietary calcium to phosphorus ratio of 1:1 and resulted in enhanced growth and bone mineralization (Gatlin and Scarpa, 1993). The change in balance between calcium and phosphorus is age-dependent in some species such as the redlip mullet (*Liza hematochiela*) (see El-Zipdeh *et al.*, 1995). Optimum levels of dietary phosphorus, however, are critical for maximum weight gain and feed efficiency. Application of elevated levels of phosphorus did not result in better growth in carp but accumulation of dorsal and body scales was observed (Schaefer *et al.*, 1995). A decreased level of dietary phosphorus resulted in an increase in lipid and a decrease in calcium, phosphorus and crude ash contents of the vertebrae of young fish (El-Zipdeh *et al.*, 1995).

The requirements for other minerals in fish remain poorly understood. A deficiency in trace elements may be linked to such disorders and conditions as retarded growth, decreased feed utilization, cataracts, exophthalmos and high mortality. A low level of dietary potassium may cause decreased total protein levels in Indian carp (*Cirrhinus mrigata*) (see Saxena and Talwar, 1996). Dietary iron can also affect haematological values and hepatic and whole-body iron concentrations. Liver vitamin C concentration and whole-body manganese decrease with increasing dietary iron (Andersen *et al.*, 1996).

Vitamin imbalance

Vitamins are essential elements for fish, and an imbalance in the diet may result in retarded growth, abnormal behaviour, physiological malfunction, impairment of metabolism and low resistance to disease (Halver and Coates, 1957). Usually, vitamin deficiency results from inadequate levels of vitamins in the feed, which can be corrected by supplementation. Vitamin deficiency may arise from disturbed environmental conditions that inhibit the absorption or use of vitamins. Fish living in toxaphene-contaminated water require higher vitamin C levels than they would otherwise (Mayer *et al.*, 1978).

Signs of hypovitaminosis are usually non-specific. Vitamin A deficiency may result in poor appetite and growth, reduced liver weight, anaemia, eye and skin haemorrhage, bulging opercula and discoloration of the body. Exophthalmos in carp also has been reported (Schaperclaus *et al.*, 1991). Dietary supplements with carotenoids as vitamin A precursors have been used to enhance reproduction and metabolism in fish. The activities of astaxanthin, canthaxanthin and retinol (vitamin A) have been investigated for UV light protection, cancer prevention and immune enhancement (Torrissen and Christiansen, 1995). Reduced growth and a high incidence of deformities may result from hypervitaminosis A (Dedi *et al.*, 1995). In turbot, high pigmentation rates were obtained by using a high level of vitamin A (500,000 IU l^{-1}) but this coincided with a greater incidence of skeletal malformations (Estevez and Kanazawa, 1995).

Fish fed diets free of riboflavin, thiamine and pantothenic acid often show significantly reduced feed intake, feed efficiency, weight gain and survival rate. Signs of riboflavin deficiency in carp are external haemorrhage on various parts of the body, nervous disorders and photophobia (Schaperclaus *et al.*, 1991). Cataracts occur in channel catfish (Murai and Andrews, 1978) and seabass (*Lates calcarifer*) (see Boonyaratpalin and Wanakowat, 1993). Channel catfish also exhibit a reduced hepatic D-amino acid oxidase activity (Serrini *et al.*, 1996).

Thiamine deficiency in carp results in body discoloration, hyperaemia of the skin and fins, muscular atrophy, exophthalmos, accelerated respiration, convulsions and equilibrium loss (Schaperclaus *et al.*, 1991). Seabass fed a thiamine-deficient diet may exhibit sluggish swimming behaviour and nervous disorders including sensitivity to shock and convulsions (Boonyaratpalin and Wanakowat, 1993). Trunk coiling has been reported in eels, and neurological disorders and dark coloration have been observed in channel catfish (see Dupree, 1966).

Deficiencies in dietary pantothenic acid may result in aberrant swimming in which the fish stay close to the water surface and show haemorrhage on the body surface, mucilaginous swelling of the gills with bulging of the opercula and difficulties in respiration (Schaperclaus *et al.*, 1991). Signs of pantothenic acid deficiency in seabass include loss of appetite and equilibrium, abnormal swimming, haemorrhage on the body, eroded fins and clubbed gills (Boonyaratpalin and Wanakowat, 1993). Within 2 weeks of adding pantothenic acid to the feed, rapid recovery and growth was noted (Boonyaratpalin and Wanakowat, 1993). Similar deficiencies have been seen in eel (*Anguilla japonica*) and channel catfish (Arai *et al.*, 1972; Murai and Andrews, 1979). The term 'nutritional gill disease' has been used to describe massive

epithelial hyperplasia and lamellar fusion, affecting the distal third of the gill filament. Affected fish compensate for the loss of the respiratory surface by increased perfusion, involvement of additional lamellae and increased branchial irrigation. Overgrowth and an increased number of gill lamellae at the tips of filaments are long-term compensatory mechanisms noted in some fish species with pantothenic acid deficiency (Ferguson, 1989).

Vitamin C (ascorbic acid) is critical to fish health. Probably through its antioxidant properties, vitamin C is involved in growth, reproduction, wound healing, stress response and disease resistance. Vitamin C acts as a cofactor in many reactions involving hydroxylating enzymes such as the synthesis of collagen, complement component C_1q and catecholamines. Through the antioxidant properties of ascorbic acid, it is also involved in tyrosine metabolism, metal ion metabolism, protection of cells from iron oxidative damage and the regeneration of vitamin E in its metabolically active form. Vitamin C affects both cellular and humoral immune processes and has an important role in maintaining the integrity of immune cells by providing protection for the cell membrane and its intracellular components from oxidation (Verlhac and Gabaudan, 1998). In gilthead seabream fed diets supplemented with vitamin C, non-specific immune response parameters including phagocytic activity, natural haemolytic complement activity and respiratory burst were enhanced (Ortuño *et al.*, 1999). Channel catfish fed diets devoid of vitamin C showed increased mortality over a period of 6–8 weeks. Gross clinical signs included a darker skin pigmentation, scoliosis, lordosis and eroded fins (Dabrowski *et al.*, 1996). Figure 8.8 shows scoliosis caused by vitamin C deficiency in a walking catfish (*C. batracus*). In carp, although no deficiency signs were noted when fed a diet lacking vitamin C (Sato *et al.*, 1978), addition of vitamin C to feed is recommended to alleviate stress.

Dietary ascorbic acid affects tissue retention of waterborne copper in carp (Yamamoto *et al.*, 1977). Ascorbic acid deficiency results in reduced serum iron, haemoglobin and haematocrit levels in channel catfish and spotted snakehead (*Channa punctatus*) (see Andrews and Murai, 1975; Agrawal and Mahajan, 1980). However, dietary ascorbic acid does not affect the mobilization of calcium and phosphorus in some fish species (Akand *et al.*, 1993).

Vitamin E (α-tocopherol) is a major metabolic antioxidant in fish. It prevents the oxidation of unsaturated phospholipids in membranes of nearly all types of cells. Vitamin E supplementation enhances production of antibodies against a variety of

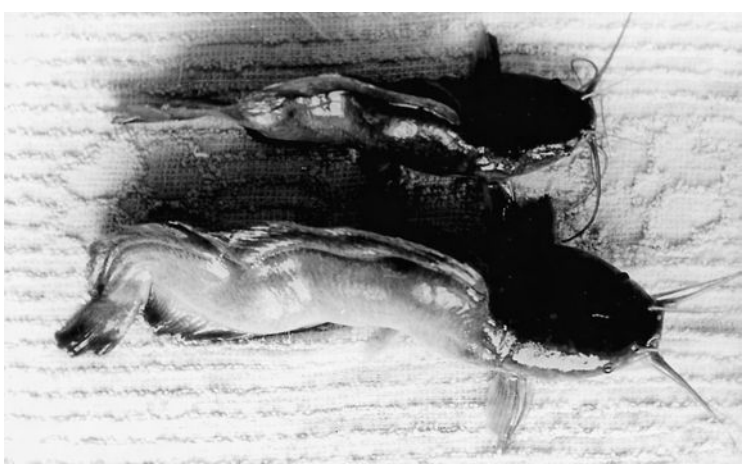


Fig. 8.8. Scoliosis in a 120-day-old walking catfish (*Clarias batracus*) due to vitamin C deficiency. (Courtesy of Dr J. Tangtrongpiros.)

antigens. It also enhances the phagocytic activity of macrophages and, thereby, disease resistance (Bendich, 1990). A condition known as 'nutritional muscular dystrophy' is caused by a vitamin E- and selenium-deficient diet in intensively cultured fish. Histopathological lesions include hyaline and granular degeneration of muscle fibres with a loss of striation. Mineralization and inflammation are usually minimal (Ferguson, 1989). Carp may show poor growth, dystrophy of the dorsal musculature, a decrease in erythrocyte and leucocyte numbers and increased mortality (Schaperclaus *et al.*, 1991). Since rancid oil in feed increases peroxides in cells and tissues, auto-oxidization of lipids occurs in cells under vitamin E-deficient conditions, resulting in free radicals from peroxides, which affect muscle fibres, hepatocytes and fat cells. Deposition of ceroid pigments from peroxidized lipids and protein is evident by the presence of fatty hepatocytes and macrophages in fish on vitamin E-deficient diets (Miyazaki, 1993). Increased vitamins E and C in the feed have been recommended to enhance immune responses against infectious diseases and environmental stress in fish culture. In rainbow trout a combination of vitamins C and E fed at high levels, together or individually, resulted in increased numbers of lymphocytes induced by concanavalin A as well as enhanced macrophage oxidative burst activity. Dietary supplementation of vitamin E, as well as vitamin C, results in increased disease resistance and survival rates (Wahli *et al.*, 1998). Vitamin E-deficient fish exposed to UV light exhibit focal white spots anterior to the dorsal fin. The caudal and pectoral fins then lose their pigmentation and become translucent. Later, extensive erosion of the caudal and pectoral fin accompanied by desquamation of the epidermis and underlying tissue of the caudal peduncle, head and snout occurs (Poston and Wolfe, 1985). Clearly, vitamin E is essential for the protection of fish against 'sunburn' caused by solar radiation.

Other signs of vitamin deficiency that have been observed in fish have involved inositol, vitamin K, choline and niacin. In carp, skin necrosis, poor growth and high mortality occurred in fish fed an inositol-deficient diet (Aoe and Masuda, 1967). Skin haemorrhage was seen in vitamin K-deficient, and lipoid degeneration of the liver in choline-deficient carp. Optimum niacin levels are important in fish diets to promote growth, improve feed conversion and prevent skin lesions induced by UV light.

Conclusion

Netpen culture of warmwater fish is clearly in a developmental stage. As species come under culture, diseases and disorders of both infectious and non-infectious origin will arise. It will be incumbent on managers and fish disease experts to identify, treat and manage the conditions with care toward protecting adjacent native stocks.

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9 Sporadic, Emerging Diseases and Disorders

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Introduction

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

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

Consequently, the role diseases play in regulating wild fish are largely unknown (Möller and Anders, 1986). The factors contributing to or altering the known status

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

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

Infectious Pancreatic Necrosis Virus in Marine-reared Atlantic Salmon

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

Impact

Currently there is a low incidence of IPNV in freshwater salmon farms and this has been achieved in part though a successful programme of broodstock testing, the destruction of infected eggs, surface disinfection of ova and other statutory measures. However, the emergence of IPN in the marine environment is having an increasing impact on the industries of Scotland and Norway. The source of the virus is speculative, and could include the transfer of infected fish from fresh water, strain differences emerging in certain areas, failure of fish to adapt to seawater and expression of low level virus or a wild reservoir. With respect to the latter, IPNV has been isolated from wild sea trout. IPNV is stable for long periods in sea and brackish waters and this could also have an impact on seawater outbreaks. At present, one serotype (Sp) appears to be responsible for the outbreaks in Scottish waters, but pathogenic and non-pathogenic forms are indistinguishable using currently available methodology. Therefore, it is difficult to determine the origin of the marine isolates. Stress may contribute to acute IPNV in marine fish, and its emergence in post-smolts would support this theory. The losses from cage sites around the Shetland Isles and in Norway in the Hitra/Frøya region are higher than the other regions where salmon farming is also concentrated. The Shetland Isles and certain regions along the Norwegian coast have a lower water temperature at smolt transfer, which is possibly a factor in the emergence

of IPN. In Norway, 90% of the losses in 1998 were attributed to IPNV, amounting to 8.3 million individual fish (T.T. Poppe, 1999, personal communication). This situation may be compounded where there is mixing of smolts of different origins, and this has been shown to increase the risk of fish developing IPN. In the UK direct losses in 1998 were estimated at £1 million, although in terms of economic loss to the industry this is higher and around £5–10 million.

Clinical signs

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

Diagnosis and control

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

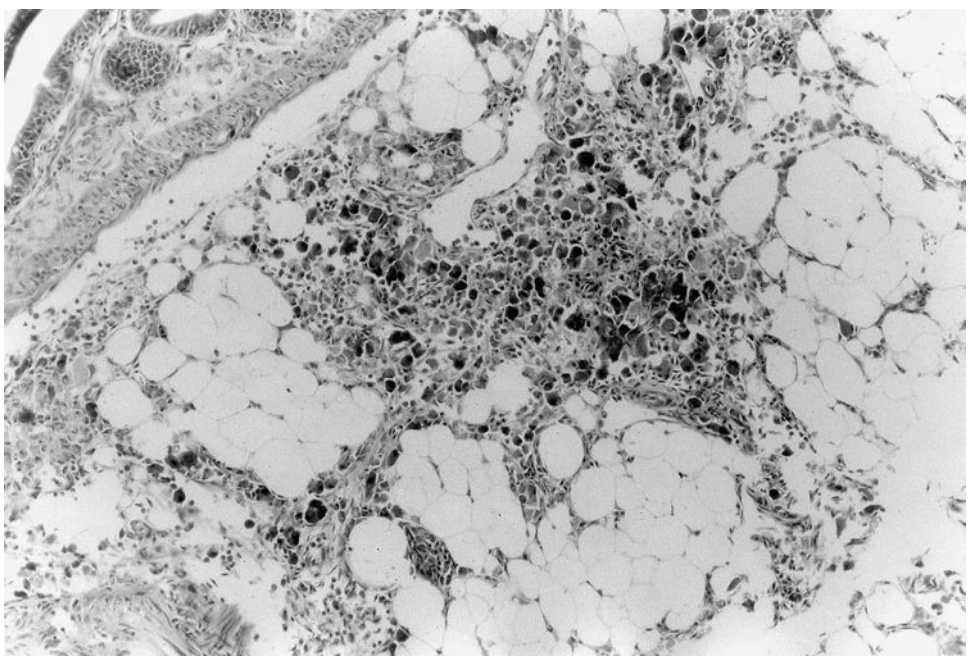


Fig. 9.1. Infectious pancreatic necrosis virus (IPNV) pathology showing a focal necrosis of the pancreatic acinar tissue with acute enteritis, necrosis and sloughing of the intestinal epithelium.

Future studies

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

Infectious Salmon Anaemia Virus

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

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

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

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

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

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

Characterization

Filtered liver homogenate used to challenge experimental fish established the cause of ISA as an infectious virus. Instability of

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

Impact

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

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

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

Transmission

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

High mortality among Atlantic salmon could indicate that this virus is a newly introduced pathogen to this species (Nylund

et al., 1997), and salmonids in other regions of the world must be considered at risk. It is noteworthy that the origin of ISA has not been identified (Nylund *et al.*, 1995; Fisheries Research Services, 1999).

Clinical signs and gross pathological changes

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

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

Histopathology

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

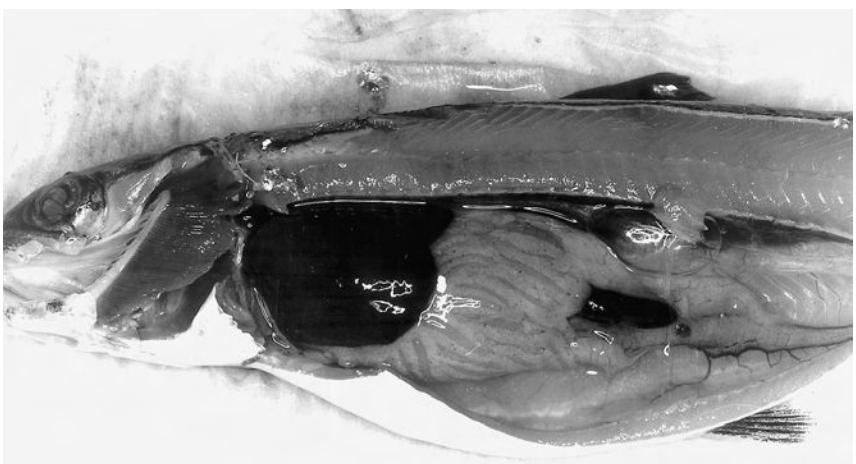


Fig. 9.2. Atlantic salmon infected with ISAV. Clear ascites, dark enlarged liver and splenomegaly are shown.

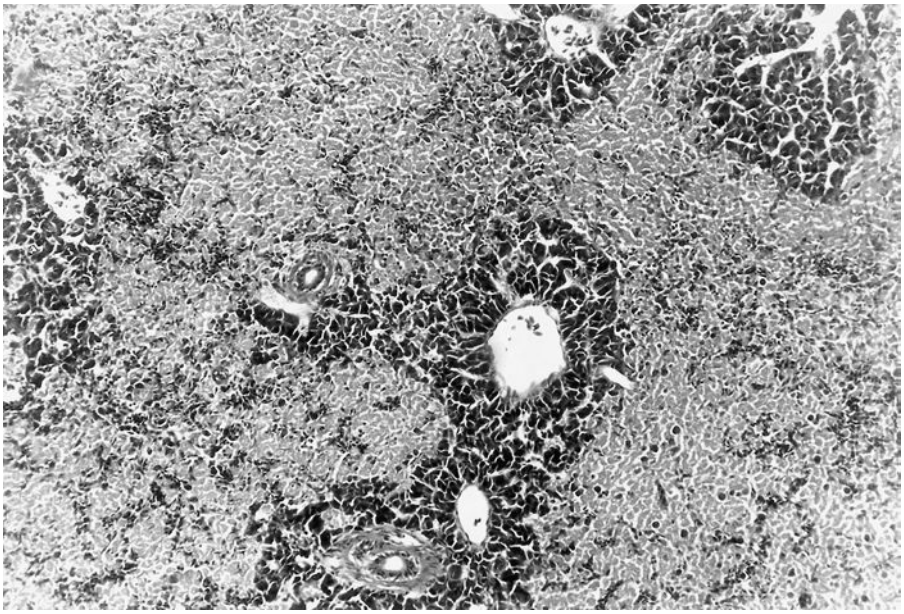


Fig. 9.3. Zonal degeneration of hepatocytes results in a multifocal confluent haemorrhagic necrosis. An anastomosing, 'bridging' necrosis typically leaves the parenchyma around the veins intact.

an increase in inflammatory cells. In the kidney, interstitial haemorrhage, necrosis, tubular degeneration and trapping of erythrocytes have been observed, contributing to a circulatory disturbance. Frank haemorrhage and desquamation of the endothelial cells may follow a marked congestion and bleeding in the lamina propria of the foregut. In experimental studies, a sudden depletion of liver glycogen has been recorded between days 14 and 18 post-infection (Speilberg *et al.*, 1995).

Ultrastructural lesions

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

Diagnostic techniques

The first isolation of ISAV was made in 1995 in a new Atlantic salmon head kidney (SHK-1) cell line (Dannevig *et al.*, 1995).

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

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

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

Risk factors

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

that waterborne transmission is important for the spread of ISA.

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

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

Nodaviruses and Nodavirus-like Viruses

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

Characterization

The family *Nodaviridae* contains two genera, and the *Betanodavirus* genera infect fish (van Regenmortel *et al.*, 2000). PNN and related viruses are the cause of viral nervous necrosis, and they belong to the family *Nodaviridae* based on biochemical characterization of the viral nucleic acid (Mori *et al.*, 1992; Munday *et al.*, 1994; Frerichs *et al.*, 1996). Electron microscopy studies show an isometric, non-enveloped virus measuring 25–34 nm in diameter. The virus is primarily found within inclusions or in the cytoplasm of cells in the brain, spinal cord and retina of infected fish (Mori *et al.*, 1991; Frerichs *et al.*, 1996; Jung *et al.*, 1996; Munday and Nakai, 1997). The virions form crystalline arrays and are packed in membranous structures (Fig. 9.4). Two pieces of single-stranded RNA make up the genome with molecular weights of

1.056×10^6 and 0.495×10^6 Da, respectively (Breuil *et al.*, 1991; Mori *et al.*, 1992). Present evidence suggests that there is more than one viral agent or strain, as Nguyen *et al.* (1994) found differences in the antigens in the coat protein gene (RNA2) (Arimoto *et al.*, 1992, 1993; Delsert *et al.* 1997). SJNNV and other fish nodavirus genotypes share a significant number of common antigentic determinants, although SJNNV is clearly distinguishable (Nishizawa *et al.*, 1999). The occurrence of two distinct isolates of nodavirus infecting seabass, *Dicentrarchus labrax*, in the Atlantic and the Mediterranean coasts of France (Thiéry *et al.*, 1999) that have a distinct genotype may suggest the absence of a species barrier. The SJNNV coat protein has been sequenced and compared with four known insect nodavirus and other fish nodaviruses causing VNN (Nishizawa *et al.*, 1995a). Their results indicated that the fish nodaviruses that cause VNN are related, but are clearly distinct from the insect nodaviruses. However, the nodavirus isolated from barramundi, *Lates calcarifer*, larvae of striped jack (*Pseudocaranx dentex*) and VNN from Japanese flounder (*Paralichchthys olivaceus*) and striped jack were suggested as antigenetically related (Munday *et al.*, 1994; Nguyen *et al.*, 1994). A new genus, *Piscinodavirus*, has been proposed to incorporate fish nodavirsus (Nishizawa *et al.*, 1995a; Delsert *et al.*, 1997).

Impact

Throughout the 1990s, a number of significant or mass mortalities attributed to PNN affected larval, juvenile and sometimes adult farmed marine fish (Boonyaratpalin *et al.*, 1996; Bovo *et al.*, 1996; Le Breton *et al.*, 1997). The fish species susceptible to PNN or picornavirus-like

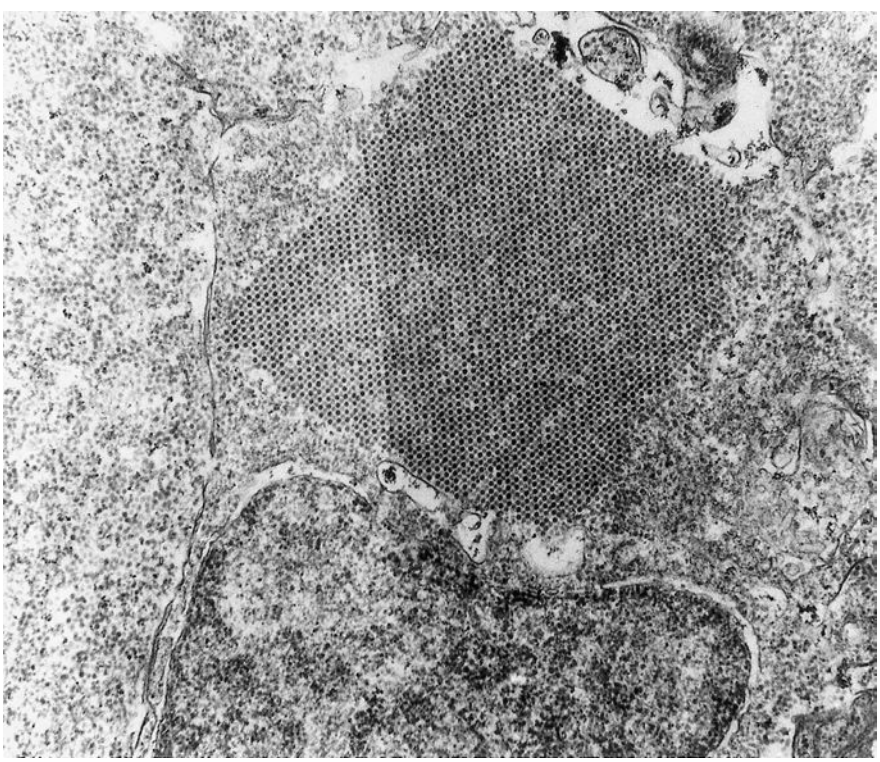


Fig. 9.4. Electron micrograph of striped jack nervous necrosis virus (SJNNV) in the retinal cells of a striped jack larva.

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

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

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

Fish	Species	Country	Reference
Atlantic halibut	*Hippoglossus hippoglossus*	Norway	Grotmol *et al*. (1995)
Atlantic salmon	*Salmo salar*	Norway	Castric (1997); Grotmol *et al*. (1997b)
Barramundi	*Lates calcarifer*	Australia	Glazenbrook *et al*. (1990)
Barfin flounder	*Verasper moseri*	Japan	Muroga (1995)
European seabass	*Dicentrarchus labrax*	Greece	Le Breton *et al*. (1997)
Greasy grouper	*Epinephelus tauvina*	Singapore	Chew-Lim *et al*. (1998)
Humpback grouper	*Cromileptes altivelis*	Indonesia	Zafran *et al*. (2000)
Japanese seabass	*Lateolabrax japonicus*	Japan	Jung *et al*. (1996)
Japanese parrotfish	*Oplegnathus fasciatus*	Japan	Yoshikoshi and Inoue (1990)
Japanese flounder	*Paralichchthys olivaceus*	Japan	Nguyen *et al*. (1994)
Kelp grouper	*Epinephelus moara*	Japan	Nakai *et al*. (1994)
Purplish amberjack	*Seriola aurata*	Japan	Muroga (1995)
Redspotted grouper	*Epinephelus akaara*	Japan	Mori *et al*. (1991)
Rock porgy	*Oplegnathus fasciatus*	Japan ?	Muroga (1995)
Seabass	*Dicentrarchus labrax*	Martinique	Bellance and Gallet de Saint-Aurin (1988)
Seabass	*D. labrax*	France	Breuil *et al*. (1991)
Seabass	*D. labrax*	Italy	Bovo *et al*. (1996)
Seabass	*D. labrax*	Tahiti	Renault *et al*. (1991)
Sevenband grouper	*Epinephelus septemfasciatus*	Japan	Fukuda *et al*. (1996)
Striped snakehead	*Channa striatus*	Greece	Frerichs *et al*. (1996)
Striped jack	*Pseudocaranx dentex*	Japan	Nguyen *et al*. (1996)
Tiger puffer	*Takifugu rubripes*	Japan	Nakai *et al*. (1994)
Turbot	*Scophthalmus maximus*	Norway	Bloch *et al*. (1991)
Yellow grouper	*Epinephelus awoara*	Taiwan	Lai *et al*. (2001)
Yellowtail	*Seriola quinquerodiata*	Japan	Muroga (1995)

suggested the development of a carrier state in this species.

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

Clinical signs

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



Fig. 9.5. Characteristic vacuolation due to piscine neuropathy nodavirus in the retina of juvenile redspotted grouper.

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

Diagnostic techniques

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

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

asymptomatic spawners of striped jack (Mushiake *et al.*, 1994). Sequence analysis of different nodavirus isolates from seabass allowed Thiéry *et al.* (1999) to design a new primer set, which amplified the coat protein gene from the Atlantic nodavirus isolates, whereas earlier primers only detected the Mediterranean isolates. Grotmol *et al.* (2000) also found a significant level of nucleotide sequence identity between RNA2 of the Atlantic halibut nodavirus and SJNNV.

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

Prevention

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

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

Recommendations

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

Fish Mycobacteriosis

Fish tuberculosis or mycobacteriosis is a systemic disease caused by *Mycobacterium* spp. Periodic outbreaks contribute to a chronic mortality in marine and freshwater fish species, which include farmed Pacific

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

Infection in Atlantic salmon

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

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

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

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

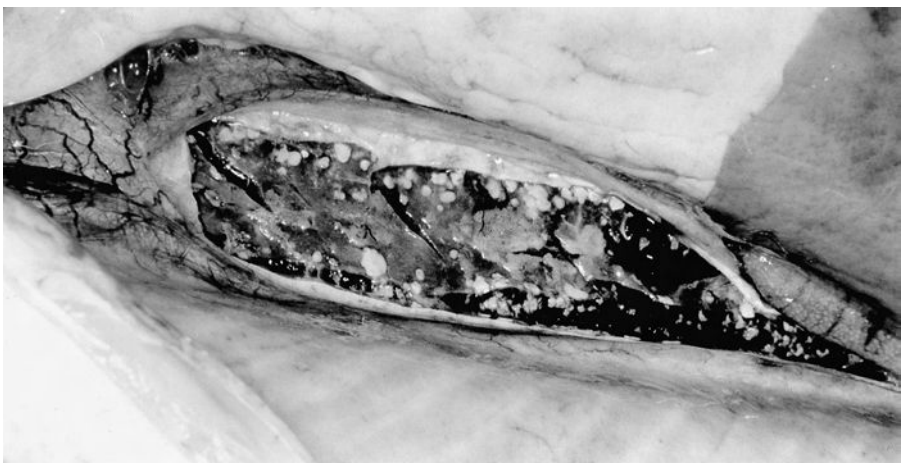


Fig. 9.6. Multiple, miliary granuloma-like nodules attributed to *Mycobacterium chelonae* throughout the enlarged kidney. Smaller granulomatous-like lesions are also present in the spleen.

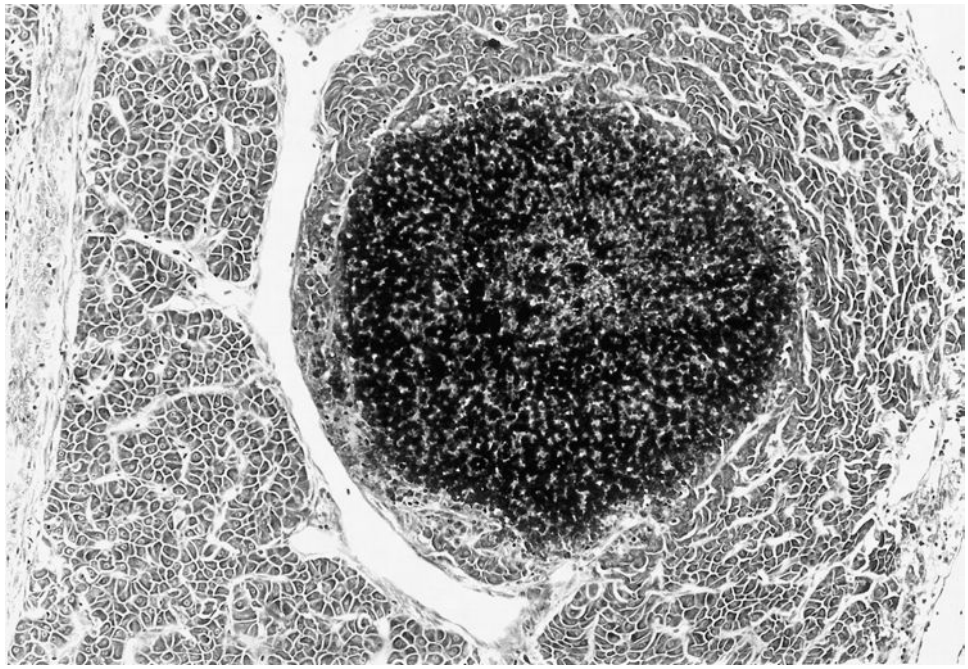


Fig. 9.7. Abundant *Mycobacterium chelonae* in densely packed nodules in the liver. There is no surrounding capsule, fibrin deposition or central necrosis.

The term 'winter ulcers' relates to the lateral lesions and the occurrence of the condition during cold water temperatures (Salte *et al.*, 1994). The condition is now known to be associated with a psychrotropic *Vibrio* sp. On the basis of infrequent records, this disease is currently considered sporadic. Vibriosis due to *Listonella anguillarum* and *Vibrio salmonicida* is discussed in Chapter 3.

Characterization

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

Impact

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

Clinical signs

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

(Bruno *et al.*, 1998b). Circular or oval, epidermal ulcers occur on scale-covered areas. Ulcers occur at various sites over the body and range from superficial to the exposure of the stratum compactum and the musculature. Internally, there are dark-brown, almost black, areas across the uncut surface of the normal or pale-coloured liver, in some cases confined to dark petechiae or ecchymotic haemorrhage 1 mm in diameter, whereas in other fish larger areas of haemorrhage were recorded (Fig. 9.8). Splenomegaly is common, occasionally with ascites within the abdominal cavity.

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

Diagnostic techniques

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

Piscirickettsiosis and *Pisrickettsia*-like Organisms

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

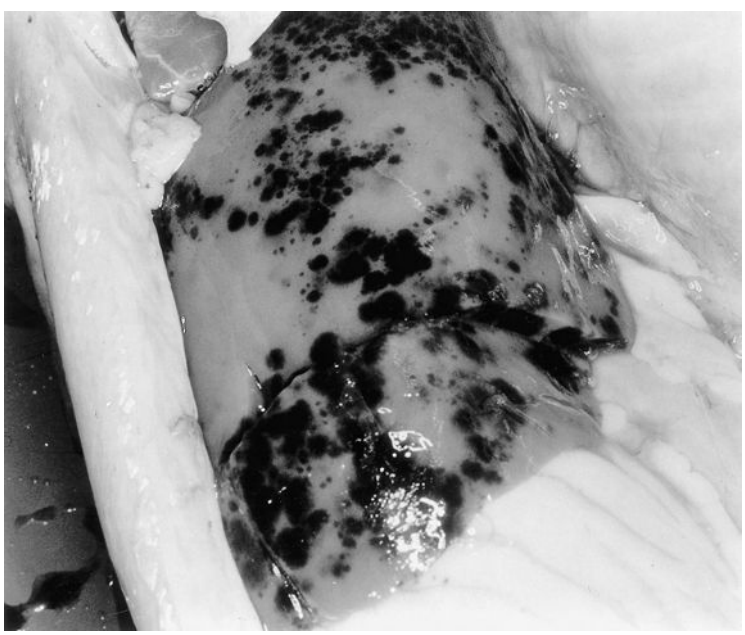


Fig. 9.8. Gross lesion in liver infected with *Vibrio viscosus* showing dark petechiae or ecchymotic haemorrhage 1 mm in diameter, spreading to larger areas of haemorrhage.

and shown to be the cause of epizootics in marine netpen-reared coho salmon (*Oncorhynchus kisutch*). The causative agent, *P. salmonis*, is an obligate bacterium, which causes a serious, systemic infection of salmonids in seawater (Fryer *et al.*, 1992) and is discussed in Chapter 3.

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

Infectious agent

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

At present there are no reports of *P. salmonis* published from feral salmonids, although Kent (1992) considered the agent was of marine origin. However, isolation from freshwater cages of coho salmon and trout in Chile suggests its presence in this environment (Gaggero *et al.*, 1995). In seawater the agent survives for several weeks between 5 and 20°C (Lannan and Fryer, 1994). However, one freeze–thaw cycle decreased the $TCID_{50}$ titre of *P. salmonis* to virtually nil (Fryer *et al.*, 1990).

Hosts and geographic distribution

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

Gross pathology

The clinical signs and gross lesions reported for piscirickettsiosis from natural and experimentally infected fish from Chile include lethargy, anorexia, respiratory distress, darkening of the skin and

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

Histopathology

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

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

Economic significance

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

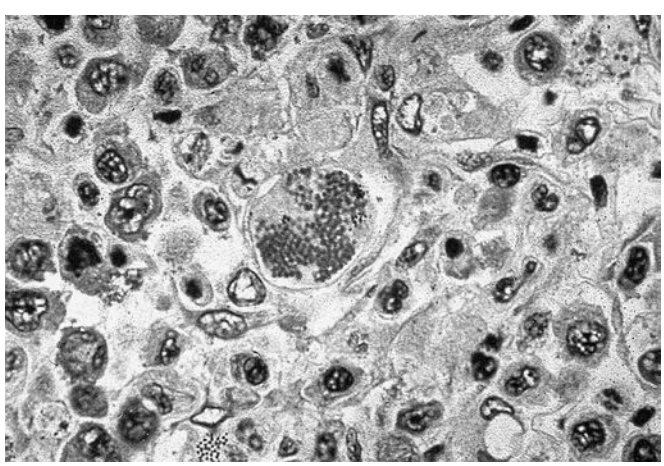


Fig. 9.9. *Piscirickettsia salmonis* infecting a variety of cells including circulating macrophages, in which they can replicate and cause cell lysis.

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

Isolation and diagnosis

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

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

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

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

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

Transmission

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

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

Management practices

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

Chemotherapy

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

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

Vaccine development

Several research programmes are directed towards developing a vaccine, and the results of an early trial have been published (Smith *et al.*, 1995). During an initial field trial, an immunoprotective effect in vaccinated fish compared with controls was achieved. However, this result is tentative as the natural challenge may have been low and the bacterium causing bacterial kidney

disease, *Renibacterium salmoninarum*, was also detected in the experimental fish. Although commercial vaccines are now available, there is little published information on their efficacy.

Recommendations

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

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

Prevention

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

Cryptobia (*Trypanoplasma*) *salmositica*

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

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

Impact

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

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

In early 2001, another outbreak occurred in preharvest chinook salmon (weighing about 3 kg) in sea cages in the same hatchery. *Cryptobia* were in large numbers in the blood and ascites fluid of moribund fish, and clinical signs (e.g. exophthalmia, anaemia, anorexia) were

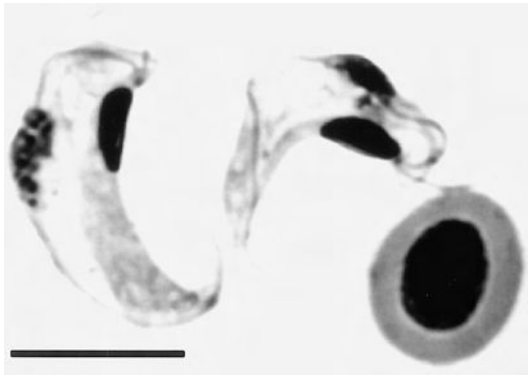


Fig. 9.10. *Cryptobia salmositica*, showing an elongated flagellum with a prominent kinetoplast close to its round nucleus. The red cell is from an anaemic fish, rounded, microcytic and hypochromic (from Woo and Poynton, 1985; courtesy of CAB International, Wallingford, UK).

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

Clinical signs and gross pathology

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

Diagnosis

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

Control and treatment

Protective strategies have been developed and they include:

1. Immunization with a live vaccine. *C. salmositica* has been attenuated and used routinely as an experimental vaccine to protect salmonids from cryptobiosis (e.g. Woo and Li, 1990; Sitjà-Bobadilla and Woo, 1994; Li and Woo, 1995, 1997; Staines and Woo, 1997). This acquired immunity lasts at least 2 years, and the protection is due to the production of complement-fixing antibodies, enhanced phagocytosis and cell-mediated cytotoxicity (Li and Woo, 1995).
2. Selective breeding of resistant fish. Some brook char (*Salvelinus fontinalis*) are naturally resistant to *C. salmositica* infection. The resistant factor(s) is controlled by a dominant Mendelian locus and is inherited by progeny of resistant fish (Forward *et al.*, 1995). The parasite is lysed in the blood of resistant char via the alternative pathway of complement activation (Forward and Woo, 1996). Both *Cryptobia*-resistant and *Cryptobia*-susceptible char respond equally well (humoral and cell-mediated immunity) to a commercially available *Aeromonas salmonicida* vaccine (Ardelli and Woo, 1995).
3. Chemotherapy. Under experimental conditions, the trypanocidal drug, isometamidium chloride (Samorin), is effective against the parasite when injected intramuscularly (1.0 mg kg^{-1} body weight) into adult rainbow trout and juvenile chinook salmon (Ardelli and Woo, 1999). The drug significantly lowered the parasitaemia, and its level in the blood peaked at 2 weeks after injection. All infected and treated chinook salmon survived while 100% of untreated fish died from cryptobiosis; the drug also had prophylactic effects in salmon (Ardelli and Woo, 2001). An antigen-capture ELISA has been developed to monitor drug levels in the blood of fish (Ardelli and Woo, 2000).

Sphaerospora dicentrarchi (Myxosoma)

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

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

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

Another outbreak occurred in seabass (about 70 g) in 1997 in a fish farm in Sicily, Italy. Mortality reached 1000 fish per day in indoor tanks, with many fish showing

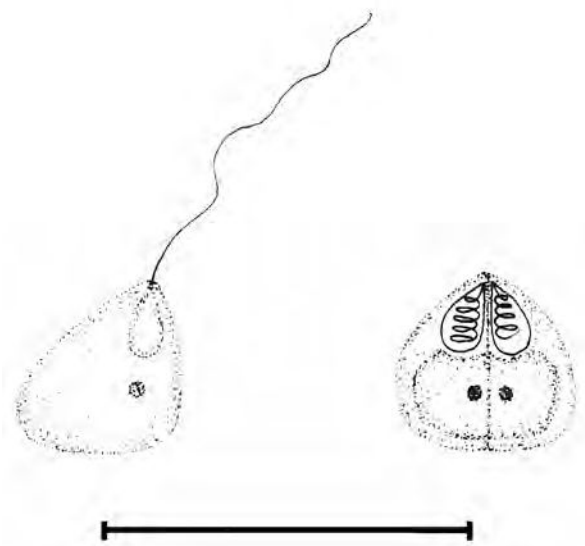


Fig. 9.11. Mature spore of *Sphaerospora dicentrarchi* showing a subspherical and slightly flattened base and bluntly pointed pole. Its surface is smooth and the suture line is straight and thin (from Sitjà-Bobadilla and Alvarez-Pellitero, 1992; courtesy of *Journal of Protozoology*).

clinical signs of the infection. Although antibiotics reduced the mortality, the more successful procedure was to increase the water flow. *S. dicentrarchi* was detected in the gut and trunk kidney, but there were also bacterial isolates from haemorrhagic areas. Fish of similar size in sea cages did not have clinical disease, but were not examined for the parasite (A. Sitjà-Bobadilla, personal communication).

Clinical signs and treatment

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

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

Fat Cell Necrosis Syndrome in Farmed Atlantic Halibut

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

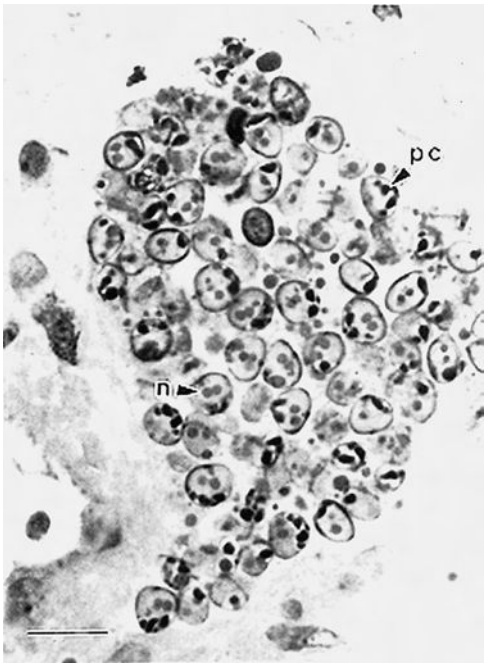


Fig. 9.12. Mature spore of *Sphaerospora dicentrarchi*. The piriform polar capsules (pc) are equal in size, and coils of the polar filament are not visible under light microscopy. The sporoplasm has two nuclei (n). (From Sitjà-Bobadilla and Alvarez-Pellitero, 1992, courtesy of *Journal of Protozoology*.)

alongside these developments (Wood *et al.*, 1996; Grotmol *et al.*, 1997b; Bricknell *et al.*, 1999, 2000). Recently, a progressive, degenerative chronic condition of the dorsal subdermal fat deposits of farmed Atlantic halibut has been reported and termed fat cell necrosis syndrome (FCNS) (Bricknell *et al.*, 1999) after the occurrence of steatitis described in common dab, *Limanda limanda* (Begg *et al.*, 2000). In both cases no infectious or malignant aetiology was identified.

The condition in halibut may be related to an imbalance between dietary oxidants and antioxidants combined with an exposure to sunlight (Smith, 1979; Bullock and Roberts, 1981; McArdle and Bullock, 1987). The lesions are similar to those described in feral-caught common dab (Begg *et al.*, 2000).

Clinical signs

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

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

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

A dietary component may be connected to this condition in common dab (Begg, 1994) due to an uncontrolled lipid peroxidation (Demopoulos, 1973). This lipid peroxidation may result from an imbalance between oxidants and antioxidants resulting in membrane damage. Such oxidative stress may occur through exposure to UV radiation, the liberation of free radicles from necrotic fat cells, or by the production of hydroperoxidases following the release of lipases (Porter, 1989). At present the cause of FCNS in Atlantic halibut has not been fully determined. However, Bricknell *et al.* (1996) noted from observations on a second, Scottish halibut farm that fish kept in lightly covered 2 m tanks in direct sunshine and fed

on a lipid-rich commercial diet (24% lipids) developed identical lesions. The fish that developed the lesions were malpigmented. These lesions resolved spontaneously when the halibut were put on to a diet containing lower levels of lipids (12%) and the exposure to direct sunlight was reduced (S. Wadsworth, 1998, personal communication). If this is the case, then reducing dietary lipids and exposure to sunlight could control outbreaks of FCNS. It is possible that increasing the levels of antioxidants, such as α-tocopherol, in the diet may also induce resistance to FCNS (Smith, 1979).

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

Disorders Due to Vaccination

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

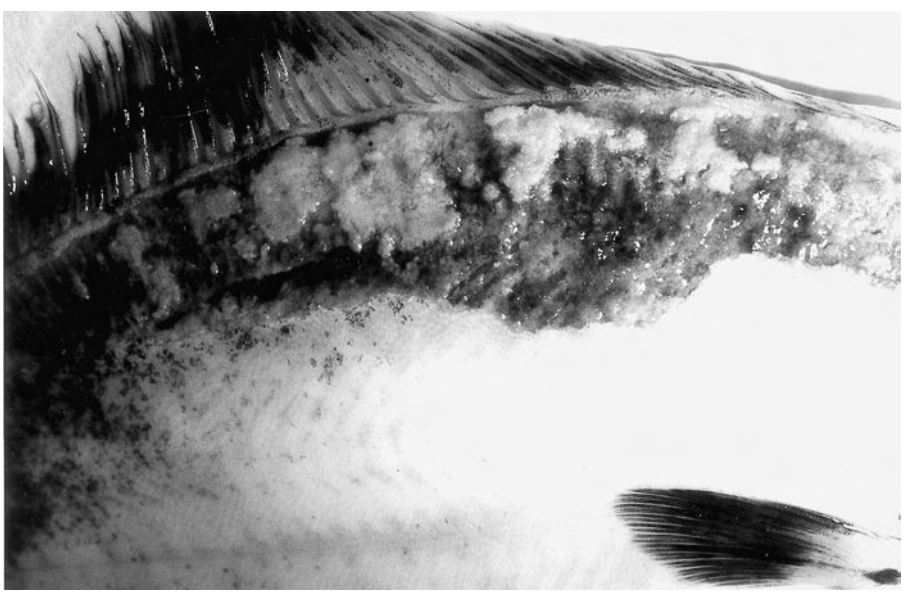


Fig. 9.13. Gross lesions typical of fat cell necrosis syndrome (FCNS) generally appear in the pterogophial region of the dorsal surface as multiple white to pale yellow-coloured spreading areas.

The most effective way to immunize a fish is by either intraperitoneal or intramuscular injection. This approach has many disadvantages and they include the use of anaesthesia and handling followed by injection. These procedures are 'stressful' and may reduce growth of fish (e.g. Pickering, 1990; Lillehaug, 1991; Lillehaug *et al.*, 1992). The reduction in growth may be as high as 4.4% in Atlantic salmon (Lillehaug, 1991) and it has been correlated to cortisol production in fish after handling and injection (Pickering, 1990).

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

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

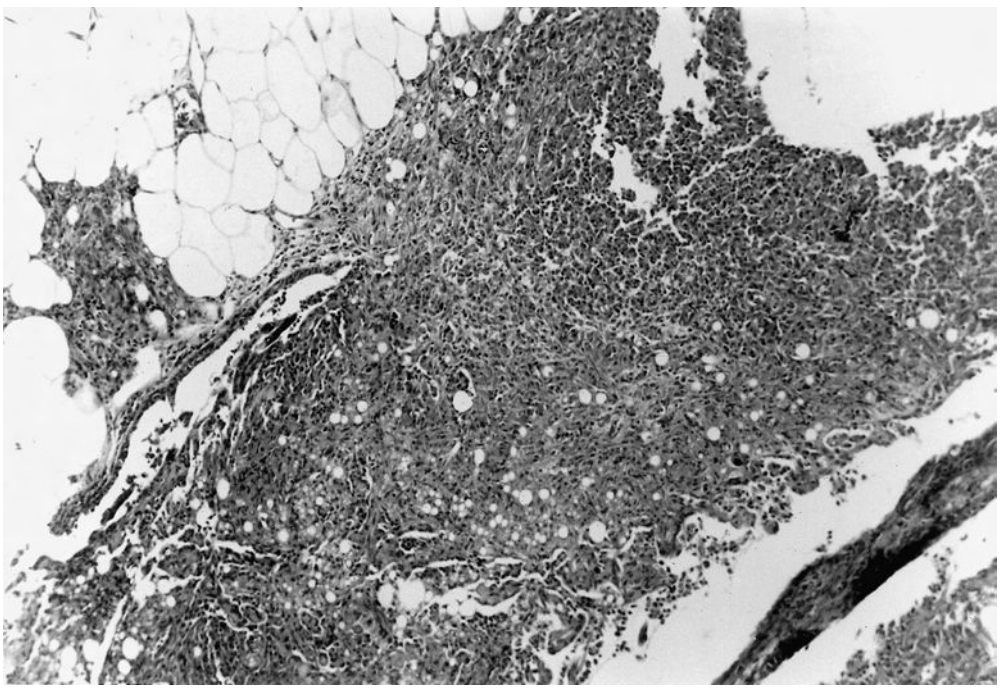


Fig. 9.14. Macrophages containing vaccine components occur in several tissues following the initial injection of vaccine. The continued presence of these cells within the abdominal cavity indicates uptake from the vaccine depot. An outpouring of a fibrin-rich exudate occurs in response to the adjuvant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

The stress on fish associated with handling/injection of vaccine, and the side effects due to the adjuvant in the vaccine are bound to be more noticeable with increased reliance on immunization as a strategy against infectious diseases.

Note Added at Proof Stage

Parvicapsula minibicornus (Myxosporea)

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

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