Australian Government

Department of Agriculture, Fisheries and Forestry

AQUAVETPLAN

Disease Strategy
Piscirickettsiosis

Version 1, 2013

AQUAVETPLAN is a series of technical response plans that describe the proposed Australian approach to aquatic animal disease incursions. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.

Standing Council on Primary Industries

This disease strategy forms part of:

AQUAVETPLAN

This strategy will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to:

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AQUAVETPLAN is available on the internet at:
www.daff.gov.au/aquavetplan

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IMPORTANT NOTE: Regulatory information for piscirickettsiosis has been removed from the OIE Aquatic Animal Health Code (OIE 2011). This code is updated annually and is available on the internet at the OIE website: **www.oie.int/en/international-standard-setting/aquatic-code/access-online**. Further details are given in Appendix 1 of this manual.

Disease Watch Hotline

1800 675 888

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency animal disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance.

Preface

This disease strategy for the control and eradication of piscirickettsiosis is an integral part of the Australian Aquatic Veterinary Emergency Plan **(AQUAVETPLAN).**

AQUAVETPLAN disease strategy manuals are response documents and do not include information about preventing the introduction of disease.

DAFF Biosecurity, part of the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), provides quarantine inspection for international passengers, cargo, mail, animals, plants, and animal or plant products arriving in Australia. DAFF Biosecurity also inspects and certifies a range of agricultural products exported from Australia.

Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable status for human, animal and plant health. Information on current import conditions can be found at the DAFF Biosecurity ICON website.[1]

This disease strategy sets out disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of piscirickettsiosis in Australia. The strategy for a response to such an incident was scientifically reviewed by the Sub-Committee on Aquatic Animal Health before being endorsed by the Animal Health Committee of the Standing Council on Primary Industries in June 2012.

The full list of AQUAVETPLAN manuals that may need to be accessed during an aquatic animal disease emergency is shown below.

Disease strategies
Individual strategies for each disease

Operational procedures manuals
Disposal
Destruction
Decontamination

Management manual
Control centres management

Enterprise manual
Includes sections on: – open systems, semi-open systems, semi-closed systems, closed systems.

This disease strategy was drafted by Kevin Ellard (primary author), Robert Cordover and Richard Morrison, in consultation with a wide range of stakeholders from aquaculture, wild-capture and recreational fishing sectors, and government

[1] www.aqis.gov.au/icon32/asp/homecontent.asp

agencies throughout Australia. Sadly, Robert Cordover passed away during the final stages of preparation; his contribution to the document is greatly appreciated. The text has been amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not necessarily reflect the views of the authors. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual has been adapted from similar manuals within AUSVETPLAN (the Australian Veterinary Emergency Plan for terrestrial animal diseases). A similar format and content have been used to enable personnel trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic animal disease emergency involving piscirickettsiosis. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents are gratefully acknowledged.

Scientific editing was by Biotext Pty Ltd, Canberra.

This version of the AQUAVETPLAN **Disease Strategy—Piscirickettsiosis** has been reviewed and approved by the following representatives of government and industry:

Government

Australian Animal Health Laboratory (CSIRO Livestock Industries)
Department of Primary Industries, New South Wales
Department of Primary Industries and Fisheries, Northern Territory
Queensland Department of Agriculture, Fisheries and Forestry
Department of Primary Industries, Parks, Water and Environment, Tasmania
Department of Fisheries, Western Australia
Department of Primary Industries, Victoria.
Department of Primary Industries and Regions of South Australia
Biosecurity Animal, Australian Government Department of Agriculture, Fisheries and Forestry

Industry

National Aquatic Animal Health Industry Reference Group (NAAHIRG)
Tasmanian Salmonid Growers Association

The complete series of AQUAVETPLAN documents is available on the internet.[2]

[2] www.daff.gov.au/aquavetplan

Contents

Tables

1 Nature of the disease

Piscirickettsiosis is a severe septicaemic condition caused by the bacterium *Piscirickettsia salmonis*, a *Rickettsia*-like organism (RLO). The disease is primarily associated with salmonid species (family Salmonidae) and is characterised by high mortality and significant production loss through inappetence.

The disease was first reported in 1989 in coho salmon (*Oncorhynchus kisutch*) farmed in net pens in Chile (Bravo & Campos 1989). The number and severity of outbreaks have since increased, and the disease has also been diagnosed in all salmonid species farmed in the region (Enriquez 1995; Olsen et al. 1997).

Losses attributed to piscirickettsiosis in the Chilean salmonid industry exceeded US$100 million in 2006 (Bustos 2006), and the condition is one of the most significant diseases threatening the salmonid industry. Disease has been reported in all stages of production, including freshwater hatcheries (Gaggero et al. 1995) and marine net pens.

Terminology used to describe disease caused by RLOs can potentially cause confusion. 'Salmon (or salmonid) rickettsial septicaemia' is a general term first coined when piscirickettsiosis was detected in Chile. Piscirickettsiosis in Chile was also referred to as 'coho salmon syndrome' and 'huito disease', but these terms are no longer in common use. Evelyn (1992) also suggested that 'parenthesis disease', which was first noted in Canada during the 1970s, is likely to have been caused by *P. salmonis*.

In this manual, the term 'piscirickettsiosis' refers specifically to disease caused by the bacterium *P. salmonis*. Diseases of salmonids caused by RLOs, including *P. salmonis,* are referred to as 'salmonid rickettsial septicaemias' (SRSs). Diseases caused by RLOs other than *P. salmonis* in fish species other than salmonids are referred to as 'rickettsial septicaemias'. Tas-RLO refers to the Tasmanian RLO that may cause disease in salmonids in Tasmania (see Section 1.1).

1.1 Aetiology

Rickettsial bacteria are a relatively small but important group of bacteria that cause disease in humans and other vertebrates, as well as in a wide range of invertebrate hosts (Buxton & Fraser 1977). *P. salmonis,* the aetiological agent of piscirickettsiosis, is a gram-negative, non-motile, intracellular bacterium (Lannan et al. 1999) belonging to the order Thiotrichales and family Rickettsiaceae (Fryer & Hedrick 2003). It is the only member of the genus *Piscirickettsia* (Fryer & Hedrick 2003).

P. salmonis isolated from Chilean coho salmon, referred to as type strain LF-89^T (OIE 2009), has been placed in the American Type Culture Collection as ATCC VR-1361 (Fryer & Mauel 1997). A number of other isolates have been identified (see Section 1.3).

P. salmonis is typically coccoid in shape and 0.5–1.5 μm in diameter (Lannan et al. 1999), and can be observed in tissues as rings or pairs of curved rods (Fryer & Maeul 1997). The organism replicates within membrane-bound cytoplasmic

vacuoles and can occur singularly, in pairs, in diffuse groups or as dense morula-like masses (Cvitanich et al. 1991).

The bacterium presents most commonly in the liver, spleen, kidney and intestine of infected fish, but may also be isolated from a range of other organs, including brain, skin, gills and muscle. Pathology induced by infection (see Section 1.4) is characterised by vasculitis and focal areas of necrosis, resulting in nodules, haemorrhaging and ulceration (Almendras et al. 2000).

In recent years, a number of RLOs that are similar to *P. salmonis* have been detected in a range of non-salmonid fish species. These organisms are either taxonomically different from *P. salmonis* or have not been adequately identified (Chen et al. 1994, 2000). One RLO (Tas-RLO) has been identified in Atlantic salmon and rainbow trout farmed in Tasmania (DPIWE 2004) and causes a disease that is clinically similar to piscirickettsiosis; however, the aetiological agent differs from *P. salmonis* (Corbeil et al. 2005). Tas-RLO has some similar morphological characteristics to *P. salmonis,* but is genetically different from it (Corbeil et al. 2005).

With the exception of Tas-RLO, *P. salmonis* has been identified as the causative agent in all reports of RLO disease in salmonids.

1.2 Susceptible species

Piscirickettsiosis is primarily a disease of salmonid species. Previously thought to be a condition affecting only coho salmon, piscirickettsiosis has subsequently been reported in all salmonid species farmed in Chile (Enriquez 1995). The disease also occurs in freshwater hatcheries (Gaggero et al. 1995). Fish of all ages, from hatchery fingerlings through to market-size fish, are susceptible to disease.

The disease has been reported in coho salmon (*Oncorhynchus kisutch*), Atlantic salmon (*Salmo salar*), rainbow trout *(Oncorhynchus mykiss*), pink salmon (*Oncorhynchus gorbuscha*), cherry salmon (*Oncorhynchus masou*) and chinook salmon (*Oncorhynchus tshawytscha*) (Bravo 1994; Bravo & Campos 1989; Fryer et al. 1992; Olsen et al. 1997).

McCarthy et al. (2005) also reported *P. salmonis* infection in European seabass (*Dicentrarchus labrax*); this is the only report of piscirickettsiosis in a non-salmonid species. In this case, serological and molecular diagnostic testing satisfied World Organisation for Animal Health (OIE) diagnostic criteria for piscirickettsiosis (OIE 2009).

Of the salmonid species listed above, coho salmon and Atlantic salmon are considered to be the most susceptible to piscirickettsiosis, and rainbow trout is relatively resistant (Cvitanich et al. 1991; Garcés et al. 1991; Smith et al. 1995).

Although Atlantic salmon and rainbow trout are farmed in large numbers in Tasmania, no cases of piscirickettsiosis have been reported. Despite this, salmonid species such as brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis)* should be considered susceptible to piscirickettsiosis.

Rickettsial septicaemia not caused by *P. salmonis* has been identified in a number of non-salmonid species (Yuksel et al. 2006). Table 1.1 provides a summary of finfish species susceptible to piscirickettsiosis and SRS.

Table 1.1a Finfish species susceptible to piscirickettsiosis

Scientific name	Common name	Pathogen	Country	Reference
Oncorhynchus kisutch	Coho salmon	*P. salmonis*	Chile	Bravo & Campos 1989; Fryer et al. 1992
Oncorhynchus tshawytscha	Chinook salmon	*P. salmonis*	Canada (Pacific)	Evelyn et al. 1998
			Chile	Fryer et al. 1992; Garcés et al. 1991
Oncorhynchus gorbuscha	Pink salmon	*P. salmonis*	Canada (Pacific)	Evelyn et al. 1998; Fryer & Mauel 1997
Oncorhynchus masou	Cherry (sakura) salmon	*P. salmonis*	Chile	Bravo 1994
Oncorhynchus mykiss	Rainbow trout	*P. salmonis*	Chile	Fryer et al. 1992; Gaggero et al. 1995
Salmo salar	Atlantic salmon	*P. salmonis*	Canada (Atlantic)	Cusack et al. 1997
			Canada (Pacific)	Evelyn et al. 1998; Gaggero et al. 1995
			Ireland	Rodger & Drinnan 1993
			Norway	Olsen et al. 1997
			Scotland	Birrell et al. 2003
Dicentrarchus labrax	European seabass	*P. salmonis*	Greece	McCarthy et al. 2005

Table 1.1b Finfish species susceptible to salmonid rickettsial septicaemia

Scientific name	Common name	Pathogen	Country	Reference
Salmo salar	Atlantic salmon	Tas-RLO	Australia (Tasmania)	Corbeil et al. 2005; DPIWE 2004
Oncorhynchus mykiss	Rainbow trout	Tas-RLO	Australia (Tasmania)	DPIWE 2004

Tas-RLO = Tasmanian *Rickettsia*-like organism

1.3 World distribution

Piscirickettsiosis has been described in salmonids farmed in Chile, Ireland, Scotland, Norway, and the Pacific and Atlantic coasts of Canada (Birrell et al. 2003; Fryer et al. 1992; Fryer & Mauel 1997; House et al. 1999; Olsen et al. 1997). McCarthy et al. (2005) reported infection of European seabass with *P. salmonis* in Greece.

Genetic comparison of isolates from various geographic regions revealed strong similarity between isolates collected in Chile (type strain LF-89^T), Canada (British Columbia) and Norway (Mauel et al. 1996). A separate isolate collected from

Atlantic salmon in Chile had larger genetic differences from LF-89^T, but the differences were not sufficient to place it as a separate species (Mauel et al. 1996).

Countries in the Northern Hemisphere that have reported piscirickettsiosis have not experienced the high mortalities and significant economic losses experienced in Chile (Skarmeta et al. 2000). Isolates of *P. salmonis* from Chile are reported to produce more severe clinical disease than those from the Northern Hemisphere (Lannan et al. 1999), despite genetic similarity. Under experimental conditions, the Canadian isolate (ATL-4-91) and Norwegian isolate (NOR-92) were less virulent than LF-89^T in coho salmon (House et al. 1999).

Non-*P. salmonis* RLOs have been isolated from salmonids in a wide range of geographic areas, including the cool temperate waters of northern Europe, Chile and Tasmania; and the warmer waters of Egypt, Mediterranean France, Greece, Colombia and Taiwan (Yuksel et al. 2006).

Neither *P. salmonis* nor the corresponding disease piscirickettsiosis have ever been reported in Australia.

1.4 Diagnosis of infection with *Piscirickettsia salmonis*

Detailed methods for diagnosing piscirickettsiosis are in the Australian and New Zealand Standard Diagnostic Procedures (ANZSDP)[3] for *P. salmonis* (Corbeil & Crane 2009). The ANZSDP, which is updated as required, should be used as a reference to confirm a presumptive diagnosis of piscirickettsiosis.

The remainder of this section relates to the use and interpretation of tests for the diagnosis and confirmation of piscirickettsiosis.

1.4.1 Field methods: clinical signs and gross pathology

Clinical signs

The clinical signs and gross pathology associated with piscirickettsiosis are similar to those associated with Tas-RLO infection in Tasmanian farmed salmon and some other fish pathogens (DPIWE 2004). Piscirickettsiosis cannot be differentiated from disease caused by Tas-RLO based solely on clinical signs or pathology. Piscirickettsiosis must also be differentiated from a range of other systemic bacterial or viral diseases of finfish (see Section 1.4.4).

In Chile, mortality rates average 20–30% in affected cages, but can be as high as 90% (Branson & Diaz-Munoz 1991). Countries in the Northern Hemisphere (see Section 1.3) have reported lower mortality rates—for example, 0.06% in Canada and Norway (Brocklebank et al. 1992; Olsen et al. 1997).

Clinical signs of piscirickettsiosis vary according to the severity and acuteness of infection. Affected fish are typically dark in colour, inappetant (although acutely affected fish may still have good body fat reserves) and lethargic. Within affected populations, a greater number of fish than usual may be observed swimming in an erratic manner near the surface and/or perimeter of tanks or net pens (Skarmeta et al. 2000).

[3] www.scahls.org.au/procedures/anzsdps2

In peracute cases, affected fish may show limited external signs, and severely affected fish may die without overt signs (Cvitanich et al. 1991; Fryer & Hedrick 2003; Turnbull 1993). Fryer and Mauel (1997) noted that, whereas coho salmon were likely to show signs of disease, Atlantic salmon held under similar conditions died without gross signs.

Chronic cases may have skin lesions—these can range from small haemorrhagic areas of 0.5 cm in diameter to white raised plaques or shallow haemorrhagic ulcers 2 cm in diameter. The most consistent external characteristic of piscirickettsiosis is pale gills due to anaemia (Fryer & Hedrick 2003).

The normal haematocrit range is 40–50% for Atlantic salmon (Cameron 1991) and 32–45% for rainbow trout (Lane 1997; Miller et al. 1983). Haematocrit levels of 18.5% are common in chronically diseased fish, but in some cases can be as low as 2% (Fryer & Hedrick 2003). Therefore, piscirickettsiosis should always be considered as a differential diagnosis in cases of infectious disease in salmonids with haematocrit levels less than 25%.

Gross pathology

Internally, ascites is a common finding in anaemic fish, and there may also be varying degrees of peritonitis. Swollen, discoloured kidneys and enlarged spleens may also be present, with petechial haemorrhage over the visceral fat, stomach, swim bladder or body musculature (Cvitanich et al. 1991; Fryer & Hedrick 2003). Olsen et al. (1997) also reported the kidneys of Norwegian Atlantic salmon being pale with inflammation and petechial haemorrhaging. Occasionally, small white foci are seen in the heart, skeletal musculature, fins and gills (Olsen et al. 1997).

Multifocal liver lesions are observed during chronic infections (Fryer & Hedrick 2003). These characteristic doughnut-shaped liver lesions, although widely recognised as characteristic of piscirickettsiosis, have only been observed in a small proportion (<20%) of affected fish (Fryer & Hedrick 2003). Liver lesions are off-white to yellow circular foci of varying sizes, up to 5–6 mm in diameter. Central or peripheral haemorrhaging occurs within the centre of foci, often raised above the liver surface. The centre of these lesions can progressively become necrotic and collapse inward, resulting in the characteristic doughnut shape. In some cases, the liver may also appear discoloured (i.e. grey–green) or have haemorrhagic spots across the surface (Cvitanich et al. 1991; Olsen et al. 1997).

1.4.2 Laboratory methods

Sample submission

For general information regarding sample collection and submission, refer to the ANZSDP *Collection and submission of samples for investigation of diseases of fin fish*.[4]

Samples must always be submitted to the relevant state or territory government veterinary laboratory. It is advisable to contact the relevant laboratory for specific instructions on appropriate sample collection and preservation. Unless otherwise advised, fixed material should be submitted for histopathology, together with fresh samples of liver, kidney and spleen suitable for cell culture and/or analysis by polymerase chain reaction (PCR).

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[4] www.scahls.org.au/procedures/anzsdps2

Fresh tissues should be collected using aseptic technique and transferred to a sterile container for transport. Fixed tissue must be preserved using 10% neutral-buffered formalin.

P. salmonis is inactivated by freezing (Fryer & Mauel 1997). Therefore, tissues collected for culture **must not be frozen**. Instead, they should be immediately placed on ice at 4 °C and transported to the receiving laboratory as soon as practicable. Transport media for tissues for cell culture **must not** contain antibiotics.

Tissues that are to be screened using PCR may be stored at –20 °C before and during transportation. If this is not possible, tissue preserved in 95% ethanol should be submitted for analysis.

Microscopy

Samples for collection

Kidney, liver and spleen should be sampled for histopathological examination (Corbeil & Crane 2009). However, it is recommended that a complete suite of fixed tissues be submitted from affected fish, including brain, gills and gut, together with any other organs showing lesions.

Smears or imprints from the kidney, liver and spleen should also be prepared (Corbeil & Crane 2009).

Histopathology

The most significant histological finding in cases of piscirickettsiosis is the presence of small (0.5–1.5 µm in diameter) pleomorphic RLOs, usually within the cytoplasmic vacuoles of host cells, although they may also be observed free in the tissue (Turnbull 1993). RLOs are basophilic and appear as amphophilic spheres in tissues stained with haematoxylin and eosin.

Histopathological changes resulting from piscirickettsiosis are considered as vasculitis with focal areas of inflammation and necrosis (Almendras et al. 2000). Changes are observed throughout internal organs, with the most prominent pathology found in the liver, kidney, spleen and intestine. Liver lesions are often severe, and RLOs can be observed in the cytoplasm of degenerating hepatocytes (Lannan et al. 1999). Infiltrating mononuclear cells accompany necrotic foci of hepatocytes. In the most chronic cases, necrotic tissue can be observed below the pale granulomatous foci.

Necrotic haematopoietic cells in the kidney and spleen are prominent during the acute phase of the disease. Necrosis precedes granulomatous inflammation (OIE 2009). Vascular and/or perivascular necrosis may also occur in the liver, kidney and spleen. Meningitis, endocarditis, peritonitis, pancreatitis and branchitis may be observed, with associated chronic inflammatory and vascular changes similar to those in the liver and haematopoietic organs (OIE 2009).

Individual or paired organisms enclosed within membrane-bound vacuoles in the cytoplasm of hepatocytes and mononuclear cells can be observed in the liver of affected fish using transmission electron microscopy. The organisms are spherical or slightly ovoid in shape, with a diameter of 0.8–1.2 µm, and bound by two

membranes: a closely apposed inner layer and a rippled outer membrane (Olsen et al. 1997).

Tissue smears and imprints

Giemsa-stained tissue smears or imprints from infected organs exhibit darkly stained pleomorphic RLOs, commonly in coccoid or ring form, with a diameter of 0.5–1.5 μm.

Culture methods

Cvitanich et al. (1991) originally described in detail the culture of *P. salmonis* in fish cells. The OIE *Manual of diagnostic tests for aquatic animals* (OIE 2009) and the ANZSDP for *P. salmonis* (Corbeil & Crane 2009) describe the diagnostic procedure for culturing *P. salmonis* using CHSE-214 cells. Briefly, cell monolayers are grown in the absence of antibiotics and inoculated with aseptically sampled homogenised kidney tissue. Cells are incubated at 15–18 °C for 28 days or until a cytopathic effect (CPE) is evident. If no CPE is observed after 28 days, cells should be incubated for a further 14 days.

Inoculation onto cell lines was previously considered essential for the culture of RLOs. However, agar supporting the growth of *P. salmonis* has since been developed. Sheep blood agar supplemented with 0.1% L-cysteine and 1% glucose (Mauel et al. 2008), and cysteine heart agar supplemented with 0.1% L-cysteine, 1% glucose and 5% sheep blood (Mikalsen et al. 2008) both provide essential nutrients and growth compounds for *P. salmonis.*

Although not described in the OIE *Manual of diagnostic tests for aquatic animals* (OIE 2009) or the ANZSDP (Corbeil & Crane 2009), agar culture is an alternative method of diagnosis when there is co-infection with another pathogenic agent (M Crane, CSIRO Australian Animal Health Laboratory, pers. comm., 2010).

Molecular techniques

Polymerase chain reaction

The PCR-based molecular diagnostic technique for the diagnosis of piscirickettsiosis was developed by Mauel et al. (1996), and is described in the OIE *Manual of diagnostic tests for aquatic animals* (OIE 2009) and the ANZSDP for *P. salmonis* (Corbeil & Crane 2009). This two-step PCR assay amplifies a target region of the small subunit ribosomal RNA gene (16S rRNA). An alternative one-step PCR assay is also available, which amplifies a target region of the internal transcribed spacer (ITS) DNA of the rRNA operon (Marshall et al. 1998).

A TaqMan® real-time PCR assay (Corbeil et al. 2003) is used to detect Tas-RLO in preference to the diagnostic methods described by the OIE. Nucleotide sequencing of a Tas-RLO isolate has revealed mismatches between the *P. salmonis* sense (PS2S) and antisense (PS2AS) PCR primers used in the PCR assay recommended by the OIE and the Tas-RLO target region of the genome (R Morrison & J Carson, Tasmanian Department of Primary Industries, Parks, Water and Environment [DPIPWE], unpublished data). The TaqMan® assay target amplicon is at the 5′ end of the large ribosomal subunit (23S rRNA).

The TaqMan® assay cannot differentiate between Tas-RLO and *P. salmonis*. Consequently, a conventional PCR assay (Marshall et al. 1998) followed by nucleotide sequencing of the amplicon(s) must be used to identify the aetiological

agent. A 19–base pair deletion in the 3′ end of the ITS amplicon will distinguish Tas-RLO from *P. salmonis*. The ITS from Tas-RLO has been partially sequenced (Genbank accession number AY578985).

DNA hybridisation

Dot-blot and in-situ hybridisation assays for detecting *P. salmonis* (Venegas et al. 2004) use the PCR primers designed by Mauel et al. (1996).

Immunohistochemical (immunoperoxidase) assays

P. salmonis identification by an immunoperoxidase assay is a standard diagnostic procedure where specific antibodies are available. Infected tissues are fixed and can be stored until later use. Fixed preparations are incubated with a primary antibody preparation that binds with specific epitopes. Following staining, any bacteria recognised by the primary antibody are identified by a colour change. The ANZSDP (Corbeil & Crane 2009) contains further information.

1.4.3 Confirmation of infection

A presumptive diagnosis of piscirickettsiosis is made following clinical and pathological observations consistent with piscirickettsiosis. *P. salmonis* is confirmed following histopathological examination and isolation in tissue culture, combined with identification by either immunofluorescence or immunoperoxidase staining and dot-blot DNA hybridisation. PCR assays are available for the rapid identification of *P. salmonis* in clinically affected animals. DNA sequencing of PCR products is required to differentiate between *P. salmonis* and Tas-RLO.

Alternative diagnostic methods to those outlined in the OIE *Manual of diagnostic tests for aquatic animals* (OIE 2009) and the ANZSDP for *P. salmonis* (Corbeil & Crane 2009) include the use of serum as a DNA template for PCR (Marshall et al. 1998) and enzyme-linked immunosorbent assay (ELISA) (Aguayo et al. 2002). Using ELISA for diagnosis would depend on the availability of appropriate capture and detection antisera.

1.4.4 Differential diagnosis

As a systemic bacterial infection, piscirickettsiosis produces a range of clinical and gross pathological signs that could occur due to infection with any one of a range of infectious agents. The clinical signs documented for piscirickettsiosis are not pathognomonic and will vary depending on the severity or stage of infection. Disease in salmonids must therefore be differentiated from other systemic bacterial or viral diseases of finfish, including:

- diseases endemic to Australia
  - disease caused by Tas-RLO
  - systemic disease caused by *Aeromonas salmonicida* biovar Acheron (atypical *A. salmonicida*)
  - systemic disease caused by *Vibrio anguillarum*
  - disease caused by epizootic haematopoietic necrosis virus
- diseases exotic to Australia
  - disease caused by non-*P. salmonis* RLOs
  - viral haemorrhagic septicaemia

- infectious haematopoietic necrosis
- infectious pancreatic necrosis
- systemic infection caused by exotic species of *Vibrionaceae* or biovars of *A. salmonicida*, including subspecies *salmonicida*.

1.5 Resistance and immunity

The immune response to *P. salmonis* is typical of that seen in infections caused by intracellular pathogens, with a poor antibody-mediated response. Although fish have been shown to react in response to many antigens of *P. salmonis*, the dominant protective antigen has not yet been identified (Birkbeck et al. 2004).

The first piscirickettsiosis vaccines were used in Chile during 1999. Although they received strong initial uptake by the Chilean salmonid industry, vaccination is reported to have had only limited success on farms (Bravo & Midtling 2007). Vaccine use has subsequently declined, and farmers rely primarily on antibiotics to control disease.

Only a limited number of experimental piscirickettsiosis vaccine trials have been reported, including trials of inactivated *P. salmonis* products and recombinant vaccines.

Preparations using whole-cell bacterins, with and without adjuvants, have been trialled, with variable results (Kuzyk et al. 2001a; Smith et al. 1995). Protection against *P. salmonis* has been established using formalin-inactivated and heat-inactivated cells, with the heat-inactivated cells providing greater protection (Birbeck et al. 2004). Birbeck et al. (2004) proposed that the protective antigen(s) are heat stable and are most likely lipopolysaccharides found within the cell wall. This is also considered to be the source of protective antigens in vaccines that are effective against other fish diseases, including vibrios and other bacterial pathogens (Evelyn 1984; Stevenson 1997). Despite a response to heat-inactivated cell vaccines, best results have been reported from fish injected with recombinant vaccines containing outer-surface proteins (Fryer & Hedrick 2003; Kuzyk et al. 2001b).

Currently, there are no anti-piscirickettsiosis vaccines available in Australia. An anti-Tas RLO vaccine that is being developed by DPIPWE may be used if cross-protection against *P. salmonis* can be demonstrated. However, a number of commercially available vaccines are marketed overseas as being effective against piscirickettsiosis (Table 1.2).

Table 1.2 Summary of commercially available anti-*P. salmonis* vaccines

Manufacturer	Name of vaccine	Number of antigens[a]
Recalcine	Ricketvac Oleo	1
Agrovet	SRS vaccine	1
Agrovet	SRS/IPNV vaccine	2
Microtek	Bayovac-SRS	1
Microtek	Bayovac-3.1	3
PHARMAQ	ALPHA JECT® 4-1	4
PHARMAQ	ALPHA JECT® micro 3	3
PHARMAQ	ALPHA JECT® micro 2	2
Novartis Animal Health	Birnagen Forte® 2	2
Novartis Animal Health	Birnagen Forte® 3	3
Novartis Animal Health	Birnagen Forte® 4	4

IPNV = infectious pancreatic necrosis virus; SRS = salmonid rickettsial septicaemia
a Monovalent or polyvalent vaccine preparation

1.6 Epidemiology

Although piscirickettsiosis has been reported primarily from marine fish farms, it has also been reported from freshwater facilities (Almendras et al. 1997; Bravo 1994; Gaggero et al. 1995).

Disease onset frequently follows the transfer of fish from freshwater hatcheries to seawater sites. In Chile, clinical signs of piscirickettsiosis are typically observed 6–12 weeks after the transfer of smolt to marine farming sites (Branson & Diaz-Munoz 1991; Fryer et al. 1992; Marshall et al. 1998). In other regions, Rodger and Drinan (1993) reported disease in Atlantic salmon 5–6 months after transfer to sea water in Ireland, and Grant et al. (1996) reported infection 3–5 months after transfer in Scotland. Disease was also diagnosed in Norwegian salmon smolt following transfer to sea water, with low cumulative mortalities lasting 1–3 months.

Piscirickettsiosis is commonly associated with environmental stressors, including fluctuations in water temperature, severe storms and algal blooms. However, significant losses have also occurred in the absence of obvious predisposing factors. See Section 1.6.4 for additional information on risk factors.

1.6.1 Incubation period

The incubation period is the period between first infection of the host by the pathogen (in this case *P. salmonis*) and first appearance of clinical signs (Thrushfield 2007). Some infectivity trials have used the presence of *P. salmonis* as an indicator of disease, but all use death as the indicator rather than clinical signs. Therefore, the information in this section refers to the period from infection to first mortality.

The incubation period for piscirickettsiosis depends on the bacterial isolate, the dose at which it is applied to the host, the route of infection, environmental factors (such as water temperature) and host factors (such as immune status, physiological status, species and age).

Smith et al. (1995) reported deaths from piscirickettsiosis as soon as 2 days following intraperitoneal inoculation of rainbow trout with *P. salmonis* (LF-89). Other studies have reported deaths 8–29 days after similar intraperitoneal inoculation in other species (Birkbeck et al. 2004; Garcés et al. 1991; Rise et al. 2004).

Fish infected via the skin, gill or oral routes (the likely routes of infection during natural horizontal spread) died 10–14 days after first infection (Smith et al. 1995). Piscirickettsiosis-related mortalities were reported in salmon as soon as 2 weeks following their introduction into infected seawater areas in Chile (Almendras & Fuentealba 1997).

Based on the above information and for the purposes of this manual, the incubation period for piscirickettsiosis under natural conditions is estimated to be 10–14 days. This period may vary depending on the host and environmental circumstances, and should only be used as a guide.

1.6.2 Persistence of the pathogen

Extracellular survival

Both temperature and salinity affect survival of *P. salmonis* outside the host. *P. salmonis* has survived for extended periods in sea water but is rapidly inactivated in fresh water (Lannan & Fryer 1994). The period of extracellular survival is also greater at cooler temperatures (5 °C), and decreases as temperature increases. Under experimental conditions, *P. salmonis* survived in sea water for at least 21 days at 5–10 °C, 14 days at 15 °C and 7 days at 20 °C. The pathogen did not persist at temperatures above 25 °C (Lannan & Fryer 1994).

Marine culture of salmonids in Australia commonly occurs in water temperatures ranging from 12 °C to 20 °C. Therefore, *P. salmonis* could survive within the marine environment for up to 2 weeks.

In comparison, *P. salmonis* is almost immediately inactivated in fresh water. Although the disease has been reported in salmon cultured in fresh water (Gaggero et al. 1995), rapid inactivation of the pathogen has the potential to limit horizontal spread and may explain why the disease is infrequently observed in fish held in fresh water (Lannan & Fryer 1994).

Infection reservoirs

The possibility of persistence of *P. salmonis* in an intermediate host is discussed in Section 1.6.3.

Inactivation

P. salmonis is a gram-negative rickettsial bacterium with no evidence of a resistant spore stage. Although specific details on susceptibility to decontamination techniques have not been documented, the AQUAVETPLAN **Operational Procedures Manual—Decontamination** indicates that this type of bacterium should be susceptible to a range of disinfection treatments.

A greater than 99% reduction in bacterial titres was reported by Fryer and Mauel (1997) following a single freeze–thaw cycle at –70 °C, suggesting that *P. salmonis* can be inactivated by freezing.

More details on decontamination are provided in Section 2.4.8, and in the AQUAVETPLAN **Operational Procedures Manual—Decontamination**.

1.6.3 Modes of transmission

Horizontal transmission

Although there is still some conjecture about the major mode of transmission of *P. salmonis* under natural conditions, direct horizontal transmission has been experimentally demonstrated in sea water and fresh water (Cvitanich et al. 1991). The pathogen enters the host through oral routes, gills or skin (Smith et al. 1999). Although intact skin and gills can be penetrated by *P. salmonis*, there is an increased risk of infection following injury to these organs (Smith et al. 1999). The bacterium may be excreted in bile, faeces and urine from live fish, making coprophagy another viable route of infection (Inglis et al. 1993; Salinas et al. 1997).

Infection is considered to occur primarily through horizontal transmission, since the bacterium is capable of surviving in marine waters for extended periods (Fryer & Hedrick 2003; Lannan & Fryer 1994). However, in fresh water, unless the bacterium is protected within host cells or other biological material, rapid inactivation makes successful horizontal transmission unlikely (Lannan & Fryer 1994).

Horizontal transmission is more likely in regions with slow-flowing water and can occur without direct contact between fish (Almendras et al. 1997).

Vertical transmission

It is not known whether vertical transmission or transmission via vectors occurs. Vertical transmission has been demonstrated under experimental conditions (Fryer & Mauel 1997).

P. salmonis has been detected in milt, eggs and coelomic fluid from infected broodstock, indicating that vertical transmission does occur (Larenas et al. 2003). Larenas et al. (2003) estimated that 10% of eggs and fry originating from one or more infected broodstock were infected with *P. salmonis*.

P. salmonis can adhere to the surface of eggs, can occur within the yolk of unfertilised eggs, and is capable of penetrating the ovum via 'piscirickettsial attachment complexes' (Larenas et al. 2003). This has serious implications for the biosecurity of hatcheries, because the surface disinfection of fertilised eggs may not inactivate all *P. salmonis* bacteria.

Intermediate hosts

It is unclear if an intermediate host is involved in the natural transmission of piscirickettsiosis. Although most rickettsial pathogens gain access to terrestrial hosts via arthropod vectors (Weiss & Moulder 1984), no intermediate vector has been identified for *P. salmonis*. However, since *P. salmonis* has been detected in the tissues of invertebrate parasites (see below), the possibility of these acting as intermediate hosts or vectors for disease transmission should not be excluded.

P. salmonis can replicate in insect- and frog-derived cell lines, suggesting that it has the potential to persist in invertebrates and non-fish poikilotherms (Birkbeck et al. 2004). The parasitic isopod *Ceratothoa gaudichaudii*, commonly associated with cultured salmon in Chile, has been identified using an indirect fluorescent

antibody test as a host for *P. salmonis* (Garcés et al. 1994); however, the importance of this parasite in the transmission of the disease is unknown. Isopod parasites sporadically occur in marine salmon culture in Tasmania (DPIPWE, unpublished data).

Reservoirs of infection in marine finfish species have been suspected. Testing of non-salmonid species in Chile failed to demonstrate evidence of reservoirs of infection in non-salmonid finfish (Garcés et al. 1994). However, *P. salmonis* infection in European seabass (McCarthy et al. 2005) suggests that such a reservoir may exist.

1.6.4 Factors influencing transmission and expression of disease

Risk factors identified for outbreaks of piscirickettsiosis are described below.

Physiological status of the host

The onset of piscirickettsiosis commonly follows the transfer of fish from freshwater hatcheries to marine sites. Smoltification—the physiological adaptation of young fish to live in sea water—can result in prolonged periods of stress. Fish not fully adapted for survival in sea water can suffer osmotic shock, resulting in immunosuppression, increased susceptibility to infection and thus increased risk of disease.

Water temperature

Research in Chile has indicated that temperature is a significant epidemiological factor in the expression of piscirickettsiosis. Atlantic salmon challenged in fresh water with *P. salmonis* had greater survival rates at 7.5–8.5 °C than those held at 16 °C (Birbeck et al. 2004)

The optimal temperature for growth of *P. salmonis* in vitro is 15–18 °C (Lannan et al. 1999), which corresponds with water temperatures reported during disease outbreaks in Chile (Branson & Diaz-Munoz 1991).

Environmental stress

Outbreaks of disease have been reported to occur in association with algal blooms (Olsen et al. 1997; Yuksel et al. 2006). Algal blooms can reduce oxygen levels in water, produce toxins and physically clog the gills. Algal cells may also cause physical damage to the gills and skin of fish, reducing their ability to maintain osmoregulation. Ongoing chronic stress may result in reduced immune responses.

Outbreaks of disease following fluctuating water temperatures and severe storms have been reported in Chile and Norway. Both have potential to cause acute stress in net-pen populations, resulting in immunosuppression.

Concurrent infection with other pathogens

Chronic infections can increase susceptibility to disease and may be a factor in outbreaks of piscirickettsiosis.

Infectious pancreatic necrosis virus has been detected in fish affected by piscirickettsiosis in Norway (Olsen et al. 1997). In Chile, co-infection with *Renibacterium salmoninarum*, the aetiological agent of bacterial kidney disease, has been reported. In the case of Tas-RLO, an aquatic reovirus has consistently been cultured from tissues of Atlantic salmon affected by SRS. This virus is considered to be an important risk factor for the expression of clinical disease of piscirickettsiosis. Caged fish that tested positive for Tas-RLO without the presence of aquatic reovirus did not display signs of clinical disease and did not have increased mortality (DPIPWE, unpublished data).

Stock handling and management

Management practices considered to increase the risk of disease transmission include:

- high stock rates for net pens
- stocking multiple year classes on particular leases or sites
- poor handling practices that cause damage to stock
- poor size grading within net pens that leads to a wide range of fish sizes and social domination within individual net pens
- poor feeding regimes.

Several husbandry practices have been reported to help reduce losses caused by piscirickettsiosis. These include rearing fish at lower densities, rearing fish in fallowed sites, and holding only single year classes of stock on individual sites (Evelyn et al. 1998).

Controlling ectoparasites may also be important to reduce skin damage and possible vector transmission.

Although the role of vertical transmission remains unclear, screening of broodstock for subclinical infection is advisable.

1.7 Impact

P. salmonis was the first rickettsial agent to be identified as the cause of fish disease and has rapidly become one of the major diseases affecting salmonid production in Chile. Losses due to mortalities attributed to SRS in Chile were reported to exceed 10 million fish in 1995 (Smith et al. 1997) and currently exceed US$100 million annually (Bustos 2006).

Losses due to piscirickettsiosis in the Northern Hemisphere have been low in comparison with Chile, but there is an increasing number of reports of disease incidence in Scotland and Norway (Olsen 2003; Reid et al. 2004).

An outbreak of piscirickettsiosis in Australia would seriously compromise the viability of the salmonid aquaculture industry. In Tasmania, the salmonid aquaculture industry has grown rapidly and is one of the most important animal production industries in the state. A severe disease event involving salmon would have major economic and social impacts in Tasmania. In other states, salmonids are largely grown in fresh water and provide important recreational fisheries. As survival of *P. salmonis* is poor in fresh water, its effects in these states would likely be less significant.

2 Principles of control and eradication

Piscirickettsiosis was first described in farmed coho salmon in Chile in 1989 (Bravo & Campos 1989) and quickly became a significant disease affecting all farmed salmonid species in the region. To date, attempts to control piscirickettsiosis in Chile have had limited success, with mortality in marine net pens ranging from 20% to 90% (Bravo & Campos 1989).

The disease has been reported in a range of salmonid species in the Northern Hemisphere, including Atlantic salmon and rainbow trout, the two salmonid species predominantly farmed within Australia.

In Australia, detection, surveillance and eradication efforts for piscirickettsiosis in Tasmania would be complicated by the presence of Tas-RLO (Tasmanian *Rickettsia*-like organism), requiring differentiation between *Piscirickettsia salmonis* and Tas-RLO.

This section provides background information to assist managers in responsible authorities make informed decisions regarding appropriate response options.

2.1 Control options

There are essentially three broad control options for *P. salmonis* in Australia:

- eradication
- regional containment and zoning
- control and mitigation of disease.

2.1.1 Eradication

Eradication as a disease response option aims to eradicate *P. salmonis* from the infected area and ensure that it does not spread to other regions within Australia.

Eradication will have the greatest long-term benefit to Australia, but it requires the highest level of commitment by both industry and government. To be effective, this strategy requires rapid deployment of significant resources in the form of finance, personnel, equipment and time. Disease eradication strategies in aquatic animals normally involve an emergency harvest and/or destruction of all infected and at-risk stock.

Depending on the circumstances of the disease outbreak, eradication may not be considered feasible or cost-effective, particularly if infection has spread to wild fish populations.

Currently, there are no formal agreements regarding compensation to owners for losses resulting from the destruction of stock as part of an eradication program for an emergency aquatic animal disease (EAAD).

2.1.2 Regional containment and zoning

Regional containment and zoning restricts *P. salmonis* to specific areas or regions where management strategies have been established (e.g. vaccination, year-class

separation, movement restrictions, partial destocking of clinical populations, stocking with resistant stock types), with the aim to reduce the prevalence of disease over time.

This option uses strict controls over the movement of live fish, fish products, equipment and personnel within and from the affected region. It also requires ongoing surveillance to determine prevalence within the designated area (see Section 2.4) and to ensure that infection has not spread to neighbouring regions.

A regional containment and zoning response strategy is normally chosen if eradication is not considered feasible because of the possibility that infection has established in wild fish populations, or because of the high financial cost associated with destruction of farmed stock as part of an eradication program.

This option is most likely to succeed when the disease outbreak is within a distinct geographic region that has physical barriers likely to restrict the spread of *P. salmonis*.

The ultimate aim of any regional containment and control program should be to eradicate disease within infected regions. This is achieved over the long term, using strategic management rather than complete destocking.

2.1.3 Control and mitigation of disease

Control and mitigation aims to decrease the incidence and severity of clinical outbreaks of piscirickettsiosis.

Although this option is unlikely to result in complete eradication of the disease, it will incur the lowest financial costs. To be effective, this option requires agreed control strategies that are uniformly enforced across the whole industry with the support of government. Although losses associated with stock destruction will be limited, this option may result in significant loss of production to industry.

The option of control and mitigation of disease should only be selected when eradication is not considered feasible and spread of the pathogen cannot realistically be restricted to a clearly defined region.

2.2 Stages of the emergency response

As with any significant exotic disease event, suspicion of piscirickettsiosis or the detection of *P. salmonis* must trigger an EAAD response using recognised emergency response principles. These principles and the agreed response format are outlined in the AQUAVETPLAN **Management Manual—Control Centres**.

There are four main phases of activation in any emergency animal disease response:

- investigation
- alert
- operational
- stand-down.

Progression from one phase to the next depends on the nature of the emergency, available information and the agreed response option.

2.2.1 Investigation phase

An investigation phase exists while preliminary activities are undertaken in response to reports of suspicion of disease. These activities are aimed at confirming or ruling out the presence of piscirickettsiosis or its causative agent (*P. salmonis*). Where there is reasonable evidence to support a diagnosis of piscirickettsiosis, quarantine measures would normally be established for the affected property, premises or region.

2.2.2 Alert phase

Once there is reasonable suspicion that an outbreak of piscirickettsiosis exists, the relevant state or territory declares an alert phase. This phase is used to inform relevant personnel, stakeholders and government agencies of the possibility of an EAAD event and allows relevant personnel to make necessary preparations. During this phase, investigations will normally be undertaken to describe the nature of the disease event and determine the area(s) affected.

2.2.3 Operational phase

An operational phase exists once the presence of piscirickettsiosis or *P. salmonis* has been established and the decision to undertake formal emergency disease control measures is confirmed.

A number of activities would normally be undertaken during the operational phase:

- *Quarantine and movement restrictions*. These involve restrictions on the movement of animals, materials, waste, personnel, vehicles and equipment; controls over water movement; and implementation of other relevant biosecurity procedures. Imposition of quarantine and movement restrictions requires rapid identification of the infected area and additional areas considered at risk. Under most circumstances, the areas to be placed under movement restriction (referred to as 'disease management areas') will extend well beyond the infected area; this area will decrease over time as a more accurate description of affected areas becomes available.
- *Destruction and disposal of diseased stock*. Rapid removal and appropriate disposal of diseased fish is a high priority for controlling the spread of disease. Infected fish are the most likely source of further infection, because pathogens will be shed into the water from live fish in mucus, faeces or urine, or from decomposing fish carcasses.
- *Emergency harvest*. Emergency harvest of fish within affected regions may be an option to reduce host biomass and allow farms to recoup a portion of production costs. Under most circumstances, emergency harvest will be the most efficient method of removing subclinical stock from production sites.
- *Treatment of affected populations*. If emergency harvest or destruction of stock are not available as immediate options, prophylactic treatment of at-risk populations may be necessary to reduce disease prevalence and shedding of the pathogen into the environment. Antibiotic treatment should occur in a strategic manner, using approved compounds and adhering to appropriate withholding periods.
- *Ongoing surveillance*. Surveillance of neighbouring and high-risk areas is required to accurately confirm that *P. salmonis* has not spread beyond the boundaries of the infected area.

- *Decontamination*. Appropriate cleaning and disinfection of infected facilities and equipment, as well as personnel and equipment moving between disease management areas, will be required.

Not all of the activities listed above will be appropriate for all outbreaks of piscirickettsiosis. Their use will depend on the type of production system involved (e.g. open, semi-open, semi-closed or closed system—see Section 2.3), as well as the response option chosen for each disease event.

2.2.4 Stand-down phase

The stand-down phase occurs once it is determined that the disease threat is no longer present or the disease is considered to be under control. A stand-down phase may also be implemented when it is considered unrealistic or not economically viable to continue with operational measures.

2.3 Aquatic animal systems

For the purpose of AQUAVETPLAN, production systems are classified into one of four categories:

- open systems
- semi-open systems
- semi-closed systems
- closed systems.

The AQUAVETPLAN **Enterprise Manual** explains each of these production types in the context of generic disease control. The following sections provide a summary of these systems, specifically in relation to controlling piscirickettsiosis.

2.3.1 Open systems

Open systems include rivers, lakes and marine ecosystems in which there is no control over fish or water movement. Wild salmonid populations in Australia occur in open freshwater systems (river and lakes) in the southern states. In Tasmania, salmonid species also occur within open marine systems.

General characteristics of open systems include:

- a comparatively large potentially infected area
- very limited or no control over water movements
- very limited or no control over stock movements
- very limited or no control over non-target fish movements
- limited or no ability to decontaminate the system
- control measures normally restricted to the movement of personnel, stock and equipment in and out of the infected area.

2.3.2 Semi-open systems

Semi-open systems are characterised by control over stock but no control over water movement. Semi-open systems (marine net pens) are used extensively for the production of farmed Atlantic salmon and rainbow trout in Tasmania, and

have previously been used to farm these species in South Australia and Western Australia.

In this type of production system, infected areas are likely to involve whole water bodies such as estuaries, lakes or bays. Disease control operations should concentrate on controlling spread of disease through the appropriate destruction and disposal of susceptible stock, together with decontamination of personnel, equipment, machinery and vessels leaving the infected area.

General characteristics of semi-open systems include:

- extremely limited or no control over water movement
- significant control over stock movements
- limited control over non-target fish species
- limited or no ability to decontaminate the environment other than pens or cages
- control measures usually restricted to personnel or equipment moving out of infected areas, destruction of stock and safe disposal of infected material.

2.3.3 Semi-closed systems

In semi-closed production systems, stock and farm areas are not in direct contact with natural waterways. Water is usually taken from an adjacent natural source and then discharged further downstream. Release of this water may be continuous or intermittent.

Freshwater semi-closed systems are commonly used for the production of juvenile salmonid species in Tasmania, which are then moved to marine sites 12–18 months after spawning. In other states, stock is normally held in semi-closed systems for the whole production cycle.

General characteristics of semi-closed systems include:

- some ability to control water movements
- ability to control stock movements
- reasonable ability to exclude non-target fish species
- reasonable ability to decontaminate the infected premises
- possible requirement to disinfect large volumes of water.

2.3.4 Closed systems

In closed systems, both the stock and the water are closely controlled, usually in tanks with attached biofiltration systems.

Closed systems are commonly used during the early stages of production of salmonids in Tasmania (i.e. incubation of eggs, fry and fingerlings), but are less common in other states.

Since infection tends to be confined to the facility and is less likely to be spread through water movement, disease eradication programs in closed systems have the greatest chance of long-term success. However, water entering these facilities is normally disinfected, but water discharged as part of normal operations is not

routinely treated. It is therefore possible, although unlikely, that piscirickettsiosis outbreaks within closed systems could involve neighbouring waterways.

General characteristics of closed systems include:

- generally associated with water recirculation facilities and use of biofiltration systems to treat recirculated water
- good control of water movements
- good control of stock movements
- ability to restrict non-target fish species
- reasonable ability to isolate and disinfect tanks
- ability to disinfect and dispose of large volumes of water, where required.

2.4 Methods to prevent spread and eliminate pathogens

2.4.1 Quarantine and movement controls

The following quarantine and movement restrictions should be implemented immediately upon suspicion of piscirickettsiosis. These measures would normally be initiated during the investigation or alert phases of an EAAD response. Restrictions may be extended following confirmation of the disease, or lifted once *P. salmonis* has been excluded as the causative disease agent.

Establishment of disease management areas

Establishment of appropriate movement control and quarantine areas during an EAAD necessitates the establishment of declared areas. The AQUAVETPLAN **Enterprise Manual** and **Management Manual—Control Centres** contain detailed information about the establishment of declared areas.

Declared areas include the following:

- *Infected area or premises*. This is a clearly defined area (or premises); it may be all or part of a premises, lease or waterway in which disease exists. An infected area may be subject to formal quarantine, control or eradication procedures.
- *Dangerous contact area or premises*. This is an area (or premises) containing susceptible species that show no signs of disease, but, because of the species' high probability of exposure to *P. salmonis*, will be subject to similar quarantine and disease control measures.
- *Suspect area or premises*. This is an area (or premises) that contains at-risk species that will be subject to quarantine and intensive surveillance.
- *Restricted area*. Restricted areas form part of a declared area around an infected area, dangerous contact area or suspect area, and are subject to surveillance and strict movement controls.
- *Control area*. This is part of a declared area in which conditions apply for the entry or exit of specified aquatic animals or fomites. Conditions applied to a control area are normally less restrictive than those for a restricted area.
- *Free area*. Free areas are typically outside the control area and make up the remaining area that is considered free from disease due its geographic isolation or the lack of a susceptible host.

- *Declared area*. This is the area made up of the combined restricted area and control area.

Some of these areas are illustrated in Figure 2.1.

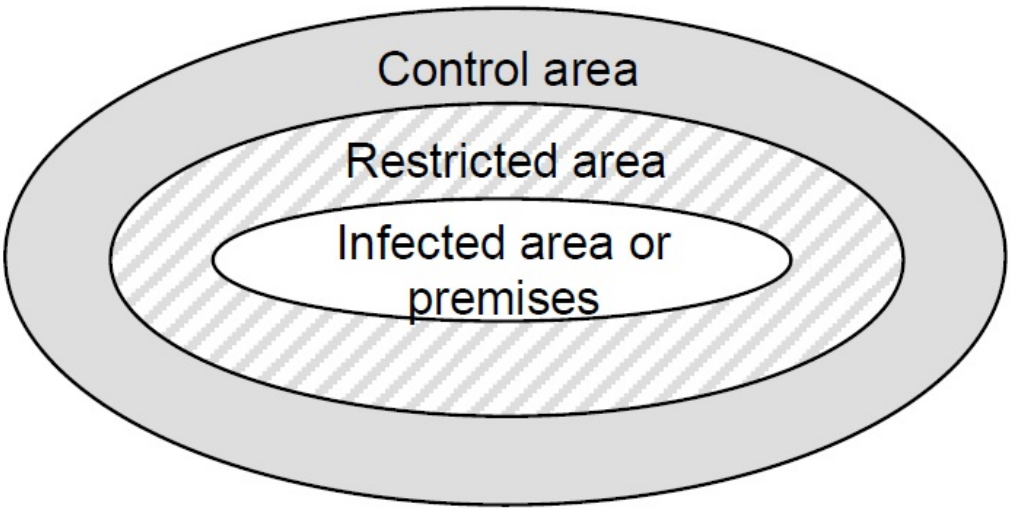

Figure 2.1 **Areas that may be designated during an aquatic animal disease emergency involving piscirickettsiosis**

Establishing declared areas during EAAD events presents difficulties beyond those involved in terrestrial animal disease control. Water movement through and around farms, within streams or rivers, and in the marine environment poses a significant risk for the spread of disease through the transfer of fomites, organic matter containing viable pathogens or wildlife reservoir host species.

If piscirickettsiosis is suspected or detected in wild fish, declared areas will include rivers, lakes, estuaries or coastlines, and will thus be more difficult to define. Consequently, it is appropriate to determine the declared area based on water catchments and the known range of wild host species.

When establishing declared areas, factors that must be considered are:

- the type of production system in which clinical disease or the pathogen is detected
- the ability of the infected premises to restrict water outflow and establish adequate biosecurity
- the potential for establishment of the disease in wild salmonid species
- recent movements of stock, equipment and personnel from or to other production areas or premises
- the proximity of other salmonid stock or production facilities within the same region or catchment.

These factors should then be used to:

- determine the potential for spread within and from the infected area

- predict the extent of infection, as realistically as possible
- identify the incident case and timeline
- attempt to determine the source of infection
- develop an agreed strategy to be undertaken during the response.

When selecting the most appropriate response option, factors that must also be considered are:

- the known geographic extent of *P. salmonis* – for example, an eradication program may be suitable for a single hatchery, whereas regional containment and zoning may be more appropriate if the infection is detected within marine farming regions
- the ability of the EAAD response program to remove infected fish from the water within a reasonable period of time
- the ability and willingness of industry to undertake emergency harvest or destruction of stock within a reasonable period of time
- the ability of the EAAD response program to safely dispose of infected stock and material
- the willingness of industry and government to consistently undertake agreed control measures over a sustained period of time.

Movement controls

During a piscirickettsiosis response program, the following movements should be assessed and, where necessary, appropriate controls should be applied:

- Movement of live salmonids within and out of the declared area.
- Movement of dead fish and salmonid products (including gametes and fertilised ova) within and out of the declared area.
- Movement of potentially contaminated equipment and personnel within or between river systems and marine farms in the restricted area.
- Movement of all susceptible species (i.e. salmonids) between different river systems, hatcheries and marine farming regions outside the declared area.

The feasibility of restrictions and the extent to which they can be enforced will depend on the location of infection, the distribution of affected enterprises, the response option chosen and available resources.

Zoning

If *P. salmonis* was to become established in specific regions of Australia, a zoning policy may be necessary to protect non-infected areas. Zones would be based on the known distribution of *P. salmonis*, potential vectors, known reservoirs of infection, and the geographical and hydrological characteristics of the water bodies or landforms. In practice, zoning will most likely rely on the identification of biogeographic barriers, and state and territory boundaries.

Surveillance and monitoring programs for piscirickettsiosis would be required to support any zoning policy.

Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines[5] and in the OIE *Aquatic animal health code* (OIE 2011).

The Tasmanian salmonid industry has an agreed approach to zoning for EAAD responses. Marine salmonid farming in Tasmania has been divided into four distinct growing regions that are well separated and can be managed separately during disease outbreaks. Disease in other states would need to be assessed according to the location of the outbreak, distribution of susceptible populations and production systems involved.

2.4.2 Tracing

Tracing is the process of retrospectively determining the method and pattern of disease spread. Tracing investigations are crucial for determining all suspect and potential locations of the disease and defining the boundaries of declared areas. The information gathered from tracing activities will also assist with deciding on the most appropriate response option.

The immediate tracing task should be to investigate sources of potentially infected material (trace-back), with the aim of determining the index case (initial site of infection). Movements out of known infected areas (trace-forward) should also be investigated to identify additional sites that are potentially affected.

Tracing should investigate:

- movements of farmed salmonid stock, including broodstock, fingerlings, smolt, gametes and fertilised ova
- natural movements of wild salmonid species
- movements of salmonid produce, including products for human consumption and processing byproducts
- movements of waste material, including faecal material, farm or hatchery mortalities, and waste from processing premises
- water sources and outflow from infected areas—this may include modelling of currents in and around marine farms, or movement of waterways associated with freshwater hatcheries and farms
- vehicle movements, particularly those carrying fish or fish products
- movement of high-risk equipment, including fish-handling equipment, bins, cages and nets
- movements of high-risk personnel who have come into contact with infected fish or material
- facilities used for processing infected fish or fish products.

2.4.3 Surveillance

Surveillance activities are necessary to define the extent of the infection and to detect any new outbreaks of disease outside the infected area. Information obtained during surveillance activities is also used to monitor the progress of ongoing disease response programs.

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[5] www.daff.gov.au/__data/assets/pdf_file/0007/146716/zoning-final-aug.pdf

There are currently no validated methods for diagnosing subclinical *P. salmonis* infections. Preliminary work suggests that polymerase chain reaction (PCR) assays could be useful in this regard, but the epidemiological sensitivity and specificity of the PCR assay has not been validated. Positive PCR results would require confirmation using additional diagnostic tests (see Section 1.4.3).

Recommendations for piscirickettsiosis surveillance programs include the following basic strategies:

- Where available, farm records should be examined in consultation with the property manager and used to help identify high-risk cages and tanks.
- Moribund fish from high-risk tanks and net pens should be examined for gross signs of systemic infection. Signs consistent with piscirickettsiosis may include pale gills, external skin lesions, paleness of internal tissues, oedema, swollen kidneys and/or spleen, bruising or doughnut-shaped lesions over the liver, and petechiation over internal surfaces. However, none of these signs are pathognomonic for the disease, and infected fish may die without overt clinical signs.
- Appropriate samples should be collected for histopathology and PCR analysis. Moribund or clinically affected fish should always be sampled in preference to fish not displaying signs (see Section 1.4.2).
- PCR should be used as the primary surveillance screening test, and histopathology should be used to confirm salmonid rickettsial septicaemia (SRS).
- In areas where RLOs are known to be endemic, further testing must occur to differentiate these from *P. salmonis* (see Section 1.4.3 and the Australia and New Zealand Standard Diagnostic Procedures for *P. salmonis*). Tas-RLO is currently known to be endemic in all salmonid marine-farming zones in Tasmania, but has not been reported in freshwater environments or mainland Australia. No other RLOs have been reported to affect finfish species in Australia.

2.4.4 Treatment of infected host species

Treatment of affected salmonid populations is an option during piscirickettsiosis EAAD response programs where at-risk stock cannot be promptly removed for emergency harvest or disposal, and the control and mitigation strategy has been selected.

In this document, treatment of infected fish is limited to the use of antibiotics. Although vaccines are an important tool in the control of disease, they are not effective in eliminating *P. salmonis* infections. (See Section 1.5 for details on the use of vaccines.)

Appropriate antibiotic treatment has the potential to reduce morbidity and mortality rates, as well as shedding of *P. salmonis* into the environment. However, antibiotic treatment may mask signs of disease and result in carrier animals that can be potential future sources of infection.

Antibiotic treatment during piscirickettsiosis control programs must be well coordinated and should only occur once surveillance activities have been completed.

Efficacy of antibiotics

Streptomycin, gentamycin, tetracycline, chloramphenicol, erythromycin, oxytetracycline, clarithromycin, sarafloxin, flumequine, doxycycline and oxolinic acid are all effective against *P. salmonis* in vitro (Cvitanich et al. 1991; Fryer et al. 1990; Inglis et al. 1993; Jones et al. 1998). *P. salmonis* has been shown to be resistant to penicillin, penicillin G and spectromycin (Cvitanich 1991; Fryer et al. 1990).

Unfortunately, most of the antibiotics listed above have shown only marginal efficacy when used as an oral medication on farms (Cassigoli 1994). Quinolones (in the form of oxolinic acid or flumequine) and oxytetracycline are the compounds most commonly used to treat piscirickettsiosis in Chile (Cassigoli 1994; Evelyn 1992), with oxolinic acid being the preferred drug (Lannan et al. 1999). Development of antibiotic resistance to oxolinic acid and oxytetracycline has been reported (Cassigoli 1994; Smith et al. 1996).

In-feed medication (oxolinic acid administered at 20 mg/kg biomass) has been highly effective in the treatment of SRS caused by Tas-RLO. Oxytetracycline administered at 100 mg/kg biomass is also effective against SRS caused by Tas-RLO (Tasmanian Department of Primary Industries, Parks, Water and Environment, unpublished data). However, oxolinic acid is currently not used in Tasmanian salmonid aquaculture due to an industry-imposed moratorium on its use. It is reserved for human medicine and thus should not be used in food-producing animals (JETACAR 1999). Oxolinic acid has been removed from fish medicine used in several other salmon-producing countries (i.e. Canada, Norway and the United Kingdom).

Available compounds

The Australian Pesticides and Veterinary Medicines Authority (APVMA) registers veterinary chemicals and issues minor-use and emergency-use permits. No antibiotics are registered in Australia for use in salmonids. Minor-use permits have previously been issued for the use of oxytetracycline and florfenicol. Although florfenicol has been used in the treatment of disease in Chile (Cassigoli 1994), information regarding its efficacy is not readily available.

Other treatments

Additional strategies to decrease the risk of vertical transmission of *P. salmonis* include antibiotic treatment of broodstock before spawning and disinfection of eggs. Broodstock should be treated with either oxytetracycline or florfenicol via intraperitoneal injection 30–60 days before spawning, to reduce the prevalence of bacteria within gametes (Bustos et al. 1994; Cassigoli 1994).

Viable fish eggs may also be surface disinfected to reduce carriage of *P. salmonis*. Povidone iodine (100 mg/L available iodine) is recommended as a routine disinfectant for the treatment of eggs following spawning, but would not be effective in treating intracellular carriage of the bacterium. Incorporating antibiotics in water during the hardening of eggs after fertilisation has also been recommended (Bustos et al. 1994).

Further details on regulations regarding the treatment of fish with veterinary chemicals are available from the APVMA and Appendix 1.

Use of any chemical (directly or indirectly) to control an animal disease is also governed by the 'control of use' legislation in each state and territory. The relevant state or territory authority should therefore be consulted for advice before the use of the chemical.

2.4.5 Treatment of host products and byproducts

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must all be considered when determining the appropriate treatment, processing and destination of potentially infected salmonid products and byproducts.

There is no evidence in the literature of *P. salmonis* causing disease in humans. The optimum temperature for growth of *P. salmonis* is 15–18 °C under experimental conditions (Fryer & Mauel 1997). No growth was reported above 25 °C (Fryer & Mauel 1997), suggesting that *P. salmonis* would not be capable of establishing in mammals. Therefore, apparently healthy fish harvested from infected populations are not considered to pose a risk to human health, subject to appropriate drug withholding periods and standard food safety procedures.

A greater than 99% reduction in *P. salmonis* has been reported from contaminated products following freezing (Fryer et al. 1992).

2.4.6 Destruction of hosts

Prompt destruction and disposal of infected stock is an important management principle for all emergency livestock disease events. During EAAD response programs, destruction of infected fish must be well planned, coordinated and humane, and have support from the relevant industry.

Before destruction programs are initiated, the AQUAVETPLAN **Operational Procedures Manual—Destruction** should be consulted and destruction options assessed according to:

- size and number of fish infected or at risk of infection
- resources available
- type of production system involved
- intended use of destroyed stock (e.g. for human consumption or disposal)
- availability of resources and facilities for slaughter, processing and disposal.

Appropriate methods of destruction may include treatment with anaesthetic agents, treatment with chemical poisons, percussion stunning, and sedation followed by exsanguination. During exsanguinations, care must be taken to contain all blood (including blood-water) to ensure that it does not enter the aquatic environment.

In many cases (particularly the destruction of salmonid stocks in marine net pens), efficient destruction and disposal cannot occur without the use of specialised equipment and the support of industry personnel. In the Tasmanian salmonid industry, the destruction of fish is best undertaken using routine stock handling methods. Unless water supplies can be shut off, fish should not be destroyed in-water, but instead should be removed live from tanks and pens before destruction.

All carcasses, waste products and other infected material must be contained during the destruction process and removed appropriately for safe disposal. The AQUAVETPLAN **Operational Procedures Manual—Disposal** and the **Operational Procedures Manual—Decontamination** should be consulted for relevant disposal and decontamination procedures.

In circumstances where destruction and disposal of infected or at-risk stock cannot occur within an acceptable period of time, consideration should be given to treating a proportion of the population with antibiotics to reduce disease prevalence (see Section 2.4.4).

Chemicals used for the destruction or treatment of fish must be approved for that purpose by the APVMA and other relevant state or territory authorities (see Appendix 1).

2.4.7 Disposal of carcasses

During an outbreak of piscirickettsiosis, fish will either die from the disease, be harvested for human consumption or be destroyed as part of the disease control program. Regardless of the destruction process, the carcasses and associated waste products need to be disposed of in an approved, biosecure manner. Contaminated products must be handled and transported to disposal sites in secure containers to avoid spillage and contamination of vehicles and equipment.

In many cases, deep burial of infected material within the infected area will be considered the best short-term option. However, depending on the type of material to be disposed of, other options should also be considered. In most cases, more than one type of disposal method will be required. Rendering, composting, ensilage, soil injection and pasture top-dressing are all options that may be considered.

For more details on the disposal of infected material, see the AQUAVETPLAN **Operational Procedures Manual—Disposal**.

2.4.8 Decontamination

Because of differences in farming enterprises, decontamination protocols will need to be developed for each specific situation by the farm manager and appropriate government personnel. Decontamination protocols should consider:

- the type of enterprise (e.g. farm, processing plant, hatchery, grow-out ponds, water source)
- the construction materials of buildings, infrastructure and equipment
- the design of the site and its proximity to other waterways or buildings
- workplace safety concerns
- the environmental impact of the various decontamination processes
- legislative requirements (e.g. workplace health and safety, environmental protection, chemical use)
- the availability of suitable chemicals and equipment.

To be effective, decontamination programs must be well planned and undertaken in a coordinated manner. Effective decontamination of equipment, structures,

vehicles and personnel requires thorough cleaning before disinfection. This aspect of decontamination is vital, and its importance must not be underestimated.

Wherever possible, cleaning and disinfection of facilities should use fresh water rather than sea water, because *P. salmonis* is rapidly inactivated in fresh water (Lannan & Fryer 1994). Cleaning activities should be structured in a way that avoids drainage into the marine environment.

See the AQUAVETPLAN **Operational Procedures Manual—Decontamination** for details of decontamination methods.

2.4.9 Vaccination

The efficacy of vaccines used overseas is variable. Nevertheless, vaccines may have some role in Australia if a control and mitigation response option is chosen. Later generation recombinant vaccines reportedly have greater efficacy than whole-cell preparations (Fryer & Hedrick 2003; Kuzyk et al. 2001b).

There are currently no piscirickettsiosis vaccines registered for use within Australia, although there is potential for commercial products to be imported at short notice if the AVPMA approved an emergency-use permit. Other permits may be required (e.g. a DAFF Biosecurity import permit and, if genetically modified, Office of the Gene Technology Regulator approval).

Further information relating to piscirickettsiosis vaccines is provided in Section 1.5.

2.4.10 Vector control

Vector-control programs aim to ensure that *P. salmonis* does not spread to areas outside the infected area and does not establish in susceptible wild populations.

Wild fish

Although controlling wild fish will be impossible in most circumstances, attempts should be made to prevent contact between farmed stock and wild fish populations. All salmonid species are considered potentially susceptible to *P. salmonis* infection.

Precautions that may help to reduce transfer of infection to wild fish populations are outlined below.

For marine farms (semi-open production systems):

- ensure that farmed stock do not escape from net pens
- where populations of salmonids have escaped, attempt to remove as many as possible using commercial fishers, netting, traps or other appropriate techniques
- promptly remove clinically infected populations to reduce shedding of *P. salmonis* into the environment
- remove moribund and deceased animals from net pens regularly (i.e. daily) to reduce bacterial shedding into the environment.

For freshwater facilities (semi-closed production systems):

- shut off untreated water outflow from the premises as soon as possible

- remove all fish from settlement ponds, and ensure that appropriate gratings are in place to avoid re-establishment of populations
- remove susceptible fish species from the immediate vicinity of the facility using netting, electrofishing or, where approved, appropriate chemical poisons
- ensure that infected material, including silt from settlement ponds, does not enter natural watercourses.

Invertebrate vectors

No intermediate hosts have been identified for *P. salmonis*, but invertebrates (i.e. copepods, isopods or molluscs) have not been ruled out as potential intermediate hosts or mechanical vectors. Parasites that compromise the integrity of the skin and gills, or result in increased stress of the host, should be considered as increased risk factors or potential vectors.

Attempts should be made to decrease contact between copepods, isopods, molluscs and at-risk fish by reducing organic build-up on nets and removing fouling from boat hulls.

Marine farms should reduce exposure of farmed stock to scavenging invertebrates and wild fish entering pens by using good feeding practices.

Birds

Net pens, raceways, tanks, ponds and mortality disposal pits may attract birds that can potentially act as physical vectors of infection through the movement of infected carcasses or organic matter. These areas must be adequately netted to restrict access by birds and other scavengers.

2.5 Environmental considerations

Environmental factors that must be taken into consideration during a piscirickettsiosis EAAD response program include the following:

- Discharge of infected or potentially infected effluent into catchment areas or natural waterways may lead to further spread of disease and the establishment of infection in wild fish populations. Facilities used to process potentially contaminated material must have adequate controls in place and must not be allowed to discharge liquid wastes without adequate treatment.
- Destruction and disposal of solid waste may have an impact on the environment and allow spread of disease. Sites and methods used to dispose of contaminated material must not allow seepage of pollutants or infected material into groundwater or natural waterways. Pits must have appropriate barriers in place to ensure that they do not present a safety risk to either people or wildlife.
- Control of wild fish species may require the use of specialised fishing techniques or chemical poisons. Appropriate permission and agreed procedures for their use must be established, and their impact must be monitored closely.
- The use of disinfectants and antibiotics could affect the surrounding environment, especially if they are used in large quantities. Minor-use or emergency-use permits will normally include instructions on required

precautions; however, appropriate state and territory authorities should be consulted.

- The relevant environmental authority for each state or territory must be consulted in all cases; regulations may vary between jurisdictions.

See the AQUAVETPLAN **Operational Procedures Manual—Decontamination** for details of decontamination methods.

2.6 Sentinel animals and restocking

Restocking with sentinel fish should only occur after all diseased and potentially exposed fish stocks have been removed from the infected site.

Fish to be used as sentinels following decontamination should be the most susceptible type available; Atlantic salmon smolt from freshwater hatcheries should be used wherever possible. Sentinel fish should come from populations known to be free from *P. salmonis* infection.

Sentinel fish should be placed in tanks or net pens and regularly monitored for signs of disease. Throughout the sentinel period, diseased or clinically affected fish should be examined as soon as they are observed. The recommended sentinel period should be at least three times the incubation period; thus, a minimum sentinel period of 6 weeks is recommended.

2.7 Public awareness

Public awareness and cooperation should be maintained through an awareness campaign incorporating the following activities:

- Maintain regular contact with relevant industry organisations. For piscirickettsiosis, relevant industry groups will include
  - the National Aquaculture Council
  - the Tasmanian Salmon Growers Association
  - the Victorian Trout Association.
- Work with industry liaison officers and industry representatives. The role of the industry liaison officer is described in the AQUAVETPLAN **Management Manual—Control Centres**.
- Erect appropriate signage and publish notifications to make the public aware of restricted areas and biosecurity procedures. Use appropriate electronic (including television) media for high-impact exposure of target groups.
- Establish good contact with the media to ensure that an appropriate and factually correct message is provided at all times.
- Prepare information brochures that provide basic information about the disease.
- Establish communication with local health authorities to ensure that information provided to the media and the general public regarding human health concerns is consistent.
- Provide regular updates of progress to the EAAD response.

Relevant industry contacts are provided in the AQUAVETPLAN **Enterprise Manual**.

2.8 Feasibility of control or eradication of piscirickettsiosis in Australia

The feasibility of controlling an outbreak of piscirickettsiosis depends on the nature and location of the outbreak, and the management strategy adopted. Feasibility of success also depends on the presence of Tas-RLO within infected areas and, where it is present, the ability to establish an effective test that quickly differentiates between Tas-RLO and *P. salmonis*. The presence of Tas-RLO significantly complicates eradication options for piscirickettsiosis and should be taken into account when determining the preferred response strategy.

As described in Section 2.1, there are three possible response options:

- eradication
- regional containment and zoning
- control and mitigation of disease.

Wherever possible, eradication is the preferred option if epidemiological investigations determine an obvious point source of infection that can be contained with minimal or no spread of the pathogen. Eradication has the highest short-term economic cost for both industry and government, none of which is covered by any formal cost-sharing or compensation agreements.

2.8.1 Response option 1: eradication

Eradication should be considered if initial epidemiological investigations indicate:

- limited spread or distribution of infection
- a clearly identified source or limited number of sources
- no apparent involvement of wild fish as reservoirs of infection.

For freshwater hatcheries and similar semi-closed facilities, eradication is considered viable and is the preferred option because of the short survival time of *P. salmonis* in fresh water, the restricted wild host range of *P. salmonis* and the ability to control water flow in these facilities.

For salmonid marine farms, successful eradication of piscirickettsiosis is considered to be much less likely, because of the high viability of *P. salmonis* in sea water (compared with fresh water), the poor control over water flow in such facilities and the much larger fish biomass involved. Eradication might be considered feasible if it can be demonstrated that reservoirs of infection have not become established.

Unexposed fish

Under the eradication option, market-size unexposed fish could be emergency harvested for commercial sale. Fish smaller than market size must be destroyed.

Exposed (or potentially exposed) clinically normal fish

Immediate destruction of exposed fish is essential to prevent further replication of the pathogen and minimise loading of the net-pen environment.

Fish not of marketable size should be immediately destroyed. On-growing undersized fish until they reach market size is not appropriate under the eradication option.

Prophylactic treatment of clinically normal fish with antibiotics is not normally considered under the eradication option, and should only be considered if available resources do not allow the rapid removal of exposed fish.

Emergency harvest of commercial-size fish for human consumption is an appropriate option but must not delay or compromise the eradication effort. Where adequate resources are not available to process all market-size fish within a reasonable period of time, fish should be destroyed and carcasses disposed of appropriately. Processing of fish from infected areas should only occur in approved processing premises with appropriate biosecurity procedures.

Clinically diseased fish

All diseased and dead fish must be removed and disposed of as soon as possible. Infected fish are considered the main source of *P. salmonis* contamination of the environment. Net pens with diseased fish must be totally depopulated, even where only a proportion of the fish display signs of clinical disease.

2.8.2 Response option 2: regional containment and zoning

It is recommended that a disease response program based on the principles of regional containment and zoning be adopted if:

- there are doubts regarding the true extent of the infection
- costs associated with eradication (e.g. costs associated with stock destruction) are considered too high
- production areas can be divided into distinct regions or catchments, and disease does not extend across all regions within a state or territory.

Disease control programs based around regional containment and zoning may be restricted to individual water catchments, specific geographic regions or individual states (e.g. Tasmania), or extend between states (e.g. Victoria and South Australia). The feasibility of such programs for the control of piscirickettsiosis can only be assessed on a case-by-case basis, but they are considered a viable option for both freshwater and marine production systems.

The ultimate goal of regional containment and zoning programs should still be eradication, but eradication may not be possible in all circumstances.

Because of the risk of disease spread associated with the transport of smolt and fingerlings, the regional containment and zoning option is not recommended where only freshwater hatcheries are involved, because these facilities will present an unacceptable risk to marine farms. In these circumstances, a disease response program based on the principles of eradication is more appropriate.

Unexposed fish

If unexposed fish can be maintained without any risk of exposure, they may be on-grown to harvest size and processed using the methods described for the eradication option.

Exposed (or potentially exposed) clinically normal fish

A successful regional containment and zoning program relies on the implementation of strict movement controls for fish, products, equipment and personnel, with the aim of preventing disease spread to uninfected zones.

Within a declared area (see Section 2.4.1), grow-out and slaughter of clinically normal fish may be feasible without further spread of infection. However, clinically normal fish should always be treated as potential carriers.

Where possible, all hatcheries and processing facilities should be located within the declared area. If marine farms within the declared area must rely on hatcheries located outside the restricted area, strict biosecurity procedures must be applied to the movement of equipment and personnel between the two areas. Fish must not be moved to areas outside the declared area when hatcheries are located within the declared area.

If harvested fish must be moved from the infected area for processing, the processing facilities should be located away from other salmon-production regions, and strict biosecurity measures must apply.

Regional containment and zoning programs for piscirickettsiosis should ultimately rely on strict regional biosecurity and changes to farming practices (e.g. attention to stock density, year-class separation, all-in-all-out stocking, vaccination programs) in infected areas. Control options may also include strategic use of antibiotics to reduce establishment of infection, particularly during smolt transfer.

Although effective vaccines are not currently available in Australia, vaccination should not be ruled out as a viable management option.

Clinically diseased fish

All diseased and deceased fish must be removed and disposed of as soon as possible. These fish, together with associated waste products, are the main source of *P. salmonis* contamination in the environment.

Appropriate biosecurity measures must be applied when removing and disposing of affected fish to avoid further contamination of the environment.

2.8.3 Response option 3: control and mitigation

Principles of the control and mitigation option involve reducing the impact of piscirickettsiosis on industry, without the overall goal of eradicating the pathogen.

This strategy relies on implementing management practices that reduce the incidence, distribution and severity of disease outbreaks. With the exception of movement restrictions for the purpose of zoning, general control measures for disease containment and zoning apply (see Section 2.8.2).

This option would not be recommended for outbreaks of piscirickettsiosis within Australia unless an effective vaccine was available.

2.8.4 Trade and industry considerations

Trade regulations, market requirements and food safety standards must be considered as part of a response program. Permits may be required from the relevant authorities to allow products from declared areas to be released and sold for human consumption.

Export markets

Although previously listed, piscirickettsiosis **does not** currently meet criteria for listing by the World Organisation for Animal Health.

DAFF Biosecurity is responsible for the health certification of all exports and should be contacted for further information (see www.daff.gov.au/aqis/export/fish).

3.1 Overall policy for piscirickettsiosis

Summary of policy

Piscirickettsiosis is an exotic, highly contagious bacterial disease that primarily affects salmonid fish species, but has been reported from one other marine fish species.

The potential impact of an outbreak of piscirickettsiosis on the Australian salmonid aquaculture sector will, to some degree, depend on the strain of *Piscirickettsia salmonis* involved. The Chilean salmonid industry has experienced significant losses, but the effect of piscirickettsiosis in other countries has not been as severe, although quantitative data regarding losses are unavailable for comparison. There are differences in virulence between the Chilean (LF-89^T), Canadian (ATL-4-91) and Norwegian (NOR-92) strains (House et al. 1999).

The optimum growth temperature for *P. salmonis* in culture is 15–18 °C. The impact of *P. salmonis* in Australia may be higher than in the Northern Hemisphere due to higher ambient water temperatures (AQIS 1999).

The majority of Australia's salmonid culture occurs in Tasmania. An outbreak of the Chilean type strain of *P. salmonis* in Tasmania is likely to have a considerable impact on the industry and state economy. Outbreaks in other states or territories would affect the industry and economy to a lesser degree.

There are no records of *P. salmonis* causing significant disease in wild salmonids. Consequently, the establishment of piscirickettsiosis was considered by the Australian Quarantine and Inspection Service's Import Risk Analysis on Non-viable Salmonids and Non-salmonid Marine Finfish to have minimal consequence for the salmonid recreational sector (AQIS 1999). *P. salmonis* has been shown to survive for only short periods in fresh water, thus limiting the potential for spread of disease in wild freshwater populations.

The appropriate response strategy following detection of *P. salmonis* in Australia depends on the nature of the outbreak and the environment in which it has occurred. The response strategy will be determined by the relevant state or territory chief veterinary officer, in consultation with the industry sector.

There are currently no government–industry cost-sharing arrangements for the aquaculture or fishing industries in Australia. Successful implementation of disease control or eradication programs will be greatly influenced by available resources. Therefore, responsibility for program costs must be agreed upon before a disease response strategy is implemented.

There are three possible response options for an outbreak of piscirickettsiosis in Australia:

- eradication
- regional containment and zoning
- control and mitigation of disease.

The type of facility or environment in which the disease outbreak occurs will largely influence the choice of response option. Other factors that will affect the decision are the spread of disease, establishment of disease in wild populations, identification of the infection source and the presence of Tasmanian RLO (*Rickettsia*-like organism) in the environment.

Each disease response option involves the use of a combination of strategies, which may include:

- quarantine and movement controls on fish, fish products and fomites in declared areas to prevent spread of infection
- destruction and safe disposal of all clinically diseased fish to prevent further bacterial shedding into the environment
- decontamination of facilities, equipment, products and personnel to eliminate the bacterium in infected premises and to prevent spread of infection
- surveillance to determine the source and extent of infection
- zoning to define and maintain regional biosecurity
- treatment of stock with appropriate veterinary medicines to reduce the incidence and prevalence of disease
- establishment of awareness campaigns to encourage cooperation by industry and the general community.

The chief veterinary officer (CVO) of the state or territory where the outbreak has occurred will be responsible for developing an emergency aquatic animal disease response plan (EAAD response plan). This plan will be submitted to the Aquatic Consultative Committee on Emergency Animal Diseases (Aquatic CCEAD), which will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

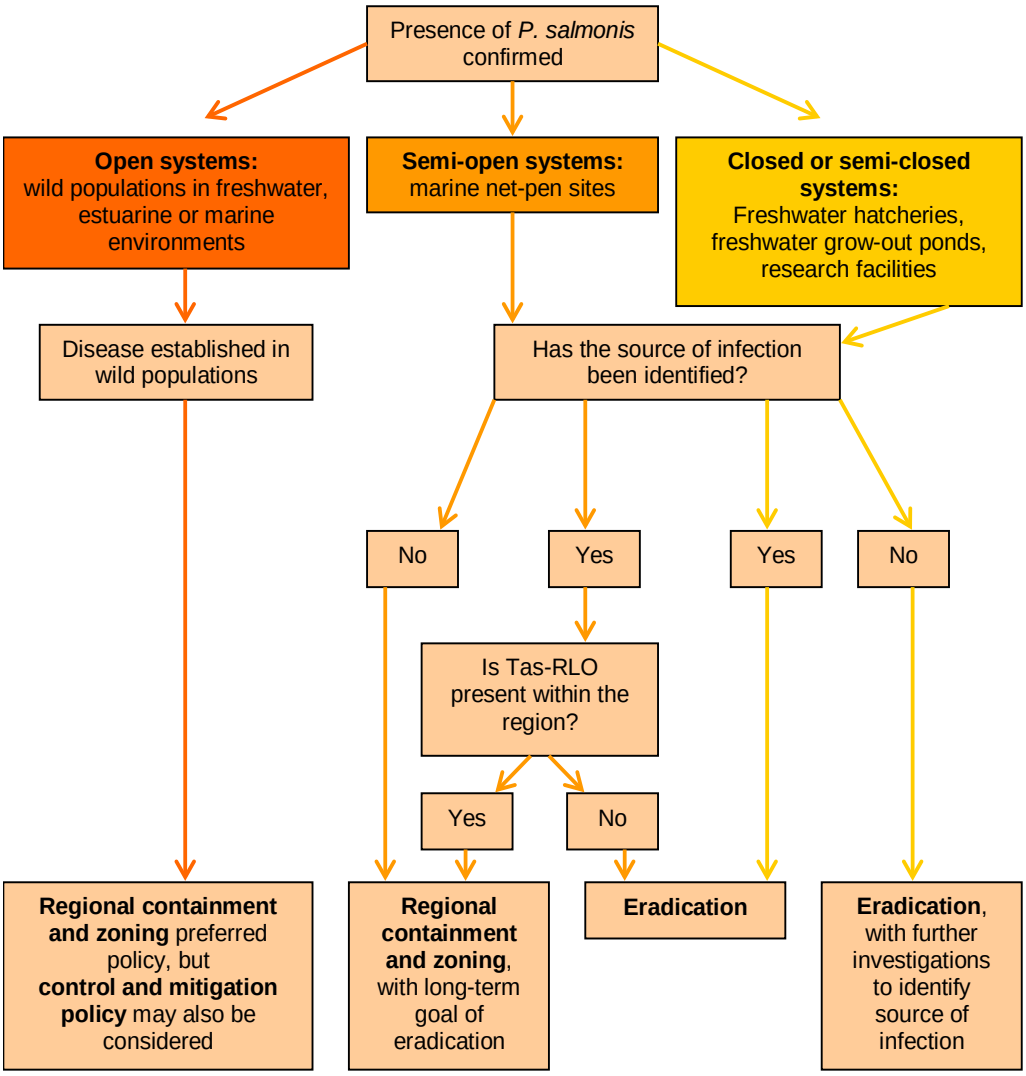
The CVO will implement disease control measures as agreed in the EAAD response plan and in accordance with relevant legislation. Decisions regarding follow-up disease response measures will be decided in consultation with the Aquatic CCEAD.

For information on the responsibilities of the CVO, state and territory disease control headquarters and local disease control centres, refer to the AQUAVETPLAN **Management Manual—Control Centres**.

3.2 Response options

The circumstances surrounding an outbreak of piscirickettsiosis will greatly influence selection of an appropriate response option and the actions that should occur on initial suspicion of piscirickettsiosis or detection of *P. salmonis* (Figure 3.1). Appropriate measures must be implemented to contain any potential spread of disease while confirmation of infection is pending. Following confirmation of *P. salmonis*, the appropriate response option should be determined (Figure 3.1).

The decision tree in Figure 3.1 is flexible; however, until the causative agent has been confirmed, a precautionary approach should be taken.



Tas-RLO = Tasmanian *Rickettsia*-like organism

Figure 3.1 **Decision matrix for appropriate response options following confirmation of *Piscirickettsia salmonis***

3.2.1 Response option 1: eradication

Eradication is recommended for outbreaks of disease in closed or semi-closed facilities (usually freshwater hatcheries). Eradication of piscirickettsiosis in semi-open production systems (usually marine net pens) is also recommended if the

source of infection is clearly identified and there is reasonable confidence that infection has not spread to, or originated from, wild fish populations.

In semi-open systems, the success of an eradication strategy will depend on the availability of resources required to undertake destocking or emergency harvest programs, together with extensive surveillance of wild and farmed populations. It is also vital that industry support is obtained and that agreement is reached on the allocation of program costs before eradication procedures begin.

If eradication is the preferred response option, the actions listed below must occur at each infected site (a number of these actions will occur concurrently):

- Quarantine and movement controls must be declared immediately, with stringent enforcement with respect to all live salmonids, salmonid products, water and vectors. These controls should be established during the investigation phase of the response and must be maintained until the aetiological agent is either eradicated (if identified as *P. salmonis*) or identified as another cause.
- A disease control centre must be established to coordinate the activities of the eradication program. As part of control centre activities, industry and other relevant agencies or authorities should be consulted and involved, where appropriate.
- Epidemiological investigations must be undertaken to determine the source of the infection, and to trace potential spread into and out of the infected area.
- Surveillance programs must be established, with the aim of defining the extent of the infection and determining whether there is any involvement of wild fish populations.
- Appropriate disease management areas (see Section 2.4.1) must be established, and movement across their borders controlled.
- All diseased fish must be immediately removed for destruction and disposal.
- All deceased fish must be removed from cages and tanks (at least daily) for disposal.
- Exposed (or potentially exposed) but clinically normal fish must also be destroyed. If there is any doubt regarding exposure, fish are to be considered infected. Market-size fish may be emergency harvested, provided that this can occur without posing a risk of further disease spread. Small fish not of market size must be destroyed and disposed of appropriately.
- If the affected premises is a semi-closed or closed system, untreated discharge water must not be released into the environment.
- Facilities, products, equipment, vehicles, boats and personnel must be decontaminated throughout the eradication process to eliminate and prevent spread of the bacterium.
- All infected products, including carcasses, waste, water and equipment that cannot be decontaminated effectively, must be disposed of in an approved and safe manner.
- Decontaminated sites must be tested with sentinel Atlantic salmon smolt before restocking. Sentinel animals should remain on site for at least 6 weeks before testing. Any fish that die or show signs of disease must be forwarded immediately for laboratory testing.

Treatment with vaccines should not occur during disease eradication programs. Antibiotics (see Section 2.4.4) would not normally be used during disease eradication programs unless available resources limit the prompt removal of at-risk populations.

3.2.2 Response option 2: regional containment and zoning

Regional containment and zoning aims to contain the pathogen to specific areas or regions. It is the preferred disease control option in semi-open or open productions systems where the infected area can be isolated with reasonable confidence, eradication of piscirickettsiosis is not considered viable or economically sustainable due to the large biomass of stock involved, Tas-RLO is present in the area, and/or infection has established in wild populations.

Measures implemented during regional containment and zoning operations include those outlined in Section 3.2.1 with the following variations:

- Destruction of all exposed (or potentially exposed) clinically normal fish is recommended but not essential. Market-size fish should be emergency harvested, provided that this can occur without posing a risk of further disease spread.
- Small fish that have not reached market size may be allowed to grow out. If the situation changes and these fish are exposed or potentially exposed, or develop clinical disease, they must be monitored and treated if clinical disease develops.

Farms in infected areas also need to implement management practices to reduce the severity and incidence of piscirickettsiosis outbreaks. The following measures should be undertaken:

- An all-in-all-out stock management strategy should be applied, with single year classes held in individual sites within infected areas.
- Farm sites should be fallowed before subsequent stock groups are introduced. *P. salmonis* may survive up to 21 days in the environment, depending on water temperature. Consequently, a fallowing period of at least 3 months is recommended (see Section 1.6.2) before new stock (including sentinels) is introduced. Counts of *P. salmonis* from highly infected areas in Chile decreased to zero, 50 days after the removal of fish (Olivares & Marshall 2010), suggesting an ideal fallowing period of 50 days before restocking. As standard conservative veterinary practice is to allow a considerable safety margin, a fallow period of 150 days has been assigned if an outbreak of *P. salmonis* were to occur in Australia.
- Fish should be maintained in low-stress environments, with low stocking densities and minimal handling.
- No live fish, including broodstock, gametes or ova, may be removed from infected areas or zones. Hatcheries within infected areas must not be allowed to supply smolt to farms located in free areas.
- Processing of harvested fish must only occur in approved biosecure premises. Infected blood–water or other effluent must be fully contained and treated.
- A vaccination program should be implemented if a suitable vaccine is available.

See Section 2.8.2 for further discussion on regional containment and zoning of disease.

3.2.3 Response option 3: control and mitigation of disease

Establishing a regional containment and zoning disease response or an eradication program will not be feasible in all circumstances. The control and mitigation option should be considered when infection with *P. salmonis* is widespread throughout large areas of the affected state or territory or established in wild fish populations, and/or the financial costs associated with zoning or eradication (e.g. costs associated with destruction) are considered too high.

As part of this response option, husbandry, management and hygiene practices should be implemented to decrease the incidence and severity of piscirickettsiosis outbreaks.

With the exception of the restrictions associated with zoning, the response options described elsewhere in this section should be implemented, with the aim of minimising the infectious load around farms and reducing exposure of fish to the bacterium.

Should an effective *P. salmonis* vaccine become available, vaccination programs would be highly recommended for inclusion in control and mitigation programs.

3.3 Criteria for proof of freedom

Proof of freedom from piscirickettsiosis may become important for trade purposes.

Freedom from *P. salmonis* can be demonstrated at the level of the aquaculture establishment, zone and country. General principles for proof of freedom at each level are given in the OIE *Aquatic animal health code* (OIE 2011*)*.

3.4 Funding and compensation

Currently, no cost-sharing agreement is in place between industry and governments for an emergency response to piscirickettsiosis.

Appendix 1 Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it is subject to the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. An import permit is also required from DAFF Biosecurity if a product containing biological material is to be sourced from overseas.

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permit. Since the assessment is a detailed process, the evaluation may take some time.

Minor-use permit system

The minor-use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. It allows the restricted use of a limited amount of a drug or chemical for a specified species when inadequate data are available to satisfy APVMA requirements for registration. Conditions are applied to the permit, which often include the collection of data related to the use of the product. The MUP system enables restricted use of a drug or chemical until sufficient data are available to enable full registration.

For example, the APVMA may set a temporary withholding period, with a wide margin of safety, for a MUP. This withholding period may be extrapolated from data relating to the use of the product in other species. In such cases, a condition of the MUP will be the collection of residue-testing data. Results are assessed by the APVMA (usually after 12 months—the duration of most permits) and used to set an accurate withholding period for the product.

Emergency-use permits

The APVMA has a permit system for the emergency use of a product that is either unregistered in Australia, or registered for use in a different species or in a different way. The APVMA will verify with the appropriate state and territory coordinators that the emergency is genuine.

For further details or permit application forms, visit the APVMA website.[6]

[6] www.apvma.gov.au

Glossary

Alert phase:
See *Stages of activation*

Animal Health Committee (AHC):
A national committee that develops science-based and nationally consistent policy on animal health issues. It reports through the National Biosecurity Committee to the Standing Council on Primary Industries. Its membership comprises the national, state and territory chief veterinary officers, and representatives from the Australian Animal Health Laboratory, Animal Health Australia, DAFF Biosecurity, the Australian Government Department of Sustainability, Environment, Water, Populations and Communities, and New Zealand.

Aquaculture establishment:
An establishment used for the culture and production of aquatic animal species. Establishments may be classified as open, semi-open, semi-closed or closed systems. Refer to the AQUAVETPLAN **Enterprise Manual** for further details.

AQUAPLAN:
AQUAPLAN is Australia's National Strategic Plan for Aquatic Animal Health. It is a comprehensive strategy to build and enhance capacity for the management of aquatic animal health in Australia.

Aquatic animal disease emergency:
An emergency situation requiring an immediate response to control an identified disease or pathogen of aquatic animals.

Aquatic Consultative Committee on Emergency Animal Diseases (Aquatic CCEAD):
A national committee called together during aquatic animal disease emergency situations, comprising the state and territory chief veterinary officers or fisheries directors, representatives from the Office of the Chief Veterinary Officer, and the chief of CSIRO Livestock Industries. The committee consults during aquatic animal disease events and provides a coordinated national approach to management of the disease event.

AQUAVETPLAN:
The Australian *Aqua*tic *Vet*erinary Emergency *Plan* is a series of manuals that outline Australia's approach to national disease preparedness and propose the technical response and control strategies to be activated in a national aquatic animal disease emergency. The manuals also provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency management plans.

See also AUSVETPLAN

Area:
A defined tract of land and/or water.

See also Premises

Australian Chief Veterinary Officer:
The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak

See also Chief veterinary officer (CVO)

Chief veterinary officer (CVO):
The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction.
See also Australian Chief Veterinary Officer

Control area:
A buffer between the restricted area and areas free from disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may reduce in size. The shape of the area may be modified according to circumstances, such as water flows, catchment limits, etc. In most cases, permits will be required to move animals and specified product out of the control area into the free area.

Dangerous contact area or premises:
An area or premises containing aquatic animals that show no signs of disease but that, because of their probable exposure to disease, will be subject to disease control measures. The type of contact that would suggest exposure will depend on the agent involved in the outbreak but, for example, may involve animal movements, or movements of nets or equipment.

Declared area:
A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include restricted area, control area, infected premises, dangerous contact premises and suspect premises.

Decontamination:
A combination of physical and chemical procedures that are used to remove soiling and inactivate the target disease organism. Includes all stages of cleaning and disinfection.

Destocking:
The process of removing some or all livestock from an aquaculture facility or natural waterway.

Destruction:
The killing by humane means (euthanasia) of infected aquatic animals and/or those exposed to infection.

Disease agent:
A general term for a transmissible organism or other factor that causes an infectious disease.

Disease management area:
A clearly defined area established to identify properties, areas or regions of differing levels of disease risk, and to enhance management of the emergency disease response through the control of stock, people, equipment, fomites or water.

Disease response plan:
See Emergency aquatic animal disease response plan

Disinfection:
The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; it applies to premises, vehicles and other objects that may have been directly or indirectly contaminated.

Disposal:
Sanitary removal of fish carcasses and fomites by burial, burning or some other process to prevent the spread of disease.

Emergency animal disease:
A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications.

Emergency aquatic animal disease response plan:
The overall plan submitted to the Aquatic CCEAD that outlines the planned response to an emergency aquatic animal disease event.

Enzyme-linked immunosorbent assay (ELISA):
A serological test designed to detect and measure the presence of antibody or antigen in a sample. The test uses an enzyme reaction with a substrate to produce a colour change when antigen–antibody binding occurs.

Exotic aquatic animal disease:
Disease affecting aquatic animals (possibly also affecting humans and other animals) not known to occur in Australia.

Fallow/fallowing:
Leaving an area unfarmed or vacant of introduced stock for a specified period (usually a season). In the case of fish, this will require all adjacent areas to fallow, depending on local conditions (currents, etc.)

Fish:
In the context of this manual, any aquatic animal within the finfish, mollusc and crustacean groups.

Fish byproducts:
Products of fish origin destined for industrial use (e.g. fishmeal).

Fomite:
Any inanimate object (e.g. water, packing, boots, equipment) that can carry the disease agent and spread the disease through mechanical transmission.

Free area:
An area known to be free from the disease agent.

Inappetance:
Lack of appetite.

Infected area or premises:
A disease management area, which may be all or part of a premises, lease or waterway, in which an aquatic animal disease emergency exists or is believed to exist. An infected area is subject to quarantine served by notice and to eradication or control procedures.
See also Disease management area

Investigation phase:
See Stages of activation

Livestock:
Any animal, including fish species, held under controlled conditions for the purposes of culture or production.

See also Stock

Local disease control centre:
An emergency operations centre responsible for the management of operations within a local designated area of responsibility.

See also State or territory disease control headquarters

Mitigation:
Reduction in severity—mitigation of the impact of disease is to decrease the severity of the impact of the disease.

Monitoring:
Routine collection of data for assessing the health status of a population.

See also Surveillance

Movement control:
Restrictions placed on the movement of animals, people and fomites to prevent spread of disease.

Operational phase:
See Stages of activation

Operational procedures:
Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.

Operations:
The activities necessary to give effect to a disease control strategy.

Petechial haemorrhage:
Tiny flat, red or purple spots in the skin or mucous membranes caused by bleeding from small blood vessels.

Piscirickettsiosis:
Disease caused by infection with the bacterium *Piscirickettsia salmonis*.

Polymerase chain reaction (PCR):
A diagnostic technique involving in vitro amplification of a specific target DNA segment to detectable levels.

Premises:
A clearly defined site, which may include a single building, property, facility or area.
See also Area

Quarantine:
Legal restrictions controlling movement to or from an area, imposed on people, animals, animal products, vehicles or other items.

Restricted area:
The disease management area around an infected area or premises that is subject to intense surveillance and movement controls.
See also Disease management area

Sentinel fish:
Fish of known health status monitored for the purpose of detecting the presence of a disease agent.

Septicaemia:
The invasion and persistence of pathogenic bacteria in the bloodstream.

Smolts:
Fish that have undergone a physiological process while in fresh water that prepares them for migration to salt water.

Stages of activation

– investigation phase exists when key members of the animal health authority are notified that an animal disease emergency may be imminent, or exists in another state or territory.

– alert phase exists when the chief veterinary officer notifies the coordinator of the state emergency services that an animal disease emergency may be imminent, or exists in another state or territory.

– operational phase exists when the chief veterinary officer notifies the coordinator of the state emergency services that an animal disease emergency exists in the state or territory.

– stand-down phase exists when the chief veterinary officer notifies the coordinator of the state or territory emergency services that an animal disease emergency no longer exists.

Stand-down phase:
See Stages of activation

Standing Council on Primary Industries:
The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Primary Industries Ministerial Council).

State or territory disease control headquarters:
The emergency operations centre that directs the disease control operations to be undertaken in a particular state or territory.

Stock:
Any animal held under controlled conditions or harvested from the wild. Includes all aquaculture and wild harvest fish species.
See also Livestock

Strategy:
The principles on which control of a disease is based.

Surveillance:
A systematic program of inspection and examination of animals or things to determine the presence or absence of an aquatic animal disease.
See also Monitoring

Survey:
A program of investigation designed to establish the presence, extent or absence of disease.

Susceptible animal/species:
An animal or species that can be infected with a particular disease.

Suspect animal:
An animal that is likely to have been exposed to an emergency aquatic animal disease such that quarantine and intensive monitoring is warranted; *or* an animal not known to have been exposed to a disease agent but showing clinical signs requiring confirmation of the diagnosis.

Suspect area or premises:
An area or premises containing suspect animals that will be subject to quarantine and intensive surveillance.

Tracing:
The process of locating animals, persons or fomites that may be implicated in the spread of disease, so that appropriate action can be taken.

Vector:
A living organism that transmits an infectious agent from one host to another. A biological vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A mechanical vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.

World Organisation for Animal Health (OIE):
The international organisation responsible for monitoring diseases in animals, including livestock; formerly known as the Office International des Épizooties.

Zoning:
The process of defining disease-free and infected areas.

Abbreviations

ANZSDP	Australian and New Zealand Standard Diagnostic Procedure
APVMA	Australian Pesticides and Veterinary Medicines Authority
Aquatic CCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
DNA	deoxyribonucleic acid
DPIPWE	Tasmanian Department of Primary Industries, Parks, Water and Environment (formerly the Department of Primary Industry and Water)
EAAD	emergency aquatic animal disease
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
RLO	*Rickettsia*-like organism
rRNA	ribosomal ribonucleic acid
SRS	salmonid rickettsial septicaemia
Tas-RLO	Tasmanian *Rickettsia*-like organism

References

Aguayo, J, Miquel, A, Aranki, N, Jamet, A, Valenzuela, PD & Burzio, LO 2002, 'Detection of *Piscirickettsia salmonis* in fish tissues by an enzyme-linked immunosorbent assay using specific monoclonal antibodies', *Diseases of Aquatic Organisms*, vol. 49, pp. 33–38.

Almendras, FE & Fuentealba, IC 1997, 'Salmonid rickettsial septicemia caused by *Piscirickettsia salmonis*: a review', *Diseases of Aquatic Organisms*, vol. 29, pp. 137–144.

Almendras, FE, Fuentealba, IC, Jones, SRM, Markham, F & Spangler, E 1997, 'Experimental infection and horizontal transmission of *Piscirickettsia salmonis* in freshwater-raised Atlantic salmon, *Salmo salar* L.', *Journal of Fish Diseases*, vol. 20, no. 6, pp. 409–418.

Almendras, FE, Fuentealba, IC, Markham, F & Speare, DJ 2000, 'Pathogenesis of liver lesions caused by experimental infection with *Piscirickettsia salmonis* in juvenile Atlantic salmon, *Salmo salar* L.', *Journal of Veterinary Diagnostic Investigation*, vol. 12, no. 6, pp. 552–557.

AQIS (Australian Quarantine and Inspection Service) 1999, 'Import risk analysis on non-viable salmonids and non-salmonid marine finfish', AQIS, Canberra, pp. 105–108.

Birkbeck, TH, Rennie, S, Hunter D, Laidler, LA & Wadsworth, S 2004, 'Infectivity of a Scottish isolate of *Piscirickettsia salmonis* for Atlantic salmon *Salmo salar* and immune response of salmon to this agent', *Diseases of Aquatic Organisms*, vol. 60, no. 2, pp. 97–103.

Birrell, J, Mitchell, S & Bruno, DW 2003, '*Piscirickettsia salmonis* in farmed Atlantic salmon, *Salmo salar* in Scotland', *Bulletin of the European Association of Fish Pathologists*, vol. 23, no. 5, pp. 213–218.

Branson, EJ & Diaz-Munoz, D 1991, 'Description of a new disease condition occurring in farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in South America', *Journal of Fish Diseases*, vol. 14, pp. 147–156.

Bravo, S 1994, 'Piscirickettsiosis in freshwater', *Bulletin of the European Association of Fish Pathologists*, vol. 14, no. 4, pp. 137–139.

Bravo, S & Campos, M 1989, 'Coho salmon syndrome in Chile', *American Fisheries Newsletter*, vol. 17, no. 2, p. 3.

Bravo, S & Midtling, Y 2007, 'The use of fish vaccines in the Chilean salmon industry 1999–2003', *Aquaculture*, vol. 270, no. 1–4, pp. 36–42.

Brocklebank, JR, Speare, DJ, Armstrong, RD & Evelyn, TPT 1992, 'Septicaemia suspected to be caused by a rickettsia-like agent in farmed Atlantic salmon', *Canadian Veterinary Journal*, vol. 33, pp. 407–408.

Bustos, P 2006, 'Growing incidence of *Piscirickettsia* infection in fish worldwide: mechanisms for prevention and control', Nutritional Biotechnology in the Feed and Food Industries: Proceedings of Alltech's 22nd Annual Symposium, Lexington, Kentucky, USA, 23–26 April 2006, pp. 397–401.

Bustos, P, Entrala, P, Montana, J & Calbuyahue, J 1994, 'Septicemia rickettsial salmonidea (SRS): estudio de transmision vertical en salmon coho (*Oncorhynchus kisutch*)', in Fundacion Chile (ed.), *Proceedings Primaer Seminario Internacional: patologia y nutricion en el desarrollo de la acuicultura*, 3–7 October 1994, Puerto Montt, pp. 33–40.

Buxton, A & Fraser, G 1977, 'The rickettsias', in A Buxton & G Fraser (eds), *Animal microbiology*, vol. 2, Blackwell Scientific Publications Ltd, Oxford, pp. 359–390.

Cameron, DE 1991, 'The evaluation of normal physiology and histology of marine cultured Atlantic salmon (*Salmo salar* L.) in Tasmania', in *Proceedings of the SALTAS Research Review*, 22 May 1991.

Cassigoli, J 1994, 'Septicaemia rickettsial del salmon', in Fundacion Chile (ed.), *Proceedings Primer Seminario Internacional: patologia y nutricion en el desarrollo de la acuicultura: factores de exito*, 3–7 October 1994, Puerto Montt, pp. 17–20.

Chen, SC, Tung, MC, Chen, SP, Tsai, JF, Wang, PC, Chen, RS, Lin, SC & Adams, A 1994, 'Systematic granulomas caused by a rickettsia-like organism in Nile tilapia, *Oreochronuis niloticus* (L.), from southern Taiwan', *Journal of Fish Diseases*, vol. 17, no. 6, pp. 591–599.

Chen, SC, Wang, PC, Tung, MC, Thompson, KD & Adams, A 2000, 'A *Piscirickettsia salmonis*-like organism in grouper, *Epinephelus melanostigma* in Taiwan', *Journal of Fish Diseases*, vol. 23, pp. 415–418.

Corbeil, S & Crane, MS 2009, *Australian and New Zealand standard diagnostic procedures*—Piscirickettsia salmonis, Sub-Committee on Animal Health Laboratory Standards, Victoria.

Corbeil, S, Hyatt, AD & Crane, M 2005, 'Characterisation of an emerging rickettsia-like organism in Tasmanian farmed Atlantic salmon *Salmo salar*', *Diseases of Aquatic Organisms*, vol. 64, pp. 37–44.

Corbeil, S, McColl, KA & Crane, MS 2003, 'Development of a Taqman quantitative PCR assay for the identification of *Piscirickettsia salmonis*', *Bulletin of the European Association of Fish Pathologists*, vol. 23, pp. 95–101.

Cusack, R, Groman, D & Jones, S 1997, 'The first reported rickettsial infections of Atlantic salmon in Eastern North America', VIIIth International Conference on Diseases of Fish and Shellfish, Edinburgh, 109 pp.

Cvitanich, JD, Garate, ON & Smith, CE 1991, 'The isolation of a rickettsia-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate', *Journal of Fish Diseases*, vol. 14, pp. 121–145.

DPIWE (Tasmanian Department of Primary Industries, Water and Environment) 2004, 'Findings of the Tasmanian Fish Health Surveillance Program 2003–

2004', internal report for the DPIWE and the Tasmanian Salmonid Growers Association.

Enriquez, R 1995, 'Actual situation of the disease produced by *Piscirickettsia salmonis* in Chile', European Association of Fish Pathologists Seventh International Conference, 10–15 September 1995 (abstract).

Evelyn, T 1984, 'Immunisation against pathogenic vibrios', in P Kinkelin (ed.), *Symposium of Fish Vaccination*, Office International des Epizooties, Paris, pp. 121–150.

Evelyn, T 1992, 'Salmonid rickettsial septicaemia', *Canadian Special Publication of Fish Aquatic Science*, vol. 116, pp. 18–19.

Evelyn, T, Kent, M, Poppe, T & Bustos, P 1998, 'Salmonid rickettsial septicemia', in ML Kent & TT Poppe (eds), *Diseases of seawater net-pen reared salmonid fishes*, Department of Fisheries and Oceans, Nanaimo, British Columbia, pp. 31–33.

Fryer, JL & Hedrick, RP 2003, '*Piscirickettsia salmonis*: a Gram-negative intracellular bacterial pathogen of fish', *Journal of Fish Diseases*, vol. 26, no. 5, pp. 251–262.

Fryer, JL, Lannan, CN, Garcés, LH, Larenas, JJ & Smith, PA 1990, 'Isolation of a rickettsiales-like organism from diseased Coho salmon (*Oncorhynchus kisutch*) in Chile', *Fish Pathology*, vol. 25, no. 2, pp. 107–114.

Fryer, JL, Lannan, CN, Giovannoni, SJ & Wood, ND 1992, '*Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes', *International Journal of Systematic Bacteriology*, vol. 42, no. 1, pp. 120–126.

Fryer, JL & Mauel, MJ 1997, 'The rickettsia: an emerging group of pathogens in fish', *Emerging Infectious Diseases*, vol. 3, no. 2, pp. 137–144.

Gaggero, A, Castro, H & Sandino, AM 1995, 'First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle', *Journal of Fish Diseases*, vol. 18, no. 3, pp. 277–280.

Garcés, LH, Correa, P, Larenas, J, Contreras, J, Oyanadel, S, Fryer, JL & Smith-Shuster, PA 1994, 'Finding of *Piscirickettsia salmonis* in *Ceratothoa gaudichaudii*', in RP Hendrick & JR Winton (eds), *Proceedings of the International Symposium of Aquatic Animal Health*, Seattle, p. 109.

Garcés, LH, Larenas, JJ, Smith, PA, Sandino, S, Lannan, CN & Fryer, JL 1991, 'Infectivity of a rickettsia isolated from Coho salmon *Oncorhynchus kisutch*', *Diseases of Aquatic Organisms*, vol. 11, pp. 93–97.

Grant, AN, Brown, AG, Cox, DI, Birkbeck, TH & Griffen, AA 1996, 'Rickettsia-like organism in farmed salmon', *Veterinary Record*, vol. 138, no. 21, p. 423.

House, ML, Bartholomew, JL, Winton, JR & Fryer, L 1999, 'Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon *Oncorhynchus kisutch*', *Diseases of Aquatic Organisms*, vol. 35, no. 2, pp. 107–113.

lnglis, V, Roberts, RJ & Bromage, NR 1993, 'Salmonid rickettsial septicaeamia (SRS)', in V Inglis, RJ Roberts & NR Bromage (eds), *Bacterial diseases of fish*, Blackwell Scientific Publications, Oxford, pp. 245–254.

JETACAR (Joint Expert Technical Advisory Committee on Antibiotic Resistance) 1999, *The use of antibiotics in food-producing animals: antibiotic resistant bacteria in animals and humans*, report of JETACAR, Australian Government Department of Health and Ageing, Canberra.

Jones, SRM, Markham, RJF, Groman, DB & Cusack, RR 1998, 'Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon (*Salmo salar*) in eastern Canada', *Diseases of Aquatic Organisms*, vol. 33, pp. 25–31.

Kuzyk, MA, Burian, J, Thornton, JC & Kay, WW 2001a, 'OspA, a lipoprotein antigen of the obligate intracellular bacterial pathogen *Piscirickettsia salmonis*', *Journal of Molecular Microbiology and Biotechnology*, vol. 3, no. 1, pp. 83–93.

Kuzyk, MA, Burian, J, Machander, D, Dolhaine, D, Cameron, S, Thornton, JC & Kay, WW 2001b, 'An efficacious recombinant subunit vaccine against the salmonid rickettsial pathogen *Piscirickettsia salmonis*', *Vaccine*, vol. 19, nos. 17–19, pp. 2337–2344.

Lane, HC 1997, 'Progressive changes in haematology and tissue water of sexually mature trout *Salmo gairdneri* Richardson, during the autumn and winter', *Journal of Fish Biology*, vol. 15, pp. 425–436.

Lannan, CN, Bartholomew, JL & Fryer, JL 1999, 'Rickettsial and chlamydial infections', in PTK Woo & DW Bruno (eds), *Fish diseases and disorders*, vol. 3, *Viral, bacterial and fungal infections*, CAB International, Wallingford (Oxon.), United Kingdom.

Lannan, CN & Fryer, JL 1994, 'Extracellular survival of *Piscirickettsia salmonis*', *Journal of Fish Diseases*, vol. 17, pp. 545–548.

Larenas, JJ, Bartholomew, J, Troncoso, O, Fernandez, S, Ledezma, H, Sandoval, N, Vera, P, Contreras, J & Smith, P 2003, 'Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum', *Diseases of Aquatic Organisms*, vol. 56, no. 1, pp. 25–30.

McCarthy, U, Steiropoulos, NA, Thompson, KD, Adams, A, Ellis, AE & Ferguson, HW 2005, 'Confirmation of *Piscirickettsia salmonis* as a pathogen in European sea bass *Dicentrarchus labrax* and phylogenetic comparison with salmonid strains', *Diseases of Aquatic Organisms*, vol. 64, pp. 107–119.

Marshall, S, Sekou, H, Vitalia, H & Orrego, C 1998, 'Minimally invasive detection of *Piscirickettsia salmonis* in cultivated salmonids via the PCR', *Applied and Environmental Microbiology*, vol. 64, no. 8, pp. 3066–3069.

Mauel, MJ, Giovannoni, SJ & Fryer, JL 1996, 'Development of polymerase chain reaction assays for detection, identification, and differentiation of *Piscirickettsia salmonis*', *Diseases of Aquatic Organisms*, vol. 26, no. 189–195.

Mauel, MJ, Ware, C & Smith, PA 2008, 'Culture of *Piscirickettsia salmonis* on enriched blood agar', *Journal of Veterinary Diagnostic Investigation*, vol. 20, no. 2, pp. 213–214.

Mikalsen, J, Skjaervik, O, Wiik-Nielsen, J, Wasmuth, MA & Colquhoun, DJ 2008, 'Agar culture of *Piscirickettsia salmonis*, a serious pathogen of farmed salmonid and marine fish', *FEMS Microbiology Letters*, vol. 278, no. 1, pp. 43–47.

Miller, WR, Hendricks, AC & Cairns Jr, J 1983, 'Normal ranges for diagnostically important hematological and blood chemistry characteristics of rainbow trout *Salmo gairdneri*', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 40, no. 4, pp. 420–425.

OIE (World Organisation for Animal Health) 2009, 'Piscirickettsiosis', in *Manual of diagnostic tests for aquatic animals*, 6th edn, OIE, Paris.

OIE (World Organisation for Animal Health) 2011, *Aquatic animal health code*, OIE, Paris.

Olivares, J & Marshall, SJ 2010, 'Determination of minimal concentration of *Piscirickettsia salmonis* in water columns to establish a fallowing period in salmon farms', *Journal of Fish Diseases*, vol. 33, no. 3, pp. 261–266.

Olsen, AB 2003, 'Oppblomstring av piscirickettsiose I Norge høsten 2002', *Fiskehelse*, no. 5, pp. 6–8.

Olsen, AB, Melby, HP, Speilberg, L, Evensen, Ø & Hastein, T 1997, '*Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway, epidemiological, pathological and microbiological findings', *Diseases of Aquatic Organisms*, vol. 31, pp. 35–48.

Reid, HI, Griffen, AA & Birkbeck, TH 2004, 'Isolates of *Piscirickettsia salmonis* from Scotland and Ireland show evidence of clonal diversity', *Applied and Environmental Microbiology*, vol. 70, pp. 4393–4397.

Rise, ML, Jones, SRM, Brown, GD, von Schalberg, KR, Davidson, WS & Koop, BF 2004, 'Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage and hematopoietic kidney response to *Piscirickettsia salmonis* infection', *Physiological Genomics*, 28 September, pp. 1–40.

Rodger, HD & Drinan, EM 1993, 'Observation of a rickettsia-like organism in Atlantic salmon, *Salmo salar* L., in Ireland', *Journal of Fish Diseases*, vol. 16, pp. 361–369.

Salinas, G, Contreras, J, Smith, P & Larenas, J 1997, 'Horizontal transmission and excretion of *Piscirickettsia salmonis* in rainbow trout (*Oncorhynchus mykiss*) in fresh water condition', VIIIth International Conference on Diseases of Fish and Shellfish, Edinburgh, p. 57.

Skarmeta, A, Henriquez, V, Zahr, M, Orrego, C & Marshall, SH 2000, 'Isolation of a virulent *Piscirickettsia salmonis* from the brain of naturally infected coho salmon', *Bulletin of the European Association of Fish Pathologists*, vol. 20, no. 6, pp. 261–264.

Smith, PA, Contreras, JR, Larenas, JJ, Aguillon, JC, Garces, LH, Pdrez, B & Fryer, JL 1997, 'Immunization with bacterial antigens: piscirickettsiosis', in R Gudding, A Lillehaug, PJ Midtlyng & F Brown (eds), *Fish vaccinology*, vol. 90, *Developments in Biological Standardization* (Basel), pp. 161–166.

Smith, PA, Lannan, CN, Garcés, LH, Jarpa, M, Larenas, J, Caswell-Reno, P, Whipple, M & Fryer, JL 1995, 'Piscirickettsiosis: a bacterin field trial in Coho salmon (*Oncorhynchus kisutch*)', *Bulletin of the European Association of Fish Pathologists*, vol. 15, no. 4, pp. 137–141.

Smith, PA, Pizarro, P, Ojeda, P, Contreras, J, Oyanedel, S & Larenas, J 1999, 'Routes of entry of *Piscirickettsia salmonis* in rainbow trout *Oncorhynchus mykiss*', *Diseases of Aquatic Organisms*, vol. 37, pp. 165–172.

Smith, PA, Vecchiola, IM, Oyanedel, S, Garces, LH, Larenas, J & Contreras, J 1996, 'Antimicrobial sensitivity of four isolates of *Piscirickettsia salmonis*', *Bulletin of the European Association of Fish Pathologists*, vol. 6, pp. 164–168.Stevenson, R 1997, 'Immunisation with bacterial antigens: yersiniosis', *Developments in Biological Standardization*, vol. 90, pp. 117–124.

Thrushfield, M 2007, *Veterinary epidemiology*, 3rd edn, Blackwell Science Ltd, Oxford.

Turnbull, JF 1993, 'Epitheliocystis and salmonid rickettsial septicaemia', in V Inglis, RJ Roberts & NR Bromage (eds), *Bacterial diseases of fish*, Blackwell Science, Oxford, pp. 235–254.

Venegas, CA, Contreras, JR, Larenas, JJ & Smith, PA 2004, 'DNA hybridization assays for the detection of *Piscirickettsia salmonis* in salmonid fish', *Journal of Fish Diseases*, vol. 27, pp. 431–433.

Weiss, E & Moulder, JW 1984, 'The rickettsias and chlamydias', in NR Krieg & JG Holt (eds), *Bergey's manual of systematic bacteriology*, vol. 1, Williams and Wilkins, Baltimore/London, pp. 687–729.

Yuksel, SA, Thompson, KD & Adams, A 2006, 'Rickettsial infections of fish', *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 6, no. 1, pp. 63–78.