AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Edition 1.0

Disease Strategy

Whirling disease

Version 1.0, 2005

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

Primary Industries Ministerial Council

This disease strategy forms part of:

AQUAVETPLAN Edition 1.0

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

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DISEASE WATCH HOTLINES

These telephone numbers connect callers to the relevant state or territory officer to report concerns about any potential emergency disease situation. Anyone suspecting an emergency disease outbreak should use this number for immediate advice and assistance.

New South Wales	**1800 043 536**	**Northern Territory**	**1800 720 002**
Queensland	**07 3830 8550**	**Victoria**	**136 186**
South Australia	**1800 065 522**	**Western Australia**	**1800 815 507**
Tasmania	**1800 005 555**		

Preface

This disease strategy for the control and eradication of whirling disease is an integral part of the **Australian Aquatic Veterinary Emergency Plan**, or **AQUAVETPLAN (Edition 1.0)**.

The strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of whirling disease in Australia. The strategy was approved by:

- the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, at meeting 04 in May 2004;
- the Aquatic Animal Health Committee of the Primary Industries Standing Committee, at meeting 04 in June 2004; and
- the Primary Industries Standing Committee, at meeting 08 in March 2005.

Whirling disease is not listed by the OIE (World Organisation for Animal Health, formerly Office International des Epizooties) in the *International Aquatic Animal Health Code* (OIE 2004). However, some countries and regions within countries require imports to be certified free from the disease.

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 **Enterprise Manual**. The full 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

Enterprise Manual
Includes sections on:
– open systems
– semi-open systems
– semi-closed systems
– closed systems

Management manual
Control centres management

Aquatic Animal Diseases Significant to Australia: Identification Field Guide by Alistair Herfort, Department of Agriculture, Fisheries and Forestry, Canberra (Herfort 2004) is a source for some of the information about the aetiology, diagnosis and epidemiology of the disease and should be read in conjunction with this strategy.

This manual was drafted by Dr Paul Hardy-Smith, with the assistance of Professor Ron Hedrick and Drs Craig Stephens and Mark Crane.

Scientific editing: Biotext Pty Ltd, Canberra.

This manual was adapted from similar manuals in AUSVETPLAN, the Australian emergency plan for terrestrial animal diseases, and from the AQUAVETPLAN **Enterprise Manual**. The format and content have been kept as similar as possible to those documents 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 is gratefully acknowledged.

The text was amended at various stages of the consultation/approval process, and the policies expressed in this version do not necessarily reflect the views of all the members of the writing group. Contributions made by others not mentioned above are also gratefully acknowledged.

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

Government	**Industry**
Commonwealth of Australia	CSIRO Division of Livestock Industries
State of New South Wales	Tasmanian Salmonid Growers' Association
State of Queensland	Tuna Boat Operators Association
State of South Australia	Pearl Producers' Association
State of Tasmania	Australian Prawn Farmers Association
State of Victoria	Pet Industry Joint Advisory Council
State of Western Australia	RecFish Australia
Northern Territory	National Aquaculture Council
Australian Capital Territory	

The complete series of AQUAVETPLAN documents is available on the internet at: http://www.affa.gov.au/AQUAVETPLAN (Accessed 11 May 2005).

Contents

Preface **3**

1 Nature of the disease **9**

1.1 Aetiology 9

Life cycle of the parasite 10
Life cycle in the fish 10
Life cycle in the worm 12

1.2 Susceptible species 13

1.2.1 Fish 13
1.2.2 Worm 13

1.3 World distribution and occurrence in Australia 14

1.4 Diagnostic criteria 14

1.4.1 Clinical signs 14
1.4.2 Pathology 15
1.4.3 Laboratory tests 16
1.4.4 Differential diagnosis 18

1.5 Resistance and immunity 19

1.5.1 Innate immunity 19
1.5.2 Adaptive immunity 20
1.5.3 Vaccination 20

1.6 Epidemiology 20

1.6.1 Historical aspects 20
1.6.2 Sources of *Myxobolus cerebralis* 21
1.6.3 Modes of transmission 24

2 Principles of control and eradication **27**

2.1 Introduction 27

2.2 Methods to prevent spread and eliminate pathogens 28

2.2.1 Quarantine and movement controls 29
2.2.2 Tracing 31
2.2.3 Surveillance 33
2.2.4 Destruction of fish 33
2.2.5 Elimination of parasite 34
2.2.6 Treatment of fish products and byproducts 35
2.2.7 Disposal of fish 35
2.2.8 Decontamination 35
2.2.9 Vaccination 37
2.2.10 Vector control 37
2.2.11 Sentinel animals and restocking 38
2.2.12 Public awareness 38

2.3 Feasibility of specific options for control in Australia ... 38

2.3.1 Eradication ... 38
2.3.2 Containment, control and zoning ... 40
2.3.3 Control and mitigation of disease ... 41
2.3.4 Emergency harvesting ... 41

2.4 Trade and industry considerations ... 41

2.4.1 Export markets ... 41
2.4.2 Domestic markets ... 41

3 Policy and rationale ... 43

3.1 Overall policy ... 43

3.2 Overview of response options ... 44

3.2.1 Option 1 — Eradication ... 45
3.2.2 Option 2 — Containment, control and zoning ... 45
3.2.3 Option 3 — Control and mitigation of disease ... 46

3.3 Strategies for control and eradication ... 46

3.3.1 Laboratory testing ... 46
3.3.2 Epidemiological investigations ... 46
3.3.3 Quarantine and movement controls ... 47
3.3.4 Treatment and vaccination ... 47
3.3.5 Destruction and disposal of fish ... 47
3.3.6 Elimination of parasite ... 48
3.3.7 Treatment of fish products and byproducts ... 48
3.3.8 Vector control ... 48
3.3.9 Public awareness ... 48

3.4 Social and economic effects ... 49

3.4.1 Export markets ... 49
3.4.2 Domestic markets ... 49

3.5 Criteria for proof of freedom ... 49

3.6 Funding and compensation ... 49

Appendix 1 Procedures for transmission of diagnostic specimens to or from AAHL ... 51

Appendix 2 Identification of *Myxobolus cerebralis* ... 53

Appendix 3 Common and scientific names of fish species mentioned in text ... 55

Glossary ... 57

Abbreviations ... 63

References ... 65

Index ... 73

Tables

Table 1 MacConnell–Baldwin numerical scale for scoring lesion severity 18

Figures

Figure 1 Life cycle of *Myxobolus cerebralis* 11
Figure 2 Establishment of specified areas to control whirling disease 30
Figure 3 Decision flow chart 45

1 Nature of the disease

Whirling disease is a disease of freshwater salmonid fish caused by the myxozoan parasite *Myxobolus cerebralis*. The parasite has never been detected in Australia, but is present in New Zealand and areas of North America, Europe, Africa and Asia.

Importantly, the parasite has two hosts — salmonids and a freshwater oligochaete worm, *Tubifex tubifex*. There are two spore stages, one released from the fish and infective for worms, the other released from worms and infective for fish. Generally, the earlier the fish is first exposed and the higher the parasite exposure dose, the greater is the severity of the disease. Water temperature has a significant influence on all stages of the parasite life cycle. Temperatures greater than 20°C are not conducive to its development or survival.

Rainbow trout (*Oncorhynchus mykiss*) is the most susceptible salmonid species. The parasite has been associated with significant declines in wild rainbow trout populations in the United States (US), and had a devastating effect on the early culturing of this species in Europe. Other salmonids are generally less susceptible.

The disease gets its name from the radical tail-chasing swimming behaviour of heavily infected fish. Clinical signs result from the presence of the parasite spores in the cartilage, causing inflammatory changes and pressure on spinal nerves. However, fish may become infected with the parasite and never show clinical signs; diagnosis of infection in these fish can be difficult.

Australian native freshwater fish are not salmonids, and are unlikely to be susceptible to whirling disease. Salmonids used in restocking programs will be susceptible.

Whirling disease is a reportable disease in Australia. State and territory governments, the recreational salmonid fishing industry and the salmonid aquaculture industry need to be adequately educated and prepared for the possible incursion of this disease. This will greatly minimise the impact of the disease on susceptible salmonid populations if the parasite is ever introduced into Australia.

1.1 Aetiology

The aetiological agent of whirling disease is the parasite *Myxobolus cerebralis* (formerly named *Myxosoma cerebralis*). *M. cerebralis* belongs to the phylum Myxozoa, class Myxosporea, order Bivalvulida, suborder Platysporina and family Myxobolidae.

Myxozoans are a diverse group of multicellular organisms characterised by spore stages with three key structural features:

- 1–7 valves or protective shells that surround the next generation of the parasite, which exists as 1–2 amoeboid infective sporoplasms, and
- 2–7 polar capsules containing polar filaments that, when extruded, aid in attaching the spore to the host tissue.

Life cycle of the parasite

Figure 1 shows the parasite's life cycle, the important elements of which are as follows.

- **Two-host life cycle**

  Salmonids

  – asexual replication only

  – susceptible salmonids are present in Australia

  Freshwater oligochaete worms (*T. tubifex*)

  – sexual and asexual replication

  – susceptible *T. tubifex* are present throughout Australia

- **Two infective stages**

  Myxospore

  – forms in the cartilage of the fish

  – infective myxospores are present in cartilage approximately 50–120 days after initial infection, depending on water temperature and salmonid species infected

  – resistant to environmental degradation — survival measured in months to years

  Actinospore or triactinomyxon

  – forms in the intestinal lining of the worm

  – infective triactinomyxons (TAMs) present 100–170 days after initial exposure, depending on temperature and strain of worm

  – susceptible to environmental degradation — survival measured in days

- **All life cycle stages significantly affected by temperature**

  – within limits, the higher the temperature, the faster the life cycle.

Life cycle in the fish

The development of *M. cerebralis* in the fish begins with attachment and penetration of the susceptible salmonid host by the waterborne infective TAM stages that have been released from the worm. Resistance to whirling disease increases with fish age (MacConnell and Vincent 2002).

TAMs attach to the skin of the fish and deposit the infective sporoplasm into the epidermis. The sporoplasm then migrates through the skin tissue. The parasite can also penetrate across the gills, through the fins and through the lining of the mouth. During the migration into the fish, the parasite cells are constantly developing and asexually replicating. If the susceptible fish are very small and there is a very high exposure dose of TAMs, there may be some effect on the fish at this stage of infection (RP Hedrick, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, pers comm, April 2003).

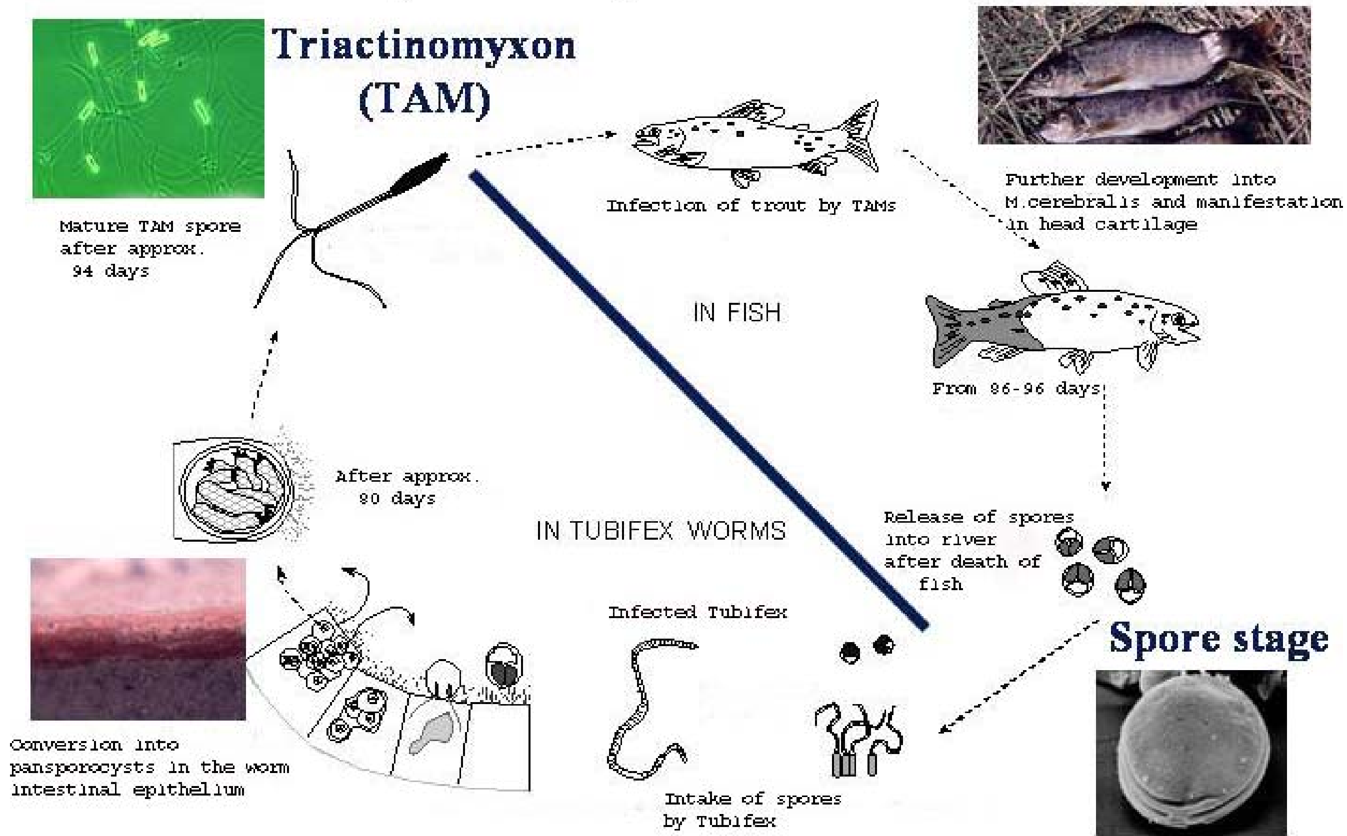

Figure courtesy of R Hedrick

Figure 1 Life cycle of *Myxobolus cerebralis*

Within 24 hours there can be numerous parasite cells deep in the skin of the fish, and by day 4 parasite cells can be found in nervous tissue. At first, the cells are found in peripheral nerves. The cells later move through ganglia to the central nervous system, replicating as they go. As early as 20 days after exposure, parasite stages can be found in the cartilage of the fish. The time needed depends on water temperature, with the optimal temperature for development being around 16–17°C (Halliday 1973b).

At this point, two parasitic cells will join to initiate sporogenesis or the formation of multicellular spores ('myxospores') in the cartilage of the fish. Cartilage throughout the body, including the cranium, spine, fins, vertebrae, ribs and operculum can be infected (Antonio et al 1998). Myxospores are elliptical and about 10 μm in diameter, and have thick protective shells.

Myxospores can take from 52 to 121 days to develop in the fish (Halliday 1973b); at 16–17°C, fully developed myxospores appear 52 days after infection. The spores remain in the cartilage and may be 'trapped' by bone as it forms around the infected cartilage.

The replication phase in the fish is significant. One TAM can potentially produce between 2800 and 7800 infective myxospores in the cartilage of the fish's head and possibly more in cartilage elsewhere (Hedrick et al 1999a, Kerans and Zale 2002).

In live fish, some myxospores escape from destroyed cartilage and are released directly into the environment or in the faeces (Nehring et al 2002). However, the most significant release of myxospores occurs when the fish dies and decomposes. The decomposition of one rainbow trout can release more than a million myxospores (Hedrick et al 1999a).

Myxospores can also survive passage through the gut of birds or other fish, and hence can be excreted in the faeces of the ingesting animal (El-Matbouli and Hoffman 1991).

Once released, myxospores are highly resistant and are able to survive in the environment for long periods, possibly years (Hoffman 1970).

Life cycle in the worm

Tubifex tubifex, the susceptible oligochaete worm host, inhabits sediments at the bottom of freshwater lakes and streams. It is present throughout Australia, including Tasmania (Pinder and Brinkhurst 2000).

The development of the parasite in the worm starts when the worm ingests the myxospore stage released from fish. The myxospore then 'hatches' and the infective sporoplasm migrates into the lining of the worm intestine. The parasite remains in the intestinal wall, multiplying and replicating (Hedrick and El-Matbouli 2002). Replication is both asexual and sexual. This therefore defines the worm as the definitive host for *M. cerebralis* (El-Matbouli and Hoffman 1998).

Around 50 days after infection, sporogenesis begins and the resulting spores (triactinomyxons, or TAMs) develop. TAMs are released into the lumen of the intestine as early as 100 days after the worm ingested the myxospores. Experimentally, peak release of TAMs was found to occur between 120 and 170 days after exposure, this being highly temperature dependent (Markiw 1986).

TAMs are very different from the myxospore released from fish. They are shaped like a grappling hook; that is, a long rod (about 146 μm) with three tails, each approximately 193 μm. While produced in very high numbers from infected worms, on release into the environment they are short lived, surviving up to 15 days. Again, this depends on temperature: the cooler the temperature, the longer the survival (El-Matbouli et al 1999).

1.2 Susceptible species

1.2.1 Fish

Susceptibility in salmonids varies between species, strains and individuals within a strain.[1] Rainbow trout is the principal salmonid host (MacConnell and Vincent 2002) and will develop darkened caudal peduncles ('black tails') with doses as low as 10 TAMs per fish, and tail-chasing swimming (whirling) with doses of 100 TAMs per fish (Hedrick et al 1999b). The dose needed for disease to develop depends on the age at which the fish are first exposed. Chinook (*O. tshawytscha*) and sockeye salmon (*O. nerka*) are also considered highly susceptible. Brook trout (*Salvelinus fontinalis*) have also shown a high prevalence of clinical signs and mortality when exposed under natural conditions. The susceptibility of Atlantic salmon (*Salmo salar*) appears to be variable.

Brown trout (*Salmo trutta*) need to be exposed to very high numbers of the infective stages (1000+ TAMS/fish) for clinical signs (black tail and skeletal deformities) to develop (Hedrick et al 1999b). Even at very high doses of 10 000 TAMs per fish, no whirling was observed in the brown trout during this study.

Of Australian native freshwater species, the largest and most widely spread are the galaxioids (also known as galaxias, native trout, jollytails and minnows). These are also the most closely related to the salmonids (M Gomon, Senior Curator, Ichthyology Department, Sciences Museum Victoria, pers comm, April 2003). A range of galaxioid species found in New Zealand are not susceptible to whirling disease (B Jones, Senior Fish Pathologist, Western Australian Department of Fisheries, pers comm, May 2003).

So far, all fish determined to be susceptible (El-Matbouli et al 1999, review in MacConnell and Vincent 2002) come from the family Salmonidae (order Salmoniformes), to which none of the Australian native fish belong. Although not confirmed, it is assumed that Australian native fish will not be susceptible. This assumption is made with reservations, as it is difficult to predict the impact of a known pathogenic agent on a new species.

1.2.2 Worm

Currently, the freshwater oligochaete worm *Tubifex tubifex* is the only worm that is known to host the parasite (Wolf et al 1986, Hedrick et al 1998, Kerans and Zale 2002).

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[1] An excellent review of salmonid susceptibilities to whirling disease is given in MacConnell and Vincent (2002).

This worm is widespread throughout Australia, but not commonly encountered (Pinder and Brinkhurst 2000). Strains of *T. tubifex* vary both in their susceptibility to infection and the level of shedding of TAMs. One strain of *T. tubifex* has even been found to be resistant to the parasite (Kerans and Zale 2002). As far as the author is aware, studies to assess the susceptibility of the Australian strains of *T. tubifex* have not been documented. The assumption is that Australian strains are susceptible, and will be capable of producing TAMs if infected with viable myxospores.

1.3 World distribution and occurrence in Australia

Whirling disease has been detected in more than 22 states in the US, as well as in Central Europe, South Africa, northeast Asia and New Zealand (Bartholomew and Reno 2002). It has never been reported in Australia.

Other *Myxobolus* species have been reported in Australia (Langdon 1990), and an actinosporean (the infective myxozoan spore released from the worm host) has been isolated from a marine oligochaete in Australia (Hallett et al 1995).

1.4 Diagnostic criteria

1.4.1 Clinical signs

In susceptible salmonids infected at an early age, clinical signs of whirling disease included erratic tail chasing (‘whirling’), blackening of the tail region, and skeletal deformities, including skull depression and spinal curvatures. Infected salmonids can also show no clinical signs. Bartholomew and Reno (2002) note that, because clinical signs are not pathognomonic (unique to this disease) and may be subtle, they might not be noted except when the disease reaches epizootic levels.

Clinical signs will vary, depending on the species of fish and the age of the fish when first infected. Generally, fish exposed when older than 9 weeks will not show clinical signs.

When fish are exposed as fry (which have a large amount of non-ossified cartilage), two clinical signs are likely. First, whirling begins about 3–8 weeks after infection. Fish may die from exhaustion or severe malnutrition. Whirling was once thought to result from cartilage deformation putting pressure on the organ of equilibrium in the fish. It is now considered to be due to lower brain stem and spinal cord compression and constriction (Rose et al 2000).

The second sign is ‘black tail’ (caudal melanosis) caused by pressure on caudal nerves controlling pigment (Halliday 1976). This may subside if fish are anaesthetised.

Infected fish that survive rarely show whirling behaviour or black tail, but may have:

- skeletal deformities — eg skull depression, misshapen jaws, shortened operculae (gill covers), spinal curvatures;
- opercular (gill cover) cysts; and
- decreased growth rate during clinical disease stage (suspected but not proven) — normal growth rate appears to resume after clinical disease subsides.

Very few or no gross clinical signs are seen in:

- rainbow trout exposed at more than 9 weeks old
- Chinook salmon exposed at more than 3 weeks (J Bartholomew, Center for Fish Disease Research, Oregon State University, pers comm, June 2003); and
- other, less susceptible salmonid species.

1.4.2 Pathology

In susceptible fish exposed at less than 9 weeks old, there can be obvious pathology. In fish exposed at older ages, pathology (gross and histological) may be minimal.

Gross lesions

Fish in the acute stage of infection may have black tail (caudal melanosis), which disappears if the fish dies.

Fish that survive the acute stage may have skeletal deformities. These are most pronounced in the cranium and spine and can vary significantly in severity. Light infections can be difficult to detect. Survivors will have no obvious internal gross lesions.

Microscopic lesions (histopathology)

The parasite initially has affinity for the skin and subsequently for the nerves and skeletal cartilage in the salmonid host. Clinical signs are directly related to the granulomatous response seen in cartilage.

In the acute stage:

- there is little cellular response in first few days.
- macrophages may be seen in subcutis, attacking residual parasitic stages; and
- nervous tissue containing sporoplasms appears normal with no tissue reaction.

In later stages:

- lysis and phagocytosis of cartilage by multicellular stages of the parasite initiate an intense inflammatory response in susceptible species;
- lesions typically contain remnant cartilage, parasites and focal to diffuse granulomatous inflammation — granulomas consist predominantly of epithelioid and mononuclear cells, fibroblasts and multinucleate giant cells from the host.

A grading system has been developed to assess the histological lesions seen in whirling disease (Baldwin et al 2000). This system is described in Section 1.4.3, Table 1.

1.4.3 Laboratory tests

Submission of specimens

If whirling disease is suspected, the chief veterinary officer (CVO) or director of fisheries of the state or territory must be notified immediately. Preliminary identification of *M. cerebralis* may be undertaken by some state/territory diagnostic laboratories (eg in Tasmania). Duplicate specimens must be consigned as soon as possible to the Australian Animal Health Laboratory (AAHL) in Geelong, Victoria.

Suspected fish specimens should initially be sent to the state or territory diagnostic laboratory. After obtaining the necessary clearance from the CVO of the state or territory of the disease outbreak and informing the CVO of Victoria (for transport of specimens to Geelong), specimens will then be forwarded to the AAHL for emergency disease testing.

The sampling methods approved by the Fish Health Section of the American Fisheries Society are currently used in Australia to diagnose whirling disease (Lorz and Amandi 1994). The procedures described for presumptive diagnosis require that whole fish be used. It is common practice in many overseas diagnostic laboratories to only inspect the gills of larger fish to detect infection in carrier fish (Lorz and Amandi 1994).

Sampling equipment may be available on site, or may be obtained from state/territory fisheries or agricultural officers (see the AQUAVETPLAN **Enterprise Manual**[2] for contact details). Equipment for collecting samples, reagents for sample preparation and facilities for chilled or frozen storage and transport of samples will be required.

Laboratory diagnosis

Currently, a positive diagnosis of whirling disease depends on both the observation of the characteristic gross clinical signs and demonstration of the presence of *M. cerebralis* myxospores in cartilage (Andree et al 2002). This is limiting, as it means whirling disease cannot be definitively diagnosed until myxospores have fully developed in the fish, which may take up to 120 days. Also, clinical signs might not be present.

In heavily infected fish (those with active infections and clinical signs), simple histological sections may be sufficient to observe myxospores. Alternatively, the gill arches of infected fish can be removed and ground up, and the homogenate observed by light microscopy.

Heads of the fish are processed by either of two approved extraction techniques, the plankton centrifuge method or the digest method, followed by microscopic examination to detect myxospores. The skeletal elements of the head are subjected to enzymatic digestion and centrifugation (Markiw and Wolf 1974), or the head is ground up directly and the homogenate is passed through a plankton centrifuge (O'Grodnick 1975a). These are the standard methods used currently by

[2] See http://www.daff.gov.au/content/publications.cfm?ObjectID=F0E292EC-426D-44E0-B1BA1F0A76DB12CC (Accessed 27 June 2005).

laboratories in Australia that test for this disease (M Crane, AAHL, pers comm, May 2003). In both procedures, the goal is to isolate myxospores for microscopic observation. The enzymatic digestion and centrifugation method is better for concentration of myxospores and clarity of sample, whereas the plankton centrifuge method, while saving time, results in a more turbid sample (Andree et al 2002).

In addition to this initial process, fish heads are placed in fixative, and after standard paraffin embedding, prepared sections are stained with haematoxylin and eosin and examined to confirm that the myxospores or developmental stages of the parasite are present in cartilage (Lorz and Amandi 1994). This is essential to diagnosis, as myxospores of other *Myxobolus* species can be found associated with head tissues of fish (Andree et al 1998).

Taxonomic identification of *Myxobolus* myxospores can be difficult, and requires the aid of an experienced parasitologist. Staining methods to aid in myxospore identification include the following:

- *Silver nitrate*. This stain is retained in the myxospores, staining them yellow/brown, but the staining methods add considerable time (3 hours) to the purification process (Wolf and Markiw 1979) and so it is not used routinely.

- *Direct fluorescent antibody test*. This serological test has proved useful, although preparation of the labelled antibodies is difficult. Some cross-reactivity has been demonstrated to *Myxobolus cartilaginis* myxospores (Markiw and Wolf 1978). The author is unaware of any published reports on the sensitivity and specificity of this test.

DNA-based tests

A number of relatively new DNA-based tests are significantly increasing the capacity for early detection of the parasite in the fish, the environment (eg the water column) and the worm. Polymerase chain reaction (PCR) tests have been developed based on parasite genomic DNA, which provides a stable target to detect the parasite throughout its life cycle (Andree et al 1998, 2002). PCR tests can detect the parasite in the fish within 2 hours of exposure, and also in the worm. New procedures to be released by the Fish Health Section of the American Fisheries Society will indicate PCR as an accepted diagnostic procedure (RP Hedrick, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, pers comm, June 2003).

AAHL has the necessary capability to conduct PCR tests on samples. However, confirmation of the diagnosis using other standard diagnostic tests would be needed before a positive isolation of whirling disease is reported.

An in situ hybridisation test has recently been developed (Antonio et al 1998) that can effectively localise all stages of the parasite in the tissues of infected worms and fish. The labelled primers for this protocol hybridise specifically to the parasite rDNA present in sections of infected tissues. It would not be difficult to develop the capacity to conduct this test in Australia (RP Hedrick, pers comm, June 2003).

Histological assessment of disease in fish

Myxospores must be associated with cartilage lesions in order to confirm a diagnosis of whirling disease. Histological assessment is costly and labour intensive but currently essential to prevent misdiagnosis. In Australia, surveillance for whirling disease is currently qualitative, a positive identification of the parasite being significant.

However, in areas where the parasite is now endemic (eg a significant number of states in the US) a more objective, quantitative measure of lesions has been developed (Hedrick et al 1999ab, Baldwin et al 2000, Andree et al 2002). Assessment by this method (shown in Table 1) is useful in evaluating the impact of *M. cerebralis* on exposed fish. Best results for this grading system are obtained when the grade is based on two head or whole-body sections per fish.

Table 1 MacConnell–Baldwin numerical scale for scoring lesion severity

Grade	Description
0	No abnormalities noted. *M. cerebralis* not present.
1	Small, discrete focus or foci of cartilage degeneration. No or few leukocytes associated with lesion.
2	Single, locally extensive focus or several smaller foci of cartilage degeneration and necrosis. Inflammation is localised. Few to moderate numbers of leukocytes infiltrate lytic cartilage.
3	Multiple foci (usually 3–4[a]) of cartilage degeneration and necrosis. Moderate numbers of leukocytes associated with lytic cartilage. Inflammation has minimal or mild impact on surrounding tissues.
4	Multifocal (usually 4 or more sites [a]) to coalescing areas of cartilage degeneration and necrosis. Moderate to large numbers of leukocytes border and infiltrate lytic cartilage. Locally extensive areas of granulomatous inflammation found, involving surrounding tissues.
5	Multifocal (usually 6 or more[a]) to coalescing areas of cartilage necrosis. Moderate to large numbers of leukocytes border and infiltrate necrotic cartilage. Granulomatous inflammation is extensive with severe impact on surrounding tissues. This classification is characterised by loss of normal architecture and reserved for the most severely affected fish. If in doubt, classify as a Grade 4.

[a] Numbers of lesions typical for sections of head, not whole body sections

Source: Baldwin et al 2000

1.4.4 Differential diagnosis

Acute whirling disease should be on the differential diagnosis list whenever young salmonids in Australia exhibit neurological signs such as tail chasing, spinning, or spiralling in the water column.

However, this must be put into perspective. A number of other diseases and conditions, identified below, can lead to the clinical signs associated with whirling disease (erratic swimming behaviour and tail chasing, caudal darkening and skeletal deformities).

Failure to correctly diagnose whirling disease would have serious consequences. Identification of a suspect case will initiate a chain of events (as outlined in the

AQUAVETPLAN **Enterprise Manual**[3] and this manual) requiring significant resources. As always, sound fish health judgment must be used in diagnosis.

Differential diagnoses for the clinical signs associated with whirling disease include:

- septicaemic conditions causing inflammatory responses in the brain and involving bacterial, viral or protozoal aetiological agents (eg *Yersinia ruckeri*);
- nutritional disorders (such as vitamin C deficiency), which have been associated with skeletal deformities in salmonids;
- early infection (eg salmonids < 5 g) with *Flexibacter* species, resulting in shortened operculae in fish that survive;
- high incubation temperatures (eg in Atlantic salmon > 8°C until first feeding) and fluctuating temperatures during incubation, causing skeletal deformities ranging from minor lesions in single vertebrae, through 'short tails' and 'humpbacks', to short body dwarfism, in which the vertebral column is compressed and ankylosed (G Baeverfjord, Research Scientist, Akvaforsk, pers comm, June 2002);
- electroshock injuries causing skeletal deformities and/or melanosis (Wolf et al 1981, Margolis et al 1996); and
- iatrogenic injection damage causing caudal melanosis, where the caudal vein has been used as the injection site.

1.5 Resistance and immunity

Salmonids can develop immunity to whirling disease, but immunity is variable both within and between species.

It is known that salmonid species' host immune response to *M. cerebralis* varies widely (Hedrick et al 1998). The high degree of variability in response to light infections suggests that the host defence and immune response can prevent infection and/or eliminate the parasite (MacConnell and Vincent 2002). However, there is still uncertainty about the mechanisms underlying the variability.

1.5.1 Innate immunity

The initial portals of entry for the waterborne infective stage of the parasite include the epidermis, respiratory epithelium and buccal cavity. Nonspecific, innate defence mechanisms are located in these areas, but their ability to prevent release of the sporoplasm from the TAM is uncertain (RP Hedrick, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, pers comm, June 2003).

[3] See http://www.daff.gov.au/content/publications.cfm?ObjectID=F0E292EC-426D-44E0-B1BA1F0A76DB12CC (Accessed 27 June 2005).

1.5.2 Adaptive immunity

There may only be a short period of about 4 days during which *M. cerebralis* is exposed to immune surveillance by the fish. This is during its migration through the epidermal layers and into the nervous tissue (El-Matbouli et al 1992).

Antibodies to the parasite have been detected (Griffin and Davis 1978), and there may be some passive but not complete protection in fish with older infections.

Cell-mediated immunity may also be important (Hedrick et al 1998). While little cellular response is seen in the first few days, macrophages soon after attack residual epithelial stages (El-Matbouli et al 1999). A strong cellular response occurs during the active feeding phase in the development of the parasite and during cartilage destruction, but does not occur once myxospores are formed (Halliday 1974).

Presporogonic and sporogonic stages associated with cartilage induce inflammation and a granulomatous response. This response may eliminate some but not all of the parasites; this varies with susceptibility of the species. In later stages a key characteristic of the disease is the formation of granulomas. The cellular immune response varies in species from an extensive, diffuse granulomatous response to the formation of well-defined, encapsulated granulomas containing few intact parasites.

1.5.3 Vaccination

There are currently no commercial vaccines available for whirling disease. It is highly unlikely that an effective vaccine will be available in the near future.

1.6 Epidemiology

1.6.1 Historical aspects

The historical spread of *M. cerebralis* and whirling disease is noteworthy.

The parasite was first detected in Germany, in 1893, in imported rainbow trout and brook trout (Höfer 1903, cited in Bartholomew and Reno 2002). The disease had a devastating effect on cultured fish of both species. Hoffman (1970) suggests that *M. cerebralis* evolved as a nonpathogenic parasite of brown trout in central Europe and northern Asia, and that susceptible North American salmonids were brought to the parasite.

The disease spread throughout Europe, probably through transfers of infected fish. Infected brown trout might not display clinical signs but can carry myxospores into hatcheries rearing susceptible rainbow and brook trout.

Until 1956, there were no reports of whirling disease in salmonids in North America. However, the parasite was then detected in brook trout at a research station in Pennsylvania (Hoffman 1962). Imported frozen European trout were implicated as the cause of the outbreak, although live brown trout were also imported from Europe during the 1950s (Bartholomew and Reno 2002). Apparently, the parasite was carried back to the home of the susceptible fish host. The disease has now spread into 22 states in the US. Spread is thought to be through the movement of infected fish from affected hatcheries.

It was not until the 1980s that *T. tubifex* was identified as an obligate host of *M. cerebralis* (Wolf and Markiw 1984). Until then, attempted control was largely based on the assumption that the life cycle was direct.

The first report of whirling disease in New Zealand was in 1971 (Hewitt and Little 1972), but it is likely that the disease had already been there for a considerable time (Boustead 1993). At the time of the report, there was a complete ban on the importation of salmonid fish in any form unless they had been heat treated, but imports of trout had occurred until the 1960s (B Jones, Senior Fish Pathologist, Western Australian Department of Fisheries, pers comm, May 2003).

Live dried food ('infusoria' culture) for tropical fish had also been imported into New Zealand from France. Hewitt and Little (1972) cited this as a possible mechanism for introduction. However, even if infected tubificids were imported with this product as suggested by Bartholomew and Reno (2002), the desiccation process is likely to have destroyed any TAMs carried by such worms.

1.6.2 Sources of *Myxobolus cerebralis*

The fish

In susceptible fish, the infective stage of the parasite is the myxospore, found in the cartilage. Section 1.1 describes the development of myxospores.

Species

M. cerebralis has been found to infect fish only of the salmonid family. While the number of nonsalmonid species challenged experimentally with triactinomyxons (TAMs) is low, the lack of any reports of epizootics and infection in nonsalmonids supports the assumption that nonsalmonids are resistant to whirling disease.

There is considerable variation in the susceptibility of salmonid species to the disease (MacConnell and Vincent 2002). This may be because the disease originated in Eurasia: the Eurasian brown trout is far less susceptible than the North American rainbow trout. In salmonid species with low susceptibility, the parasite replicates poorly.

There is also considerable variability within species. Different strains of rainbow trout have shown significant differences in susceptibility to infection. Some strains of rainbow trout established in Europe are showing signs of resistance to whirling disease (Vincent 2002, El-Matbouli et al 2003).

Non-indigenous salmonids form the basis of commercial fish farming and recreational industries in some Australian states. The most commercially significant are the Atlantic salmon, the rainbow trout and the brown trout. There are also small populations of quinnat (Chinook) salmon in Victoria and brook trout (brook char) in New South Wales and Tasmania (Allen et al 2002).

Susceptibility of Atlantic salmon, rainbow trout and brown trout strains in Australia is unknown, but assumed to be similar to that documented in these species elsewhere. Based on this assumption, rainbow trout in this country are likely to be highly susceptible to whirling disease. The susceptibility of Atlantic salmon to the disease is still unclear (MacConnell and Vincent 2002). The species is variously classified as intermediate in susceptibility (clinical disease common at high parasite doses, but greater resistance to disease at low doses), to low

susceptibility (partial resistance, clinical disease rare and develops only when exposed to very high parasite doses). It is assumed that the brown trout in Australia are likely to be partially resistant to whirling disease, with clinical disease rare and only developing when fish are exposed to very high parasite doses.

Susceptible species such as rainbow trout, given the same exposure dose of TAMs, have significantly more infective myxospores within the cartilage than less susceptible species (Hedrick et al 1999a). Once infected, this species will have the potential to rapidly build up myxospore numbers in the environment. Susceptible species can also be infected with a much lower TAM dose than less susceptible species.

In New Zealand, *M. cerebralis* was detected in rainbow trout being reared in outdoor raceways at a hatchery, but not in brown trout reared at the same hatchery. Whirling behaviour was observed in some of the rainbow trout (Boustead 1993). The parasite has also been detected in Chinook salmon in New Zealand.

Nonsalmonids are considered to be resistant to infection with *M. cerebralis*. When challenged, the distantly related salmonid Arctic grayling (*Thymallus arcticus*) was considered 'invulnerable' (Hedrick et al 1999a). Australian native freshwater species are also assumed to be resistant (see Section 1.2).

Age of fish

The age at which fish are first exposed to the TAM is critical to the development of the disease. In rainbow trout, exposure to TAMs when the fish is less than 9 weeks old can result in clinical whirling disease and death (Ryce 2003). If infected when greater than 9 weeks old, there is a high probability that rainbow trout will still become infected but clinical disease will be minimal and the number of myxospores produced per TAM will be lower. The likely reasons for the age susceptibility are:

- higher cartilage:bone ratio in young fish; and
- little innate and no acquired resistance in the young fish.

This age effect is also present in other salmonid species. For example, in Chinook salmon, the critical age beyond which overt clinical disease will not develop is 3 weeks (J Bartholomew, Center for Fish Disease Research, Oregon State University, pers comm, June 2003).

Temperature

The entire life cycle of the parasite is temperature dependent. Within the fish, the development of viable myxospores in the cartilage is affected by temperature. Experimentally, myxospores were found in cartilage at 52 days after infection at 17°C, but this was delayed to 120 days at 7°C (Halliday 1973b). Temperatures greater than 20°C can also delay development (El-Matbouli et al 1992).

Exposure dose

In general, disease severity increases with increasing parasite dose (MacConnell and Vincent 2002). A low dose is considered to be 100–200 TAMs per fish, whereas

a high dose is 1000–2000 TAMs per fish (Hedrick et al 1999ab). Experimental exposures of high doses (1000 and 10 000 TAMs per fish) can overwhelm the innate resistance of a less susceptible species such as brown trout. Unlike rainbow trout, brown trout do not show increasing parasite burden (myxospore concentration) with increasing exposure dose.

Survival of the myxospore

The myxospore survives well in the environment, probably because of its hard shell (Kerans and Zale 2002). After release from the fish, myxospores can survive at least 5 months at 13°C (El-Matbouli and Hoffmann 1991) and anecdotally for many years. Myxospores can survive the passage through the gut of birds (Taylor and Lott 1978) but possibly not through the gut of tubificid worms other than *T. tubifex*. The spore survives freezing at –20°C for 3 months (El-Matbouli and Hoffmann 1991), but was killed in fish smoked at 66°C for 40 minutes (Wolf and Markiw 1982).

Myxospores are probably dispersed in water currents, and therefore may settle preferentially in areas such as backwaters and other areas where the current is slow.

The worm

In susceptible worms, the infective stage of the parasite is the triactinomyxon (TAM). TAMs develop in the lining of the intestine, from where they are released. Section 1.2 describes the development of TAMs.

Species

Currently, the oligochaete worm, *Tubifex tubifex* is considered the only susceptible worm host for *M. cerebralis* (Wolf et al 1986, Hedrick et al 1998, Kerans and Zale 2002). This worm is globally distributed, including Australia (Pinder and Brinkhurst 2000, Beauchamp et al 2001). While the worm is considered widespread in this country and found in a wide variety of habitats, it is not commonly encountered (Pinder and Brinkhurst 2000).

Experimental data suggests that populations of *T. tubifex* differ in degree of host competency, as measured by output of TAMs. Some lineages of *T. tubifex* have even been found to be resistant to infection by *M. cerebralis* (Beauchamp et al 2002, Rasmussen et al 2003).

In the laboratory, infected worms can live for up to 2 years (Gilbert and Granath 2001).

As far as the author is aware, the susceptibility of Australian strains of *T. tubifex* to *M. cerebralis* has not been studied. This may be a worthwhile area for research. For the purpose of this manual, it is assumed that the Australian strains are susceptible to infection and have the ability to produce viable TAMs.

Temperature

Temperature has a significant influence on the output of TAMs from susceptible worms and on the time taken by the parasite to develop to the TAM stage (El-Matbouli et al 1999, Kerans and Zale 2002). At 8°C, TAMs were released 170 days after exposure to myxospores; at 15°C, TAMs were released after 89–90 days.

Water temperature also influenced the prevalence of infection in worm populations. At 9°C, prevalence of infection was 11.4%; at 17°C, prevalence was 22.2%. This difference may relate to the development rate of the parasite in the worm and/or to the level of feeding activity of the worm.

No infected worms were detected above 20°C. This may be significant in Australia, assuming the strains of worm in this country are of similar susceptibility.

Environment

In the northern hemisphere, *T. tubifex* is not a common species. However, it is often found in marginal sites, such as those with heavy organic pollution or sedimentation, or in highly oligotrophic waters (Brinkhurst 1996). This may bode well for Australia, provided the ecology of our natural waterways remains healthy and unconducive to heavy population with *T. tubifex*.

T. tubifex is a freshwater species that can tolerate salinities of up to 10 parts per thousand. Mature worms can encyst and survive short periods of drought.

Survival of the triactinomyxon

Compared to myxospores, TAMs are short lived in the environment. Temperature significantly influences survival time. TAMs can survive temperatures below 7°C for up to 7 days, and at 12.5°C for up to 5 days, but survive only 24 hours at 23–24°C (Markiw 1992). Aging of TAMs in the environment also affects their ability to infect fish.

Drying and freezing kills TAMs. Salinities greater than 20 parts per thousand killed 80% of TAMs within an hour (Kerans and Zale 2002).

TAMs are neutrally buoyant, so their potential for dispersion in the water column is high, although this is not borne out by research. As discussed above, high temperatures will rapidly inactivate TAMS, as will high salinities. This is significant because TAM density will influence infection rates in the fish, especially in the early stages of an outbreak.

Other possible reservoirs

Water

Both myxospores and TAMs can be found in the water column. The resistance to environmental degradation of both spore stages is discussed above.

Farm equipment and personnel

Farm equipment and personnel that might come in contact with the bottom of ponds or mud containing myxospores could carry infection to susceptible *T. tubifex* worms elsewhere.

1.6.3 Modes of transmission

Horizontal transmission

The modes of transmission of *M. cerebralis* have been discussed. Whirling disease is a disease of freshwater fish, and can only be transmitted in fresh water. Fish infected in fresh water can remain infected after moving to salt water. If infected fish return to fresh water (eg broodstock returning to spawn), myxospores may be released and infection established in worm populations.

It is important to note that *M. cerebralis* was detected in adult steelhead (anadromous rainbow trout), sockeye and Chinook salmon returning to rivers in Oregon in the US. Currently, there is no evidence that the parasite has become established in these areas, even with several years of intense monitoring (Engelking 2002).

Vertical transmission

Salmonid eggs from *M. cerebralis* infected broodstock have been shown to be free of whirling disease (O'Grodnick 1975b). There is potential for mechanical transmission of myxospores and TAMs in packing material used for egg transport.

2 Principles of control and eradication

2.1 Introduction

Myxobolus cerebralis has never been reported in Australia.

If *M. cerebralis* is introduced, there are essentially three broad control options available:

- **Eradication**

  This control option aims to eradicate *M. cerebralis* from Australia. This is the highest level of control measure and cost.

- **Containment, control and zoning**

  Containment, control and zoning include measures to contain the parasite to areas with endemic 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. This is the lowest level of control measure and cost.

The basic principles of eradication and other control responses are described in the AQUAVETPLAN **Enterprise Manual** and the AQUAVETPLAN **Control Centres Management Manual**. The **Enterprise Manual** details state/territory legislation relating to disease control and eradication.

If *M. cerebralis* is introduced into Australia, its introduction will most likely be discovered by confirmation of *M. cerebralis* in wild and farmed rainbow trout showing clinical signs of whirling disease.

The circumstances of the discovery will affect the choice of control option. For example, if wild trout with clinical signs are discovered, the parasite is quite likely to have been introduced well before (possibly months to years) and to be well established in the area in worm and fish hosts.

Whatever the scenario, the general strategies to control whirling disease will include:

- rapid detection and identification of infection;
- rapid definition of the nature and extent of the problem;
- rapid implementation of control measures;
- prevention of parasite spread, by controlling stock and water movement within and between farms;
- management, where possible, to avoid water containing infective spores (eg change from above-ground water to well water, move fish to salt water);

- elimination of worm habitat (eg change to concrete raceways); and
- good management practices and maintenance of high hygiene standards.

Our ability to use particular strategies may be limited. For example, it may be difficult to rapidly detect infection, and to rapidly define the nature and extent of the problem.

Given such limitations, the most appropriate control option will depend on the:

- expertise and capabilities of fish health management and response personnel;
- location and presence or absence of reservoirs of infection (eg *Tubifex* worm host, wild salmonids), knowledge of which will depend on our ability to identify such areas and correctly identify the species of worm;
- chances of successful eradication;
- level of risk accepted for future spread of infection (eg the risk associated with grow-out of infected populations);
- short-term costs of control and disruption to production;
- long-term costs of production, with or without the presence of the parasite; and
- long-term costs of control should the parasite become endemic.

2.2 Methods to prevent spread and eliminate pathogens

This description of methods for the control and/or eradication of *M. cerebralis* uses the following terms:

- *infected fish* — a fish, which might or might not show clinical signs, whose cartilage contains mature or maturing *M. cerebralis* myxospores;
- *infected worm* — a *Tubifex tubifex* oligochaete in which there are mature or maturing *M. cerebralis* triactinomyxons (TAMs);
- *suspect fish* — any salmonid fish that could potentially have been exposed to viable TAMs;
- *suspect worms* — any *T. tubifex* worms that could potentially have been exposed to viable myxospores.

Live salmonids cannot legally be imported into Australia, so any fish showing clinical signs of whirling disease and confirmed positive for *M. cerebralis* must have become infected through contact with viable TAMs released from a susceptible *Tubifex* worm.

If *M. cerebralis* is detected in a live fish, it is likely that other fish are infected and that there is an infected worm population, either in the area of detection or in an area from which the infected fish has been moved. In such a case, our ability to prevent spread and eliminate the parasite may already be significantly compromised.

2.2.1 Quarantine and movement controls

Quarantine and movement restrictions that could be implemented immediately upon suspicion of the isolation of *M. cerebralis* are:

- establishment of specified areas (see Figure 2 and the AQUAVETPLAN **Enterprise Manual** for more details), which will include:
  - an *infected area or premises*
  - a *restricted area* surrounding an infected area or premises
  - a *control area*, which is a buffer between the restricted area and free areas (together, these three areas form the *declared* area; the free area is the area outside the declared area and may include large areas of Australia in which *M. cerebralis* remains unassessed);
- bans on the movement of live fish, *T. tubifex* worms and aquatic plants out of declared areas (but because of the freshwater life cycle of the worm host, movement of fish into salt water may be deemed acceptable);
- bans on the movement of live fish and *Tubifex* worms into disease-free areas from areas where *M. cerebralis* is considered present;
- bans on the movement of *all* oligochaete worms;
- restrictions or bans on the release of fish and *Tubifex* worms into river or freshwater lake systems in designated areas;
- restrictions or bans on the movement of fish and *Tubifex* worms between different river systems in designated areas; and
- restrictions or bans on the use and movement of equipment within and between river systems.

The threshold for establishment of specified areas will depend on the circumstances and on information available about the disease outbreak or isolation of the parasite. The potential movement of infective myxospores and TAMs along waterways must be considered.

It is important to note that quarantine measures must take into account water movements eg a river flowing out of an infected area can carry viable spores (either TAMs or myxospores) from the area, as can effluent water flowing out of a fish farming operation.

The feasibility of the restrictions and bans and the extent to which these are to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

The implementation of restrictions can significantly help in the early stages of control of a disease outbreak. Imposing restrictions also buys time while the true extent of the problem is assessed.

The benefit from restrictions should be weighed against their costs.

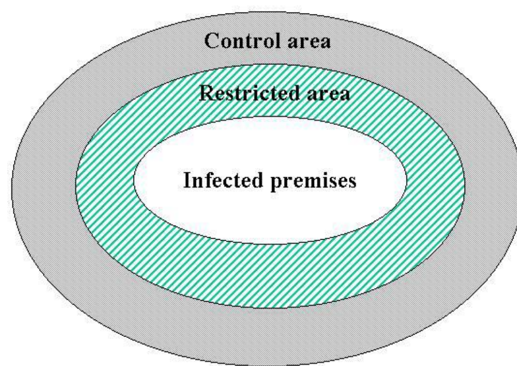

Figure 2 Establishment of specified areas to control whirling disease

Semi-open fish farming systems

Whirling disease is a disease of fish inhabiting fresh water. However, fish reared and exposed to TAMs in fresh water could remain infected when transferred to salt water. Myxospores released directly from fish into the marine environment are unlikely to find a susceptible worm host, as *T. tubifex* does not tolerate salinities above 10 parts per thousand (Pinder and Brinkhurst 2000).

Fish contained in cages moored in estuaries or sheltered areas of the sea could escape into the wild. If infected fish escape and return to fresh water, there is a potential for disease transfer, although a US study in Oregon looking at infected anadromous salmonids did not support this assumption (Engelking 2002).

There are no effective treatments to ensure freedom from myxospores in fish transferred to semi-open systems (Staton et al 2002, Wagner 2002, Schisler et al 2003).

Semi-closed fish farming systems

Semi-closed systems have slightly more control over inputs and outputs than semi-open systems. However, farms differ in the extent to which their input and output water can be contained. Because semi-closed systems are not designed to be self-contained, preventing inflows or outflows may adversely affect production. Most of the Australian salmon and trout farms are based on flow-through systems. Influent water comes from a surface water source, usually a freshwater river or stream. These farms usually have limited facilities to hold and/or treat influent and effluent water.

Myxospores are extremely resistant to physical and chemical inactivation; on the other hand, treatment of influent water in semi-closed systems may be possible to exclude the less resistant TAMs. Ultraviolet irradiation, sand filtration and ozonation are options (see also Section 2.2.8).

Control and treatment of output water to eliminate TAMS are possible in theory, but would not be feasible on most farms. A better option would be to remove worms by eliminating any potential habitat for them (for example, by lining earthen ponds with concrete).

Treatment to ensure inactivation of myxospores in effluent water is also not considered feasible. Daily removal of dead fish ('morts'), which is possible but not universal in semi-closed systems, will reduce the number of myxospores released from decomposing carcases.

Fish input and output may be controlled, but some movement restrictions could significantly interrupt production. Fish inputs into freshwater farms may be from on-site hatcheries or from other freshwater or marine farms (eg broodstock).

Fish are also able to enter farm waterways and possibly ponds via intake water from rivers, or may be carried onto a farm by birds.

Zoning

Whether zoning is a viable option for whirling disease will depend on the circumstances. Zoning may be possible if the parasite is isolated in only one hatchery that has the ability to disinfect effluent, or in a landlocked lake population of trout. For some salmonid populations in Australia (such as the small trout population in Western Australia), geographical zoning is feasible.

Zoning must consider the two-host life cycle of *M. cerebralis*. Covertly infected fish populations can become established and are very difficult to detect, and infected worms can live for up to two years in the laboratory and possibly longer in the wild. It can be difficult to identify *T. tubifex* and to determine if a *T. tubifex* population is infected.

Human activity (eg fishermen moving between areas carrying mud on their boots) could also transfer the parasite.

These factors will make it very difficult to protect disease-free zones from infection unless they are surrounded by significant natural barriers.

If zoning is attempted, surveillance and monitoring will be needed to support it.

Principles of zoning for infected and non-infected areas in Australia are outlined in the **AQUAPLAN Zoning Policy Guidelines**.[4]

2.2.2 Tracing

If *M. cerebralis* is isolated in Australia, the first step in determining the most appropriate control option will be to conduct an epidemiological investigation. This will determine:

- the source and point of entry of the parasite (allowing any further introduction to be prevented);
- the *possible* locations of the parasite and worms (even without confirming that parasite is present in those locations, which may be very difficult); and

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[4] See http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717 (Accessed 27 June 2005).

- how to quickly prevent any further parasite spread from the infected location.

The nature of whirling disease imposes some limitations on epidemiological investigation, including:

- the delay between the exposure of susceptible fish and the development of clinical signs of disease (3–8 weeks depending on water temperature);
- the absence of clinical signs in some infected fish;
- the considerable delay (>50 days depending on water temperatures) between exposure and the development of mature spores, which are needed to confirm diagnosis, in the cartilage of fish;
- difficulty confirming infection in a fish and in a population of fish; and
- difficulty confirming infection in *Tubifex* worms or in a population of worms.

A thorough and comprehensive epidemiological investigation requires the availability of trained personnel, with the required skills and resources.

As a guide, the following should be traced.

Fish

All movements of the infected fish should be traced back to help establish the origin of the outbreak, and whether the infected fish were exposed to TAMs at their current location, were brought there after exposure, or both.

All contacts with infected fish should be traced forward.

Any recent movement of broodstock, smolts or fish destined for restocking should be noted.

Water

Both the water source for the infected site and the destination for effluent water from the site should be noted. If the site is in salt water, fish must have been exposed in fresh water.

Equipment, vehicles and personnel

Because myxospores can be carried in mud, all movement of equipment, vehicles and personnel should be traced.

***Tubifex* worms**

Habitat suitable for *Tubifex* worms should be identified, and any local movement of these worms (eg by anglers for bait) should be noted.

Neighbouring fish populations

Fish farms on the same watercourse or in the same watershed may already be infected. Some freshwater operations slaughter and process fish on site, and processing waste, if discharged into the watershed, could be a source of

myxospores — and of further infection downstream if susceptible *Tubifex* worms are present. Maps showing neighbouring fish farms and waterways, and hydrographic data, will be needed for monitoring the potential spread of the parasite. Further sources of infection may be identified if a number of facilities share common water. The upstream and downstream locations of susceptible fish species should be noted.

The state/territory fisheries or agriculture agency will have information on the location of farms and wild fish populations at risk of infection (see the AQUAVETPLAN **Enterprise Manual**[5] for contact details).

2.2.3 Surveillance

Surveillance, by screening for clinical signs and by laboratory testing, is necessary to:

- define the extent of infection;
- detect new outbreaks;
- establish restricted and control areas to which quarantine and movement restrictions are applied;
- establish disease-free and infected areas or zones for a whirling disease zoning program; and
- monitor the progress and success of an eradication strategy if one is implemented.

Because of the inadequacies in currently approved diagnostic tests for whirling disease (see Section 1.4.3), the development of effective surveillance will be difficult. Newer tests, such as those based on polymerase chain reaction (PCR) could significantly improve this situation. The Australian Animal Health Laboratory (AAHL) is currently the only Australian laboratory able to use PCR to detect the parasite.

These limitations should be acknowledged when deciding on a control option if *M. cerebralis* is isolated in Australia.

2.2.4 Destruction of fish

While no current treatments effectively eliminate infection from fish, some have reduced myxospore counts in fish. These include the antibiotic fumagillin, but fumagillin is not registered for use in fish in Australia. Treatment of infected fish is therefore unlikely to be an option.

Slaughter must be both hygienic and humane. Although the exact process by which myxospores are expelled from living fish is not known, it is unlikely that the choice of slaughter method will affect the numbers of myxospores shed during slaughter. However, one possibility is that myxospores in peripheral tissues such

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[5] See http://www.daff.gov.au/content/publications.cfm?ObjectID=F0E292EC-426D-44E0-B1BA1F0A76DB12CC (Accessed 27 June 2005).

as the fins may be shed through abrasion of epidermal surfaces, and crowding and rough handling of fish might increase shedding.

Methods used to anaesthetise and/or slaughter fish include:

- chemical anaesthesia;
- ice slurry;
- stunning (with or without subsequent bleeding); and
- carbon dioxide narcosis (with or without subsequent bleeding).

Fish can be captured on lines and then stunned, and raceways can be netted to crowd fish for slaughter.

When chemical anaesthesia is used, the water level in the tank is lowered and an anaesthetic solution is added to the water. Aqui-S® is the only anaesthetic currently fully registered for use on some fish species in Australia and has no withholding period. The dose of can be varied, depending on whether the fish are to be harvested or euthanased and disposed of.

The most appropriate method of slaughter depends on the following factors:

- the size and number of fish;
- the deadline for slaughter, which depends on the pressure of infection and the chance of containing the disease;
- the destination of the fish (human consumption or disposal);
- the availability of slaughter facilities (site, equipment and other resources); and
- the experience and availability of personnel.

See the AQUAVETPLAN **Destruction Operational Procedures Manual**[6] for details of destruction methods for fish.

2.2.5 Elimination of parasite

Changing production systems from earthen ponds to concrete raceways will eliminate the worm's habitat and effectively remove worms.

Physical alteration of habitats has been attempted overseas to remove 'hot spots', or areas of streams with higher densities of *T. tubifex*. Such measures have significant engineering and other costs.

[6] See http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906 (Accessed 27 June 2005).

Bayluscide (a molluscide; 5,2′-dichloro-4′-nitrosalicylanilide) was found to reduce worm densities by 73–82% (Kowalski and Bergersen 2003). This compound is toxic to fish at the doses used to kill worms.

2.2.6 Treatment of fish products and byproducts

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

M. cerebralis can survive well in dead fish, even when frozen at –20°C for up to 3 months. Brined fish also retain viable spores, although hot-smoking at 66°C for 40 minutes inactivates spores.

The species of salmonid affected will influence the number of potential myxospores per fish. Infected rainbow trout carry more myxospores in the cartilage than less susceptible species, such as brown trout, so rainbow trout byproducts have a higher risk.

M. cerebralis is not transmitted vertically, but myxospores and TAMs could be transmitted mechanically in packing material and in fluid surrounding fish eggs during transport.

2.2.7 Disposal of fish

Because myxospores are highly resistant, correct disposal of carcases is critical. Rapid removal of morts from ponds or raceways is also essential to minimise myxospore release and prevent exposure of susceptible *Tubifex* worms. Burial sites must be chosen carefully to ensure that there is no contact with waterways or with birds or animals that could carry myxospores to *Tubifex* habitats.

Myxospores are *not* directly infectious to fish, so infected fish are not an immediate source of infection for uninfected fish.

See the AQUAVETPLAN **Disposal Operational Procedures Manual**[7] for details of disposal of fish carcases.

2.2.8 Decontamination

Because the myxospore is highly resistant, decontamination of equipment, materials, personnel, tanks and buildings must adequately inactivate this stage of the parasite or ensure that viable myxospores do not enter freshwater waterways containing susceptible tubificid hosts. Wash water entering a freshwater habitat may contain viable myxospores if not disinfected before release.

Decontamination requires thorough cleaning before disinfection.

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[7] See http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5 (Accessed 27 June 2005).

Myxospores

Compounds and methods found to be effective in killing myxospores (reviewed in Wagner 2002) include:

- calcium hydroxide at more than 0.5% for 24 hours;
- calcium oxide (quicklime) or potassium hydroxide at more than 0.25% for 24 hours;
- chlorine at 1600 ppm (parts per million) for 24 hours or 5000 ppm for 10 minutes (chlorine at such high concentrations could be hazardous for the operator); and
- heating for 10 minutes at 90°C.

Thorough drying of ponds may be effective. Contaminated mud dried for 13–19 months was incapable of inducing infection when the pond was restocked.

TAMs

TAMs are not nearly as resistant as myxospores. For example, they are readily inactivated by drying or freezing for at least an hour (Wagner 2002).

Other methods of TAM inactivation include:

- temperatures above 75°C for at least 5 minutes (TAMs can survive 7–8 days at 7°C);
- chlorine concentrations of 130 ppm for more than 1 minute at water temperatures from near freezing to room temperature, with total hardness levels from 10 to 500 mg/L;
- hydrogen peroxide at 10% for 10 minutes; and
- povidone–iodine at 5000 ppm active iodine for 60 minutes.

Stringent decontamination of saltwater facilities from which fish previously infected in fresh water are removed will probably not be required, because myxospores released in salt water will be unlikely to find a *Tubifex* host before dying. For the same reason, a processing plant discharging into salt water is unlikely to need stringent decontamination unless there is potential traffic between the plant and freshwater habitats, or birds or animals are likely to carry carcases or offal to freshwater habitats.

Because of differences between farming enterprises, disinfection protocols for freshwater facilities may need to be determined case by case. This would involve the farm manager, the state/territory CVO and/or director of fisheries, and possibly the environmental authorities. The disinfection protocol should take into consideration the factors outlined in Section 1.6, and particularly:

- the source and location of infection;
- the type of enterprise (eg use of underground water as opposed to river, lake or stream water);
- the design of the site and its proximity to other waterways;
- the environmental impact of the disinfectant protocol; and

- the availability of approved, appropriate and effective disinfectants.

Contaminated facilities that have earthen ponds can break the life cycle of the parasite by eliminating habitat for the worm host, for example by converting to concrete raceways.

Environmental considerations

Environmental considerations in the control of whirling disease include the following:

- Discharge of infected or potentially infected effluent into freshwater catchment areas or natural waterways may lead to further spread of infection and could lead to the establishment of reservoirs of infection in *T. tubifex*, wild fish populations and waterways.
- Disinfectants can adversely affect the environment, especially if they are used in larger than normal quantities or concentrations, as is possible in a disease control program. The local environmental protection agency may need to be consulted (see the AQUAVETPLAN **Enterprise Manual**[8]).
- The destruction and disposal of infected carcases or material will have an environmental impact, which must be minimised while dissemination of infection is prevented.
- *T. tubifex* is found in high numbers in polluted streams (Pinder and Brinkhurst 2000). Ensuring pristine stream ecology may help to keep numbers of this species low.

2.2.9 Vaccination

There are no commercially available vaccines against *M. cerebralis*.

2.2.10 Vector control

Myxospores can survive passage through the gut of birds, which can therefore carry the disease. Open-air tanks, ponds and especially processing facilities and areas where carcases are disposed of may attract birds and must be covered, for example by nets or tank roofs.

The persistence of myxospores makes possible the spread of infection through mud carried on boats, trailers, boots or other items, which should be thoroughly cleaned before they leave an infected area. The requirement to clean equipment could include anglers leaving a lake known to be infected with *M. cerebralis*.

Because myxospores can survive the passage through the gut of fish, wild predatory fish can carry myxospores even if the predator is not infected.

Movement of infected *T. tubifex* worms between waterways (for example, by anglers for bait) could transmit infection.

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[8] See http://www.daff.gov.au/content/publications.cfm?ObjectID=F0E292EC-426D-44E0-B1BA1F0A76DB12CC (Accessed 27 June 2005).

2.2.11 Sentinel animals and restocking

Rainbow trout are considered to be the fish species most susceptible to infection. Therefore, young rainbow trout can be used as sentinel fish to determine the presence or absence of the parasite. This is routine in countries where whirling disease is present.

While Atlantic salmon and brown trout can be infected with *M. cerebralis*, and myxospores can form in the cartilage of these species, clinical disease is rare and morbidity low. Therefore, restocking with less susceptible species such as these, using fish older than 9 weeks, may be an option in some areas to maintain production.

If *T. tubifex* is removed from a facility, and there is no likelihood of TAMs re-entering the facility, restocking can begin immediately.

Restocked fish on previously infected farms must be free of covert or overt infection or disease. Given the current limitations in diagnostic tests, this may be difficult to confirm. If areas are declared free of *M. cerebralis*, fish introduced into those areas must also be free from infection.

2.2.12 Public awareness

A public awareness campaign must emphasise education, surveillance and cooperation from industry and the community in order to control potential outbreaks of whirling disease in Australia.

2.3 Feasibility of specific options for control in Australia

The feasibility of control of an outbreak of whirling disease or isolation of *M. cerebralis* in Australia depends both on the circumstances of the outbreak or isolation and on the control strategy adopted: eradication; containment, control and zoning; or control and mitigation of the disease.

2.3.1 Eradication

Once wild populations are infected, the conventional wisdom is that *M. cerebralis* cannot be eliminated. Failed eradication efforts in areas of the world where the parasite is now endemic (eg many states in the US) have been documented in the literature. Efforts have included such drastic measures as chlorinating an entire stream. As Wagner (2002) states, 'The best management is to avoid infecting negative waters.'

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, has no point source, is unable to be contained and is present or potentially present in wild fish species, lakes or rivers. This is due to:

- *M. cerebralis*' ability to spread rapidly and establish reservoirs of infection that would be impossible to eradicate in wild fish populations;

- the parasite's ability to produce covert infections that are difficult to detect;

- the ability of infected wild fish to transmit and establish infection in freshwater habitats; and
- close contact between and relative lack of control over farmed fish, wild fish and water in Australian salmonid farming operations using semi-open or semi-closed systems.

Eradication measures must ensure that there is no further exposure of unexposed fish populations to TAMS and no further spread of infection via the release of myxospores into the environment.

In certain circumstances, it may be possible to eradicate *M. cerebralis* from a particular fish farming facility. This has been achieved overseas (Anderson 1993). The principles ensure that:

- source water is either free of TAMs or is treated to make it so; and
- there are no potential habitats for *Tubifex* worms in the facility.

Unexposed fish

Immediate destruction of unexposed fish populations in a declared area would be a drastic option if the fish were not likely to have been exposed to TAMs. *M. cerebralis* cannot be transmitted directly from fish to fish, and the development of the parasite in the worm host requires a significant time before infective TAMs are released (more than 90 days, depending on temperature).

Young (pre-market sized) unexposed fish may be allowed to grow out, provided there has been no possibility of infection (ie exposure to TAMs) and there is no likelihood of future infection. This may be difficult to confirm. Older fish that have had no possible exposure to infection (also difficult to confirm) may be emergency harvested and slaughtered for human consumption.

Alternatively, fish may be transferred to salt water to remove the possibility of their exposure to TAMs. This is a viable option only if the fish will tolerate such salinity changes (eg smolted Atlantic salmon) and saltwater facilities are available and suitable.

Exposed or potentially exposed, but clinically normal fish

Normal or controlled grow-out of exposed or potentially exposed but clinically normal farmed salmonids could be used in an eradication program, provided myxospores released during grow-out are prevented from coming into contact with *Tubifex* worms. Grow-out in salt water may achieve this, as may grow-out in facilities that send effluent water to ground or can otherwise prevent contact with worms. These fish are safe for human consumption.

Immediate destruction of these fish populations will not be effective in an eradication program if effluent water or escaped infected fish have spread myxospores to downstream worm habitats.

There is no need for immediate destruction if the fish are in salt water.

Clinically diseased fish

Clinically diseased fish are safe for human consumption, and may be emergency harvested under some circumstances (see Section 2.3.4).

In fresh water, if there is no possibility of disinfecting or redirecting effluent water during emergency harvesting, the removal, destruction and disposal of diseased and dead fish is essential to prevent myxospores from transmitting infection.

If fish are to be slaughtered and disposed of, burial sites should be chosen carefully to ensure that there is no contact with waterways or with birds or animals.

2.3.2 Containment, control and zoning

A zoning program might or might not be used as part of a containment and control program. Zoning will rely on movement restrictions on exposed or potentially exposed fish to prevent infection spreading to uninfected zones, and will take into account water movement and the possible waterborne spread of the two spore stages of the parasite.

The feasibility of movement restrictions will depend on farm management practices, the extent to which infection has already spread and the location of reservoirs of infection. Feasibility will be assessed at the time of the outbreak.

Zoning is discussed further in Section 2.2.1.

Unexposed fish

Control options for unexposed fish are the same in containment programs as in eradication programs (see Section 2.3.1).

Exposed or potentially exposed, but clinically normal fish

If young fish are allowed to grow out, they must be treated as infected during the grow-out period.

In a declared area, normal or controlled grow-out and slaughter may be feasible without further spread of infection. However, to prevent spread of infection, final products must be processed to the degree required for the designated market.

Immediate destruction of the fish is an option in a containment, control and zoning strategy, as it can help decrease the infectious load on a site and minimise the spread of infection. However, if susceptible *Tubifex* hosts are also infected, destruction of fish may have no overall benefit. Removing both hosts by destroying fish and eliminating *Tubifex* habitat may be a viable option.

Clinically diseased fish

In a containment and control program, the only real option for clinically diseased fish is immediate destruction, unless effluent water can be contained or disinfected or the fish can be moved to salt water, in which case controlled grow-out is possible.

2.3.3 Control and mitigation of disease

The aim of a control and mitigation strategy is to reduce the impact of disease. Therefore, in such a program all the options listed for the containment, control and zoning strategy (Section 2.3.2 above) apply, except for zoning.

2.3.4 Emergency harvesting

Emergency harvesting of fish is unlikely to result in further transfer of infection, provided there is no possibility of untreated processing waste, especially skeletal elements, being released into freshwater habitats.

Strict control measures necessary to prevent further spread of infection during emergency harvesting include:

- disinfection of all equipment and personnel involved to eliminate the risk of transferring myxospores off site;
- quarantine restrictions on infected sites, including of personnel, equipment and vehicles;
- processing on or off site, as long as waste and fish carcases cannot come into contact with freshwater *Tubifex* habitats;
- holding, treatment and safe disposal of slaughter and processing effluent, including holding water and waste offal; and
- treatment of the product to prevent the spread of infection.

2.4 Trade and industry considerations

In countries where whirling disease is endemic, the salmonid farming and recreational angling industries are affected. In Australia, it is unlikely that other aquaculture industries would be affected by this disease.

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

2.4.1 Export markets

Whirling disease is endemic in many countries (see Section 1.3), and is not listed by the World Organisation for Animal Health, formerly Office International des Epizooties (OIE, formerly Office International des Epizooties). However, some countries require imports to be certified free from the disease, and some have requirements for particular regions (for example, some states of the US). The Australian Quarantine and Inspection Service (AQIS) should be contacted for further information about current export market requirements.

2.4.2 Domestic markets

A cautious approach to the salvage of exposed or potentially exposed product for the domestic market is needed. Decisions about the release of salmonids or

salmonid products to the domestic market will depend on the control strategy implemented.

If eradication is the chosen strategy, product releases to the domestic market must carry no potential for the spread of *M. cerebralis*. This may be difficult.

If a strategy of containment, control and zoning is used, releases must not risk the spread of viable *M. cerebralis* to areas or zones declared free of whirling disease.

If whirling disease becomes endemic in Australia and a strategy of control and mitigation of disease is implemented, requirements for the release of exposed or potentially exposed product will be less stringent.

3 Policy and rationale

3.1 Overall policy

Whirling disease has the potential to cause significant mortality and morbidity in farmed and wild salmonid populations in Australia. Fish can only be infected with parasite spores released from the susceptible *Tubifex* worm host. Rainbow trout are particularly susceptible, especially if infected when less than 9 weeks old.

It takes 3–8 weeks from infection for clinical signs to develop in susceptible fish, but fish may show no clinical signs. Epidemiological investigation may be hindered because it can be difficult to confirm infection in fish or worms.

There are three potential control options:

- ***Option 1 — eradication of the parasite*;**
- ***Option 2 — containment, control and zoning* of the parasite to areas with endemic infection, prevention of further spread and protection of uninfected areas; or**
- ***Option 3 — control and mitigation of disease* through management practices that decrease the incidence and severity of the disease.**

The control policy chosen will depend on the circumstances of the outbreak and/or isolation of the parasite. The director of fisheries and/or the CVO of the state or territory in which the parasite is isolated will decide the policy.

It is important that the chosen policy can evolve with the changing situation. For example, a choice of containment, control and zoning in the short term does not preclude eradication as a long-term policy.

Strategies that may be used under these options include:

- ***quarantine and movement controls* on fish, fish products, *Tubife*x worms, equipment and other things in declared areas to prevent spread of infection;**
- ***prevention* of access by predators (eg birds) to infected fish;**
- ***destruction and disposal* of clinically diseased and dead fish to prevent further myxospore release into the environment;**
- ***alteration* of the *Tubifex* worm habitat to eliminate the worm host (eg by replacing earthen ponds with concrete raceways);**
- ***decontamination* of infected premises to inactivate the resistant myxospore stage of the parasite and prevent spread to *Tubifex* worms;**
- ***surveillance* to determine the extent of possible infected worm and fish hosts, and to provide proof of freedom from the parasite;**
- ***zoning* to define and maintain infected and parasite-free zones;**
- ***restocking* with older, less susceptible fish or less susceptible species unlikely to develop clinical disease; and**
- ***education* of the public, aquaculturalists and government.**

If *Myxobolus cerebralis*, with or without clinical disease in fish, is confirmed in Australia, the director of fisheries and/or the CVO of the state or territory in which the isolation occurs will be responsible for implementing disease control measures in accordance with relevant legislation. The aquatic Consultative Committee on Emergency Animal Diseases (aqCCEAD) will be convened to discuss response options and the agreed management strategy will then be implemented by the state or territory involved. The detailed control measures will be determined using the principles of control and eradication and epidemiological information about the isolation and/or outbreak.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel during the various stages of activation of AQUAVETPLAN, see the **Control Centres Management Manual**.[9]

3.2 Overview of response options

One of three control options may be chosen to control whirling disease:

- **Eradication**

  This control option aims to eradicate *M. cerebralis* from Australia. This is the highest level of control measure and cost.

- **Containment, control and zoning**

  Containment, control and zoning include measures to contain the parasite to areas with endemic 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. This is the lowest level of control measure and cost.

Whichever control option is chosen, the strategies for control and eradication in Section 3.3 will be applied immediately to prevent the spread of infection. Additional measures that might be applied in non-eradication programs are listed in Sections 3.2.2 and 3.2.3.

The flow chart in Figure 3 is designed to aid decision making. The initial decision may need to be made on very limited epidemiological information, and might be modified as more information becomes available. For example, if whirling disease is confirmed in farmed fish, eradication in the short term is unlikely to be an option. However, in the long term (possibly years), eradication may be possible by using various management options, such as stocking fish species with lower susceptibility to the disease.

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[9] See http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=9A235104-7AF3-46C7-BED132208D1826DA (Accessed 27 June 2005).

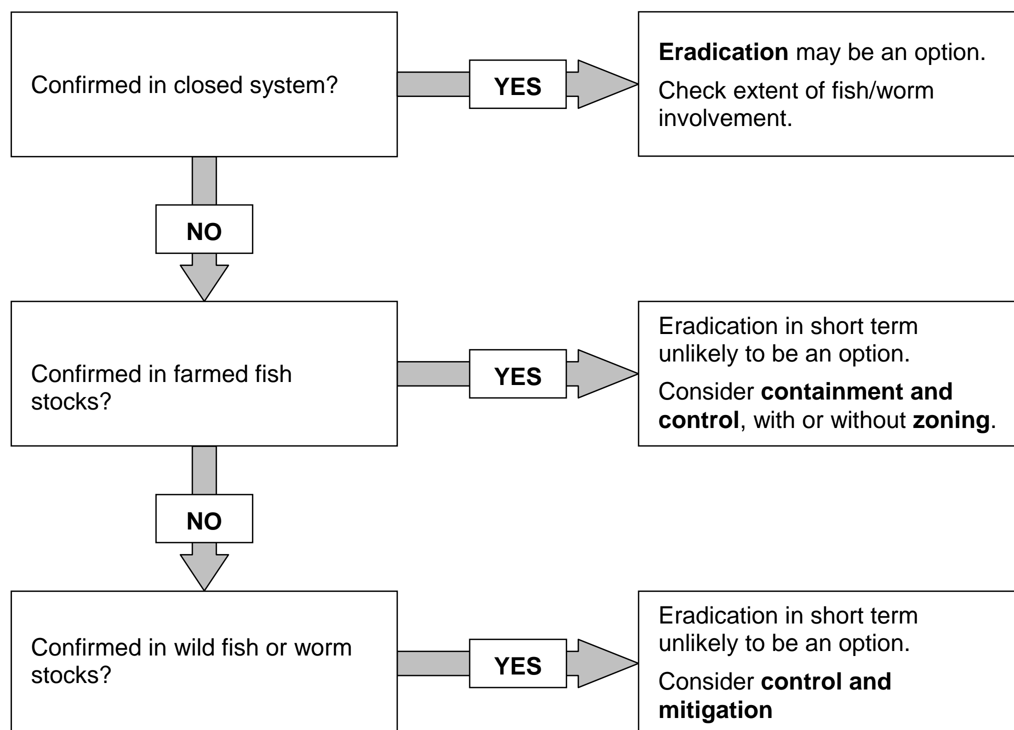

Figure 3 Decision flow chart

3.2.1 Option 1 — Eradication

If epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the parasite (eg a closed system, such as an aquarium or fully recirculating system), an eradication strategy may be successful and will be attempted. Compared with containment or mitigation, eradication can have the highest short-term economic costs, but these would be outweighed by long-term benefits if *M. cerebralis* were to be eradicated.

Successful eradication in the short term will be less likely if the parasite is confirmed in:

- wild fish stocks that are not in a landlocked lake system; or
- a fish farm or hatchery from which effluent water returns to a stream or lake system holding wild salmonids and susceptible *Tubifex* hosts.

In an eradication program, the strategies for control and eradication listed in Section 3.3 will be applied from the outset and continued until further notice.

3.2.2 Option 2 — Containment, control and zoning

The strategies listed in Section 3.3 will be applied as soon as an outbreak of whirling disease is confirmed or *M. cerebralis* is isolated in Australia.

If eradication is subsequently discounted as a control strategy, containment and prevention of further spread to protect and maintain uninfected areas will become the preferred option.

Additional measures to be taken under this control option are:

- implementation of a zoning program (the poor sensitivity and specificity of available diagnostic procedures, case definitions and sampling bias must be considered before zoning is implemented);
- management options to reduce the severity and incidence of infection; and
- restocking with less susceptible fish species (eg Australian native species) or preventing the exposure of salmonids under 9 weeks old to triactinomyxons (TAMs) in order to significantly reduce the incidence of clinical disease.

3.2.3 Option 3 — Control and mitigation of disease

The strategies listed in Section 3.3 will be applied as soon as an outbreak of whirling disease is confirmed or *M. cerebralis* is isolated in Australia.

If the control option chosen is control and mitigation of the disease, measures tailored to the management of the disease in affected areas (detailed in Section 2.3.2, but not including zoning) will be implemented.

3.3 Strategies for control and eradication

3.3.1 Laboratory testing

Suspicion of whirling disease is classified as a disease outbreak emergency in the AQUAVETPLAN **Enterprise Manual**. The state or territory CVO or director of fisheries must be notified immediately. Preliminary identification of *M. cerebralis* may be undertaken by some state/territory diagnostic laboratories, but duplicate specimens must be consigned to AAHL the same day. AAHL will conduct primary or index case diagnostic testing as far as is practical. Samples must be submitted from the state or territory laboratory unless the laboratory, in consultation with the CVO, has given prior approval for a direct submission.

The CVO of Victoria must be informed before specimens are transported through Victoria to AAHL (see Appendix 1).

The testing laboratory should be contacted immediately to ensure that samples are collected correctly, and that sample collection techniques satisfy the requirements of the laboratory.

3.3.2 Epidemiological investigations

An initial epidemiological investigation, including tracing and surveillance, must begin immediately upon suspicion or confirmation of the isolation of *M. cerebralis* in Australia (for details of tracing, see Section 2.2.2)

3.3.3 Quarantine and movement controls

Quarantine and movement controls must be implemented on anything capable of transmitting the infective stages of the parasite (both the TAM, released from the worm, and the myxospore, released from the fish). Consideration must be given to the likelihood of the parasite spreading into the local *Tubifex* worm population through the spread of myxospores and TAMs down waterways. If clinical signs of disease are present, such spread is likely to have been occurring for over three months.

Control areas (see Section 2.2.1) should be established as quickly as possible, even if epidemiological information is initially limited, and refined later as more information becomes available.

Items that could transmit *M. cerebralis* include:

- myxospores (highly resistant stage) in or on
  - fish, both farmed and wild
  - soil from ponds or anywhere where there has been decay of dead fish
  - equipment and vehicles from infected premises or areas
  - water flowing out of infected premises and effluent from such areas as processing facilities
  - birds and other predators feeding on carcases or infected fish
  - personnel
- TAMs (less resistant stage) in
  - *Tubifex* worms
  - soil containing *Tubifex* worms (including soil on aquatic plants)
  - water flowing out of infected premises.

3.3.4 Treatment and vaccination

There are no treatments for whirling disease that effectively eliminate the parasite from the fish and worms. There are no vaccines for the disease.

3.3.5 Destruction and disposal of fish

The purpose of destroying fish is to limit further spread of disease by preventing the release of myxospores.

Clinically affected fish are unlikely to recover to a marketable product, will be the biggest potential source of infective myxospores, and should be humanely destroyed and disposed of.

If such fish cannot be effectively segregated from fish showing no clinical signs, the entire population may need to be destroyed. The exception is where there is no possibility of myxospores released from such populations contacting a *Tubifex* worm host (for example, salmonids growing in a saltwater environment where there is no possibility of fish escaping and travelling back into fresh water).

The decision on whether or not to destroy potentially exposed fish showing no clinical signs will be made when adequate epidemiological information is available and a control option is chosen.

Destruction and disposal operations must be carried out in a way that ensures no possibility of spread of the parasite. See the **AQUAVETPLAN Destruction** and **Disposal Operational Procedures Manuals** for details.

3.3.6 Elimination of parasite

Removing fish from areas where there is habitat suitable for the *Tubifex* worm host (eg organic-rich pond bottoms) will potentially decrease the ongoing infective challenge to those fish. This may not be possible during the initial response. Elimination of these areas and replacement with habitat unsuitable for the worm (eg concrete raceways) will reduce the number of TAMs being released from those areas.

TAMs can also enter facilities with intake water where there are infected *Tubifex* worms upstream of the intake. Facilities drawing water from wells are not at such risk.

3.3.7 Treatment of fish products and byproducts

The treatment of fish products and byproducts must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product.

In the initial response, harvested fish can be safely frozen until infection is definitively diagnosed or ruled out.

Filleting fish or hot-smoking eviscerated fish at 66°C for 40 minutes are acceptable processes to manage the risks (see Section 2.2.8). The release of smoked fillets to the market will depend on which control option is chosen, and so should be delayed during the initial response.

While processing fish at a processing plant that discharges to salt water may reduce the potential for spread of infection, transporting fish to such a plant may entail risk and should be avoided in the initial response if possible.

Any harvesting or processing equipment used must be treated as contaminated and disinfected accordingly (see the AQUAVETPLAN **Destruction** and **Disposal Operational Procedures Manuals**).

3.3.8 Vector control

In the initial response, effective control to prevent predators (eg birds, rodents) eating or carrying infected carcases away from infected premises is essential to limit spread of the parasite.

3.3.9 Public awareness

In the early stages of an outbreak investigation, education and public relations, especially with the media, is critical. The use of trained officers as media contact points is critical to effective communication with stakeholders and the public. A vital aspect of disease control will be to satisfy the public (especially such groups

as fishers) by all available means that the authorities are in the position to take, and are taking, all necessary measures to control the situation. It must also be clearly stated that this disease holds no health risk for humans.

3.4 Social and economic effects

Australia's aquatic animal health status for whirling disease will change if whirling disease is isolated from Australian animals. This change may only be temporary if the parasite is successfully eradicated.

3.4.1 Export markets

If whirling disease is isolated in Australia, its isolation will be reported to the OIE, although whirling disease is not a notifiable disease. Industries exporting salmonid products will need to confirm and comply with the requirements of countries importing Australian product.

Increased monitoring and surveillance, with comprehensive sampling of fish populations in affected industries, may be subsequently required to satisfy proof of freedom requirements for importing countries.

Permits may be required from the relevant authorities to allow products derived from disease control programs to be released and sold for human consumption.

The Australian Quarantine and Inspection Service (AQIS) should be contacted for the most current information about export market requirements.

3.4.2 Domestic markets

Decisions about the release of fish or fish products to the domestic market will depend on the control strategy implemented (see Section 2.3).

3.5 Criteria for proof of freedom

Proof of freedom from whirling disease may be important for trade. Proof of freedom can be demonstrated at the aquaculture establishment, zone and country levels.

3.6 Funding and compensation

There are currently no cost-sharing arrangements in place for aquatic animal diseases.

Appendix 1 Procedures for transmission of diagnostic specimens to or from AAHL

Veterinary Committee protocols: submission of specimens to AAHL

As far as is practical, the Australian Animal Health Laboratory (AAHL) will do all primary or index case diagnostic testing for emergency disease incidents. This aligns with AAHL's national role and obligations to lead the development and improvement of testing for exotic and emergency diseases.

Category 2 or Category 3 diagnostic submissions should only be sent to AAHL from state government laboratories unless the government laboratory, in consultation with the state chief veterinary officer (CVO), has given prior approval for a direct submission. The state government laboratory does not have to be government owned or operated, but does have to be government approved.

Where a state laboratory wants to commence its own testing for the presence of a suspected emergency disease, parallel specimens will be consigned to AAHL that same day. The inclusion of AAHL at the earliest time in a suspected emergency disease outbreak is likely to add value to the outcome. However, as a disease emergency unfolds, significant diagnostic work will usually be undertaken at the relevant state laboratories in consultation with AAHL.

Where the diagnosis of a suspected zoonotic disease in a human is concerned, AAHL will report the results formally to the hospital submitting the specimen. If a positive result is found, the relevant state chief medical officer or delegated health officer will be advised prior to notifying the relevant state chief veterinary officer.

In new or emergency disease situations (that do not appear to involve exotic disease) diagnostic material will be forwarded to AAHL at the earliest possible time. To coordinate the process of sending any specimens overseas, specimens will be forwarded to AAHL for dispatch to an appropriate overseas laboratory. The state CVO or state veterinary laboratory personnel may wish to discuss the appropriate laboratory to send the material.

The national responsibilities of government laboratories for emergency disease diagnoses necessitate that diagnostic systems, particularly those of AAHL, are regularly challenged to maintain fully operational systems.

Except where exotic disease screening technology has been specifically transferred elsewhere, screening tests for exotic disease exclusion should be done at AAHL.

The submission of a regular flow of specimens to AAHL will help to provide key information about freedom from exotic and emerging diseases and so facilitate international livestock movement negotiations.

Types of specimens for submission to AAHL

There are three categories of specimens:

- *Category 1* – Routine submission: no suspicion of exotic or emerging diseases, eg specimens for quarantine testing.

- *Category 2* — Submissions for exotic or emerging disease exclusion: remote likelihood of presence of such disease.

- *Category 3* – Submissions for exotic or emerging disease diagnosis: high level of suspicion of such disease.

In the terminology of the AQUAVETPLAN **Control Centres Management Manual**, specimens in Category 2 align with the *Investigation Phase* while specimens in Category 3 align with the *Alert Phase.*

Dispatch of specimens

Category 1 specimens — The submitter will contact the AAHL duty veterinarian (or, in the case of aquatic animal specimens, the officer-in-charge at the AAHL Fish Diseases Laboratory [AFDL]) and advise by phone or fax the details of the dispatch and testing required.

Category 2 specimens arising from the Investigation Phase — The submitter will:

- inform the director of the laboratory making the submission, who will advise the state or territory CVO; and
- inform the AAHL duty veterinarian (or the officer in charge, AFDL) as for *Category 1.*

Category 3 specimens arising from the Alert Phase — The submitter will:

- obtain the approval of the state CVO before dispatch;
- advise the AAHL duty veterinarian (or the officer in charge, AFDL), the Victorian CVO, and the Victorian Australian Quarantine and Inspection Service (AQIS) Veterinary Officer by phone or fax of full details of dispatch to facilitate passage of the specimens through (usually) Melbourne Airport, collection by AAHL personnel and quick passage to AAHL; and
- ensure that the state or territory CVO notifies the director of AAHL or the director's delegate.

All specimens must be accompanied by a completed specimen advice note. These are available from the CSIRO website or can be sent on request.

The Victorian AQIS Veterinary Officer can only facilitate transfer of specimens through Melbourne Airport to AAHL personnel when full information of the specimens and transport details are given in advance. The Victorian AQIS Veterinary Officer only needs to be notified when the specimens are being flown through Tullamarine airport.

Appendix 2 Identification of *Myxobolus cerebralis*

The following methods are used for the identification of *Myxobolus cerebralis*, the aetiological agent of whirling disease, at CSIRO AFDL.

Spore detection method

The spore detection method detects the microscopic spores of *M. cerebralis* in cartilage. The procedure involves dissection of sections of cartilage from the heads of fish, freeing the soft tissues from the cartilage, followed by homogenisation, digestion with pepsin and examination of the preparation for the presence of *M. cerebralis* spores.

The procedure is a modification of the method outlined in *Fish Health Protection Regulations Manual of Compliance* (DFO 1984). Identification of *M. cerebralis* spores is based on the morphological characters described by Lom and Hoffman (1971).

PCR method

The polymerase chain reaction (PCR) method is based on that of Epp and Wood (1998) and Andree et al (1998), and can be applied to a variety of sample types, including whole fry, bone and cartilage, *Tubifex* worms, isolated liver, kidney, spleen, blood and faeces, filtered water, pepsin–trypsin digest residues, and purified myxospores.

Primer sequences

PM9 = 5′- GCA TTG GTT TAC GCT GAT GTA GC -3′ 23mer

PM4 = 5′- GGC ACA CTA CTC CAA CAC TGA ATT TG -3′ 26mer

Preparation of DNA from fish tissues

QIAGEN DNeasy Tissue Kit (Cat: 69504) is used to extract nucleic acids from whole fry and from bone, cartilage and gills from older fish.

The samples are boiled and defleshed using a scalpel, and bone and cartilage is vortexed with glass beads in the tissue lysis buffer and proteinase K solution supplied with the QIAGEN tissue kit. The preparation is incubated at 55°C, with occasional vortexing, for one hour or until digestion is complete. Following centrifugation, the aqueous supernatant is used for DNA extraction using the commercial kit.

Appendix 3 Common and scientific names of fish species mentioned in text

Common name	**Scientific name**
Arctic grayling	*Thymallus arcticus*
Atlantic salmon	*Salmo salar*
Brook trout	*Salvelinus fontinalis*
Brown trout	*Salmo trutta*
Chinook salmon, quinnat salmon	*Oncorhynchus tshawytscha*
Rainbow trout	*Oncorhynchus mykiss*
Sockeye salmon	*Oncorhynchus nerka*

Glossary

Anadromous fish	Fish species that hatch and live initially in freshwater (as fry), undergo smolt and migrate to seawater (for 'grow-out') and then return to freshwater to spawn. *See also* Catadromous fish
Aquatic Animal Health Committee	A committee comprising representatives of the Australian government, Australian state and territory governments, the major aquaculture, wild capture, aquarium and recreational fishing industries and a CSIRO representative. The committee provides advice to Primary Industries Ministerial Council on aquatic animal health matters, focusing on technical issues and regulatory policy. *See also* Primary Industries Ministerial Council
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. *See also* Chief veterinary officer
AQUAVETPLAN	*A*ustralian A*qua*tic *Vet*erinary Emergency *Plan*. A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. *See also* AUSVETPLAN
AUSVETPLAN	*Aus*tralian *Vet*erinary Emergency *Plan*. 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.
Catadromous fish	Fish species that hatch and live initially in seawater (as fry), undergo smolt and migrate to freshwater (for 'grow-out') and then return to seawater to spawn. *See also* Anadromous fish
Chief veterinary officer (CVO)	The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. *See also* Australian Chief Veterinary Officer
Compensation	The sum of money paid by government to an owner for stock that are destroyed and property that is compulsorily destroyed because of an emergency animal disease.

Control area	A buffer between the restricted area and areas free of 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, eg 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	Clinically inapparent infection betanodavirus that is transmissible and that may eventually lead to clinical disease.
Dangerous contact animal	A susceptible animal that has been designated as being exposed to other infected animals or potentially infectious products following tracing and epidemiological investigation.
Dangerous contact premises or area	That which has had a direct, and possibly infectious, contact with an infected premises/area. The type of contact will depend on the agent involved in the outbreak but, for example, may involve animal movements or net/equipment movements.
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	Includes all stages of cleaning and disinfection.
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; applies to premises, vehicles and other objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of fish carcases and things by burial, burning or some other process so as to prevent the spread of disease.
Emergency animal disease	A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications. *See also* Endemic animal disease, Exotic animal disease

Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. *See also* Emergency animal disease, Exotic animal disease
Enterprise	*See* Risk enterprise
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with the disease.
Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. *See also* Emergency animal disease, Endemic animal disease
Fish byproducts	Products of fish origin destined for industrial use (eg fishmeal).
Fish products	Fish meat products and products of fish origin (eg eggs) for human consumption or use in animal feeding.
Free area	An area known to be free of the disease agent.
Infected premises or area	The area in which the disease has been confirmed. Definition of an 'infected area' is more likely to apply to an open system, such as an oceanic fish farm lease.
Mitigation	Reduction in severity of a disease to reduce its impact.
Monitoring	Routine collection of data for assessing the health status of a population. *See also* Surveillance
Movement control	Restrictions placed on the movement of fish, people and other things to prevent the spread of disease.
OIE Aquatic Code	OIE *International Aquatic Animal Health Code* (OIE 2004). Published on the internet at: **http://www.oie.int/eng/normes/fcode/a_index.htm** (Accessed 11 May 2005).
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Owner	Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).
Polymerase chain reaction (PCR)	A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also *Reverse transcriptase PCR (RT-PCR)* and *Nested RT-PCR*
Premises or area	A production site, which may range from an aquarium to an aquaculture lease in the open ocean.

Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or positive antibody titre) at a given point in time.
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, 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.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). *See also* Specificity
Sentinel fish	Fish of known health status monitored for the purpose of detecting the presence of a specific disease agent.
Serotype	A subgroup of microorganisms identified by the antigens carried (as determined by a serology test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). *See also* Sensitivity
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible fish	Fish that can be infected with a particular disease.

Suspect fish	Fish that may have been exposed to an emergency disease such that its quarantine and intensive surveillance, but not pre-emptive slaughter, is warranted. *or* Fish not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis.
Suspect premises or area	Temporary classification of premises or area containing suspect fish. After rapid resolution of the status of the suspect fish contained on it, a suspect premises is reclassified either as an infected premises (and appropriate disease-control measures taken) or as free from disease. The reason for the suspicion varies with the agent; however, it may involve clinical signs or increased mortality.
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.
Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.
Vaccine	Modified strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
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.

Abbreviations

AAHL	Australian Animal Health Laboratory
AFDL	AAHL Fish Diseases Laboratory
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
aqCCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
AUSVETPLAN	Australian Veterinary Emergency Plan
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Department of Agriculture, Fisheries and Forestry (Australian Government)
DNA	Deoxyribonucleic acid, rDNA = ribosomal DNA
ELISA	enzyme-linked immunosorbent assay
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PCR	polymerase chain reaction
RNA	ribonucleic acid
SDCHQ	state or territory disease control headquarters
TAM	triactinomyxon

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Index

abbreviations, 63
aetiology, 9–13
clinical signs of disease, 14–15
compensation. *See* cost sharing
control and eradication, 25–42
  containment, 40, 45–46
  decontamination, 35–37
  destruction of fish, 33–34, 47–48
  disposal, 35
  elimination of parasite, 34–35, 48
  epidemiological investigations, 46
  eradication, 38–40, 45
  feasibility in Australia, 38–42
  general principles, 27–28
  initial response, 46–49
  mitigation, 46
  mitigation of disease, 41
  policy, 42–49
  public awareness, 38, 48–49
  quarantine, 29–31, 47
  response options, 44–46
  restocking, 38
  sentinel animals, 38
  social and economic effects, 49
  surveillance, 33
  tracing, 31–33
  trade and industry considerations, 41–42
  treatment of products, 35, 48
  vaccination, 37, 47
  vector control, 37, 48
  zoning, 31
cost sharing, 49
decontamination, 35–37
destruction of fish, 33–34, 47–48
diagnosis, 14–19
  clinical signs, 14–15
  differential, 18
  DNA-based tests, 17
  gross lesions, 15
  histological assessment, 18
  histopathology, 15
  laboratory diagnosis, 16–17
  laboratory tests, 16–18
disposal of fish, 35
epidemiological investigations, 46
epidemiology, 20–25
eradication. *See* control and eradication
feasibility of control in Australia, 38–42
fish products, treatment, 35, 48
funding. *See* cost sharing
glossary of terms, 57
histological assessment, 18
histopathology, 15
history of transmission, 20–21
immunity, 19
  adaptive, 20
  innate, 19
  vaccination, 20
laboratory diagnosis, 16–17
laboratory tests, 16–18
  submission of specimens, 16, 46
lesions, diagnostic, 15
movement controls. *See* quarantine
Myxobolus cerebralis
  elimination, 34–35, 48
  modes of transmission, 24
  sources, 21–24
occurrence in Australia, 14
pathology, 15
proof of freedom, 49
public awareness, 38, 48–49
quarantine, 29–31, 47
references, 65
resistance. *See* immunity
restocking, 38
sentinel animals, 38
social and economic effects, 49
sources of *Myxobolus cerebralis*, 21–24
specimen submission, 16
submission of specimens, 16, 46
surveillance, 33
susceptible species, 13–14
tracing, 31–33
trade implications, 41–42
transmission of *Myxobolus cerebralis*, 24
treatment of fish products, 35, 48
vaccination, 20, 37, 47
vectors, 37, 48
world distribution, 14
zoning, 31, 45