

AQUAVETPLAN

Disease Strategy

Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*)

Version 2, 2009

AQUAVETPLAN is a series of manuals that outline Australia's approach to national disease preparedness and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency.

Primary Industries Ministerial Council

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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Being a guide only, outbreaks or suspected outbreaks must be assessed on a case by case basis and expert advice should be obtained to determine the most appropriate management plan in response to the risk.

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 the disease furunculosis is an integral part of the **Australian Aquatic Veterinary Emergency Plan**, or **AQUAVETPLAN**.

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

The Australian Government Department of Agriculture provides quarantine inspection for international passengers, cargo, mail, animals, plants, and animal or plant products arriving in Australia, and inspection and certification for 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 human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture ICON website.¹

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of furunculosis in Australia. The strategy was scientifically reviewed by the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, before being endorsed by the Aquatic Animal Health Committee of the Primary Industries Standing Committee on 30 June 2009.

Furunculosis is not listed by the World Organisation for Animal Health (OIE) in the *Aquatic Animal Health Code*² but is on 'Australia's national list of reportable diseases of aquatic animals'.³

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the AQUAVETPLAN **Enterprise Manual**. The list of AQUAVETPLAN manuals that may need to be accessed in an emergency is shown below:

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Disposal

Destruction

Decontamination

Enterprise manual

Includes sections on:

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

¹ <<http://www.agriculture.gov.au/biosecurity/import/icon-icd> >

² < www.agriculture.gov.au/_data/assets/word_doc/0003/346521/reportable-aquatic-diseases.doc >

³ < The complete series of AQUAVETPLAN documents is available on the internet at www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan >

Management manual

Control centres management

*Aquatic animal diseases significant to Australia: identification field guide*⁴ is a source of information about the aetiology, diagnosis and epidemiology of infection with furunculosis and should be read in conjunction with this strategy.

The first edition of the furunculosis manual, written by Drs Iska Sampson and Eva-Maria Bernoth, was published in 2001. The second edition of this manual was prepared by Drs Paul Hardy-Smith, Rob Jones, Robin Vandegraaff and Craig Stephen. The authors were responsible for reviewing the first edition of the strategy, in consultation with stakeholders from aquaculture, recreational fishing and government sectors throughout areas of Australia in which there are aquatic species susceptible to furunculosis. However, the text was 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 was adapted from similar manuals in AUSVETPLAN (the Australian veterinary emergency plan for terrestrial animal diseases). The format and content have been kept as similar as possible to these documents, in order to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. 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.

⁴ < [http://www.agriculture.gov.au/animal-plant-health/aquatic/guidelines-and-resources/aquatic animal diseases significant to australia identification field guide](http://www.agriculture.gov.au/animal-plant-health/aquatic/guidelines-and-resources/aquatic%20animal%20diseases%20significant%20to%20australia%20identification%20field%20guide) >

The revised manual has been reviewed and approved by the following representatives of government and industry:

Government

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CSIRO Livestock Industries
Department of Primary Industries,
New South Wales
Department of Business, Industry and
Resource Development, Northern
Territory
Queensland Primary Industries and
Fisheries
Primary Industries and Resources of
South Australia
Department of Primary Industries,
Parks, Water and Environment,
Tasmania
Department of Primary Industries,
Victoria
Department of Fisheries, Government
of Western Australia
Office of the Chief Veterinary Officer,
Australian Government Department of
Agriculture

Industry

NSW Confederation of Freshwater Anglers
Tasmanian Salmonid Growers Association
Ltd
Tasmanian Association for Recreational
Fishing
Victorian Trout Association

The complete series of AQUAVETPLAN documents is available on the internet.⁵

⁵ < www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan >

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1 Nature of the disease

Furunculosis is a highly contagious, bacterial disease capable of causing high levels of morbidity and mortality in salmonid fish, particularly in unvaccinated populations. All age groups of salmonids in both fresh water and salt water are susceptible.

Furunculosis is exotic to Australia.

1.1 Aetiology

The aetiological agent of furunculosis in salmonids is the bacterium *Aeromonas salmonicida* subspecies *salmonicida*, which is commonly known as the 'typical' strain of *A. salmonicida*. This strain is exotic to Australia. For the purpose of this manual, furunculosis will be used to denote both infection and disease with *A. salmonicida* subspecies *salmonicida* (referred to as *A. salmonicida* subsp. *salmonicida* throughout this document) in salmonids and not infection or disease with other subspecies (biovars).

This is a critical distinction, as the 'typical' strain of the bacterium is exotic to Australia while other subspecies of the bacterium, commonly called 'atypical' strains, are not.⁶ Atypical strains will not be discussed except to highlight where their presence may complicate diagnosis and surveillance of the typical strain.

A. salmonicida subsp. *salmonicida* is a non-motile, gram-negative rod of the family *Aeromonadaceae* (NCBI 2008). This was the first biovar of the species recognised and subsequently designated as a subspecies. A characteristic of this bacterium is the formation of a brown, diffusible pigment on tryptone soy agar (TSA) although this characteristic is found in some atypical strains as well. *A. salmonicida* subsp. *salmonicida* has been shown to be genetically homogeneous (O'hici, Olivier & Powell 2000).

1.2 Susceptible species

Furunculosis may affect all species and all ages of both freshwater and marine salmonids, with brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) being particularly susceptible.

⁶ Currently there are four atypical strains of *Aeromonas salmonicida*: *A. salmonicida* subsp. *achromogenes*, *A. salmonicida* subsp. *masoucida*, *A. salmonicida* subsp. *smithia* and *A. salmonicida* subsp. *pectinolytica*. Atypical strains are a genetically heterogeneous group (O'hici, Olivier & Powell 2000). As with the typical strain, all subspecies except *A. salmonicida* subsp. *pectinolytica*, are pathogenic for fish. Three atypical biovars of these strains exist in Australia: in goldfish (*Carassius auratus*) (Carson & Handler 1988), greenback flounder (*Rhombosolea tapirina*) (Whittington et al. 1995) and farmed Atlantic salmon (*Salmo salar*) in Tasmania (J Carson, Tasmania DPIWE, pers. comm. 2008).

In Australia, all wild and farmed salmonid species, including Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) are considered susceptible to the disease.

Non-salmonid species of fish, in both freshwater and marine environments, are also susceptible to infection with *A. salmonicida* subsp. *salmonicida* (Table 1). Some of these findings were associated with an outbreak of furunculosis in salmonids; for example, where non-salmonid 'cleaner fish' (fish used to 'clean' the salmon of external parasites) have been held in farmed salmon sea cages in which salmon have furunculosis.

The importance of this is that although it is likely that the greatest impact of the disease will be on salmonid fish, non-salmonid fish species are capable of being infected and may transfer the pathogen.

A. salmonicida subsp. *salmonicida* is not a human pathogen.

Table 1 Examples of non-salmonid species susceptible to *Aeromonas salmonicida* subsp. *salmonicida*

Scientific name	Common name	Natural or experimental infection	Genus present in Australia?	Reference
<i>Anarhichas lupus</i>	Wolf-fish	Natural	No	Lillehaug, Lunestad & Grave (2003)
<i>Anguilla rostrata</i>	Eel	Natural	Yes	Hayasaka & Sullivan (1981) ^a
<i>Gadus morhua</i>	Atlantic cod	Natural/ experimental (limited)	No	Hjeltne et al. (1995); Lillehaug, Lunestad & Grave (2003)
<i>Hippoglossus hippoglossus</i>	Halibut	Natural/ experimental (limited)	No	Hjeltne et al. (1995); Lillehaug, Lunestad & Grave (2003)
Labridae spp.	Wrasse	Experimental	Yes	Hjeltne et al. (1995)
<i>Notropis cornutus</i>	Common shiners and other freshwater baitfish	Natural	Yes	Ostland, Hicks & Daly (1987)
<i>Petromyzon marinus</i>	Sea lamprey	Natural	No	El Morabit, García-Márquez & Santos (2004)
<i>Psetta maxima</i>	Turbot	Natural	No	Lillehaug, Lunestad & Grave (2003)
<i>Sander lucioperca</i>	Pike perch	Experimental	No	Siwicki et al. (2006)
<i>Sparus aurata</i>	Sea bream	Natural	Yes	Real et al. (1994)

^a This paper reports eels as being infected with 'furunculosis', but the bacterium isolated from diseased eels was *A. salmonicida* with no differentiation as to subspecies.

1.3 World distribution

Furunculosis is enzootic in northern European salmonid-producing countries, including Norway, Scotland and Ireland, as well as in North America, South Africa and Japan. It is an economically significant disease in these regions, although improved management and husbandry practices (including vaccination) have led to decreased mortality rates and outbreaks of clinical disease.

There has been no occurrence of furunculosis in Australia or New Zealand, and vaccination specific for this disease is not practiced.

1.4 Confirmation and differential diagnosis

1.4.1 Confirmation

For the purposes of this manual, confirmation of furunculosis will require the following:

- Where there are clinical signs and gross pathology in salmonids
 - a. observation of clinical signs and gross pathology consistent with furunculosis
 - b. presumptive identification of *A. salmonicida* subsp. *salmonicida* at the jurisdictional veterinary laboratory
 - c. confirmation of *A. salmonicida* subsp. *salmonicida* by CSIRO Australian Fish Diseases Laboratory (AFDL). AFDL has evaluated and validated polymerase chain reaction (PCR) tests for detection and identification of *A. salmonicida* isolates (Byers, Gudkovs & Crane 2002; Byers et al. 2002).
- Where there is suspected subclinical (covert) infection in salmonids
 - a. If clinical disease can be induced using a stress test, confirmation of *A. salmonicida* subsp. *salmonicida* by CSIRO AFDL using a combination of cellular and colonial morphology, and biochemical characteristics.
 - b. If clinical disease cannot be induced, a **presumptive** diagnosis of furunculosis can be made using molecular and immunological tests to detect *A. salmonicida* subsp. *salmonicida* as described above.

It is possible that *A. salmonicida* subsp. *salmonicida* may be isolated from a non-salmonid fish. In this case, CSIRO AFDL will confirm its presence, probably using PCR tests and sequence analysis. By definition, *A. salmonicida* subsp. *salmonicida* in a non-salmonid species is **not** called furunculosis. However, confirmation of the pathogen in this manner will still invoke the same disease response options as would the finding of furunculosis.

1.4.2 Differential diagnosis

Clinical signs observed during outbreaks of furunculosis are not specific for this disease. Therefore, differential diagnoses could include any fish disease that has the ability to cause similar, if not the same, clinical signs accompanied by high morbidity and mortality in salmonid fish. The most likely differential diagnoses are bacterial septicaemic and haemorrhagic viral conditions.

Enzootic diseases include:

- vibriosis – in particular caused by *Vibrio anguillarum*
- epizootic haematopoietic necrosis (EHN) – caused by EHN virus
- septicaemic conditions caused by atypical *A. salmonicida*.

Exotic diseases include:

- viral haemorrhagic septicaemia (VHS) – caused by VHS virus
- infectious haematopoietic necrosis (IHN) – caused by IHN virus
- vibriosis caused by a number of exotic *Vibrio* species.

Non-infectious conditions can cause clinical signs that resemble those observed in furunculosis. These include trauma from, for example, grading or electrocution. However, it is unlikely that these signs will be associated with high morbidity and mortality in the population.

Further information on both confirmation and differential diagnosis of furunculosis are provided in Appendix 1.

1.5 Resistance and immunity

The immune system of fish includes both innate and adaptive immunity. In comparison to mammals, the innate immune system in fish is generally more highly developed than the adaptive immune system (Watts, Munday & Burke 2001; Whyte 2007). In healthy fish, non-specific, innate defence mechanisms are immediately available to help prevent pathogenic invasion. These mechanisms include:

- physical barriers – scales, skin and associated mucous layers
- bioactive molecules – antimicrobial peptides, complement, lectins, antibodies, lysozyme, cytokines and other bacteriolytic enzymes (often found within mucous layers)
- inflammatory response cells – phagocytic cells and other leucocytes.

A. salmonicida subsp. *salmonicida* is a facultative intracellular pathogen. This intracellular attribute may allow this bacterium to temporarily avoid the host immune system once it has successfully invaded the fish host (Dacanay et al. 2003).

The specific defence mechanisms of the adaptive immune response are delayed relative to the innate immune response. This active immune response involves both the production of specific antibodies (in fish this is immunoglobulin M only, as fish are not known to possess immunoglobulin G) and the activation of leucocytes.

Salmonid populations do not gain any long-term, specific immunity to *A. salmonicida* subsp. *salmonicida* after an outbreak of furunculosis. Thus, the adaptive immune response of fish does not protect against recurring episodes of furunculosis after natural infection.

Genetic resistance to furunculosis has been shown to have high heritability (Gjedrem 2000). Some populations of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) have been selected for their heritable, innate resistance to furunculosis (Cipriano et al. 2002), which was linked to increased serum bactericidal activity (Hollebecq et al. 1995).

Vaccination, however, does lead to the production of antibodies against both cellular and soluble antigens of *A. salmonicida*. Vaccination also stimulates cellular immunity (Håstein, Gudding & Evensen 2005). Most vaccines use oil-based adjuvants because they confer superior protection and duration of protection compared to other adjuvants (Håstein, Gudding & Evensen 2005). Potent adjuvants like oil can cause intense local tissue reactions in the fish, which can be a downside of the vaccination process (see Figure 1).

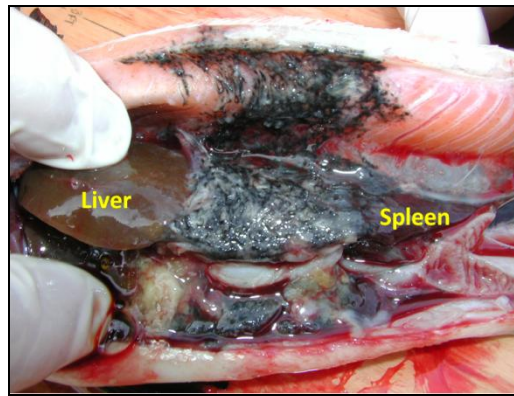


Photo: P Hardy-Smith.

Figure 1 Peritoneal cavity of an Atlantic salmon showing severe local tissue reaction, including extensive melanisation in response to injection with an oil-based vaccine. Although not a typical response, it does demonstrate the severity of the reaction to the oil adjuvant.

Vaccines are included in furunculosis management strategies overseas to provide protection against clinical furunculosis. However, there are no vaccines available that prevent or eliminate covert infections of *A. salmonicida* subsp. *salmonicida* (Hiney 1999; Hiney, Smith & Bernoth 1997). Vaccination does not fully protect a population although, anecdotally, the protective effect of vaccines against furunculosis in the field has been reported to range from 'acceptable' to 'very good' (Håstein, Gudding & Evensen 2005).

Håstein, Gudding and Evensen (2005) note that both typical and atypical *A. salmonicida* have antigenic characters in common. Vaccination of fish against *A. salmonicida* subsp. *salmonicida* may to some extent provide protection against infection with atypical *A. salmonicida*. There are no furunculosis vaccines currently approved for use in Australia. However, there is a bivalent vaccine available under a minor use permit (MUP number 9793) that is currently being used in Tasmania. This vaccine contains an inactivated atypical strain of *A. salmonicida*. Whether Tasmanian Atlantic salmon vaccinated against an atypical strain will be protected against challenge from the typical strain is not known.

A list of furunculosis vaccines available overseas at the time of writing is provided in Appendix 2.

Leucine metabolites (e.g. β -hydroxy- β -methylbutyrate) and probiotics (e.g. *Lactobacillus* spp.) significantly reduced mortalities due to furunculosis when fish were challenged with *A. salmonicida* subsp. *salmonicida*, indicating a significant innate response against this pathogen (Nikoskelainen et al. 2001; Siwicki et al. 2006). However, using these products in the face of a furunculosis outbreak is not

recommended, as this was experimental work and the efficacy of this approach in practice has not been shown.

1.6 Epidemiology

The epidemiology of furunculosis is not fully understood, but many factors are involved in the development of the disease following infection with *A. salmonicida* subsp. *salmonicida* (Smith 1997).

A key aspect of the epidemiology of furunculosis is that covert infections are common and may persist in fish populations for months. Stressors can cause covert infections to progress to clinical disease. Covert infections in salmonids or other fishes are a major factor in managing disease, particularly in the spread of infection.

There are a number of native marine and freshwater salmoniformes in Australia (e.g. Galaxiidae, Retropinnidae). Their distribution is more extensive than that of introduced salmonids. The susceptibility of native salmoniformes to furunculosis is not known, but needs epidemiological consideration as they may potentially have a profound effect on the spread of the disease.

1.6.1 Incubation period

At 14 °C, the period from exposure of naive fish to *A. salmonicida* subsp. *salmonicida* (by cohabitation with infected fish) to bacterial shedding can be as short as three days. Death can occur as soon as two days later (i.e. at five days post-exposure; Ogut & Reno 2005).

At lower temperatures, the time between infection and death may be prolonged. This may be due to the effects of temperature on pathogen multiplication and host defence mechanisms (Groberg et al. 1978).

In Australia, summer water temperatures in both fresh water and salt water where salmonids are farmed may exceed 18 °C. Hence, it is reasonable to assume that the incubation period for furunculosis could be as short as three days in many parts of Australia where salmonids are present. In winter the incubation period may be longer.

If wild salmonids in Australia are affected by furunculosis, it is possible that deaths due to the disease may not be observed, at least in the early stages. This must be considered when determining how long the disease has been present in wild populations if furunculosis is confirmed.

1.6.2 Persistence of the pathogen

General properties

A. salmonicida subsp. *salmonicida* is considered to be capable of surviving in a pathogenic form outside its host in marine, brackish and freshwater environments, and for prolonged periods (many months) in some waters and sediment (Hiney et al. 2002). The pathogen is also known to persist in animal reservoirs, as summarised in Table 2.

However, the period of survival reported in the scientific literature differs greatly. Reasons for the differences include:

- that cell survival is highly dependent on the composition and structure of the sediment within which survival is determined (Hiney et al. 2002)
- that *A. salmonicida* subsp. *salmonicida* is likely to survive in a non-culturable but viable state (as noted in Section 1.4.3), thus eluding common methods of detection (Pickup et al. 1996)
- the difficulties associated with isolating the pathogen from contaminated environmental samples (Hiney, Smith & Bernoth 1997).

Hiney et al. (2002) note that because survival is highly dependent on many factors, no single figure for survival time in the environment should be used as an estimate for risk assessment.

Table 2 Reservoirs and potential transport hosts of *Aeromonas salmonicida* subsp. *salmonicida*

Reservoirs and transport hosts	Marine farms	Freshwater farms and hatcheries
Breeding stock from marine farms (covert infections)	na	Yes
Farm equipment	Yes	Yes
Other infected fish (cultured or wild) in the water system	Yes	Yes
Personnel	Yes	Yes
Smolts from hatcheries (covert infections)	Yes	na
Transfer of infection between closely neighbouring farms (sediment water, birds)	Yes	Yes
Water transfer from infected hatcheries to marine farms	Yes	na

na = not applicable

Source: Smith (1997).

Live fish

Covertly infected wild and farmed fish may act as reservoirs of *A. salmonicida* subsp. *salmonicida* (Bernoth 1997; Ferguson 1988). This includes both salmonid and non-salmonid fish. Continuous shedding of bacteria and re-infection can maintain the infection without additional introduction of the bacterium (Hiney, Smith & Bernoth 1997).

The epidemiological relationship between wild and farmed fish is unclear, although covertly infected wild fish present in rivers supplying freshwater farms appear to influence the incidence of covert infections in hatchery fish populations (McCarthy 1977). Stress experienced by these wild fish populations during spawning and smoltification might account for seasonal fluctuations in the frequency of stress-inducible furunculosis infections in hatchery fish populations (Hiney, Smith & Bernoth 1997). Indirect evidence (Jarp et al. 1993) and epidemiological models (Johnsen & Jensen 1994) suggest that covertly infected migratory fish that escape from marine pens may transfer infection to freshwater fish or their environment by migrating upstream into rivers.

Other vertebrate animals

Animals that may come in contact with infected fish should be considered potential transport hosts capable of spreading viable *A. salmonicida* subsp. *salmonicida*. For example, sea birds and rodents around land-based farms could carry the lipophilic aggregates of free *A. salmonicida* subsp. *salmonicida* cells (Enger 1997) and transfer the bacteria to the fish.

Aquatic invertebrates

Marine plankton, protozoa and other ectoparasites such as copepods (e.g. salmon louse) and branchiurans may act as reservoirs of *A. salmonicida* subsp. *salmonicida* (Nese & Enger 1993). Bivalve molluscs can acquire *A. salmonicida* subsp. *salmonicida* via filter feeding and then act as a source either directly or via translocation (Starliper 2001).

Water

The bacterium is considered to be capable of surviving in a pathogenic form outside its host in marine, brackish and freshwater environments (Hiney et al. 2002).

Survival times for *A. salmonicida* subsp. *salmonicida* in fresh water and sea water vary greatly, being from 2 to 63 days and 2 to 24 days respectively (Hiney 1994). Other studies have shown a 90% reduction in the number of colony-forming cells after 1.4–2.2 days in sea water and 3.4 days in brackish water or fresh water (Enger 1997; McCarthy 1977; Rose, Ellis & Munro 1990). Survival time is dependent on many factors including temperature, salinity and the presence of organic matter.

Biofilms

A. salmonicida subsp. *salmonicida* is capable of adhering to solid surfaces (Carballo, Seoane & Nieto 2000), and hence may be present in the biofilm but not detectable in the water column. Biofilms are microenvironments that can be important in protecting bacteria from lethal factors.

Sediment

Sediment is an important environmental reservoir of *A. salmonicida* subsp. *salmonicida* as the pathogen can survive and retain its pathogenicity in faecal and food waste sediment at the bottom of sea cages, freshwater tanks or in pond mud (Hiney 1994; Munro & Hastings 1993). For example, *A. salmonicida* subsp. *salmonicida* was detectable in non-sterile pond mud for at least 29 days (McCarthy 1977), retaining its pathogenicity in such mud for 6–9 months (Plumb 1999). Viable *A. salmonicida* subsp. *salmonicida* cells were detected for more than 105 days when the pathogen was exposed to organic particulate matter (Sakai 1986).

In the absence of overt disease, *A. salmonicida* subsp. *salmonicida* can persist in marine salmon farms for periods of up to six months (Smith et al. 1982).

Hiney et al. (2002) showed that *A. salmonicida* subsp. *salmonicida* in a sediment–water mix remains viable for as long as 276 days.

Farm equipment

During an outbreak of furunculosis, it is likely that farm equipment will become contaminated with *A. salmonicida* subsp. *salmonicida*.

A. salmonicida subsp. *salmonicida* can survive for up to six days on both wet and dry contaminated fish nets (McCarthy 1977). In Sweden, contaminated equipment has been implicated in the spread of furunculosis to uninfected sites on at least two occasions (Wichard, Johansson & Ljungberg 1989). The surface of the equipment may be important; one study indicated that *A. salmonicida* subsp. *salmonicida* attached to plastics in much higher numbers than to stainless steel (Carballo, Seoane & Nieto 2000).

1.6.3 Modes of transmission

Horizontal spread

A. salmonicida subsp. *salmonicida* is shed into the environment primarily by clinically diseased and dead fish, which are the main environmental source of this pathogen. Shedding from live, clinically affected fish is primarily via faeces and urine, and from furuncular lesions (Enger et al. 1992; McCarthy 1977; Novotny 1978). It may also be shed from reservoirs of infection, such as resuspended infected sediment.

There are three potential portals of entry of *A. salmonicida* subsp. *salmonicida* into the fish: gills, skin and gastrointestinal tract.

Branchial (i.e. gill) colonisation with *A. salmonicida* subsp. *salmonicida* is frequently observed in infected fish (Ferguson 1988; Hiney 1994). The pathogen is often present on the external surfaces of infected fish and can readily invade via any form of skin lesion. *A. salmonicida* subsp. *salmonicida* is also able to colonise the intestinal lumen and cross the intestinal wall (Jutfelt et al. 2006; Ringø et al. 2004).

Horizontal transmission of furunculosis may occur if fish are exposed to:

- other fish (direct fish-to-fish contact); the risk of transmission occurring via this route increases with
 - increased stocking densities (Ogut & Reno 2004)
 - fish crowding in one area of the enclosure
 - handling procedures
 - the presence of small, wild fish
 - introduced or naturally present 'cleaner' fish
- fresh water infected with *A. salmonicida* subsp. *salmonicida* (McCarthy 1977). Hatcheries can minimise outbreaks of furunculosis if their intake water is free from *A. salmonicida* subsp. *salmonicida* (Needham & Rymes 1992; P Hardy-Smith, pers. obs.)
- sea water infected with *A. salmonicida* subsp. *salmonicida* (Hiney 1994). The pathogen can potentially spread through the water column to neighbouring marine farms (Turrell & Munro 1988); this is dependent on water currents, density of fish in farms and pathogen load in the water
- lipid-rich bacterial aggregates found at the water surface; these adhere to birds or to food pellets dropped into the water and form aerosols in high winds (Enger 1997; Enger & Thorsen 1991)

- invertebrates such as sea lice (e.g. *Lepeophtheirus salmonis*) (Nese & Enger 1993) and bivalve molluscs (Starliper 2001). *L. salmonis* is not present in Australia but it is possible that ectoparasites found in Australia, such as branchiurans (e.g. *Argulus* spp.) and copepods (e.g. *Caligus* and *Ergasilus*) could become vectors for the transmission of *A. salmonicida* subsp. *salmonicida*.

Vertical spread

There is evidence that the pathogen is associated with surface contamination of fertilised eggs (Cipriano et al. 2001).

It is standard practice in farming areas where furunculosis is endemic to perform surface disinfection on all fertilised eggs using an iodine preparation. Cipriano et al. (2001) reported that an effective regime to prevent transmission of egg associated *A. salmonicida* subsp. *salmonicida* is to disinfect eggs twice; once using 50 mg/L active iodine for 30 minutes and then using 100 mg/L active iodine for 10 minutes.

1.6.4 Factors influencing transmission and expression of disease

Predisposing factors that lead to the development of clinical furunculosis are primarily those that cause the fish to be stressed, leading to elevated plasma cortisol levels and consequent leukocytopenia and immunosuppression. This makes the fish more susceptible both to primary infection and to the progression of clinical disease from covert infection with *A. salmonicida* subsp. *salmonicida*.

Endogenous factors

- *Smoltification*. Atlantic salmon (*Salmo salar*) are anadromous and 'smolt' at any time from one to three years of age. Smoltification is a process of extensive physiological change in preparation for the marine environment and can cause a prolonged period of stress.
- *Spawning*. The stress of spawning can also increase a salmonid's susceptibility to furunculosis.

Exogenous factors

- *Elevated temperature*. This is considered a primary factor influencing the onset of clinical furunculosis. Water temperatures of 15–20 °C (as experienced in late spring, summer and early autumn in southern Australia) correlate with increased clinical signs of furunculosis as a direct result of temperature stress (Lillehaug, Lunestad & Grave 2003; Sako & Hara 1981), and also with more rapid growth of *A. salmonicida* subsp. *salmonicida* (Malnar, Teskeredzic & Coz-Racovac 1988; Pickering 1997). Groberg et al. (1978) showed that at 3.9 °C and 6.7 °C, mortality in fish infected with *A. salmonicida* varied from 2% to 26% among three salmonid species (*Oncorhynchus mykiss*, *O. kisutch* and *O. tshawytscha*), whereas at 20.5 °C, 93–100% of these fish died within 2–3 days. This paper did not specify a subspecies of *A. salmonicida* but the disease was reported as being furunculosis.
- *Low levels of dissolved oxygen*. Low dissolved oxygen can cause respiratory distress and may induce a classical stress response leading to infection and clinical furunculosis. Likewise, oxygen supersaturation can also predispose fish to infection, clinical disease and death.

- *Poor water quality.* This is closely associated with the onset of bacterial infections in fish. Exposure of fish to high ammonia levels, chlorine, pesticides, metal pollution, sewage sludge and other organic matter or respiratory waste can result in suppression of the immune system and greater susceptibility to infection.
- *Physical damage to the skin and gills.* Physical damage resulting from, for example, rough handling, predator attack, algal blooms and ectoparasites can lead to infection with *A. salmonicida* subsp. *salmonicida* (Morgan, Rhodes & Pickup 1993; Pickering 1997).
- *Improper timing of transfer of fish from fresh water to sea water.* In Atlantic salmon, the 'smolt window' (i.e. time during which fish are physiologically prepared for the transition from fresh water to salt water) is relatively narrow and does not necessarily occur at the same time for all fish within a population. It can be difficult to judge when a population of anadromous salmonids is capable of surviving the transfer from fresh water to seawater. 'Pre-smolts' who are not yet ready for transfer can suffer osmotic shock and severe immunosuppression if transferred into a full marine environment. Likewise, 'post-smolts' will also suffer osmotic stress before reverting back to a freshwater physiological state.
- *Management practices.* Management practices that may trigger a transient stress response in fish (Pickering & Pottinger 1989) include:
 - high stocking densities
 - grading
 - handling and hauling (transporting)
 - netting
 - injection and smolt transfer
 - lighting
 - inadequate predator protection
 - poor or inadequate nutrition, especially vitamin C deficiency.

Fish exposed to these transient stresses are less susceptible to bacterial infection than those exposed to chronic stresses, such as poor water quality (Pickering 1997).

Transmission of furunculosis is directly related to stocking density (Ogut & Reno 2004). Reducing stocking density (and hence stress) has reduced mortalities during a furunculosis outbreak (Glenn & Taylor 2006).

Stopping feeding can also reduce mortalities, although the reduction in mortality is often transitory (P Hardy-Smith, pers. obs.).

Psychological stress associated with social domination is one of the most potent forms of chronic stress in subordinate fish.

1.7 Impact of the disease

The Australian salmonid industry includes commercial farming, hatcheries, tourism and recreational fishing.

Salmonids are not native to the southern hemisphere, but have been introduced into Australia over the last 150 years. Salmonid imports have been prohibited under quarantine regulations since 1975. There are five species of exotic salmonids in Australia:

- Atlantic salmon (*Salmo salar*)
- brook trout (*Salvelinus fontinalis*)
- brown trout (*Salmo trutta*)
- chinook (quinnat) salmon (*Oncorhynchus tshawytscha*)
- rainbow trout (*O. mykiss*).

Of these species, brown, brook and rainbow trout have established self-sustaining populations where suitable conditions exist (Kahn et al. 1999). Wild populations of Atlantic and chinook salmon are supported by regular release of hatchery-bred fish.

Farmed salmonid production in Australia for the financial year 2007–08 was \$299.3 million (ABARE 2009). This was produced by over 80 farms across Australia. Most production was in Tasmania (\$290.9 million), which was predominantly Atlantic salmon.

In 1999, the recreational salmonid fishing sector was estimated to be worth approximately \$234 million. In Tasmania, New South Wales and Victoria, salmonid fishing activity is significant. Trout fishing is important in Western Australia and South Australia (McIlgorm & Pepperell 1999). McIlgorm and Pepperell (1999) estimated a value of \$1025 million (highest estimate) arising from the total collapse of expenditure nationally due to disease over a five-year period, assuming a 6% discount rate. These estimates are 'worst case scenarios'.

Therefore, there is considerable value in these industries, particularly in Tasmania where salmon farming is one of the most important animal production industries. If furunculosis was to become endemic in Australia, the value of these industries will decrease. The extent of the reduction would depend on the extent of the establishment of the disease. For example, if the disease became established in Tasmania, the reduction in value could conceivably be in the millions of dollars.

2 Principles of control and eradication

This section provides background information to enable the choice of the most appropriate response option following detection of furunculosis or *Aeromonas salmonicida* subsp. *salmonicida* in Australia.

Furunculosis can cause high mortality and morbidity in susceptible salmonid populations. The disease is exotic to Australia, but if introduced it has the potential to have a serious impact on the salmonid farming industry and wild salmonid populations.

Furunculosis is highly contagious. Covert infection is a feature of the disease. Depending on conditions, the pathogen can remain viable for months in the environment. *A. salmonicida* subsp. *salmonicida* can also survive in a non-culturable but viable (NCBV) state, which restricts the range of effective measures for monitoring and surveillance. Molecular biology techniques may be able to detect NCBV material if present.

There are essentially three disease response strategies available to minimise the impact of this disease if exclusion strategies were unsuccessful in keeping furunculosis out of Australia:

- Eradication—eradication of furunculosis and *A. salmonicida* subsp. *salmonicida* from Australia. This is the preferred response option, which is the highest level of response measure and may also incur the highest cost in the short term. It is acknowledged that eradication may not be possible if the disease is present in wild fish populations. Currently in Australia, there are no formal agreements in place to compensate for stock losses where destruction of fish is carried out as a means of eradicating a disease.⁷
- Containment, control and zoning—containment of the disease and bacterium to areas with identified infection, prevention of further spread and protection of uninfected areas.
- Control and mitigation of disease—the implementation of management practices that decrease the incidence and severity of clinical outbreaks. In the short term this is the lowest level of response measure and may incur the least cost. However, it may also incur significant cost in the long term through its impact on production.

The basic principles of eradication and other response options are described in the AQUAVETPLAN **Enterprise Manual** and the AQUAVETPLAN **Control Centres Management Manual**. Appendix 1 of the AQUAVETPLAN **Enterprise Manual** lists the state/territory legislation relating to disease control and eradication.

⁷ Fish farms that are insured against stock losses will generally be able to claim for loss of fish where fish have died from disease, but will not be able to claim for loss of fish where fish have been destroyed as part of a disease control strategy.

If furunculosis or *A. salmonicida* subsp. *salmonicida* is detected, the general principles for any response option include:

- rapid detection and identification of disease and infection
- rapid definition
 - of the nature and extent of the outbreak
 - of the extent of exposure of wild fish populations and environmental reservoirs
 - and implementation of response measures
- prevention of bacterial spread by controlling stock and water movement, within and between farms
- ensuring good management practices and biosecurity control measures.

Selecting the most appropriate option will depend on:

- the system(s) in which furunculosis is detected (e.g. semi-open system, closed system)
- the location and presence (or absence) of reservoirs of infection. This must acknowledge the limitations of detecting the pathogen in the environment and in salmonids with covert furunculosis, and the potential for non-salmonids to be infected with *A. salmonicida* subsp. *salmonicida*
- short-term costs of the response measure and disruption to production, acknowledging that no formal compensation mechanism currently exists if fish are destroyed
- long-term costs of production with or without the presence of the pathogen
- long-term costs of control should the pathogen become endemic.

These factors are all influenced by whether farmed or wild fish (or both) are affected, as described below.

2.1 Aquatic animal systems

For the purpose of aquatic animal disease control in Australia, four systems are used to describe the methods used to farm aquatic animals and for wild aquatic animals. These systems are open, semi-open, semi-closed and closed. The AQUAVETPLAN **Enterprise Manual** fully explains each of these systems in the context of generic disease control.⁸ The following sections provide a summary of these systems in the context of furunculosis.

⁸ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan>>

2.1.1 Open systems

Fish growing wild in rivers, lakes and the ocean are considered to be in an open system, as there is no control over fish or water movement. In Australia, Victoria, South Australia, New South Wales and Tasmania have wild salmonid populations growing in open systems.

2.1.2 Semi-open, semi-closed and closed systems

The main systems involved in salmonid aquaculture are semi-open and semi-closed systems with a small amount of culture in closed systems for juvenile fish.

Semi-open systems

Semi-open systems are systems where there is control of fish movement but no control of water flow or the aquatic environment (e.g. cage-pen culture in estuaries or lakes). Although there may be control of the farmed aquatic species in these systems, there is no control over wild aquatic species that may be in close contact with the farmed species.

Cages can become damaged, thereby allowing fish to escape into the wild. Wild fish species are able to swim in and out of the cages at any time if the fish are small enough.

Fish and feed wastes can enter the environment directly, as can any pathogen shed by infected fish.

Semi-closed systems

Semi-closed systems are systems where there is control of fish movement and some control of water flow (e.g. raceway culture using water drawn off a river).

These systems are not designed to be self-contained, and so preventing inflow or outflow of water will have adverse effects on the fish. Although control and treatment of discharge water is possible, it is unlikely to be feasible due to the large volumes of water involved.

A potential hazard is the risk of wild fish being able to enter farm waterways, and possibly ponds, via intake water from the rivers. Farmed fish can also escape, and if water continues to discharge pathogens may enter the environment.

Pathogens can potentially be spread by predators in both semi-open and semi-closed systems.

Closed systems

Closed systems are those where there is total control over water and fish (e.g. a recirculation hatchery system). These systems are used to a small degree in salmonid culture for early growing of fingerlings. These systems present the simplest scenario for control of furunculosis.

2.2 Methods to prevent spread and eliminate pathogens

To prevent the spread of *A. salmonicida* subsp. *salmonicida*, quarantine and movement controls need to be implemented. Knowledge gained from zoning, tracing and surveillance measures contributes to effective management of pathogen spread.

2.2.1 Quarantine and movement controls

The following quarantine and movement restrictions should be implemented immediately upon suspicion of furunculosis where there is:

- disease in salmonids causing high morbidity and mortality with evidence of extensive haemorrhage in affected fish, with or without furuncles
- suspicion or confirmation (i.e. if the bacterium is isolated from a non-salmonid fish) of *A. salmonicida* subsp. *salmonicida*.

Establishment of quarantine areas

When furunculosis is suspected, specified areas should be established (Figure 2; see the AQUAVETPLAN **Enterprise Manual**, Section A for more details), including:

- *declared area* – includes the restricted area and control area
- *infected premises or area* – a clearly defined area, which may be all or part of a premises, lease or waterway in which an emergency aquatic animal disease exists
- *restricted area* – area around infected premises or area
- *control area* – a buffer between the restricted area and free areas
- *free area* – non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of *A. salmonicida* subsp. *salmonicida* remains unassessed).

Note that if furunculosis is detected or suspected in wild fish, there may be limited ability to define the extent of the 'infected area' due to difficulty in determining the potential range of movement of wild fish.

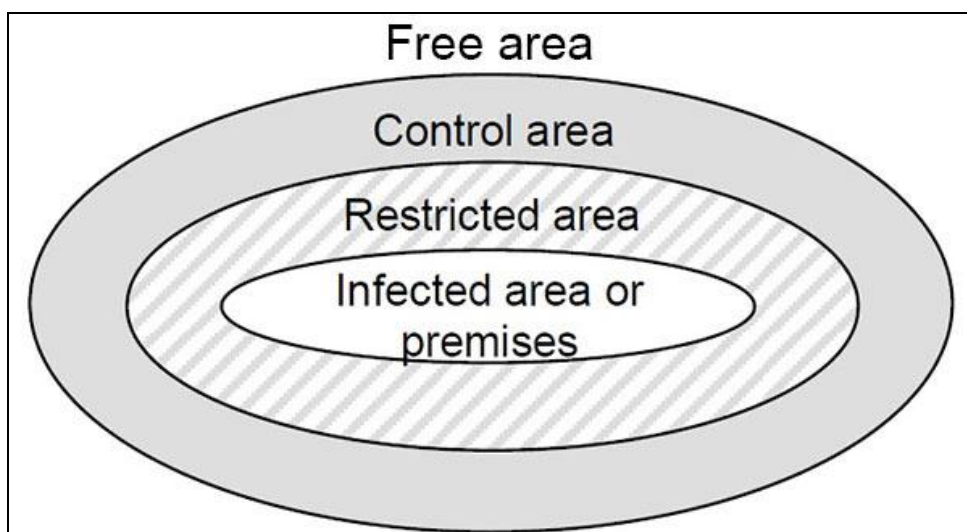


Figure 2 Establishment of specified areas to control furunculosis

When declaring quarantine areas, consider:

- other salmonid farms in the area
 - for freshwater hatcheries, other farms within the same watershed or linked by movement of vehicles, equipment and personnel
 - for marine operations, other farming operations within a distance in the order of kilometres (based on epidemiological risk factors as outlined in Jarpe and Karlsen (1997) and McClure, Hammell & Dohoo (2005), which, though not specifically about furunculosis, provide very useful information regarding risk factors and spread of disease)
- the presence of native or introduced fish populations susceptible to furunculosis
- the presence of potential vectors (e.g. wild fish, copepods and bivalves)
- environmental factors, such as the direction and strength of water flow
- live fish transportation between and within freshwater and marine operations (including smolts going out to cages, broodstock and marine cage towing)
- fish harvesting and transportation to processing plants
- discharge of processing plant effluent
- transportation of consumer-ready products
- disposal of dead fish
- disposal of waste products from processing plants
- recreational fishing activities.

Movement controls

The feasibility of restrictions and bans and the extent to which these are able to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen (i.e. whether the aim is to eradicate the disease agent or to control its spread).

Implementation of bans and restrictions will be a dynamic process. Implementation will be determined by the location and extent of the ability to define the disease outbreak (where clinical furunculosis is present) and the distribution of infected fish and reservoirs (where covert furunculosis is suspected or *A. salmonicida* subsp. *salmonicida* is detected in a non-salmonid fish) – or both.

Movement controls include banning:

- the movement of ALL live fish into or out of declared area(s)
- the release of fish into river systems or marine locations in declared area(s)

- the movement of fish between different river systems, between marine farm locations, and between marine and freshwater farm locations within the declared area(s)
- recreational fishing in the declared area(s)
- or restricting the use and movement of equipment and personnel within and between river systems (or farms) and marine farms within the declared area(s).

2.2.2 Zoning

If furunculosis were to become endemic in specific regions of Australia, a zoning policy specific for the disease and for the bacterium *A. salmonicida* subsp. *salmonicida* may be necessary to protect non-infected areas and to prevent further spread of infection. Zones would be based on the distribution of any vectors or reservoirs present or suspected (if appropriate), the geographical and hydrological characteristics of water bodies and landforms, and predictions of the most likely method of spread of infection. Zoning may rely on the identification of biogeographic barriers. A corresponding surveillance and monitoring program for furunculosis would be required to support the zoning policy.

Principles of zoning for infected and non-infected zones in Australia are outlined in the *AQUAPLAN zoning policy guidelines*.⁹ Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the *Aquatic animal health code* (OIE 2009; Chapter 4.1).¹⁰

Zoning for furunculosis could be difficult depending on where it is detected. Covertly infected fish populations can become established and are very difficult to detect. Reservoirs of infection can become established in the environment and in wild fish populations, and are unlikely to be successfully eradicated. Once established in a river system or in a migratory wild fish population, infection is easily spread. These factors make it very difficult to protect furunculosis-free zones.

2.2.3 Tracing

Tracing a disease outbreak is the process of retrospectively determining the method and pattern of disease spread and finding the source of the outbreak. Tracing investigations form part of the outbreak investigation, and are crucial in determining all confirmed and potential locations of the disease, as well as defining restricted and control areas. The information gathered from tracing will assist in shaping the most appropriate response action. A full outbreak investigation, which includes tracing, must be conducted as soon as possible after the detection of furunculosis.

The immediate steps required are to trace-back all contacts with infected fish, premises and sites (to establish the source of the outbreak) and to trace-forward all

⁹ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/guidelines-and-resources>>

¹⁰ <<http://www.oie.int/international-standard-setting/overview/>>

contacts with infected fish, premises and sites (to establish the current location and potential spread of infection).

The presence or absence of predisposing factors must be examined when determining the duration of the outbreak and estimating the time and source of initial infection. It is possible that a covert infection may have been present for some time before clinical disease becomes apparent. Much information may also come from a detailed investigation regarding what is happening on the affected farm or area.

As a guide, items that must be traced are:

- fish
 - farmed fish, including broodstock, fingerlings, smolts, fish for stocking waterways, fish for farm dams etc.
 - wild fish, including both salmonid and non-salmonid species (acknowledging that it is not possible to trace individual wild fish)
- fish products—eggs, fish for consumption, effluent and waste products from slaughter and processing
- water—input and output
- vehicles—fish transport vehicles, feed trucks, visitors' cars, boats
- equipment—fish cages, nets, other floating installations, tools and instruments
- personnel—farm workers, sales and feed representatives, tradespeople, veterinarians, scientists, technicians and visitors
- processing facilities—particularly if there is a possibility that infected fish have been harvested and sent for processing.

Neighbouring fish populations

Neighbouring fish farms and processing plants may become or may already be infected depending on a number of factors, including their location, and the severity and duration of infection on the infected premises.¹¹

Maps identifying the location of neighbouring fish farms, processing plants, waterways and hydrographic data are helpful to monitor the potential spread of the pathogen.

The location of susceptible wild fish species and vectors should also be confirmed both upstream and downstream of the infected freshwater site. In the marine environment, knowledge of tidal and current patterns in the affected area is

¹¹ It is also worthwhile to use the investigation of neighbouring fish farms and processing plants to alert these facilities about the outbreak and provide immediate education on prevention, including implementation of risk management strategies.

critical. Further sources of infection may be identified if a number of facilities share common water.

For information on the location of farming establishments and wild fish populations at risk of infection, contact the relevant state and territory fisheries or agriculture agency (see the AQUAVETPLAN **Enterprise Manual**, Appendix 5 for contact details).

2.2.4 Surveillance

Surveillance is necessary to:

- define the extent of the infection
- detect new outbreaks
- establish restricted and control areas to which quarantine and movement restrictions can be applied
- establish infected and non-infected areas or zones for a furunculosis zoning program
- monitor the progress and success of an eradication or control strategy.

At present, there is no recognised surveillance program either in Australia or overseas that is specific for *A. salmonicida* subsp. *salmonicida*. Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the *Aquatic animal health code* (Chapter 1.4; OIE 2009).¹²

Confirmation of furunculosis in Australia is defined in Section 1.4.1 of this manual. It would be based on observation of clinical signs in salmonids, and then isolation and confirmed identification of *A. salmonicida* subsp. *salmonicida*. Diagnosing subclinical infections is difficult, but the stress-inducible furunculosis (SIF) test may be of value.

As also noted in Section 1.4.1, it is possible that the first detection of *A. salmonicida* subsp. *salmonicida* in Australia may be from a non-salmonid fish. The detection of *A. salmonicida* subsp. *salmonicida* in this way would invoke the same disease response as would detection of clinical or covert furunculosis.

2.2.5 Treatment of infected host species

In an outbreak, treatment of fish can decrease morbidity and mortality and can reduce the shedding of pathogen into the environment.

Farmed fish can be effectively treated for furunculosis using antibiotics, generally administered via the feed. When medicating populations of fish (e.g. salmon in sea cages), preparation and distribution of medicated feed can take a number of days, particularly if the medication is milled into the feed; hence, there is generally a lag period between the decision to treat and feeding the medication to the fish.

¹² <http://www.oie.int/eng/normes/fcode/en_sommaire.htm>

Considerations for the use of antibiotics for the treatment of furunculosis include:

- the cost of treatment, including preparation, labour, administration and equipment
- the fact that sick fish do not eat, so in-feed medication will assist in protecting asymptomatic fish, but not fish with clinical disease. The lag period required for preparation and distribution of feed may mean that the number of sick fish will continually increase before the feed arrives at the farm
- that treatment will not clear a farm of infection – covert infections are likely to persist even with treatment
- the necessity to apply withholding periods, so treatment of fish populations close to slaughter may not be feasible
- that multiple drug-resistant strains of *A. salmonicida* subsp. *salmonicida* can develop.

In the event of an outbreak it is essential that samples be collected for culture and sensitivity testing using an approved method before the administration of any treatment (Hawke et al. 2006a,b). This will ensure that the treatment does not preclude pathogen isolation and culture, and will also ensure that the most appropriate and effective antibiotic treatment is selected.

The decision to treat the fish (or not) will also depend on the circumstances. For example, eradication will be the preferred response option if furunculosis is detected in Australia. If furunculosis is detected in a farmed population of salmonids, the decision may be made to slaughter out this population. However, this may take weeks to months, and during this time there will be considerable shedding of *A. salmonicida* subsp. *salmonicida*. Treatment may assist in decreasing this shedding during the slaughter-out process, provided the lag period between deciding to treat and getting medicated feed to fish is kept short.

Antibiotic treatment may be limited to incidences when there is no alternative control method (e.g. use of antibiotics to prevent loss of fish stocks during a severe furunculosis outbreak where large numbers of fish are still clinically healthy).

Minor use permits exist in Australia for treatment of salmonids using oxytetracycline and florfenicol, both of which have been used to treat furunculosis overseas. In particular, florfenicol has clinically been shown to be very effective for this disease overseas (P Hardy-Smith, pers. obs.). The withholding period for oxytetracycline is 1600 degree days¹³ for initial treatment and 1000 degree days for florfenicol. Further details are available from the Australian Pesticides and Veterinary Medicines Authority (APVMA).¹⁴

¹³ 'Degree days' equals the number of days multiplied by the water temperature, hence 1000 degree days may be 100 days at an average water temperature of 10 °C or 50 days at an average water temperature of 20 °C.

¹⁴ <www.apvma.gov.au>

Multiple drug-resistant strains of *A. salmonicida* subsp. *salmonicida* have been detected overseas (McIntosh et al. 2008). While drug-resistant *A. salmonicida* subsp. *salmonicida* remains in the environment, attempts to eradicate infection through the continued use of antibiotics will be unsuccessful. This is because as antibiotic concentrations decrease in the fish, reinfection can occur (McCarthy 1977).

2.2.6 Treatment of host products and byproducts

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered when determining the treatment and processing, and the destiny of fish products and byproducts.

A. salmonicida subsp. *salmonicida* can survive in dead fish, even when frozen for up to 50 days (Ferguson 1988). Therefore, there may be a risk of the dissemination of infection to uninfected areas if there is a possibility that the infected frozen product could come in contact with and be transferred to susceptible fish species in those areas.

Furunculosis is not a recognised zoonosis, so infected fish may be consumed after the treatments and appropriate drug withholding times outlined above.

Viable fish eggs may be surface disinfected to prevent mechanical transmission. Surface disinfection of eggs is discussed in Section 1.6.3.

2.2.7 Destruction of hosts

Destruction of fish must be both hygienic and humane as outlined in the AQUAVETPLAN **Destruction Manual**.¹⁵ There must be no spillage of infectious waste. Increased bacterial shedding may occur if fish are stressed at slaughter, therefore the least stressful methods should be used.

There are many different methods to anaesthetise or destroy fish, all of which have limitations. These methods are discussed in the AQUAVETPLAN **Destruction Manual** and include using standard harvesting procedures, and chemical anaesthesia and euthanasia.

The most appropriate method of destruction depends on the:

- size and number of fish
- deadline for slaughter – this depends on the pressure of infection and the risk of further spread
- resources available for slaughtering fish – a slaughter-out policy may require crews to work around the clock
- type of system

¹⁵ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan>>

- destination—human consumption or disposal. Although not a human pathogen, furunculosis can result in unsightly lesions within and on the fish carcase
- slaughter facilities—site, equipment and methods available
- experience and availability of personnel.

Any chemicals used must be approved for that use by the APVMA.

In addition, any chemical that is used directly or indirectly for the control of an animal disease is governed in its use by relevant 'control of use' legislation in each state and territory. The relevant state or territory authority (in most cases this is the veterinary registrar within the relevant state department of primary industry or agriculture) should also be consulted for advice before the chemical is used.

2.2.8 Disposal of fish deemed to be infected

In an outbreak of furunculosis, fish will either die from the disease, be harvested for human consumption or be destroyed. Disposal of the fish will depend on the cause of death.

For more details on the disposal of fish, see the **AQUAVETPLAN Disposal Manual**.¹⁶

2.2.9 Decontamination

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, the state or territory chief veterinary officer and/or the director of fisheries. The protocol should take into consideration the factors outlined in Section 1.6, in particular:

- the source and location of infection
- the type of enterprise (e.g. farm, processing plant, hatchery, grow-out ponds)
- water source
- the construction materials of the buildings and structures on the site
- the design of the site and its proximity to other waterways or buildings
- current disinfection and biosecurity protocols
- workplace safety concerns
- environmental impact of the disinfectant protocol
- legislative requirements (occupational health and safety, environmental protection, chemical use)
- availability of approved, appropriate and effective disinfectants.

¹⁶ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan>>

Effective decontamination of equipment, materials, tanks and buildings requires thorough cleaning before disinfection.

The ability of *A. salmonicida* subsp. *salmonicida* to remain viable for long periods in sediments must be considered.

For more information on decontamination methods, disinfectants and their indications, see the AQUAVETPLAN **Decontamination Manual**.¹⁷

2.2.10 Vaccination

Vaccination is used overseas in the management (but not elimination or eradication) of furunculosis. Vaccination is discussed in Section 1.5. In Australia, the use of vaccination in the control of furunculosis would depend upon the choice of response strategy.

Eradication

Vaccination is not appropriate for the eradication of furunculosis because:

- it does not prevent or eliminate covert infections of fish (Hiney, Smith & Bernoth 1997) – such fish are capable of transmitting infection to non-infected populations
- vaccinated fish may interfere with surveillance and monitoring programs, as they may test falsely positive for the bacterium if a diagnostic test that does not differentiate between viable and non-viable bacteria is used (e.g. polymerase chain reaction techniques).

Containment, control and zoning

Vaccinated fish populations may test falsely positive to immunological or DNA-based tests since such tests are currently unable to distinguish between the presence of the vaccine or a natural infection (Hiney, Smith & Bernoth 1997; Høie et al. 1993). Therefore, vaccination may interfere with surveillance and control programs if such tests are used. However, bacterial culture will distinguish between viable pathogen and killed vaccine agent. Vaccination may be useful for fish in areas outside the infected area to provide an additional layer of protection.

Control and mitigation of disease

If furunculosis became endemic in Australia, vaccination would be a useful management tool to increase population immunity, as it has been in overseas countries.

Although no vaccines for the typical strain of *A. salmonicida* are available in Australia, a vaccine containing an atypical strain of *A. salmonicida* is currently available and being used. It is not certain whether this vaccine would confer a level of immunity against the typical strain that causes furunculosis.

¹⁷ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan>>

A list of commercially available vaccines for the typical strain, which are available overseas, is given in Appendix 2. Details of how to obtain approval for use of these vaccines in Australia are provided in Appendix 3.

2.2.11 Vector control

A potential problem with the control of furunculosis is the control of biological vectors, such as wild fish, birds and aquatic invertebrates (e.g. copepods and molluscs). 'Cleaner fish' (mentioned in Section 1.2) are not routinely used in Australia.

Wild fish

Controlling wild fish is impossible in many areas. Attempts can be made to prevent contact between wild fish and farmed fish, but there is limited ability to do this in marine farming operations.

Birds

Sea pens, raceways, open air tanks and ponds may attract birds and so must be covered (e.g. using nets or tank roofs) to prevent access by birds, which can potentially spread disease.

Copepods and molluscs

Attempts can be made to decrease contact between copepods and molluscs, and farmed fish (e.g. by keeping organic buildup on nets to a minimum and removing fouling from boat hulls).

2.3 Environmental considerations

Environmental considerations in the control of furunculosis include the following:

- Discharge of infected or potentially infected effluent into catchment areas or natural waterways will lead to further spread of infection. This could also lead to the establishment of reservoirs of infection in wild fish populations and waterways. If eradication of furunculosis from a farm is chosen as the response option, it is likely that discharge of infected effluent will cease.
- The destruction and disposal of infected carcasses and other material may have an impact on the environment. This impact must be minimised to ensure that infection is not spread.
- The use of disinfectants and antibiotics could impact on the environment, especially if used in larger than normal quantities or concentrations as is possible in a disease control situation. Minor use permits for oxytetracycline and florfenicol provide instruction on what local environmental monitoring is required when these antibiotics are used.

2.4 Sentinel animals and restocking measures

Following an outbreak, fish species known to be more susceptible to furunculosis may be restocked as sentinel fish. Brook trout is considered the most susceptible species in fresh water, and Atlantic salmon the most susceptible species in sea water. However, before stocking with sentinel fish, it is important to determine

whether or not there are further risks associated with translocating fish between areas.

A. salmonicida subsp. *salmonicida* can survive in sediments for months, as discussed in Section 1.6.2. Fallowing time required before restocking will need to be assessed on an individual basis. The OIE *Aquatic animal health code* (OIE 2009; Chapter 4.4)¹⁸ also provides guidance on fallowing in aquaculture. The fallowing period will depend on the number of sites with confirmed diagnoses, the features of the sites (including season) and the extent of the outbreak. If entire sites or leases cannot be fallowed at once, the usefulness of this procedure is reduced.

In Scotland, where furunculosis is endemic, it has been common practice since the late 1970s to completely destock, disinfect and allow salmon-farm sites to fallow. This decreases the infection pressure on the site by removing the main sources of infection and allowing separation of year classes. A fallowing period of up to one month, and cleaning of nets and equipment, allows restocking without risk of re-infection providing other sources of infection are also managed (Munro 1988). This practice does not eliminate infection but appears to be an effective disease control measure where the aetiological agent is endemic.

For eradication, restocked fish must be free of covert or overt infection or disease, confirmed by laboratory testing of an acceptable sample of the restocked population. If areas are declared free of furunculosis, fish introduced into those areas must also be free from infection.

2.5 Public awareness

The appropriate industry organisations in each state and territory should be contacted by the primary industries or fisheries department using either internal databases of stakeholders or the National Aquaculture Council's (NAC) network – for example, the Tasmanian Salmonid Growers Association (TSGA) and the Victorian Trout Association (VTA). Industry awareness and support for implemented control measures is essential in a management program. A public awareness campaign should be implemented and must emphasise education, surveillance and cooperation from industry and the community in order to control potential outbreaks of furunculosis in Australia. Such campaigns should emphasise that furunculosis does not pose a human health risk. It is likely that industry and government will collaborate in the implementation of this campaign.

¹⁸ <<http://www.oie.int/international-standard-setting/overview/>>

2.6 Feasibility of control or eradication of furunculosis in Australia

The feasibility of eradicating or controlling an outbreak of furunculosis or incursion with *A. salmonicida* subsp. *salmonicida* will depend on the surrounding circumstances. Essentially, as outlined in Section 2.1, there are three response options:

- Eradication—eradication of furunculosis and the pathogen *A. salmonicida* subsp. *salmonicida* from Australia.
- Containment, control and zoning—containment of the disease and pathogen to areas with enzootic infection, prevention of further spread and protection of uninfected areas.
- Control and mitigation of disease—the implementation of management practices that decrease the incidence and severity of clinical outbreaks.

2.6.1 Response option 1: Eradication

The eradication option is directed at removing the risk of exposure of unexposed fish populations to the pathogen and preventing further spread of infection.

Eradication may be feasible if initial epidemiological investigations reveal:

- a limited focus or distribution of infection
- an apparent point source or limited point sources
- no apparent involvement of wild fish or reservoirs in the environment
- that containment is economically and technically feasible.

Depending on the level of exposure, different fish populations require different response strategies.

Clinically diseased populations of fish

These fish, along with infectious waste, are the main source of *A. salmonicida* subsp. *salmonicida* in the environment. Immediate destruction and disposal of all clinically affected populations of fish and dead fish is essential to the success of an eradication strategy. Emergency harvesting is not an option for these fish, although some of the equipment used for harvesting (e.g. pumps, percussion stunning devices) may be used in the removal of fish.

There is currently no mechanism or government-industry cost-sharing arrangement to compensate farmers for the destruction of fish.

If *A. salmonicida* subsp. *salmonicida* is confirmed in a non-salmonid species and the preferred option is eradication, then clinically diseased populations of fish must be destroyed.

Disposal methods should be chosen carefully to ensure there is no contact of the dead fish with waterways or vectors.

Exposed or potentially exposed, clinically normal populations of fish

These fish are safe for human consumption and emergency harvesting may be the quickest option to remove market-size fish in the shortest time. Emergency harvesting of fish involves a risk of further transfer of infection, which may jeopardise the success of an eradication strategy. A final decision about the use of emergency harvesting will need to be made on a case-by-case basis.

Fish that are not at market size must be destroyed. Growing fish until they reach market size is not an option, as these fish could spread infection to wild or feral fish stocks.

For both clinically diseased and exposed (or potentially exposed) populations of fish, strict control measures to prevent further spread of infection during emergency harvesting and destruction must be implemented and followed. This includes:

- disinfection or decontamination of all equipment and personnel involved in harvesting, slaughter and processing
- application of quarantine restrictions and procedures to the infected site, including personnel, equipment and vehicles
- ensuring only on-site processing where possible
- implementation of strict movement and disinfection procedures to the transport of fish to off-site processing plants. These will then become infected sites, and quarantine procedures will apply to these sites
- holding, treatment and safe disposal of slaughter and processing discharge, including holding water and waste offal
- ensuring that the final product will not result in the spread of infection; this may require placing restrictions on which products may be released to the market place (e.g. allowing skinless fillets but not whole fish).

If *A. salmonicida* subsp. *salmonicida* is confirmed in a non-salmonid species and the preferred option is eradication, then all exposed or potentially exposed fish must be destroyed and disposed of in a manner that prevents further spread of disease.

Unexposed populations of fish

Where epidemiological evidence suggests that the possibility of exposure of fish populations to disease is very low, young (i.e. pre-market sized) unexposed populations of fish may be allowed to grow out. However, these fish must be closely monitored during the growing-out period. Strict on-farm transportation and processing biosecurity protocols are still important.

Market-sized unexposed fish may be emergency harvested and slaughtered for human consumption.

Destruction of unexposed fish populations located within a declared area or within a destocking area will decrease the chance of spread of infection to fish stocks and prevent propagation of the disease. Compensation is not formally assured if these fish are destroyed.

2.6.2 Response option 2: Containment, control and zoning

This response option is more likely than response option 1 (eradication) if:

- *A. salmonicida* subsp. *salmonicida* is confirmed or suspected to be present in wild fish populations
- there are doubts regarding the extent of the infection
- the extent of infection means that the cost associated with the eradication is deemed too high. Compensation is not formally assured for stock that is destroyed.

Containment of disease requires zoning. The feasibility of a zoning program can only be assessed at the time of the outbreak, taking into account the required movement restrictions on fish, people, vehicles, boats and market access for the fish products and byproducts.

Clinically diseased populations of fish

These fish, along with infectious wastes, are considered to be the main source of *A. salmonicida* subsp. *salmonicida* in the environment, and constitute the greatest risk for spreading the infection to uninfected zones.

Clinically diseased populations of fish should be destroyed to prevent further spread of disease. Antibiotic treatment for these populations will reduce mortality, but will not eliminate infection and hence is not recommended.

Exposed or potentially exposed, clinically normal populations of fish

A successful zoning program relies on the implementation of movement restrictions on exposed or potentially exposed populations of fish that prevent infection spreading to uninfected zones.

In a declared area, controlled grow out and slaughter may be feasible without further spread of infection, although fish should be treated as if infected.

Antibiotic treatment of a fish population may be considered feasible and appropriate where epidemiological evidence suggests possible exposure to disease. Treatment can be cost-effective and used as a temporary measure to prevent losses of fish until other preventive practices can be implemented. Antibiotic-resistant strains of *A. salmonicida* subsp. *salmonicida* have become established in some regions overseas and this must be considered when deciding to treat with antibiotics.

Treatment will also mean that antibiotic residues may be present in the fish if they are destroyed. Fish cannot be harvested for human consumption within the withholding period for the antibiotic used.

Destroying fish is an option for containment, control and zoning, as it can decrease the infectious load on a site and minimise the spread of infection. There is currently no established mechanism of compensation for fish that are destroyed, hence destroying fish for this purpose must be carefully considered.

Unexposed populations of fish

Control options for unexposed populations of fish are the same as those outlined for eradication (Section 2.6.1). The implementation of a zoning program and associated control measures to maintain uninfected zones would be necessary.

Vaccination of unexposed fish is an option if it is desirable to move unexposed fish into a region where they may be exposed to disease. For example, the translocation of young salmonids from a freshwater hatchery that does not have disease, to a marine site where disease is known to be present. In this case, vaccination against furunculosis must occur before fish are translocated, and with sufficient time for the vaccine to increase immunity of the fish to the disease.¹⁹ Vaccination does not provide total protection, so these fish must be closely monitored for evidence of infection with naturally occurring *A. salmonicida* subsp. *salmonicida*. The use of culture methods will allow detection of live *A. salmonicida* subsp. *salmonicida* if present in sufficient numbers, but will not be suitable for the detection of covert infection. Strains of bacteria used in commercially available vaccines are killed and will therefore not grow on culture.

2.6.3 Response option 3: Control and mitigation of disease

The principles of control and mitigation of disease are to reduce the impact of furunculosis. This involves implementation of management practices that decrease the incidence, distribution and severity of clinical outbreaks.

The general control measures for containment, control and zoning described in Section 2.6.2 also generally apply in the control and mitigation of disease, except for the measures associated with zoning. In this response option, zoning is not considered and hence neither are the strict restrictions placed on movement of exposed or potentially exposed populations of fish.

All efforts should still be made to prevent the spread of disease and minimise the risk of exposure in naïve populations. Likewise, a vaccination strategy to vaccinate unexposed populations may be implemented, particularly where there is a risk of exposure.

If a clinical disease outbreak occurred, treatment with antibiotics is justified and encouraged to reduce overall mortality in affected populations of fish.

2.7 Trade and industry considerations

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

In countries where furunculosis is endemic, the only industries that have been affected by the disease are the salmonid farming industries. However, it is

¹⁹ The time required for immunity to develop in the fish will depend on water temperature. Commercially, at temperatures >10 °C, a minimum period of two weeks is necessary.

impossible to predict whether other aquatic species in Australia would also be affected.

2.7.1 Export markets

Some countries may have import conditions in place related to *A. salmonicida* subsp. *salmonicida*, such as requiring certain salmonid products (e.g. live fish) to be certified free of *A. salmonicida* subsp. *salmonicida*.

The Australian Department of Agriculture should be contacted for further information regarding health certification of exports.

2.7.2 Domestic markets

A cautious approach is required for the harvest of exposed or potentially exposed product for the domestic market, due to the potential for further spread of infection. As previously stated, *A. salmonicida* subsp. *salmonicida* is not a zoonosis. Therefore, provided normal seafood safety practices are followed, there are no public health risks associated with harvesting infected fish for human consumption. If healthy, potentially infected or infected fish are destined for human consumption, the chief medical officer and health authority of the relevant state or territory should be notified that there are no human health concerns associated with *A. salmonicida* subsp. *salmonicida*, and that furunculosis is not a zoonotic disease.

Decisions regarding the release of fish or fish products to the domestic market will depend on the response strategy implemented.

3 Preferred Australian response options

3.1 Overall policy for furunculosis

Furunculosis is an exotic, highly contagious bacterial disease of salmonid fish. The disease has the potential to cause serious economic loss in the farmed salmonid industries in Australia due to the morbidity and mortality expected in these naive populations. The disease also has the potential to cause morbidity and mortality in wild salmonid populations and possibly in native salmoniform species.

The policy of control of an outbreak of furunculosis in Australia depends upon both the nature of the outbreak and the response strategy adopted. The choice of response options will be decided by the chief veterinary officer of the state or territory or the director of fisheries (or both) in which the outbreak occurs. This decision will be made in consultation with the relevant industry sector where major aquaculture industries may be affected. Epidemiological investigation will be used to assist in the decision.

There are currently no government–industry cost-sharing arrangements to cover the costs of control operations or to compensate for destroyed stock. Successful implementation of a control or eradication program may not be possible without first establishing agreement on these costs.

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

- *option 1—eradication* of furunculosis from Australia. This is the preferred option
- *option 2—containment, control and zoning* of the pathogen to areas with endemic infection, prevention of further spread and protection of uninfected areas
- *option 3—control and mitigation of disease* by implementing management practices that decrease the incidence and severity of clinical outbreaks.

Each of these control options involves the use of a combination of strategies, which may include:

- *quarantine and movement controls* on fish, fish products and items in declared areas to prevent spread of infection
- *destruction and safe disposal* of all clinically diseased or dead fish as soon as possible, to prevent further bacterial shedding
- *decontamination* of facilities, products and equipment to eliminate the bacterium on infected premises and to prevent spread of infection
- *surveillance* to determine the source and extent of infection and to provide proof of freedom from the disease
- *zoning* to define and maintain infected and disease-free zones
- *treatment of infected fish* by antibiotic use or vaccination (or both)
- an *awareness campaign* to encourage cooperation by the industry and the community.

An uncontrolled outbreak of furunculosis would cause short-term and long-term production losses with consequent dislocation and economic losses in the salmonid farming industry and associated production, sales and export industries. It will therefore be necessary to act immediately to control or eradicate the disease.

The chief veterinary officer (CVO) or director of fisheries in the state or territory in which the outbreak occurs (or both) will be responsible for developing an emergency animal disease response plan. This plan will be submitted to the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD), who will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

CVOs or directors of fisheries (or both) will implement the disease control measures as agreed in the emergency animal disease response plan and in accordance with relevant legislation. They will make decisions on follow-up disease response measures in consultation with AqCCEAD. The detailed response measures adopted will be determined using the principles of control and eradication (see Section 2), epidemiological information about the outbreak and the financial feasibility of the option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN **Control Centres Management**.²⁰

3.2 Response options

The circumstances surrounding an outbreak of furunculosis will greatly influence selection of the most suitable response option. Figure 3 details the actions that should occur immediately on suspicion of furunculosis. In this situation, it is critical that measures are taken to contain any potential spread of disease while laboratory tests are done to confirm infection. These measures are clearly identified in Sections 2.2 and 2.3.

It is important to note that suspicion of disease may not always precede confirmation of *A. salmonicida* subsp. *salmonicida*. For example, it is possible that the first knowledge of the pathogen's presence in Australia may be confirmation of the pathogen from a routine health sample taken from a non-salmonid fish (e.g. an imported ornamental fish species while still in quarantine).

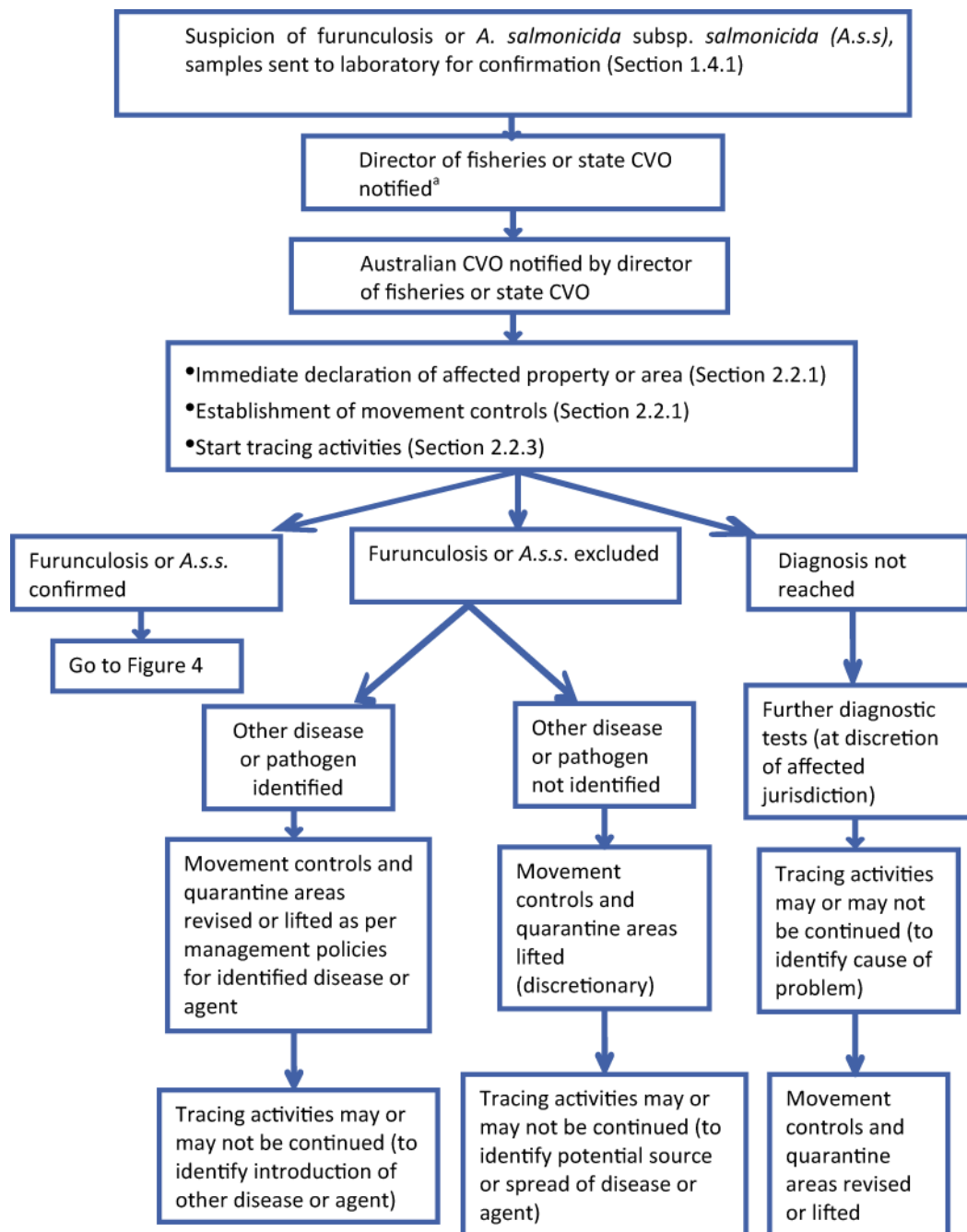
If furunculosis or the presence of *A. salmonicida* subsp. *salmonicida* is confirmed, refer to Figure 4, which has been developed to help identify the most appropriate response option. This decision tree is flexible, and highly dependent on the specific situations experienced. Although eradication is the preferred option, the decision to choose eradication must be taken carefully as:

- it will involve destruction of fish for which compensation is not assured
- it may be difficult to determine the extent of the infection given the potential for reservoirs of infection in the environment and the difficulties associated with culturing the pathogen from covertly infected fish.

If epidemiological evidence suggests no further spread in a closed system, such as a recirculation aquaculture system or an ornamental fish tank, then responding to the finding by eradication is reasonably straightforward and desirable.

²⁰ <<http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan>>

In any system where there is potential contact with wild fish, it is likely that some exposure may have occurred by the time the disease is confirmed. In these circumstances, a decision must be made as to whether it is too late to attempt eradication (i.e. whether the 'horse', or in this case 'fish', has already bolted).



A.s.s = *A. salmonicida* subsp. *salmonicida*; CVO = chief veterinary officer

^a As appropriate in the affected jurisdiction.

Figure 3 Flowchart of activities to follow when furunculosis is suspected

The number of fish affected may influence the decision to eradicate stock. For example, if a small salmonid hatchery containing a few broodstock and some fingerlings is affected, the short-term cost associated with destroying these fish will not be as great as the cost associated with destruction of a sea pen of Atlantic salmon near harvest weight. The cost of these fish will be hundreds of thousands of dollars if one pen is affected, and potentially millions of dollars if multiple pens are affected.

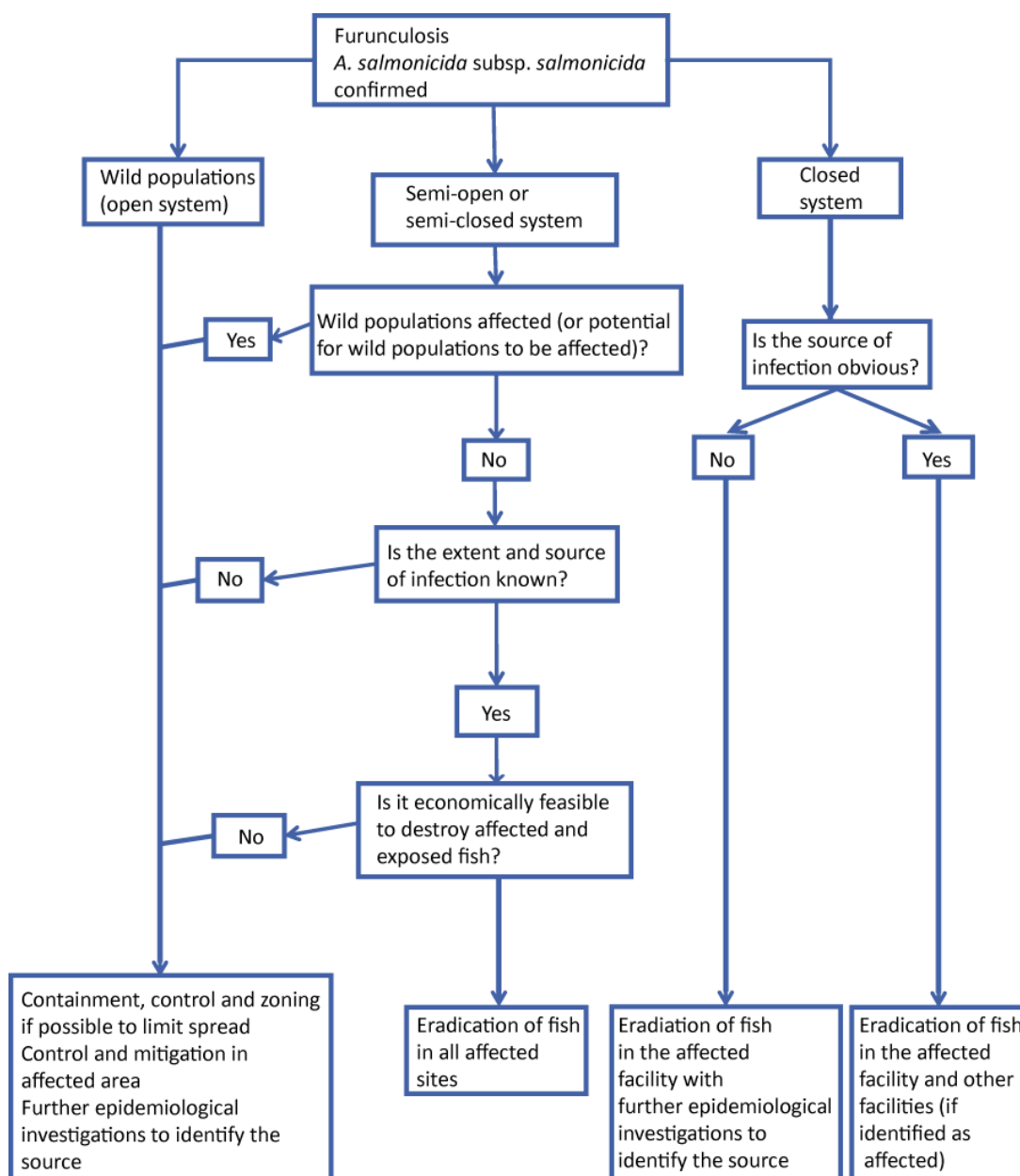


Figure 4 Choosing the preferred response option if furunculosis or *Aeromonas salmonicida* subsp. *salmonicida* is confirmed

3.2.1 Option 1—Eradication

If the response option of eradication is chosen, at each site identified as infected there must be:

- an immediate outbreak investigation, particularly tracing of potential spread onto and out of premises
- zoning to define the declared area and disease-free areas
- quarantine and movement controls or restrictions on fish, fish products, water and any other vectors (including fomites) located within declared areas to prevent further spread of infection, as discussed in Section 2.2
- removal of untreated discharge water from a semi-closed or closed system; untreated discharge water must not be released into the environment
- destruction of all **clinically diseased fish**; emergency harvest is not an option
- destruction of all **exposed or potentially exposed, clinically normal fish** from the water. If there is any doubt as to exposure, fish are to be treated as exposed. Market-size fish may be emergency harvested provided it can be done in a manner that will not pose a risk of further disease spread. Small fish may not be allowed to grow out
- destruction of any other non-salmonid fish that may be involved
- harvesting of **unexposed fish** that are of market size. Small fish that have not reached market size may be allowed to grow out, provided there is no risk of exposure. If the situation changes and these fish are exposed or potentially exposed, then they must be removed immediately
- ongoing decontamination of facilities, products, equipment, vehicles, boats etc. throughout the eradication process to eliminate the bacterium from infected premises and to prevent spread in declared areas
- education and disease awareness for those involved in, or affected by, the eradication process.

Treatment with antibiotics and vaccination are not options for eradication.

For further discussion on eradication of disease, refer to Section 2.6.1.

3.2.2 Option 2—Containment, control and zoning

If the response option of containment, control and zoning is chosen, measures implemented include those outlined in Section 3.2.1 above, with the following variations:

- Destruction of all **exposed or potentially exposed, clinically normal fish** from the water is an option, but treatment with antibiotics will achieve reduction in pathogen release and will avoid costly destruction of stock. Market-size fish should be emergency harvested, provided it can be done in a manner that will not pose a risk of further disease spread. Small fish may be allowed to grow out, but should be treated if clinical disease develops.

- Destruction of other cultivated, non-salmonid fish that may be involved, as these fish cannot be released from the infected premises or infected region alive and hence cannot be sold.
- Harvesting of **unexposed fish** that are of market size. 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, then they must be monitored and treated if clinical disease develops.

In addition, the following measures may also be taken:

- Sites may be fallowed until the disease is contained.
- Unexposed fish that may be exposed or potentially exposed can be vaccinated. Vaccination against furunculosis must occur before fish are translocated, and allow sufficient time for the vaccine to increase the immunity of the unexposed fish population to the disease.
- In areas where there is uncertainty as to whether infection remains, restocking with sentinel fish stocks may be useful.

For further discussion on containment, control and zoning of disease, refer to Section 2.6.2.

3.3.3 Option 3—Control and mitigation of disease

If the response option of control and mitigation of disease is chosen, there must be:

- implementation of husbandry, management and hygiene practices that aim to reduce stress on fish and minimise bacterial load to decrease the incidence and severity of furunculosis outbreaks
- vaccination of unexposed fish that may potentially be exposed
- treatment with antibiotics of clinically affected, exposed and potentially exposed fish to reduce the morbidity and mortality associated with outbreaks of disease
- fallowing of sites to decrease the infectious load on the site between batches of fish.

For further discussion on control and mitigation of disease refer to Section 2.6.3.

3.3 Criteria for proof of freedom

Proof of freedom from furunculosis, which may be important for trade, can be demonstrated at the aquaculture establishment, zone and country level. Criteria for proof of freedom at each level are given in the OIE *Aquatic animal health code* for the various OIE-listed diseases (OIE 2009).

Furunculosis is not an OIE-listed disease. However, the criteria for demonstration of proof of freedom for listed diseases can be used as a guide for Australia to demonstrate proof of freedom from furunculosis, if required.

3.4 Funding and compensation

There are currently no national cost-sharing agreements in place for emergency responses to furunculosis. It is the responsibility of the users of this publication to seek advice in relation to any relevant funding or compensation arrangements within the relevant jurisdiction.

Further information on the impact of the disease is provided in Section 1.7.

Appendix 1 Identification and confirmation of *Aeromonas salmonicida* subsp. *salmonicida*

The following describes the methodology that would be used to isolate and identify *Aeromonas salmonicida* subsp. *salmonicida*. An initial presumptive diagnosis may be made at a jurisdictional diagnostic laboratory. Confirmatory testing will be performed at the CSIRO Australian Fish Disease Laboratory (AFDL).

Upon suspicion of disease, at least six preferably live, moribund fish should be transferred to the jurisdictional diagnostic laboratory. For full details of the collection and submission of specimens, refer to 'Collection and submission of samples for investigation of diseases of finfish' by Judith Handlinger.²¹ Samples can also be taken on site by trained staff. To avoid transmission of any disease, all equipment should be disinfected before and after transport.

For investigation of an index case, all precautions should be taken to minimise risk of transporting pathogens between locations, including decontamination or changing of equipment and clothes between sampling sites. Presumptive identification of furunculosis by molecular tests or distinctive histology will indicate that culture must be undertaken immediately to confirm the presence of viable pathogen. Prepared samples (swabs, cultures, dried smears) should also be sent to the CSIRO Australian Animal Health Laboratory (AAHL) for confirmation of isolated material.

Surveillance testing will require sampling of moribund fish where available, and sampling in the same way as above. Screening using polymerase chain reaction (PCR) would be acceptable for surveillance purposes, with rigorous record keeping. Samples should be retained to culture any samples returning positive PCR results. Any surveillance program should cover the entire area with a view to determining the extent of any new outbreaks. Surveillance testing may also involve regular sampling of smolts from hatcheries to ensure they are free of the pathogen before transfer to cages, as well as other species including native salmoniformes (if present), which may carry the agent.

Where whole fish are submitted, external lesions are sampled with the use of a sterile bacteriological inoculating loop at the edge of the active lesion. Swabs from external lesions may contain many contaminants. Kidney sampling is preferable, as pigmented typical *A. salmonicida* can be readily isolated from kidney tissue of dead or moribund fish by aseptic procedures. Where the pathogen is suspected, some asymptomatic infections may be detected using enzyme-linked immunosorbent assay (ELISA) on samples from the lower intestine (Hiney, Kilmartin & Smith 1994).

²¹ <<http://www.scahls.org.au/Procedures/Pages/Aquatic-ANZSDPs.aspx>>

Diagnosis of furunculosis or infection with *Aeromonas salmonicida* subsp. *salmonicida*

Furunculosis should be suspected where there is disease in salmonids causing high morbidity and mortality with evidence of extensive haemorrhage in affected fish. Furunculosis is a misnomer as development of furuncles rarely accompanies the disease state and is more likely to be seen as an advanced chronic condition.

Note that the state or territory chief veterinary officer must be notified immediately of any suspected incidents of furunculosis.

Diagnosis of furunculosis, where there is accompanying evidence of disease (clinical furunculosis), or infection with the pathogen with no evidence of disease (subclinical or covert furunculosis), is made using clinical signs and gross pathology (where present). Laboratory methods, particularly culture and molecular biology techniques, will confirm the presence of the pathogen or pathogen nucleic acid. Molecular biology techniques offer rapid and sensitive determination of presence of pathogen nucleic acid.

In addition, it is possible that *A. salmonicida* subsp. *salmonicida* may be isolated from a non-salmonid fish. In this case, confirmation of *A. salmonicida* subsp. *salmonicida* is made using laboratory methods including culture and molecular biology techniques. As noted in Section 1.4.1, *A. salmonicida* subsp. *salmonicida* in a non-salmonid species is not called furunculosis.

Clinical signs

Clinical signs associated with furunculosis are summarised in Table 3. However, these signs may be seen with almost any septicaemic infectious disease of fish, either viral or bacterial. Thus, clinical signs alone are not sufficient to make a diagnosis of furunculosis (Bernoth 1997; Plumb 1999). Furuncles are not a consistent clinical sign of the disease.

Furuncles are likely to be present where there are high mortality rates caused by the disease. If furuncles are present, they may develop into open, crater-like wounds (Figure 5).



Photo: P Hardy-Smith.

Figure 5 External appearance of a large 'furuncle' under the skin of an Atlantic salmon with furunculosis; inset shows open furuncle

Table 3 Clinical signs of furunculosis^a

Clinical signs	Clinical course of disease		
	Peracute infection	Acute infection	Subacute (chronic) infection
Septicaemia	<ul style="list-style-type: none"> • dark colour • lethargy • tachybranchia 	<ul style="list-style-type: none"> • dark colour • inappetance • lethargy • tachybranchia • haemorrhage at base of fins 	<ul style="list-style-type: none"> • dark colour • inappetance • lethargy • pale, congested gills • serosanguinous fluid at nares or vent • injected sclera • congested blood vessels at base of fins
Furuncle development	No	Possible	Possible, and if present, are large and scarred
Exophthalmia	Possible	Possible	Possible
Morbidity or mortality	Very high mortality	High mortality	Low mortality, high morbidity

^a The intestinal form of furunculosis, not detailed in this table, typically has a very low mortality rate with prolapse of the anus as the sole clinical sign.

Gross pathology

In some affected individual fish there may be no obvious external signs of disease, but gross pathology may be evident on examination of the internal organs and musculature of the fish. For example, extensive haemorrhaging may only be obvious once the abdominal cavity is opened (Figure 6).

Gross pathology (Table 4) is not specific for furunculosis. Petechial haemorrhages can occur throughout the serosal surfaces of the viscera. The swim bladder can be especially affected, with diffuse and ecchymotic haemorrhaging in diseased fish (P Hardy-Smith, pers. obs.). The petechiation of visceral surfaces and liquefaction of the kidney appear to be the result of bacterial proteolytic enzymes (Munro & Hastings 1993).

Note that if covertly infected fish are present, they will show neither clinical signs nor gross pathology.

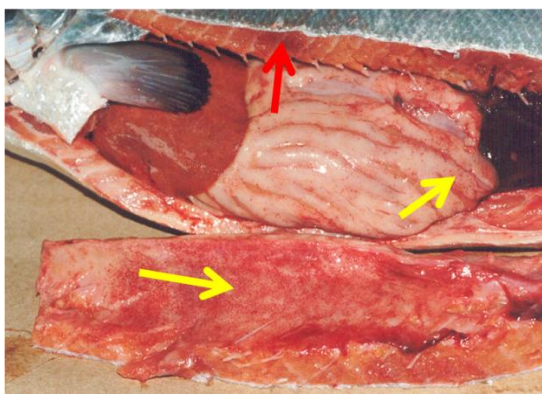


Photo: P Hardy-Smith.

Figure 6 Opened peritoneal cavity of an Atlantic salmon with furunculosis showing extensive haemorrhage in peritoneal fat and wall (yellow arrows) and within musculature (red arrow); this salmon presented with no external clinical signs

Table 4 Furunculosis—gross pathology

Gross signs	Clinical course of disease		
	Peracute infection	Acute infection	Subacute (chronic) infection
Vasocongestion	Peripheral	na	na
Haemorrhage	Yes, extensive: <ul style="list-style-type: none"> • petechial <ul style="list-style-type: none"> – parietal peritoneum – visceral peritoneum – myocardium • focal <ul style="list-style-type: none"> – gills 	Yes, extensive: <ul style="list-style-type: none"> • kidneys • spleen • liver • stomach and intestine • swim bladder • skin lesions 	Yes: <ul style="list-style-type: none"> • muscular (e.g. epicardium)
Enlarged organs	Yes: <ul style="list-style-type: none"> • spleen • kidneys Liquefaction may also be observable in these tissues	Possible, especially spleen Liquefaction may also be observed in the kidney and spleen	Possible, especially spleen
Visceral congestion	na	Yes	Yes (peritoneum)
Furuncles	No	Yes (skin or muscle)	Yes (muscle)

na = not applicable

Sources: Austin & Austin (1999); Ferguson (1988); Frerichs & Roberts (1989); P Hardy-Smith (pers. obs.); McCarthy & Roberts (1980); Munro & Hastings (1993).

Microscopic pathology

The microscopic lesions observable in furunculosis are provided in Table 5. Although not specific for furunculosis, the furuncular lesions are readily recognisable at a microscopic level, and the microcolonies in vascular endothelia are suggestive of furunculosis. Numerous eosinophilic granulocytes may be found in the intestinal submucosa, and to a lesser extent in the gills.

Table 5 Furunculosis—microscopic pathology

Microscopic signs	Clinical course of disease		
	Peracute infection	Acute infection	Subacute infection
Bacterial colonisation	Small colonies in: <ul style="list-style-type: none"> • branchial mesenchyme • myocardium • anterior kidney • spleen 	Seen anywhere, especially: <ul style="list-style-type: none"> • heart (myocardial degeneration) • kidney (possible macrophage involvement; renal tubular degeneration) • spleen (vascular collapse, splenomegaly) • gills (lamellar thrombosis) • skin (subdermal colonies) 	Conspicuous infection, especially: <ul style="list-style-type: none"> • heart (myocardial degeneration; fibrinoid degeneration) • spleen (vascular collapse, splenomegaly)
Inflammatory infiltrate	No	Yes	Yes
Necrosis	Limited	Toxic haematopoietic necrosis Ellipsoidal necrosis (spleen)	Only heart and spleen

Sources: Austin & Austin (1999); Ferguson (1988); Frerichs & Roberts (1989); McCarthy & Roberts (1980); Munro & Hastings (1993).

Laboratory tests

Specimens required

Confirmation of diagnosis can be made by culture and identification of *A. salmonicida* subsp. *salmonicida* from infected fish. Moribund, clinically affected fish that have been euthanased immediately before sampling provide the best possibility of detecting *A. salmonicida* subsp. *salmonicida* by culture and for ensuring well-preserved tissues for microscopic examination.

If fish show signs consistent with clinical furunculosis, the kidney should be sampled using a sterile swab. Duplicate air-dried impression smears of organs or lesions that are swabbed should also be prepared at the same time for an indirect fluorescent antibody test (IFAT) and Gram staining (M Crane, CSIRO AFDL, pers. comm., April 2008). It is important to clearly label the swab with the sample site.

Fixed tissues, cultures and impression smears should be forwarded to the jurisdictional diagnostic laboratory. Cultures can be submitted using Stuart or Amies transport media as *A. salmonicida* subsp. *salmonicida* will remain viable for 48 hours in these media (Cipriano & Bullock 2001).

If it is not possible to take a sample of the kidney using a sterile swab in the field, then six moribund fish showing clinical signs should be selected and euthanased before submitting these fish whole, on ice, to the jurisdictional veterinary laboratory. Where samples are unlikely to reach the laboratory within 24 hours, frozen specimens could also be submitted, although these are not ideal. Where frozen fish are submitted, it is recommended that fixed tissues are also collected from cohort fish, if possible before freezing, and submitted with the frozen samples.

Although it is likely that an outbreak of furunculosis in Australia will result in fish showing clinical signs of disease (and hence provide a high likelihood that that *A. salmonicida* subsp. *salmonicida* will be isolated) it is also possible that *A. salmonicida* subsp. *salmonicida* could be present in infected fish that do not show clinical signs (e.g. in wild salmonids where the initial outbreak went unobserved). It can be difficult to isolate *A. salmonicida* subsp. *salmonicida* from these covertly infected fish. The reasons for this remain unclear, but it is likely that *A. salmonicida* subsp. *salmonicida* can survive in a non-culturable but viable state in the fish, thus eluding common methods of detection (Pickup et al. 1996).

Culture methods

Culture and identification of *A. salmonicida* subsp. *salmonicida* takes approximately five days to complete. Characterisation of the cultured bacterium is achieved by phenotyping using biochemical tests, colonial morphology and absence of motility.

Austin and Austin (1999) note that *A. salmonicida* subsp. *salmonicida* may be readily recovered from the kidney of diseased fish on standard non-selective bacteriological agar media. Clinical experience supports this—growing a pure culture of *A. salmonicida* subsp. *salmonicida* from the kidney of a fish with clinical furunculosis is usually successful (P Hardy-Smith, pers. obs.).

Methods used for the culture and characterisation of *A. salmonicida* subsp. *salmonicida* at CSIRO AFDL are provided below.

Stress test

If furunculosis is suspected, but bacterial culture results are negative (which may be possible, e.g. if covert infection is present in a salmonid population exposed to the pathogen), a stress test may be conducted to determine whether the fish are harbouring the pathogen. The stress test has been used widely in Ireland, Scotland and eastern Canada.

The stress test aims to induce clinical disease in fish that are covertly infected by exposing fish to stressors. One stressor is increased water temperature. This stressor may be combined with the use of corticosteroids via injection (e.g. triamcinolone) or bath administration (e.g. prednisolone acetate) (Hiney, Smith & Bernoth 1997). Once clinical disease becomes apparent, samples are taken for bacterial culture.

Bullock and Stuckey (1975) found that the combination of injecting covertly infected fish with corticosteroids and exposing these fish to increased temperatures was more effective for detecting covert infection compared to using either temperature or corticosteroid injection alone. Interestingly, the temperature used to stress fish was 18 °C, a temperature to which salmonids growing in Australia are exposed during summer.

The disadvantages of stress tests are that they are time-consuming (taking up to 18 days), logistically demanding and involve the destruction of individual fish. Furthermore, different populations and even different individual fish within a population can respond differently to a standard stressing regime. For example, in one study, fish that were free of any specific bacterial infection were unable to survive even 24 hours after the administration of the stressors (Olivier 1992). Consequently, the results can be unreliable (Hiney, Smith & Bernoth 1997), and it is unlikely that the stress test would be applied as molecular biology techniques are now available to detect the pathogen.

Bacteriological techniques

Isolation

A. salmonicida grows readily on standard non-selective bacteriological agar. Separate tissues are plated onto SBA (Columbia agar base containing 5–7% (v/v) defibrinated sheep's blood), TSA-C (tryptone soy agar containing 0.01% (w/v) Coomassie brilliant blue) and BHIA (brain-heart infusion agar). Cultures are incubated in air at 22 °C (20–25 °C is acceptable) and examined daily for up to 7 days.

Cultures from external lesions typically exhibit a heavy, mixed growth of flora that may overgrow slow-growing *A. salmonicida* colonies. The appearance of colonies surrounded by a dark-brown, diffusible pigment is considered indicative of *A. salmonicida* subsp. *salmonicida*. However, non-pigmented strains are common and other bacterial species such as *A. hydrophila* and *A. media* may produce brown pigment. Isolates of *A. salmonicida* subsp. *salmonicida* have the ability to disassociate into different colony types. Pigment production should therefore only be regarded as a useful guide for initial colony selection. Any small, circular, 1–2 mm, low convex, entirely cream or buff-coloured colonies, particularly those that may be moved over the surface of the agar with a sterile inoculating loop, should be selected for identification.

A gram-negative isolate with colonial and cellular morphology consistent with that described for *A. salmonicida* subsp. *salmonicida* is presumptively identified as *A. salmonicida*. Further characterisation is used to confirm the isolate as *A. salmonicida*. Presumptive identification of *A. salmonicida* follows findings of:

- a gram-negative isolate with colonial and cellular morphology consistent with that described for *A. salmonicida* subsp. *salmonicida*
- oxidase positive and catalase positive (usually)
- non-motile (usually)
- fermentative
- resistant to the vibriostat 0/129
- PCR positive by either the Beaz-Hidalgo et al. (2008), the Gustafson, Thomas and Trust (1992) or Mooney et al. (1995) method
- IFAT positive with specific antiserum.

An interim report is generated once the presumptive diagnosis is made.

Sample preservation of presumptive isolates

Presumptive isolates or isolates that remain suspect are subcultured onto two agar plates of the same media used for primary isolation. After incubation at 25 °C for 2–3 days, or when good growth is obtained, the growth from one plate is confirmed for purity, harvested into tryptone soy broth with 10% glycerol and stored at –80 °C. The growth from the remaining plate is used for further characterisation testing, including biochemical testing, western blot analysis, PCR and 16S rDNA sequencing.

Characterisation (phenotyping)

Characterisation is by cellular and colonial morphology, biochemical profiling and temperature tolerance tests.

Methods for phenotypic characterisation are based on those previously outlined by Balows et al. (1991), Barrow and Feltham (1993) and MacFaddin (2000). As the medium may have effects on biochemical reactions (Dalsgaard et al. 1998) this must be taken into account when using these tests.

Biochemical characterisation of strains (from five geographic areas) and the reference strain of *A. salmonicida* subsp. *salmonicida* have been well documented by Dalsgaard et al. (1994, 1998). All strains were positive for the following fermentation of carbohydrates and glycosides: glycerol, L-arabinose, glucose (except one Canadian strain), glucose gas, fructose, galactose, mannitol, mannose, maltose, trehalose (variable reactions but all positive), dextrin, glycogen, starch, aesculin and salicin. Activities towards amino acids, proteins and lipids indicated that all strains were positive in lysine and most were positive in arginine, and all were indole, calf serum and rainbow trout serum negative. Full characterisation tests are reported in Dalsgaard et al. (1994).

Note that, with the exception of growth at 37 °C, all tests are incubated at 25 °C. Some fastidious strains may require supplementation of basal media (e.g. with haeme), at least for initial isolation and for growth when performing these tests (e.g. Ishiguro et al. 1986).

Detection of *Aeromonas salmonicida* subsp. *salmonicida* DNA using PCR

PCR tests can be used to detect *A. salmonicida* subsp. *salmonicida* DNA. Current PCR-based assays cannot distinguish between live and dead bacteria. Tissues or isolates for PCR are prepared by DNA extraction. A range of DNA dilutions is then prepared and amplified with the appropriate PCR primers according to described methods (Gustafson, Thomas & Trust 1992; Miyata, Inglis & Aoki 1996; Mooney et al. 1995). These methods were combined and modified by AFDL to detect 99% of all *A. salmonicida* isolates (Byers, Gudkovs & Crane 2002; Byers et al. 2002).

A PCR protocol targeting the ferric-siderophore receptor has been designed to detect *A. salmonicida* in fish tissues for the diagnosis of furunculosis (Beaz-Hidalgo et al. 2008). This method is non-destructive, and when compared to PCR methods previously reported, this protocol recognised all 69 *A. salmonicida* strains evaluated. However, this method is not specific to subspecies.

Identification and differentiation of *Aeromonas salmonicida* subsp. *salmonicida* using PCR and 16S rDNA sequencing

Molecular biology methods test for the presence of the pathogen's nucleic acids but do not indicate viability of the pathogen, hence bacteriological and pathology tests would also be required.

PCR tests have been modified and used in combination to differentiate and identify *A. salmonicida* subsp. *salmonicida* (Byers, Gudkovs & Crane 2002; Byers et al. 2002). In addition to PCR differentiation techniques, sequencing the 16S rRNA gene is a commonly used molecular approach for identifying bacteria. Primers used for sequencing the 16S rDNA gene have been described (Dorsch & Stackebrandt 1992). The bacterial sequence obtained can then be checked for matches in the non-redundant bacterial database using the BLASTn program. This database is maintained by the Australian National Genomic Information Service.

Immunoassay

Immunological techniques can also be used to identify and confirm *A. salmonicida* subsp. *salmonicida* infection, as well as an alternative to stress tests to detect covert infection. Immunological methods include serum agglutination, IFAT and ELISA. These techniques are unlikely to be used except where molecular techniques yield unexpected results requiring further detailed investigation.

Western blotting provides a valuable comparison of isolates by analysis of patterns of activity towards a range of antisera. At present, Australian isolates of atypical *A. salmonicida* can be divided into two different but homogeneous groups. Western blotting, therefore, has the potential to quickly identify new, potentially exotic strains. Denatured peptide samples are separated by electrophoresis on polyacrylamide gels, then transferred to nitrocellulose and blotted using reference antisera raised against endemic and exotic isolates (see Byers, Gudkovs & Crane 2002). A range of isolates, including type and reference cultures, are cultured and processed in parallel as a basis for comparison (M Crane, CSIRO AFDL, pers. comm., February 2008).

Appendix 2 Vaccination

Vaccines against *Aeromonas salmonicida* subsp. *salmonicida* are commercially available in Europe, the United States and Canada. They are not currently registered for use in Australia.

The administration of injectable vaccines can be stressful on fish as it may require netting and anaesthesia. Fish can experience a stress-induced immunosuppression following injectable vaccine administration (Hiney, Smith & Bernoth 1997). Oil-based adjuvanted injectable vaccines are the most widely used as they give optimal and consistent protection against clinical furunculosis (Håstein, Gudding & Evensen 2005). Intraperitoneal vaccine administration is more commonly used, to minimise carcass damage caused by intramuscular vaccination—the resulting internal adhesions may present some issues for both broodstock and the removal of abdominal contents at processing (Midtlyng 1997). Immersion vaccines are available and becoming more common. They are generally used for smaller fish, with protection only lasting for 2–3 months, and are then followed by an injection vaccine. Table 6 gives details of the main commercial furunculosis vaccines available overseas, many of which are immersion and injection vaccines.

Vaccination protocols

Size of fish at vaccination

Size is an important factor when determining the most effective time for vaccine administration. Salmonids weighing less than 1 g are not immunocompetent and should not be vaccinated. Once they reach at least 5 g they are considered of suitable size to mount an effective response to vaccination (Scott 1993). However, it is generally not until they reach at least 20–25 g that injectable vaccines are used (P Hardy-Smith, unpublished data). Therefore, immersion vaccines may be used to temporarily vaccinate fry in high-risk areas until they reach at least 20 g.

Timing of vaccination

Although the overall response to vaccination is dependent on the size of the fish, the time taken for antibodies to reach protective levels is also temperature dependent (Scott 1993). Temperature extremes delay the responses to vaccination: low temperatures reduce the immune responsiveness of salmonids, and high temperatures cause a stress response resulting in immunosuppression and complete vaccine failure (Pickering 1997; Secombes & Olivier 1997). Hence, where possible, fish should be vaccinated when temperatures are within the preferred physiological temperature range for that species to allow optimal immune response.

Fish should be vaccinated two to four months before smolt transfer, so that maximum protection from the vaccine coincides with the time of greatest stress on the fish (Håstein, Gudding & Evensen 2005).

Operator self-injection

Vaccinators who accidentally inject themselves typically suffer severe reactions with swellings and intense pain at the site of injection and require hospital treatment. People who have injected themselves once are not allowed to administer injectable vaccines again, as a second accidental injection is potentially life threatening.

Masking

Vaccinated fish may test falsely positive to immunological or DNA-based tests since such tests are currently unable to distinguish between the presence of the vaccine or a natural infection (Hiney, Smith & Bernoth 1997; Høie et al. 1993). Therefore, vaccination may interfere with surveillance and control programs if such tests are used.

Table 6 A selection of commercially available furunculosis vaccines, as of May 2008

Manufacturer	Vaccine name
Intervet Pty Ltd < aqua.intervet.com >	Compact™ Fur-IPN Norvax® Compact 4 Norvax® Compact 6 Norvax® Minova 4WD Norvax® FurVib Norvax® Minova 6
Novartis Animal Health < www.livestock.novartis.com/aqua.html >	Furogen (immersion and injection)
Schering-Plough Animal Health (Aquaculture) < www.spaquaculture.com >	Aquavac Furovac (immersion and injection)
Syndel Laboratories Ltd < www.syndel.com/Vaccines-C27.aspx >	ALPHA JECT 4000 Polyvalent vaccine including <i>A. salmonicida</i> subsp. <i>salmonicida</i> . Injectable only

Note: This table is intended as a guide only, and was compiled from information available at the time of writing. Vaccine manufacturers or distributors should be contacted directly for their latest information and specific details of vaccine use.

Appendix 3 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 must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. In addition, an import permit is required from the Department of Agriculture 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 permits. Since the assessment process is so detailed, the evaluation may take some time to complete.

Minor use permit system

The minor use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. The system was devised by the APVMA for Australia, and allows the restricted use of a limited amount of a drug or chemical in 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 aims to enable 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 have been 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 from the data are assessed by the APVMA (usually after 12 months—the duration of most permits) and used to more accurately set a 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 for a different use pattern. 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.²²

²² <www.apvma.gov.au>

Glossary

Anadromous fish	Fish species that hatch and live initially in fresh water (as fry), and migrate to sea water (for 'grow-out') and then return to fresh water to spawn.
Animal products	Meat products and products of animal origin (e.g. eggs) for human consumption or use in animal feeding.
Aquatic Animal Health Committee	A committee comprising representatives of the Australian Government; state and territory governments; the major aquaculture, wild capture, aquarium and recreational fishing industries; and CSIRO. The committee provided advice to the Primary Industries Ministerial Council on aquatic animal health matters, focusing on technical issues and regulatory policy. <i>See also</i> Primary Industries Ministerial Council
AQUAVETPLAN	<i>Australian Aquatic Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. <i>See also</i> AUSVETPLAN
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. <i>See also</i> Chief veterinary officer
AUSVETPLAN	<i>Australian Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.
Carcase	The body of an animal slaughtered for meat, after removal of the offal etc.
Catadromous fish	Fish species that hatch and live initially in sea water (as fry), and migrate to fresh water (for 'grow-out') and then return to sea water to spawn.
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. <i>See also</i> Australian Chief Veterinary Officer
Compensation	The sum of money paid by government to an owner for stock and/or property that is destroyed, possibly compulsorily, because of an emergency animal disease.
Composting	Decomposition of infected material by microorganisms. Suitable only where there is a small risk of fomite spread.

Control	Reduction in morbidity and mortality from disease by measures intended to interfere with the unrestrained occurrence of disease.
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.
Covert infection	Infection that is clinically inapparent, which may be transmissible and can eventually lead to clinical disease.
Dangerous contact premises or area	An area or premises containing aquatic animals that show no signs of disease but which, 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.
Destruction	The killing by humane means (euthanasia) of infected aquatic animals and/or those exposed to infection. <i>See also</i> Stamping out.
Disease agent	A general term for a transmissible organism or other factor that causes an infectious disease.
Disinfectant	A chemical used to destroy disease agents outside a living animal.
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 things by burial, burning or some other process so as to prevent the spread of disease.
Ecchymotic haemorrhages	Bleeding or bruising in the skin or a mucous membrane in the form of small round spots or paintbrush-like red-purplish discoloration.

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. <i>See also</i> Endemic animal disease, Exotic animal disease
Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. <i>See also</i> Emergency animal disease, Exotic animal disease
Enterprise	<i>See</i> Risk enterprise
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.
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with the disease.
Exophthalmia	Protrusion of the eyeball from the orbit, caused by disease or injury.
Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. <i>See also</i> Emergency animal disease, Endemic animal disease
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, to be of use depending on local conditions (currents etc.)
Fish byproducts	Products of fish origin destined for industrial use (e.g. fishmeal).
Fish products	Fish meat products and products of fish origin (e.g. eggs) for human consumption or use in animal feeding.
Fomite	Any inanimate object (e.g. water, packing, boots, equipment) that can carry the agent and spread the disease through mechanical transmission.
Free area	An area known to be free from the disease agent.
Furuncle	A boil-like inflammatory sore or lesion. More precisely, an area of focal liquefactive necrosis in the axial musculature.
Gram stain	Gram's stain reaction divides bacteria into two major groups, gram positive or gram negative, based on their cell wall structure.
Haemorrhage	The escape of blood from a ruptured vessel, causing bleeding.
Hyperaemia	An excess of blood in an area.

High-risk material	Animal wastes that constitute or are suspected of constituting a serious health risk to animals or humans.
Inappetance	Lack of appetite.
Infected premises or area	A defined 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, or in which the infective agent of that aquatic animal disease exists or is believed to exist. An infected area is subject to quarantine served by notice and to eradication or control procedures.
Leukocytopenia	Abnormally reduced numbers of white blood cells (leukocytes) in the bloodstream.
Local disease control centre	An emergency operations centre responsible for the command and control of field operations in a defined area.
Low-risk waste	Animal wastes that do not constitute a serious risk for the spread of disease to humans or animals.
Mitigation	Reduction in severity (e.g. 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. <i>See also Surveillance</i>
Movement control	Restrictions placed on the movement of fish, people and other things to prevent the spread of disease.
OIE Aquatic Code	<i>OIE Aquatic Animal Health Code</i> (OIE 2009), viewed 2009, < www.oie.int/eng/normes/fcode/en_sommaire.htm >
OIE Aquatic Manual	<i>OIE Manual of Diagnostic Tests for Aquatic Animals</i> (OIE 2009), viewed 2009, < www.oie.int/eng/normes/fmanual/A_summry.htm >. Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines).
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Petechial haemorrhage	Tiny flat, red or purple spots in the skin or mucous membranes caused by bleeding from small blood vessels.
Polymerase chain reaction (PCR)	A diagnostic technique involving in vitro amplification of a specific target DNA segment to detectable levels.
Premises or area	A production site for aquatic animals that may range from an aquarium to an aquaculture lease in the open ocean.
Primary Industries Ministerial Council	The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Agriculture and Resource Management Council of Australia and New Zealand).

Quarantine	Legal restrictions imposed on a place, fish, vehicles, or other things, therefore limiting movement.
Restricted area	The area around an infected premises (or area), likely to be subject to intense surveillance and movement controls. It is likely to be relatively small. It may include some dangerous contact premises (or area) and some suspect premises (or area), as well as enterprises that are not infected or under suspicion. Movement of potential vectors of disease out of the area will, in general, be prohibited. Movement into the restricted area would only be by permit. Multiple restricted areas may exist within one control area.
Risk enterprise	A defined livestock or related enterprise, which is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots, and garbage depots.
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.
Serosa	A smooth membrane consisting of a thin layer of cells that excrete a fluid, known as serous fluid. Serous membranes line and enclose several body cavities (serous cavities), where they secrete a lubricating fluid that reduces friction from muscle and organ movement.
Serosanguinous fluid	Fluid composed of blood and serum.
Smoltification	Physiological metamorphosis that facilitates the fish's survival in the marine environment.
Smolts	Fish that have undergone a physiological process while in fresh water that prepares them for migration to salt water.
Stamping out	Eradication procedures based on quarantine and destruction of all infected animals and animals exposed to infection.
State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, which may involve testing samples of a population.
Susceptible animal	An animal that can be infected with a particular disease.

Suspect premises or area	Where the emergency disease is suspected but not yet confirmed. The reason for the suspicion varies with the agent; however, it may involve clinical signs or increased mortality.
Tachybranchia	Rapid gill movement.
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.
Transport	The biosecure removal of aquatic animals, aquatic animal carcasses or parts of aquatic animals from the infected aquaculture establishment to the site of disposal.
Vasocongestion	Enlarged congested blood vessels.
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.
Zoning	The process of defining disease-free and infected areas.
Zoonosis or zoonotic disease	Disease transmissible from animals to humans.

Abbreviations

AFDL	Australian Animal Health Laboratory Fish Diseases Laboratory (formerly Australian Fish Health Reference Laboratory, AFHRL)
APVMA	Australian Pesticides and Veterinary Medicines Authority
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
IFAT	indirect fluorescent antibody test
MUP	minor use permit
OIE	Office International des Épizooties (World Organisation for Animal Health)
PCR	polymerase chain reaction

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