FINAL REPORT

Aquatic Animal Health Subprogram – Whirling Disease – A Disease Strategy Manual

P. Hardy-Smith
Panaquatic Health Solutions

June 2004

FRDC Project No. 2002/651

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2002/651	**Whirling Disease – A Disease Strategy Manual**

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Outcomes achieved to date:

The final draft of Whirling Disease – A Disease Strategy Manual is now complete. Chapter One of this Manual describes the disease. Chapter Two discusses the Principles for Control of this disease, and Chapter Three details the Preferred Control Policy that will be used if the disease is detected in Australia.

The preparation of this Manual has involved consultation with industries and governments in States where an outbreak of this disease could occur i.e. Tasmania, Victoria, New South Wales, South Australia and Western Australia. Some States have larger populations of susceptible fish species than others, and the degree of consultation relative to each State has reflected this.

During the consultation process, the disease has been discussed and outbreak scenarios considered. This has therefore raised the level of understanding and awareness of this disease with government and industries in these States. This is considered a significant outcome achieved to date.

In addition, some of the key personnel responsible for the control aquatic animal disease in States with susceptible fish populations have reviewed the draft Manual (in some cases more than once) and forwarded useful and pertinent comments to the Principal Investigator. Having such personnel consider the disease and control options has been proactive in preparing Australia for a possible incursion of this disease.

The draft Manual has been reviewed by a number of international peers. Such collaboration is important in fostering relations that may become very useful should whirling disease ever be detected in Australia.

Non technical summary:

Whirling disease is the 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. Whirling disease is a reportable disease in Australia.

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, and had a devastating effect on the early culturing of this species in Europe. Other salmonids are generally less susceptible.

The parasite has two hosts – salmonids and a freshwater oligochaete worm, *Tubifex tubifex*. This worm lives in the sediments at the bottom of freshwater streams, rivers, lakes and ponds, and is ubiquitous in Australia.

In the lifecycle of this parasite there are two spore stages, one released from the fish that is infective for worms, the other released from worms that is infective for fish. While the spore released from worms is short lived in the environment, the spore released from fish is extremely resistant and capable of remaining viable in the environment for years.

Infection with the parasite may lead to clinical signs of disease in susceptible fish. These signs relate to the presence of parasite spores in the cartilage of these fish and include radical tail-chasing behaviour of heavily infected fish ('whirling'). Fish will eventually die if severely infected. Generally the earlier a susceptible fish is first exposed (e.g. < 9 weeks old in rainbow trout), the greater is the severity of the disease, depending on parasite exposure dose. Fish may also become infected with the parasite and never show clinical signs; diagnosis of infection in these fish can be difficult.

Water temperature has a significant influence on all stages of the parasite lifecycle. Temperatures greater than 20ºC are not conducive to its development or survival.

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

The first line of defence against whirling disease is the continued implementation of preventative customs and quarantine measures to minimize the risk of importing the parasite into Australia.

If *M. cerebralis* was introduced into Australia, there are a number of ways this introduction could be detected. These range from:

- The identification of myxospores in product imported from a country in which *M. cerebralis* is endemic (e.g. New Zealand); to,
- Confirmation of *M. cerebralis* in wild and farmed rainbow trout showing clinical signs of the disease.

There are essentially three broad options available to control the disease. These options are:

1. ***Eradication***
   - Eradication of *M. cerebralis* from Australia. This is the highest level of control measure and likely to be the most costly.
2. ***Containment, control and zoning***
   - Involves containment of the parasite to areas with enzootic infection, prevention of further spread and protection of uninfected areas.
3. ***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 likely to be the least costly.

Conducting a comprehensive epidemiological investigation is important in deciding upon a control strategy. With respect to whirling disease, there are limitations to conducting such an investigation. These limitations include:

- The delay between exposure of susceptible fish (3-8 weeks depending on water temperature) and development of clinical signs of disease in these fish;
- The delay between exposure of susceptible fish to infective spores released from worms and the development of matures spores in the cartilage of fish (>50 days depending on water temperatures). Matures spores are needed to confirm diagnosis of infection with *M. cerebralis*;
- Difficulty confirming infection in a fish and in a population of fish;
- The limited number of personnel in Australia capable of positively identifying *Tubifex tubifex*.
- Difficulty in confirming infection in *Tubifex* worms or in a population of *Tubifex* worms;
- The fact that infected fish may never show clinical signs.

The preferred control policy in Australia is to eradicate *M. cerebralis* if it were detected here. Depending on circumstances, this may not be an option, at least in the short term. If eradication is considered to be not an option, then either Option (2) or (3) given above will be chosen.

It is important that the chosen policy is dynamic to allow evolution of the strategy with the changing situation e.g. choosing containment, control and zoning in the short term does not preclude adopting eradication as a long-term policy.

Strategies, which may be used under these options, include:

- *Quarantine and movement controls* on fish, fish products, *Tubife*x worms and things in declared areas to prevent spread of infection;
- *Prevention* where possible of predators and scavengers (e.g. birds) gaining access 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 e.g. changing earthen type ponds to concrete raceways;
- *Decontamination* of facilities to inactivate the resistant myxospore stage of the parasite on infected premises and to 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 acknowledging limitations in diagnostic capabilities.
- *Zoning* where possible 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

Acknowledgments

The Principal Investigator acknowledges the contribution of Dr Ron Hedrick, Dr Craig Stephens and Dr Mark Crane for their help in reviewing the initial drafts of the document. The Principal Investigator also acknowledges the help of key stakeholders from around Australia who provided comments and critique during the preparation of the Manual, and to those key stakeholders that reviewed the final draft of the Manual. The assistance of the library staff and Ms Nette Williams at AAHL was also greatly appreciated as was the support provided by members of the Aquatic Animal Health Subprogram.

Background

In the May 2000 Budget, the Federal Government announced its *Building a National Approach to Animal and Plant Health* program. This initiative seeks to maintain Australia's status as a sought after supplier of high quality, 'clean, green' agricultural produce. Within this initiative, funds were made available to Agriculture, Fisheries and Forestry – Australia (AFFA) as administered funds for the Program *Emergency Management Planning* for aquatic animal diseases. As per an Agreement between AFFA and the Fisheries Research and Development Corporation (FRDC), these monies are administered by the FRDC on AFFA's behalf. The FRDC's vehicle for delivery is the FRDC Aquatic Animal Health Subprogram.

During December 2001 and January 2002, stakeholders from industry and governments in Australia nominated their priorities for projects under this Program. On 15 February 2002, the Subprogram's Steering Committee and Scientific Advisory Committee met to evaluate the nominations. Through this process, the *Whirling Disease – Disease Strategy Manual* was approved as a priority. This Manual is one of a total of nine disease strategy and Operational manuals approved as priorities through this process.

These Manuals will form part of a series that are being developed under *Australia's National Strategic Plan for Aquatic Animal Health* (AQUAPLAN) and collectively will be known as AQUAVETPLAN.

Need

Whirling disease is the disease of freshwater salmonid fish caused by the myxozoan parasite *Myxobolus cerebralis*. The parasite is present in New Zealand and areas of North America, Europe, Africa and Asia. Whirling disease has never been detected in Australia, and is a reportable disease here. The intermediate host of the parasite, a *Tubifex* worm, is present in Australia. The parasite is difficult to detect in fish. Infective spores released from fish can survive for considerable periods in the environment. These spores can also survive the passage through the gut of birds.

There is a significant population of salmonids in Australia that would be susceptible to this disease were an outbreak to occur. These populations are valuable both commercially and recreationally.

For these reasons it is important to have in place options for dealing with an outbreak of the disease to minimize the impact of such an outbreak should it occur. It is also highly beneficial that such options have been considered and agreed to by relevant stakeholders before the emergency occurs. Such options would acknowledge this disease may be insidious in its onset so may go undetected for a long time.

While the Federal Government encourages a detection and eradication culture based on constant vigilance and a readiness to tackle any emergency, prior to this Manual there were no accepted national guidelines for dealing with an outbreak of an exotic parasitic disease in finfish in Australia.

Hence the need to develop a Disease Strategy Manual for Whirling Disease that includes information on all potentially susceptible species, describes details about the disease, response options and the preferred, nationally agreed upon approach to its control. This is supported by the FRDCs Subprogram's Steering Committee who, through consultation with stakeholders from industry and governments determined as a priority the need for this Manual.

Objectives

1. To develop a consensus between government and industry on a preferred control policy for Whirling disease should an outbreak of this disease occur in Australia
2. Preparation of a stakeholder endorsed final version of Whirling Disease - Disease Strategy Manual for submission to AQUAPLAN Business Group (ABG)/Scientific Advisory Committee (SAC) which incorporates this preferred control policy. This manual will enhance the capability of both terrestrial and aquatic animal health professionals to identify and efficiently manage an emergency response in the event of a suspect or confirmed incursion of whirling disease in Australia.

Methods

1. Current literature on the disease was reviewed. This review was done in collaboration with the Australian Animal Health Laboratory (AAHL), using the facilities available to AAHL for such review.
2. Attendance at the 9th Annual Whirling Disease Symposium 'Managing the Risk', Bell Harbour Conference Centre, Seattle by the Principal Investigator in February 2003. This Symposium consisted of two intense days on management of whirling disease.
3. Consultation with international fish health professionals involved in research on and/or managing of this disease and disease outbreaks. Professionals included Dr Ron Hedrick, of the Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Professor Barry Hill, Chief Adviser for Fish and Shellfish Health for the UK Ministry of Agriculture, Fisheries and Food (MAFF) and Dr Craig Stephens, a veterinary fish epidemiologist who prepared a paper for the United States (US) Whirling Disease Foundation which reviewed the state of whirling disease in the US and recommended future research and actions.
4. Preparation of the initial draft of Sections 1 (nature of the disease) and 2 (Principles of control and eradication) of the Manual. This was prepared after reviewing the process used in developing the first Disease Strategy Manual (the AQUAVETPLAN Furunculosis Disease Strategy Manual) to identify what were considered the pros and cons of this process. The positive aspects were, where possible, incorporated into the preparation of this Manual.
5. Distribution of the initial draft to a considerable number of industry and government stakeholders (as identified in consultation with AFFA) and to nominated peers (e.g. R. Hedrick, B. Hill). Draft was distributed electronically. Feedback was not as extensive as hoped. As a suggestion, it possibly would have been better to limit the number of stakeholders to whom the draft was sent, to individually discuss the review with them prior to forwarding the draft, and to follow up with each of them after they had sufficient time to review. This was done in a later step (Step 7 below) and worked very well.

6. A number of workshops were held with relevant government and industry stakeholders in Tasmania, Victoria and South Australia. Appendix A contains a copy of the agenda for these workshops. The aim of these workshops was to go through draft sections 1 and 2 and gain consensus for section 3 (response options for the control of the disease and the preferred control option(s)). The workshops involved discussing feasible scenarios surrounding the detection of whirling disease in Australia[1]. Stakeholders in Western Australia, Australian Capital Territory and New South Wales were individually consulted at this stage.
7. The draft Section 3 (Preferred Control Policy in Australia) was then prepared. A small number of key stakeholders (including the Government aquatic animal veterinarians in Tasmania, Victoria and South Australia, and the Project Leader of the AAHL Fish Diseases Laboratory) reviewed the draft. Time availability has been a critical factor for these reviewers; fortunately most have completed the review and forwarded comments back to the Principal Investigator.
8. The Final Draft for Endorsement was then prepared and the final report written in preparation for circulation.

Results/Discussion

The first key objective of this project has now been completed. Consensus has been reached on the preferred control policy for whirling disease should this disease be detected in Australia. Unlike the preferred control policy in many terrestrial animal diseases (e.g. Rabies, Foot and Mouth disease) the preferred policy was not simply to take all measures to eradicate the disease. While it was agreed that this was the **ideal** policy, it was also agreed that the actual policy adopted depended on the circumstances surrounding the outbreak/detection. Eradication was not always considered to be an option. The chosen policy could also change as more information became available during the outbreak investigation.

The second key objective, stakeholder endorsement of the final version of Whirling Disease - Disease Strategy Manual for submission to AQUAPLAN Business Group (ABG)/Scientific Advisory Committee (SAC) has been completed.

Benefits and adoption

This project has ensured that there is now a working Whirling Disease - Disease Strategy Manual in place. This specifically details the disease, known susceptible and potentially susceptible species, response options and the preferred, nationally agreed-upon approach to its control should an outbreak occur. Federal and State authorities adopting this Manual now have a guide to how deal with an outbreak of this disease in Australia. This will help considerably in the effective management and control of the outbreak.

The submission of the final document is the culmination of a significant process. This process, as outlined in "Methods" above, involved considerable consultation with relevant government and industry stakeholders who would be affected by such an outbreak and who will be in positions of responsibility should such an outbreak occur. Hence there is already a significant increase in the understanding and awareness of this disease, and the possible options for dealing with an emergency should an outbreak occur. Benefits have also resulted from the planned workshops with key stakeholders and their inclusion in the review process. This has ensured they have ownership of the Manual which will significantly benefit the response should an emergency occur.

The Manual also highlighted the fact that there are limitations to conducting a comprehensive epidemiological investigation should an outbreak of this disease occur in Australia, or should the

[1] As the Principal Investigator was also preparing the draft Manual on Viral Haemorrhagic Septicaemia (VHS), where relevant this disease was also discussed to minimise the time required by stakeholders to attend these workshops.

causative pathogen, *Myxobolus cerebralis*, be detected here. Some of the limitations identified were:

- The delay between exposure of susceptible fish (3-8 weeks depending on water temperature) and development of clinical signs of disease in these fish;
- The considerable delay between exposure of susceptible fish to infective spores released from worms and the development of matures spores in the cartilage of fish (>50 days depending on water temperatures). Matures spores are needed to confirm diagnosis of infection with *M. cerebralis*;
- Difficulty confirming infection in a fish and in a population of fish;
- Difficulty confirming infection in *Tubifex* worms or in a population of *Tubifex* worms;
- The fact that infected fish may never show clinical signs.

These limitations could hamper control of the disease should it be detected in Australia, and reinforce the need to maintain adequate preventative customs and quarantine measures as part of the process to ensure Australia remains free of the disease.

Having this Manual available benefit the private sector that farm commercially susceptible species by potentially minimizing the impact of the disease on such industries should an outbreak occur. The exact value of this benefit is difficult to calculate, but as value of these industries grow, so too does the potential impact.

Having this Manual should also minimize the impact of the disease on potentially susceptible wild finfish species e.g. trout and native fish populations.

Further Development

This Manual is a working document. It is recommended that it be reviewed on a regular basis to ensure information is kept up to date. Ideally the Manual would be reviewed annually.

It is the opinion of the Principal Investigator that the current format of the AQUAVETPLAN manuals should be reviewed.

The manual has been prepared for formal endorsement by Australia's Aquatic Animal Health Committee (AAHC) and Primary Industries Standing Committee (PISC).

Planned outcomes

The Project's outputs have contributed to the planned outcomes as identified in **Benefits and Adoption** above.

Conclusion

Whirling disease is the 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. Whirling disease is a reportable disease in Australia. This disease could have a significant impact on salmonid populations in this country if it were ever detected here.

Prior to the development of this Manual, there was no nationally agreed to approach to control this disease in Australia. As this disease is caused by a parasite, this Manual is also the first aquatic animal disease strategy manual detailing control of a parasitic disease in fish.

It is hoped that whirling disease is never detected in Australia. If it is detected, there is now a Manual that should ensure a far more rapid, comprehensive and accepted approach to control of the disease, and minimise its impact.

KEYWORDS: **Whirling disease, *Myxobolus cerebralis*, disease strategy manual**

Note: Outcomes are the results, impacts or consequences of actions by the FRDC and its R&D partners on the fishing industry and Australia's economic, environmental and social resources. **Outputs** are the goods and services (mainly knowledge, processes and technology) that the FRDC and It's R&D partners produce for external organisations and individuals.

Appendix A:

Basic agenda of workshops held with relevant industry and government stakeholders to discuss whirling disease and Viral Haemorrhagic Septicaemia (VHS)

10.00AM Welcome and a brief summary of the Aquavetplan manuals, and how they fit into the Commonwealth Aquaplan strategy (PH-S)

10.15AM Summary of the current status and strategy in the State to fish health emergencies (local speaker)

10.30AM Brief summary of Whirling Disease and consideration of 2 scenarios where Whirling Disease is isolated in the State:

1) Confirmation of *M. cerebralis* myxospores in Atlantic salmon that have been routinely sampled for export purposes at a commercial hatchery. No evidence of clinical signs of disease.

2) Anglers reporting deformities in wild trout in one of the States lakes, and further investigation confirming the presence of *M. cerebralis* myxospores in the cartilage of these affected trout.

Areas to consider are:

- Eradication OR
- Containmnent, control and zoning OR
- Control and mitigation of disease.

What would you like to see happen in this State?

11.30AM Brief summary of Viral Haemorrhagic Septicaemia and consideration of 2 scenarios where VHSV is isolated in this State:

1. VHSV isolated from Atlantic salmon in saltwater as part of routine testing – no clinical signs of disease. For the purposes of this discussion the area of isolation was given as a particular region in the State.

2. VHSV isolated from Atlantic salmon broodstock in freshwater at spawning – accompanied by some haemorrhaging. Some morbidity and mortality.

Again areas to consider are:

- Eradication OR
- Containmnent, control and zoning OR
- Control and mitigation of disease.

What would you like to see happen in this State?

12.30PM Wrap up and lunch.

Appendix B:

Aquatic Animal Health Subprogram – Whirling Disease – A Disease Strategy Manual

Draft manual for endorsement by Australia's Aquatic Animal Health Committee (AAHC) and Primary Industries Standing Committee (PISC).

Appendix B:

AUSTRALIAN AQUATIC ANIMAL DISEASE EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy Manual

WHIRLING DISEASE

(Myxobolus cerebralis)

DRAFT FOR ENDORSEMENT

CONTENTS

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 lifecycle. Temperatures greater than 20ºC 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, 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 behavior of heavily infected fish. Clinical signs relate to the presence of the parasite spores in the cartilage causing inflammatory changes and pressure on spinal nerves. 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 this 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 this disease on susceptible salmonid populations if the parasite is ever introduced into Australia.

1.1 Aetiology

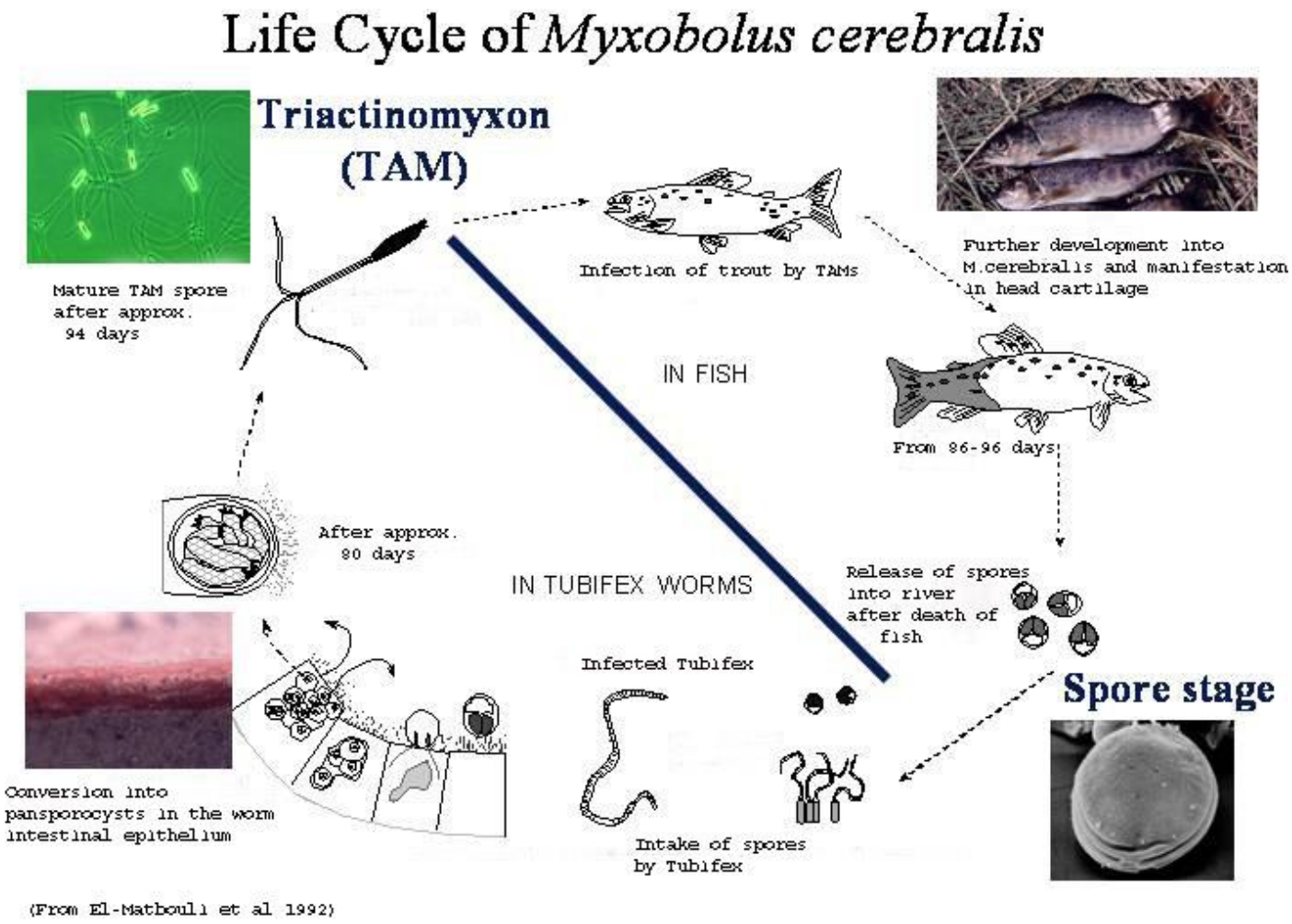
The aetiological agent of whirling disease is the parasite Myxobolus cerebralis (formerly named Myxosoma cerebralis).

M. cerebralis belongs to the phylum Myxozoa, Class Myxosporea[1]. Myxozoans are a diverse group of multicellular organisms characterised by spore stages with three key structural features:

(i) 1-7 valves or protective shells that surround (ii) the next generation of the parasite existing as 1-2 amoeboid infective sporoplasms and (iii) 2-7 polar capsules that contain polar filaments that, when extruded, aid in attaching the spore to the host tissue.

[1] Phylum **Myxozoa**, Class **Myxosporea**, Order **Bivalvulida**, Suborder **Platysporina**, Family **Myxobolidae**

Figure 1 Lifecycle of *Myxobolus cerebralis* (Whirling Disease) (courtesy of R. Hedrick)



1.2 Lifecycle of the parasite (see Figure 1)

The important elements of the lifecycle of this parasite are:

1/ Two-host lifecycle

(A) Salmonids

- *Asexual replication only*
- *Susceptible salmonids are present in Australia*

(B) freshwater oligochaete worms, T. tubifex

- *Sexual and asexual replication*
- *T. tubifex is present throughout Australia*

2/ Two infective stages

(A) 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*

(B) Actinospore, or triactinomyxon (TAM)

- *forms in the intestinal lining of the worm*
- *infective TAMs present 100-170 days after initial exposure depending on temperature and strain of worm*
- *susceptible to environmental degradation, survival measured in days*

3/ All lifecycle stages significantly affected by temperature

- *within limits, the higher the temperature, the faster the lifecycle*

1.2.1 Lifecycle 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 triactinomyxon (TAM) stages. These TAMs have been released from the worm. Resistance to whirling disease increases with fish age (MacConnell and Vincent, 2002).

TAMs attach to the skin of susceptible 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 (R.P. Hedrick, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, personal communication.).

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 and multiplying as they go. As early as 20 days post exposure parasite stages can be found in the cartilage of the susceptible fish. This is dependant on water temperature, optimal temperature for development being around 16-17°C (Halliday 1973). At this point two parasitic cells will join to initiate sporogenesis or the formation of 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 can take from **52-121 days** to develop in the fish (Halliday 1973b). At 16-17°C fully developed myxospores appear 52 days post infection. These spores are elliptical in shape, are multicellular, have thick protective shells and are approximately 10 μm in diameter. Myxospores 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 2800-7800 infective myxospores in the cartilage of the fish' head and possibly more in cartilage elsewhere (Hedrick et al 1999, 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 1999).

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

Once released, myxospores are highly resistant and are able to survive in the environment for long periods, possibly years (Hoffman 1990).

1.2.2 Lifecycle 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 post 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 120-170 days post 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 i.e. a long rod (approx. 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 (Markiw 1992, El-Matbouli et al 1999).

1.3 Susceptible species

1.3.1 Fish:

Susceptibility in salmonids varies amongst species, strains and individuals within a strain[2]. Rainbow trout (*Oncorhynchus mykiss*) 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 1999). This 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. 1999). Even at very high doses of 10 000 TAMs per fish there was never any evidence of tail chasing swimming (whirling) observed in the brown trout during this study.

With respect to 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 of Sciences Museum Victoria, personal communication). So far all fish determined to be susceptible to this disease (El-Matbouli et al 1999, review in MacConnell and Vincent, 2002) come from the family Salmonidae (Order Salmoniformes) (Nelson 1994) to which none of the Australian native fish belong. Although not confirmed, it is assumed that Australian native fish will not be susceptible to this disease. There is reservation in making this assumption as it is difficult to predict the impact of a known pathogenic agent on a new species.

1.3.2 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)

This worm is widespread throughout Australia, though 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 the Australian species is susceptible, and will be capable of producing TAMs if infected with viable myxospores.

[2] An excellent review of salmonid susceptibilities to whirling disease is given in MacConnell and Vincent 2002

1.4 World distribution and occurrence in Australia

Whirling disease has been detected in more than 22 states in the United States of America as well as Central Europe, South Africa, North East Asia and New Zealand. It has never been reported in Australia[3].

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).

1.5 Diagnostic criteria

1.5.1 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. "Since clinical signs are not pathognomonic, and may be subtle, they might not be noted except when the disease reaches epizootic levels (Bartholomew and Reno 2002)".
The clinical signs of whirling disease will vary depending on when the fish are first infected.

When exposed as fry[4] (i.e. large amount of non-ossified cartilage present)

- 'whirling' signs (erratic chasing of tail) first appear approximately 3- 8 weeks post infection. Fish may die due to exhaustion and/or severe malnutrition. This swimming behaviour was thought to be due to 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);
- 'black tail' (caudal melanosis) due to pressure on caudal nerves controlling pigment (Halliday 1976). This may subside if fish are aneasthetised.

If infected fish survives:

Survivors rarely show whirling behaviour or black tail but may have:

- Skeletal deformities – e.g. skull depression, misshapen jaws, shortened operculae (gill covers), spinal curvatures;
- Opercular (gill cover) cysts; and
- Decreased growth rate during clinical disease stage (not proven but suspected). Normal growth rate appears to resume after clinical disease subsides.

When exposed > 9 weeks old[5]:

[3] Reviewed in Bartholomew and Reno 2002
[4]This is dependent on salmonid species but as a generalisation fish exposed when >9 weeks of age will not show clinical signs
[5] 9 weeks refers to rainbow trout. In Chinook salmon, clinical signs may not develop in fish exposed when older than 3 weeks (J. Bartholomew, Center for Fish Disease

- Very few or no gross clinical signs.

1.5.2 Pathology

In susceptible fish exposed < 9 weeks old, there can be obvious pathology. In fish exposed > 9 weeks old, pathology (gross and histological) may be minimal.

Gross lesions:

In the acute stage:

- Black tail (caudal melanosis) during acute stage (this disappears if the fish dies);
- If fish remain alive, then erratic swimming behaviour – tail chasing, 'whirling', especially when startled.

In survivors:

- Skeletal deformities – most pronounced in the cranium and spine, can vary significantly in severity and light infections can be difficult to detect;
- No obvious internal gross lesions.

Microscopic lesions (histopathology)

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

In acute stages:

- Little cellular response in first few days;
- Macrophages may be seen in subcutis attacking residual epithelial stages; and,
- Nervous tissue containing parasite appears normal with no tissue reaction.

Later stages:

- Lysis and phagocytosis of cartilage by trophozoites initiate an intense inflammatory response in susceptible species;
- Lesions typically contain remnant cartilage, developmental and sporogonic stages of the parasite, and focal to diffuse granulomatous inflammation. Granulomas consist predominantly of epithelioid and mononuclear cells, fibroblasts and multinucleate giant cells.

A grading system has been developed to assess the histological lesions seen in whirling disease (Baldwin et al. 2000). This system is described in Table 1 Section 1.5.3.3.

1.5.3 Laboratory tests

If whirling disease is suspected in a State or Territory, the corresponding State/Territory Chief Veterinary Officer (CVO) or Director of Fisheries must be

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Research, Oregon State University, personal communication.) and other less susceptible salmonid species

notified immediately. Preliminary identification of M. cerebralis may be undertaken by some State/Territory diagnostic laboratories (e.g. Tasmania). Duplicate specimens must be consigned to the Australian Animal Health Laboratory (AAHL) as soon as possible.

Suspected fish specimens should initially be sent to the State or Territory diagnostic laboratory. After obtaining the necessary clearance from the Chief Veterinary Officer (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 exotic disease testing.

Submission of specimens

Currently, in Australia the sampling methods approved by the Fish Health Section of the American Fisheries Society are used in the diagnosis of 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 AQUAVETPLAN **Enterprise Manual**[6] 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.

1.5.3.1 Laboratory diagnosis

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

In heavily infected fish (active infections with clinical signs) simple histological sections may be sufficient to observe myxospores. Alternatively, the gill arches of infected fish can be removed and ground, 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 directly and the homogenate is passed through a plankton centrifuge (O'Grodnick 1975). These are the standard methods used currently by laboratories in Australia that test for this disease (M. Crane, Australian Animal Health Laboratory, personal communication). In both procedures, the goal is to isolate myxospores for microsopic observation. The enzymatic digestion and centrifugation method is better for concentration of myxospores and clarity of sample, whereas the plankton

[6] http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=00499893-720A-4F46-9CB9D85A876398B7

centrifuge method, while time saving, 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 there are other *Myxobolus* myxospores that 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:

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

ii. Direct Fluorescent Antibody Test (DFAT). This serological test has proved useful, though preparation of the labeled 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.

1.5.3.2 DNA based tests

There are a number of relatively new DNA based tests that are significantly increasing the ability for early detection of the parasite in the fish, the environment (e.g. water column) and in 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 lifecycle (Andree et al 1998, 2002). This test can detect the parasite in the fish within 2 hours of exposure, and also in the worm. The new procedures to be released by the Fish Health Section of the American Fisheries Society will indicate PCR as an accepted diagnostic procedure (R.P. Hedrick, personal communication).

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

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 section of infected tissues. It would not be difficult to develop the capacity to conduct this test in Australia (R.P. Hedrick, personal communication).

1.5.3.3 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 (e.g. a significant number of states in the USA) a more quantitative measure of lesions has been

developed (Hedrick et al 1999ab, Baldwin et al 2000, Andree et al 2002). Assessment by this method is useful in evaluating the impact of *M. cerebralis* on exposed fish.

Table 1 outlines this objective, quantitative method for measurement of lesions. 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*) 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*) 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*) 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 characterized by loss of normal architecture and reserved for the most severely affected fish. If in doubt, classify as a Grade 4

* numbers of lesions typical for sections of head, not whole body sections

1.5.4 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 spiraling in the water column.

However, this must be put into perspective. As identified below, there are a number of other diseases/conditions that can lead to the clinical signs associated with whirling disease (erratic swimming behaviour and tail chasing, caudal darkening and skeletal deformities). Failing to diagnose whirling disease would have serious consequences. Identifying a suspect case will initiate a chain of events (as outlined in the AQUAVETPLAN **Enterprise Manual**[7] and this Manual) requiring significant resources. As always, sound fish health judgment must come into any diagnostic decision making.

The differential diagnoses that should be considered when the clinical signs associated with whirling disease are seen in salmonids in Australia include:

- Septicaemic conditions that cause inflammatory responses in the brain (e.g. *Yersinia ruckeri*). The aetiological agent causing the associated inflammation may be bacterial, viral or protozoal.

[7] http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=00499893-720A-4F46-9CB9D85A876398B7

- Nutritional disorders e.g. vitamin C deficiency have been associated with skeletal deformities in salmonids.
- Early infection (e.g. salmonids < 5g) with *Flexibacter* species resulting in shortened operculae in fish that survive.
- High incubation temperatures (e.g. in Atlantic salmon > 8°C until first feeding) and fluctuating temperatures during incubation causing skeletal deformities, ranging from minor lesions in single vertebrae, through 'shorttails' and 'humpbacks', to short body dwarfism, in which the vertebral column is compressed and ankylosed (Grete Baeverfjord, Research Scientist, Akvaforsk, personal communication).
- Electroshock injuries causing skeletal deformities and/or melanosis (Wolf et al 1981, Margolis et al 1996)
- Iatrogenic injection damage causing caudal melanosis where the caudal vein has been used as the injection site.

1.6 Fish defence mechanisms, resistance and immunity potential

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

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

1.6.1 Innate immunity

The initial portals of entry for the waterborne infective stage of the parasite include the epidermis, respiratory epithelium and buccal cavity. Non-specific, innate defense mechanisms are located in these areas but their ability to prevent release of the sporoplasm from the TAM is uncertain[8]. (Pickering 1997, R.P. Hedrick, personal communication)

The ability to prevent sporoplasm release by these mechanisms is poor in susceptible species such as rainbow trout, judging by the effectiveness of infection in this species. While there is little cellular response seen in the first few days, macrophages are seen soon after attacking residual epithelial stages (El-Matbouli et al 1999). The parasite also elicits a strong cellular response. This response occurs during the active feeding phase during 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 a well defined, encapsulated granuloma containing few intact parasites.

[8] Research in this area is ongoing (R.P. Hedrick, pers.comm.)

1.6.2 Active immunity

With *M. cerebralis*, there may only be a short period of approximately 4 days when the parasite 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 with antibody transfers from fish with older infections. Cell mediated immunity may also be important in this disease (Hedrick et al 1998).

1.6.3 Vaccination

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

1.7 Epidemiology

Salmonids and T. tubifex, the two hosts required to complete the lifecycle of M. cerebralis are present in Australia. Presence of parasitic infection does not necessarily mean that disease occurs[9].

1.7.1 Historical aspects:

When considering the epidemiology of the parasite *M. cerebralis* and whirling disease the historical spread of the parasite is noteworthy.

M. cerebralis was first detected in Germany in 1893, in imported rainbow trout *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) and brook trout *Salvelinus fontinalis* (Höfer 1903 in Bartholomew and Reno 2002). The disease had a devastating effect on both cultured rainbow and brook trout in which it was found. Hoffman (1970) suggests that *M. cerebralis* evolved as a non-pathogenic parasite of brown trout *Salmon trutta* in central Europe and northern Asia. In this case it seems the susceptible fish (i.e. salmonids of North American origin) were brought to the parasite. The disease spread throughout Europe, probably due to transfers of infected fish. Brown trout, though infected, may 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, at this time the parasite was detected in brook trout at a research station in Pennsylvania (Hoffman 1962). Imported frozen European trout were implicated as the cause of the outbreak though imports of live brown trout from Europe were also occurring during the 1950's (Bartholomew and Reno 2002). It would seem that the parasite was carried back to the home of the susceptible fish host.

The disease has now spread into 22 states in the USA. Spread is thought to be through the movement of infected fish from affected hatcheries. It was not until the 1980s that the freshwater oligochaete *Tubifex tubifex* was identified as an obligate host of *M. cerebralis* (Wolf and Markiw, 1984). Prior to this, attempted control was largely based on the assumption that the lifecycle 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 been there for a considerable time prior to this report (Boustead 1993). While at the time of the Hewitt and Little report there

[9] Bartholomew and Reno 2002

was a complete ban on the importation of salmonid fish in any form unless heat treated, imports of trout had occurred up until the 1960s but the precise details are unknown (B. Jones, Senior Fish Pathologist, Department of Fisheries, Government of Western Australia, personal communication). Live dried food for tropical fish had also been imported from France. The authors cited this as a possible mechanism for introduction. However, even if infected tubificids were imported with this product as suggested in Bartholomew and Reno (2002) the dessication process is likely to have destroyed any triactinomyxons (TAMs) that such worms may have carried.

1.7.2 Sources of *M. cerebralis*:

1.7.2.1 The fish

In susceptible fish, the infective stage of the parasite is the myxospore, found in the cartilage. Section 1.2.1 describes the development of myxospores.

Species:

M. cerebralis has only been found to infect fish of the salmonid family. Whilst the actual number of non-salmonids challenged with triactinomyxons (TAMs) is low (complete list still being compiled), the lack of any reports of epizootics and infection in non-salmonids supports the assumption that non-salmonids are resistant to this disease.

There is considerable variation in the susceptibility of salmonid species to the disease (MacConnell and Vincent, 2002). This may be due to the disease originating in Eurasia. Hence, species such as brown trout, a salmonid of Eurasian origin, is far less susceptible than rainbow trout, a salmonid of North American origin. In species with low susceptibility there is poor replication of the parasite within the fish.

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

Non-indigenous salmonids form the basis of commercial fish farming and recreational industries in a number of states of Australia. The most commercially significant of them is the Atlantic salmon (*Salmo salar*) the rainbow trout (*Oncorhynchus mykiss*) and the brown trout (*S. trutta*). There are also small populations of quinnat (Chinook) salmon (*O. tshawytscha*) in Victoria and brook char (trout) (*Salvelinus fontinalis*) in NSW and Tasmania (Allen et al 2002).

Susceptibility of Atlantic salmon, rainbow trout and brown trout strains in Australia is unknown. It is assumed susceptibilities of the Australian populations will 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 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 lesser susceptible species (Hedrick et al 1999). Once infected this species will have the potential to rapidly build up myxospore numbers in the environment. Also, susceptible species can be infected initially with a much lower TAM dose than lesser susceptible species.

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

Non-salmonids 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. 1999***)***. Australian native freshwater species are also assumed to be resistant (see Section 1.3.1).

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:

i. Higher cartilage:bone ratio in young fish

ii. Little innate and no aquired 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 clinical overt disease will not develop is 3 weeks (J. Bartholomew, Center for Fish Disease Research, Oregon State University – personal communication).

Temperature:

The entire lifecycle 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 post infection at 17°C, but this was delayed to 120 days at 7°C (Halliday 1973). Temperatures greater than 20° C can 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 1999a,b). Experimental exposures of high doses (1000 and 10000 TAMs per fish) can overwhelm the innate resistance of a less susceptible species such as brown trout. Unlike rainbow trout, brown trout show a lack of increasing parasite burden (myxospore concentration) with increasing exposure dose.

1.7.2.2 Survival of the myxospore:

The myxospore survives well in the environment. This is most likely due to its hard shell (Kerans and Zale, 2003). 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 non *T. tubifex* worms . The spore survives freezing at –20°C for 3 months (El-Matbouli and Hoffmann 1991). Smoking of fish at 66°C for 40 minutes was effective at killing spores (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.

1.7.2.3 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 it is released. Section 1.2.2 descibes 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). Whilst 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. For the purpose of this Manual, it is assumed that the Australian strains are susceptible to infection with *M. cerebralis*, and have the capability of producing viable TAMs[10].

Temperature:

Temperature has a significant influence on the output of TAMs from susceptible worms and the development time of the parasite to TAM stage (El-Matbouli et al. 1999, Kerans and Zale, 2002). At 8°C, TAMs were released 170 days post exposure to myxospores. At 15°C, TAMs were released 89-90 days post exposure. Water temperature also influenced the prevalence of infection in worm populations. At 9°C prevalence of infection was 11.4%, and at 17°C prevalence was found to be 22.2%. This difference in prevalence may be due to the development rate of the parasite in the worm and also the activity rate of the worm in feeding.

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.

[10] This may be a worthwhile area for research.

Environment:

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

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

1.7.2.4 Survival of the triactinomyxon (TAM)

TAMs are short lived in the environment relative to the myxospore stage. Temperature significantly influences survival time. In temperatures <7°C, survival can be up to 7 days, at 12.5°C up to 5 days, whereas at 23-24°C survival is only 24 hours (Markiw 1992). Aging of TAMs in the environment also affects their ability to infect fish.

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

TAMs are neutrally buoyant so their potential for dispersion in the water column is high though this is not borne out by research; TAM densities declining rapidly over short distances. TAMs appear to be removed from the water column or deactivated quickly. This is significant since TAM density will influence infection rates in the fish, especially in the early stages of an outbreak.

1.7.2.5 Other possible reservoirs of M. cerebralis

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 coming into contact with the bottom of ponds or mud containing myxospores could be a source of infection to susceptible *T. tubifex* worms elsewhere.

1.7.3 Modes of transmission

1.7.3.1 Horizontal:

The modes of transmission of *M. cerebralis* have been discussed. Whirling disease is a disease of freshwater fish, and can only be transmitted in freshwater. Fish infected in freshwater can remain infected in saltwater. If infected fish return to freshwater (e.g. 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 *Oncorhynchus mykiss*), sockeye *O. nerka* and Chinook salmon O. *tshawytscha* returning to rivers in Oregon. Currently there is no evidence that the parasite has become established in these areas, even with several years of intense monitoring (Engelking, 2002).

1.7.3.2 Vertical:

Salmonid eggs from *M. cerebralis* infected broodstock have been shown to be free of whirling disease (O'Grodnick 1975A). There is the potential for mechanical transmission of myxoxpores 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.

The first line of defense against whirling disease is the continued implementation of preventative customs and quarantine measures. The reader is referred to The Australian Quarantine Inspection Service (AQIS) 1999 Import Risk Analysis (IRA) on Non-Viable Salmonids and Non-Salmonid Marine Fish[11], and further Policy Memoranda that have been released subsequent to the publication of this IRA.

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

1. ***Eradication***
    - eradication of *M. cerebralis* from Australia - highest level of control measure and cost.
2. ***Containment, control and zoning***
    - containment of the parasite to areas with endemic infection, prevention of further spread and protection of uninfected areas.
3. ***Control and mitigation of disease***
    - the implementation of management practices that decrease the incidence and severity of clinical outbreaks - 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 Centre Management Manual** (AFFA 2001a,c).

If *M. cerebralis* were to be introduced into Australia, there could be a range of scenarios as to how this introduction could be discovered. These range from:

- The identification of myxospores in product imported from a country in which *M. cerebralis* is endemic (e.g. New Zealand); to:
- Confirmation of *M. cerebralis* in wild and farmed rainbow trout showing clinical signs of the disease.

The circumstances around which the discovery is made will affect which control option is considered. For example, in scenario (i) there may not have been a possibility of release of those myxospores into the environment, and hence no possibility for infection of *Tubifex* worms, a necessity to continue on the lifecycle of the parasite. Destruction of the product may be all that is required to prevent any spread of the disease. In scenario (ii), if clinical signs are evident then the parasite is quite likely well established in the area in both worm and fish hosts. In this scenario

[11] Prepared by SA Kahn, PT Beers, VL Findlay, IL Peebles, PJ Durham, DW Wilson and SE Gerrity and available from the Agriculture, Food and Fisheries – Australia (AFFA) website http://www.affa.gov.au/content/publications.cfm?ObjectID=7472F78A-091E-4A25-8F87FE38128EB7CA

the original introduction of the parasite into Australia may have occurred well before its detection (possibly months or even years).

Whatever the scenario, there are general strategies that would be used in the control of this disease. These are:

- 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;
- Avoidance where possible of water containing infective spores (e.g. change from above ground water to well water, moving fish to saltwater);
- Elimination of worm habitat (e.g. change to concrete raceways);
- Good management practices and maintenance of high hygiene standards.

There are limitations in our ability to satisfy these principles in the control and/or the eradication of *M. cerebralis*. For example, it may be difficult to rapidly detect infection, and to rapidly define the nature and extent of the problem.

Acknowledging 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 (e.g. *Tubifex* worm host, wild salmonids), which is dependent on the ability to identify such areas and correctly identify the species of worm;
- Chances of success of eradication;
- Level of risk accepted for future spread of infection (e.g. 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.

The reader is referred to the AQUAVETPLAN **Enterprise Manual** (AFFA 2001c) for State/Territory legislation relating to disease control and eradication.

2.2 Methods to prevent spread and eliminate pathogens

In this description of methods for the control and/or eradication of *M. cerebralis*, the following terms are used:

- *Infected* fish – a fish whose cartilage has mature or maturing *M. cerebralis* myxospores – these fish may or may not show clinical signs;

- *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.

It is important to note that if *M. cerebralis* is detected in Australia in live fish, depending on the circumstances it is likely that there will be more than one infected fish. It is also possible that there will be an infected worm population, either locally, or in the area from which the infected fish have been moved (if the fish have come from elsewhere)[12]. Thus the ability to prevent spread and eliminate the parasite may already be significantly compromised.

2.2.1 Quarantine and movement controls

Acknowledging the fact that there may have been considerable time between detection of the parasite and when a fish became infected, the quarantine and movement restrictions that could be implemented immediately upon suspicion of the isolation of *M. cerebralis* are:

A. Establishment of specified areas (Figure 1)(see AQUAVETPLAN **Enterprise Manual, Section A** for more details):
   - *declared area* - includes restricted area and control area
   - *restricted area* - area around infected premises or area
   - *control area* - a buffer between the restricted area and free areas
   - *free area* - non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of *M. cerebralis* remains unassessed).

   The threshold for establishment of specified areas will depend both on the circumstances and on information surrounding the outbreak/isolation. The potential movement of infective myxospores and TAMs along waterways must be considered.

B. Bans on the movement of live fish and *T. tubifex* worms out of infected areas into areas where *M. cerebralis* is considered absent. This would rely on the ability to accurately identify *T. Tubifex*. This is difficult even for trained personnel[13]. Due to the freshwater lifecycle of the worm host, movement of fish into saltwater may be deemed acceptable;
C. Bans on the movement of live fish and *Tubifex* worms into disease-free areas from areas where *M. cerebralis* is considered absent;
D. Restrictions or bans on releasing fish and *Tubifex* worms into river or freshwater lake systems in designated areas;

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

[13] It may be decided to simply ban movement of **all** oligochaete worms.

E. Restrictions or bans on the movement of fish and *Tubifex* worms between different river systems in designated areas;
F. Restrictions or bans on the use and movement of equipment within and between river systems.

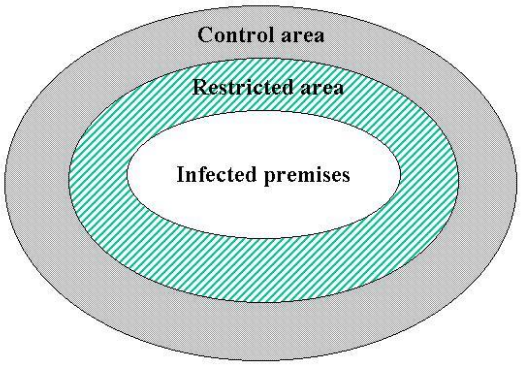
It is important to note that quarantine measures must take into account water movements e.g. a river flowing out of an infected area can carry viable spores (either TAMs or myxospores) from the area, as can effluent water flowing out of a fish farming operation.

The feasibility of the restrictions and bans and extent to which these are 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 extent that such restrictions are imposed should be weighed against the impact such imposition will bring about.

Figure 2 Establishment of specified areas to control whirling disease



2.2.1.1 Zoning

Whether or not zoning is a viable option for whirling disease will depend on the circumstances. Zoning may be possible if the parasite is isolated to one hatchery that has the ability to disinfect effluent, or if the parasite is isolated in a landlocked lake population of trout. There are some salmonid populations in Australia (e.g. the small trout population in Western Australia) where zoning on a geographical basis is feasible.

Zoning must consider the 'two host' lifecycle of this parasite. Covertly infected fish populations can become established and are very difficult to detect. Infected worms can live for up to two years in the laboratory – possibly longer in the wild. Not only is there some difficulty identifying *T. tubifex* itself, it can also be difficult to determine whether or not a population of *T. tubifex* is infected with *M. cerebralis*. Human activity (e.g. fishermen moving between areas with mud on their boots) could also transfer the parasite. These factors would make it very difficult to protect whirling disease-free zones, unless there are significant natural barriers around that zone. A corresponding surveillance and monitoring program for whirling disease will also be required to support a zoning policy.

Principles of zoning for infected and non-infected zones in Australia are outlined in the **AQUAPLAN Zoning Policy Guidelines**.[14] This document is based on terrestrial diseases, though many of the principles still apply.

2.2.1.2 Semi-open systems

Whirling disease is a disease of fish inhabiting the freshwater environment. However, fish initially reared in freshwater and exposed to TAMs could remain infected when transferred to the marine environment. Myxospores released directly from fish into the marine environment are unlikely to encounter a susceptible worm host. In addition, *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 freshwater, there is a potential for disease transfer[15].

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).

2.2.1.3 Semi-closed systems

Semi-closed systems have slightly more control than semi-open systems. However, there are differences between farms in the extent to which input and output water can be contained. These systems are not designed to be self-contained; preventing inflow or outflow of water may have adverse effects on production. Most of the salmon and trout farms currently in Australia are based on flow through systems. Influent water comes from a surface water source, usually a freshwater river or stream. These farms generally 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 may be possible to exclude the less resistant TAMs. Ultraviolet irradiation, sand filtration and ozonation are options that may be considered in treating influent water that enters a semi-closed production system (see Section 2.2.9).

Output water control and treatment are possible in theory for the TAMs. Under the current management practices of most farms such treatment would not be feasible. A better option would be to remove worms by eliminating any potential habitat for them (e.g. lining earthen ponds with concrete).

Treatment to ensure inactivation of myxospores in effluent water is also not considered feasible. Daily removal of dead fish will reduce myxospore release from the decomposing carcasses. Daily "mort" (mortality) removal is possible in semi-closed systems, but not always carried out.

Fish input and output may be controlled, however some movement restrictions could significantly interrupt farm management practices and production. Fish inputs into freshwater farms may be from on-site hatcheries or from other freshwater or marine

[14] http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717

[15] It should be noted that a study in Oregon looking at infected anadromous salmonids did not support this assumption (Engelking 2002).

farms (e.g. broodstock). Fish are also able to enter farm waterways and possibly ponds via intake water from the rivers. Fish may also be carried onto a farm by birds.

2.2.2 Tracing

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

(i) where the parasite came from as quickly as possible to ensure any further introduction can be prevented;

(ii) where all the *potential* locations are for the parasite and worms (even without confirming that parasite is present in those locations, which may be very difficult);

(iii) how to quickly prevent any further parasite spread from the infected location.

In the case of whirling disease, there are limitations to conducting a comprehensive epidemiological investigation. Some of these limitations include:

- The delay between exposure of susceptible fish (3-8 weeks depending on water temperature) and development of clinical signs of disease in these fish;
- The considerable delay (>50 days depending on water temperatures) between exposure of susceptible fish to infective spores released from worms and the development of matures spores in the cartilage of fish. Matures spores are needed to confirm diagnosis of infection with *M. cerebralis*;
- Difficulty confirming infection in a fish and in a population of fish;
- Difficulty confirming infection in *Tubifex* worms or in a population of *Tubifex* worms;
- That infected fish may never show clinical signs.

A thorough and comprehensive epidemiological investigation requires trained personnel. These personnel must have the skills and be capable of spending the time to conduct such an investigation. Before conducting such an investigation, the availability of trained personnel must be confirmed.

As a guide, the following should be traced:

1. Fish:

   All movements of the infected fish should be traced-back to help establish the origin of the outbreak, whether the infected fish were exposed to TAMs at the current location, or had been imported into the infected location post TAM exposure, or both;

   Trace-forward all contacts with infected fish;

   Determine if there has been any movement of broodstock, smolt or fish destined for restocking.

2. Water:

Determine the water source for the site at which the infected fish have been diagnosed and the destination of effluent water from the site. If the site is saltwater, fish must have come from a freshwater site to be exposed to TAMs. It is unlikely that myxospores released directly into saltwater will encounter a freshwater *Tubifex* host.

3. Equipment/vehicles/personnel:

   There is potential for myxospores to be carried in mud. Hence all movement of equipment/vehicles/personnel should be traced. It should be acknowledged that infection may have been present for a considerable period prior to detection.

4. *Tubifex* worms:

   Habitat suitable for the existence of these worms needs to be identified. In addition, whether or not there is local movement of these worms (e.g. by anglers for bait). Because *T. Tubifex* is widespread, it is likely that if there is suitable habitat this species will be present.

2.2.3 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. Waste from such processing, if discharged into the freshwater, could be a source of myxospores. Infection could be established downstream if susceptible *Tubifex* worms are present. Maps with the location of neighbouring fish farms, waterways and hydrographic data are necessary to monitor the potential spread of the pathogen. The location of susceptible fish species should also be noted both upstream and downstream of the infected site. Further sources of infection may be identified if a number of facilities share common water. Both myxospores and TAMs (which are neutrally buoyant) can travel considerable distances in the water column.

Predators such as birds can also carry infected fish to neighbouring waterways. Myxospores can survive passage through the gut of birds.

For information on the location of farming establishments and wild fish populations at risk of infection, contact the relevant State/Territory fisheries or agriculture agency (see AQUAVETPLAN **Enterprise Manual**[16] for contact details).

2.2.4 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/zones for a whirling disease zoning program;

[16] http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=00499893-720A-4F46-9CB9D85A876398B7

- monitor the progress and success of an eradication strategy if implemented.

Because of the inadequacies in currently approved diagnostic tests for this disease, the development of effective surveillance will be difficult. Newer technologies such as tests based on Polymerase Chain Reaction (PCR) could significantly improve this situation. Currently in Australia the Australian Animal Health Laboratory (AAHL) is the only laboratory with PCR capabilities for detecting this parasite.

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

2.2.5 Treatment of infected fish

There are currently no treatments that effectively eliminate infection from fish. There are a number of treatments that have shown some effectiveness in reducing myxospore counts in fish. These include the antibiotic fumagillin. Fumagillin is not registered for use in fish in Australia. Treatment of infected fish is not considered an option.

2.2.6 Destruction of fish

Slaughter must be both hygienic and humane. It is unlikely that the chosen slaughter method will influence shedding of the myxospore stage from the fish during this period, although the exact process by which myxospores are expelled from the living fish is still unknown. One possibility is that myxospores residing in peripheral tissues such as the fins may well be shed through abrasion of epidermal surfaces. Hence crowding and rough handling of fish may increase this means of release.

There are many different methods to anaesthetise and/or slaughter fish, all of which have limitations. Some methods used include the following:

- chemical anaesthesia — the water level is lowered in a tank of fish, then an anaesthetic solution is added to the water in the tank before slaughter (Aqui-S® in the only anaesthetic currently fully registered for use in Australia, and has no withholding period). The dose of anaesthetic can be varied depending on whether the fish are to be subsequently harvested or immediately euthanased;
- ice slurry;
- stunning (with or without subsequent bleeding);
- carbon dioxide narcosis (with or without subsequent bleeding);
- caught on a line and stunned with a blow to the head;
- netting a raceway to crowd fish before slaughter.

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

- size and number of fish;
- deadline for slaughter — depends on the pressure of infection and the ability to contain the disease;
- destination — human consumption or disposal;
- slaughter facilities — site, equipment and methods available;

- experience and availability of personnel.

2.2.7 Elimination of worms

Physical altering of habitats has been attempted oversees to remove 'hot spots' – areas of streams identified as having increased density of *T. tubifex*. Significant engineering and cost can be associated with such measures. These studies are ongoing.

A molluscide, Bayluscide (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. Changing from earthen ponds to concrete raceways will eliminate the worm's habitat, and effectively remove worms in these areas.

2.2.8 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 though hot-smoking at 66°C for 40 minutes inactivates spores.

The species of salmonid affected will influence the number of potential myxospores per fish. Rainbow trout (*Oncorhynchus mykiss*) can if infected have a high burden of myxospores in the cartilage. Thus fish products and byproducts from rainbow trout will potentially have higher myxospore burdens than for other less susceptible species such as brown trout (*Salmo trutta*).

The following extract is taken from the *Animal Quarantine Policy Memorandum 1999/70* published by the Australian Quarantine and Inspection Service (AQIS) on 21 October 1999. It provides the arrangements for importation of specified thermally treated salmonid products into Australia from countries including those in which *M. cerebralis* occurs:

The product must be '… processed in accordance with one of the minimum time/temperature parameters set out below:

- for salmonid roe: the product was washed and treated at 65°C for 30 minutes;
- for skin-on fillets[17] (where the weight of individual pieces is greater than 450g): 65°C for 30 minutes;
- for head-on eviscerated trout: 66°C for 40 minutes[18].'

Eggs:

[17] 'Skin-on fillets of less than 450 g weight may be imported under the conditions that apply to uncanned, non-specified thermally treated product (refer *Animal Quarantine Policy Memorandum 1999/69*, 20 October 1999).'

[18] 'For countries in which *Myxobolus cerebralis* is not reported, head-on trout must be cooked at a minimum of 65˚C for 30 minutes. At this time AQIS recognises that *Myxobolus cerebralis* is not reported from Canada.'

M. cerebralis is not transmitted vertically. Myxospores and TAMs could potentially be mechanically transmitted in packing material and in any fluid surrounding the eggs during transport.

2.2.9 Disposal

Correct disposal of carcasses is critical due to the highly resistant nature of myxospores. Rapid removal of carcasses ('morts') from ponds or raceways is also essential to both minimise myxospore release and prevent exposure of susceptible *Tubifex* worms. Burial sites must be chosen carefully to ensure there is no contact with waterways or birds/animals that could transmit the myxospores to *Tubifex* habitats.

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

See the AQUAVETPLAN **Operational Procedures Manual (Destruction** and **Disposal)**[19] for details of destruction and disposal of fish carcasses.

2.2.10 Decontamination

Due to the resistant nature of the myxospore, effective 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 tubifex hosts. Decontamination requires **thorough cleaning** before disinfection. However, wash water entering a freshwater habitat may contain viable myxospores if not disinfected prior to release.

Myxospores

Compounds that have been found to be effective in killing myxospores (reviewed in Wagner 2002) include:

- Calcium hydroxide at more than 0.5% for 24 h
- Calcium oxide (quicklime) or potassium hydroxide at more than 0.25% for 24 hours
- Chlorine at 1600ppm for 24 h or 5000ppm for 10 min[20]
- Heating for 10 min at 90ºC

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, drying or freezing for at least an hour will readily inactivate TAMs (Wagner 2002).

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[19] Destruction Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906
Disposal Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5

[20] Using chlorine at such high concentrations could be potentially hazardous for the operator

Other methods of TAM inactivation include:

- Temperatures >75ºC for at least 5 minutes (at 7ºC TAMs can survive 7-8 days)
- Chlorine concentrations of 130 ppm for >1 minute[21]
- Hydrogen peroxide at 10% for 10 minutes
- Povidone-iodine at 5000ppm active iodine for 60 minutes

Stringent decontamination of saltwater facilities from which fish need to be removed (i.e. fish infected in freshwater, then transferred to saltwater) will not likely be required. It would be difficult for myxospores released into the saltwater environment to come in contact with a freshwater tubifex host while still viable.

Likewise, processing plants discharging into saltwater are unlikely to need stringent decontamination unless there is potential traffic between the facility and freshwater, or the potential for birds/animals to carry carcasses or offal to freshwater habitats.

Due to differences in farming enterprises, disinfection protocols of freshwater facilities may need to be determined on an individual basis. This would involve the farm manager, the State/Territory CVO and/or Director of Fisheries and possibly environmental authorities. The protocol should take into consideration the factors outlined in Section 1.6, in particular the:

- Source and location of infection;
- Type of enterprise (e.g. facilities using underground water versus those using groundwater from river, lake or stream);
- Design of the site and its proximity to other waterways;
- Current disinfection/treatment protocols;
- Environmental impact of the disinfectant protocol;
- Availability of approved, appropriate and effective disinfectants.

As mentioned in 2.2.6 above, contaminated facilities using earthen ponds can also break the lifecycle of the parasite by eliminating habitat for the worm host e.g. converting to concrete raceways.

2.2.11 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.
- The use of disinfectants could impact on the environment, especially if used in larger than normal quantities or concentrations. This is possible in a disease control situation. The local environmental protection agency

[21] this level of chlorine was found to be effective at water temperatures ranging from near freezing to room temperature with total hardness levels from 10-500mgl^{-1}

may need to be consulted — see the AQUAVETPLAN **Enterprise Manual**[22]**.**

- The destruction and disposal of infected carcases/material will have an impact on the environment. This impact must be minimised while ensuring that there is no dissemination of infection.
- *T. tubifex*, is found in high numbers in polluted stream habitats (Pinder and Brinkhurst 2000). Therefore ensuring pristine stream ecology may help in maintaining low numbers of this worm species.

2.2.12 Vaccination

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

2.2.13 Predator control

- *Birds* — myxospores can survive the passage through the guts of birds, so they can act as a carrier of this disease. Open air tanks, ponds and especially processing facilities and areas where carcasses are disposed of may attract birds and must be covered (e.g. using nets or tank roofs).
- *Fomites* — the persistence of myxospores makes it possible that infection could be spread by personnel through mud carried on boats, trailers, boots or other items. Hence such items should be thoroughly cleaned before leaving an infected area (this could include anglers leaving a lake known to be infected with *M. cerebralis*).
- *Wild fish* — wild fish can act as carriers of myxospores through predation on infected fish (myxospores can survive the passage through the gut of fish) or if infected themselves, hence spreading the parasite through freshwater waterways by their own movement.
- *Worms* — transporting infected *T. tubifex* worms between waterways (e.g. by anglers for bait) could transmit infection.

2.2.14 Sentinel and restocking measures

Rainbow trout (*O. mykiss*) are considered the most susceptible fish to infection. Young rainbow trout may be stocked as sentinel fish to determine the presence or absence of the parasite. This is done routinely in countries where whirling disease is present.

While Atlantic salmon (*S. salar*) and brown trout (*S. trutta)* can be infected with the parasite, and myxospores can form in the cartilage of these fish, clinical disease is rare and morbidity low. Hence restocking with less susceptible species (and when fish are older e.g. >9 weeks) may be an option in some areas to maintain production.

Removing all salmonids from an area is the only way to ensure there are no susceptible fish hosts in which the parasite can complete its lifecycle.

If *T. tubifex* is removed from a facility, and there is no possibility of TAMs entering the facility, restocking can commence immediately[23].

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[22] http://www.affa.gov.au/content/publications.cfm?category=Animal%20fixand%20Plant%20Health&ObjectID=00499893-720A-4F46-9CB9D85A876398B7

[23] This may incur risk as in farming there seems to never be the “no possibility” situation.

For eradication in farming facilities, restocked fish 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. Again, determining an area free of the parasite may be very difficult.

2.2.15 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. This is absolutely essential.

2.3 Feasibility of control in Australia

The feasibility of control of an outbreak of whirling disease or isolation of *M. cerebralis* in Australia depends upon both the nature of the outbreak and/or parasite isolation and the control management strategy adopted.

2.3.1 Option - Eradication

If wild populations are affected, the conventional wisdom is that *M.cerebalis* cannot be eliminated once it is established. Failed eradication efforts from areas of the world where the parasite is now endemic (e.g. many States in the United States) have been documented in the literature including taking of 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 nor 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 in wild fish populations that would be impossible to eradicate;
- *M. cerebralis*' ability to produce covert infections that are difficult to detect;
- The ability of infected wild fish to transmit and establish infection in rivers and other freshwater habitats;
- Close contact between, and relative lack of control over, farmed and wild fish populations, and water in Australian salmonid farming operations (both semi-open and semi-closed systems);
- Experience in affected countries that eradication is unsuccessful once reservoirs of infection become established in wild fish populations and the natural environment.

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

(i) Ensure source water is either free of TAMs or there is the potential to adequately treat incoming water;

(ii) Ensure there are no potential habitats for *Tubifex* worms within the facility.

The option chosen must ensure that there is:

(i) No further exposure of unexposed fish populations to TAMS;

(ii) No further spread of infection via the release of myxospores into the environment.

2.3.1.1 Unexposed fish

Young (pre-market sized) unexposed fish may be allowed to grow-out provided there has not been a risk of infection (i.e. exposure to TAMs) and that there is no risk of future infection. This may be difficult to confirm. Older fish that have had no possible exposure to infection (again difficult to confirm) may be emergency harvested and slaughtered for human consumption. Alternatively, fish may be transferred to saltwater to remove the possibility of exposure of these fish to TAMs. This is only a viable option when:

(i) Fish will tolerate such salinity changes;

(ii) Saltwater facilities are available and suitable.

Immediate destruction of unexposed fish populations located within a declared area is a drastic option if such fish are not at risk from exposure to TAMs. *M. cerebralis* cannot be transmitted directly from fish to fish, and there is a significant period of time for development of the parasite to occur within the worm host before infective TAMs are released (>90 days, depending on temperature).

2.3.1.2 Exposed or potentially exposed, clinically normal fish

Normal or controlled grow-out of exposed or potentially exposed, clinically normal farmed salmonid populations could be used when eradication is considered the best option provided any myxospores potentially released during the grow out period of these fish are prevented from coming in contact with tubifex worms. Grow out in saltwater may achieve this where fish can tolerate such salinities (e.g. smolted Atlantic salmon), and where the facilities exist. Facilities that have the ability to send effluent water to ground or where there is no possibility of contacting areas of worm habitat is another option.

There are no effective treatments that destroy all myxospores in the fish host. The species of salmonid affected will influence the number of potential myxospores that may be released per fish. Rainbow trout (*O. mykiss*) are considered the most susceptible species. Infected fish of this species will potentially have a high burden of myxospores than other salmonid species, such as Atlantic salmon (*S. salar*) or brown trout (S. *trutta*).

These fish are safe for human consumption as are infected fish. Emergency harvesting of fish, has a low risk of further transfer of infection and is a feasible option providing there is no risk of processing waste, especially skeletal elements, being released into freshwater habitats. This may jeopardise the success of an eradication strategy.

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

- Disinfection of all equipment/personnel involved in harvesting, slaughter and processing to eliminate the risk of transferring myxospores off site;
- Quarantine restrictions and procedures apply to the infected site, including personnel, equipment and vehicles. This is aimed at myxospores, as it is unlikely that TAMs would be transferred off site by this route;

- Processing — possible on or off site provided waste and fish carcasses cannot come in contact with freshwater *Tubifex* habitats;
- Holding, treatment and safe disposal of slaughter/processing effluent (includes holding water and waste offal);
- Ensuring that the final product will not result in the spread of infection;
- Disinfection of effluent water.

Immediate destruction of these fish populations will not be effective in eradication of the parasite if there is the possibility that effluent water or escaped infected fish from the facility have spread myxospores to downstream worm habitats.

There is no need for immediate destruction if the fish are located in saltwater.

2.3.1.3 Clinically diseased fish

If the diseased fish are located in freshwater, and if there is no possibility of disinfecting or redirecting effluent water from the facility, removal, destruction and disposal of diseased and dead fish is essential to prevent myxospores transmitting infection. Tubifex worms may also be infected, and will remain infected for the life of the worm, which can be up to two years.

If clinically infected fish are to be removed, burial sites should be chosen carefully to ensure there is no contact with waterways or birds/animals.

2.3.2 Option 2 - Containment, control and zoning

2.3.2.1 Unexposed fish

Control options for unexposed fish are the same as those outlined for eradication in Section 2.3.1. The implementation of a zoning program, and associated control measures, to maintain uninfected zones would be necessary. For zoning see Section 2.2.1.1.

2.3.2.2 Exposed or potentially exposed, clinically normal fish

A zoning program will rely on the implementation of movement restrictions on exposed or potentially exposed fish that prevent infection spreading to uninfected zones. The zoning program must also take into account water movement and possible spread of the two spore stages of the parasite by this means. The feasibility of these restrictions will depend on farm management practices, the extent to which infection has already spread and the location of reservoirs of infection. Feasibility can only be assessed at the time of the outbreak, taking into account movement restrictions required on fish, people, vehicles, boats and also market access for the fish products and byproducts. Even then assessment may be limited. If young fish are allowed to grow-out, they must be treated as infected.

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 (e.g. if destined for domestic human consumption in areas free of *M. cerebralis*, products must be processed so that they do not contain viable myxospores).

There are no effective treatments that destroy all myxospores in the fish host. Treatment is not considered a viable option in Australia.

Immediate destruction of the fish is an option for containment, control and zoning, 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 must be carefully considered as to what the overall benefit is of removing one host but not the other. Removing both hosts by destruction and elimination of *Tubifex* habitat if this is possible (e.g. cleaning/drying ponds, or conversion to concrete raceways) may be a viable option.

2.3.2.3 Clinically diseased fish

Clinically diseased fish, along with infectious wastes, are considered to be the main source of myxospores in the environment. They constitute the greatest risk for spreading the infection to uninfected zones due to the resistant nature of myxospores.

The only real option for clinically diseased fish if effluent water cannot be contained or disinfected is immediate destruction or to transport to saltwater for grow out. It is unlikely that myxospores would contact a susceptible tubifex host in this environment. Escaped, infected fish may be a risk if they return to freshwater.

2.3.3 Option 3 - Control and mitigation of disease

The principles of control and mitigation of disease are to reduce the impact of disease. Therefore, all options listed in Section 2.3.2 for containment, control and zoning apply, except for the measures associated with zoning.

2.3.4 Trade and industry considerations

In countries where whirling disease is endemic, industries affected include the salmonid farming and recreational angling industries. It is unlikely that other aquatic farming industries in Australia 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.

Export markets

Whirling disease is endemic throughout many states of the United States of America, Central Europe, South Africa, North East Asia and the east coast of the south island of New Zealand. It is not listed by the Office International des Épizooties (World Organisation for Animal Health) (OIE). However, there are some countries that require imports to be certified free from whirling disease. Some countries also have regional requirements that differ within the country; for example some states of the United States. For further information regarding export market requirements, contact AQIS[24].

Domestic markets

A cautious approach is required for the salvage of exposed or potentially exposed product for the domestic market. The myxospore is highly resistant and can survive for long periods. Decisions regarding the release of salmonids or salmonid products to the domestic market will depend on the control strategy implemented.

[24] http://www.affa.gov.au/outputs/quarantine.html

Eradication

If eradication is considered, decisions relating to the release of product for domestic market must ensure there is no potential for the spread of *M. cerebralis*. This may be difficult.

Containment, control and zoning

The release of exposed, or potentially exposed salmonid product to the domestic market must be carefully controlled to ensure there is no potential spread of viable *M. cerebralis* to areas/zones declared free of whirling disease.

Control and mitigation of disease

Requirements for the release of exposed or potentially exposed salmonid product to the domestic market will be less stringent if whirling disease becomes endemic in Australia.

3 Preferred control policy in Australia

3.1 Overall policy for whirling disease

Whirling disease has the potential to cause significant mortality and morbidity in farmed and wild salmonid populations in Australia. Rainbow trout are particularly susceptible to the disease, especially if infected when < 7 weeks old.

It takes 3-8 weeks post infection for clinical signs to develop in susceptible fish; many infected fish may never show clinical signs. Fish can only be infected with spores released from the worm host. Epidemiological investigation may be hindered because it can be difficult to confirm infection in fish or worms.

The preferred policy is to eradicate *M. cerebralis* if it were isolated in Australia. Depending on circumstances, this may not be an option, at least in the short term. Two other control options that may be chosen are:

- ⇒ ***containment, control and zoning*** **of the parasite to areas with endemic infection, prevention of further spread and protection of uninfected areas; or**
- ⇒ ***control and mitigation of disease*** **by implementing management practices that decrease the incidence and severity of the disease.**

The control policy chosen will depend on the nature of the outbreak and/or isolation of the parasite. The Director of Fisheries and/or the CVO of the State/Territory in which the parasite is isolated will decide the control option(s).

It is important that the chosen policy be dynamic to allow evolution of the strategy with the changing situation e.g. choosing containment, control and zoning in the short term does not preclude adopting eradication as a long term policy.

Strategies which may be used under these options include:

- ☞ ***quarantine and movement controls*** **on fish, fish products, *Tubifex* worms and things in declared areas to prevent spread of infection;**
- ☞ ***prevention*** **where possible of predators (e.g. birds) gaining access 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 e.g. changing earthen type ponds to concrete raceways;**
- ☞ ***decontamination*** **of facilities to inactivate the resistant myxospore stage of the parasite on infected premises and to 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 acknowledging limitations in diagnostic capabilities.**
- ☞ ***zoning*** **where possible to define and maintain infected and parasite-free zones; and**
- ☞ ***restocking*** **with older, less susceptible fish or less susceptible species unlikely to develop clinical disease.**
- ☞ ***education*** **of public, aquaculturalists and government**

If *Myxobolus cerebralis* with or without disease is confirmed in Australia, the Director of Fisheries and/or the CVO of the State/Territory in which the isolation(s)

occurs will be responsible for implementing disease control measures (in accordance with relevant legislation), and will make ongoing decisions on follow-up disease control measures in consultation with the aquatic Consultative Committee on Emergency Animal Diseases, the State/Territory and Commonwealth governments and representatives of the affected industry(s). The detailed control measure adopted will be determined using the principles of control and eradication and epidemiological information about the isolation +/- outbreak.

The flowchart diagram depicted in Figure 3 (page 38) gives some possible scenarios and is designed to help in the initial decision making process. This decision may need to be made on very limited epidemiological information. As more information becomes available, there may be modification to the initial response[25].

As indicated by this diagram, there are three control options that may be chosen to control whirling disease. In summary, these three options are:

- *Eradication* — eradication of *M. cerebralis* from Australia (highest level of control measure and cost).
- *Containment, control and zoning* — containment of 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 (lowest level of control measure and cost).

For a description of the notification arrangements, order of procedures, management structures and roles of personnel during the various stages of activation upon suspicion of an incidence of whirling disease in Australia, refer to the AQUAVETPLAN **Control Centres Management Manual**[26].

3.2 Initial response

3.2.1 Laboratory testing

If whirling disease is suspected in a State or Territory, the corresponding State/Territory Chief Veterinary Officer (CVO) or Director of Fisheries must be notified immediately. Preliminary identification of *M. cerebralis* may be undertaken by some State/Territory diagnostic laboratories (e.g. Tasmania) however duplicate specimens must be consigned to the Australian Animal Health Laboratory (AAHL) that same day.

3.2.1.1 Submission of specimens

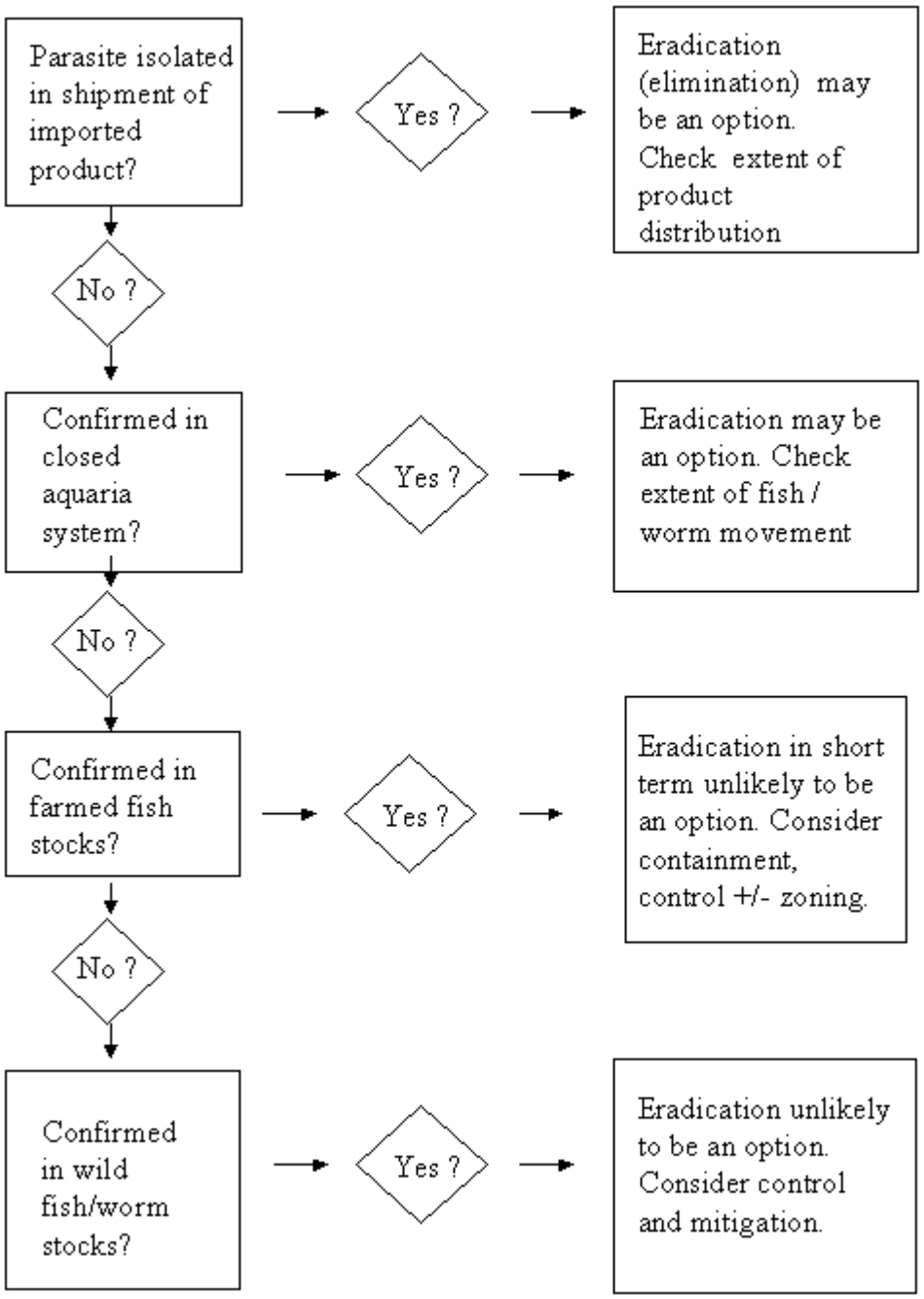
Suspicion of whirling disease is classified as a disease outbreak emergency under the definition given in the AQUAVETPLAN **Enterprise Manual** (AFFA 2001a). If an exotic disease outbreak is suspected, primary or index case diagnostic testing will be done at AAHL as far as is practical. This aligns with AAHLs national role and

[25] For example, if whirling disease is confirmed in farm fish, eradication in the short term is unlikely to be an option. However, in the long term (possibly years), eradication may be an option through incorporation of different management options (e.g. stocking fish species with lower susceptibility to the disease) depending on the circumstances of the outbreak

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

obligations to lead the development and improvement of testing for exotic and emergency diseases.

Figure 3 - Decision flowchart[27]



[27] Note that in this flowchart "farmed fish" refers to fish farmed in semi open or semi closed systems

The CVO of Victoria must be informed before specimens from suspected whirling disease incidents are transported through Victoria to AAHL (see Appendix A – Veterinary Committee Protocols - Procedures For Transmission of Diagnostic Specimens to or from AAHL).

As samples would be classified as Category 2 or 3, such samples must be submitted from State Government laboratories unless the Government laboratory, in consultation with the State CVO, has given prior approval for a direct submission.

It is recommended that the testing laboratory be contacted directly to ensure that samples are collected correctly, and sample collection techniques satisfy the requirements of the laboratory.

3.2.2 Epidemiological investigation

A comprehensive epidemiological investigation, including tracing and surveillance, must be initiated immediately on suspicion or confirmation of the isolation of *M. cerebralis* in Australia. As outlined in **Section 2.2.2.**

3.2.3 Quarantine and movement controls

Quarantine and movement controls must be implemented on anything capable of transmitting the infective stages of the parasite i.e. the spore stage in, and released from, the worm which is infective for fish (the triactinomyxon, or TAM stage), and the spore stage in, and released from fish, which is infective for worms (the myxospore stage).

Control areas (Section 2.2.1) should be established as quickly as possible. However, in the early stages there may only be limited epidemiological information on which to make a decision. Consideration must be given to the likelihood of the parasite spreading into the local *Tubifex* worm population. Control areas should be refined as more information becomes available.

Some of the possible items that could transmit *M. cerebralis* are:

A. Myxospores (highly resistant stage) in/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
   - Water flowing out of infected premises[28] and effluent from such areas as processing facilities
   - Birds and other predators feeding on carcasses or infected fish
   - Personnel

B. Triactinomyxon (less resistant stage)in:
   - *Tubifex* worms
   - Soil containing *Tubifex* worms
   - Water flowing out of infected premises

[28] The spread of myxospores and triactinomyxons down waterways must be taken into consideration in establishing control areas, acknowledging that if clinical signs of disease are present, such spread is likely to have been occurring for over 3 months.

3.2.4 Treatment of fish and worms

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

3.2.5 Vaccination of fish

There are no vaccines for whirling disease.

3.2.6 Destruction of fish

The objective of destroying fish is to limit further spread of disease through prevention of myxospore release and spread. Disease can also spread through release of the triactinomyxons (TAM) stage from the worm host. Myxospores are infective for worms and not fish; TAMs are directly infective for fish.
Clinically, affected fish are unlikely to recover to a marketable product. These fish will be the biggest source of infective myxospores. Fish that exhibit clinical signs of whirling disease and from which the parasite has been confirmed should be humanely destroyed and disposed of in such a manner to ensure no possibility of spread of myxospores. See the **AQUAVETPLAN Operational Procedures Manuals – Destruction** and **Disposal** for details[29]. 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[30].
The decision on whether or not to destroy potentially exposed fish showing no clinical signs will need to be made when more epidemiological information becomes available, and a control option is chosen.

3.2.7 Alteration of habitat to destroy the *Tubifex* worm host

Removing fish from areas where there is habitat suitable for the *Tubifex* worm host (e.g. organic rich pond bottoms) will potentially decrease the ongoing infective challenge on those fish. This may not be possible during the initial response. Elimination of these areas and replacement with habitat unsuitable for propagation of the worm (e.g. concrete raceways) will reduce the concentration of triactinomyxon (TAM) spores 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.

3.2.8 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 a definitive diagnosis is made on whether the fish are infected or not. Filleting fish and hot-smoking fillets

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[29] Destruction Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906
Disposal Manual: http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5

[30] For example, salmonids growing in a saltwater environment where there is no possibility of fish escaping and travelling back into freshwater.

at 66°C for 40 minutes is an acceptable process to ensure inactivation of myxospores (see Section 2.2.7). The release of smoked fillets to the marketplace will depend on which control option is chosen, so should be delayed during the initial response.

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

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

3.2.9 Predators

Effective predator control to prevent predators (e.g. birds, rodents) eating or carrying infected carcasses away from the infected premise(s) is essential to limit spread of the parasite in the initial response.

3.2.10 Education and media

In the early stages of an outbreak investigation education and public relations, especially with the media, is critical. Diseases such as whirling disease have the potential for sensationalism, especially considering the significant coverage the disease has had in the United States. Hence, providing the true facts, in a clear and concise way, is important to minimise this and the time required to deal with media that would be better spent dealing with the outbreak. 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 taking, and are in the position to take, all measures to control the situation. It must also be clearly stated that this disease holds no health risk for humans.

3.3 Control options

The following describes in detail the three strategies that may be chosen to control an outbreak of whirling disease, or isolation of *Myxobolus cerebralis* in Australia. In all three strategies it is assumed that measures described in **Section 3.2 Initial Response** have been adopted where possible.

3.3.1 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 (e.g. in a closed system such as an aquarium or fully recirculating system), an eradication strategy may be successful and should be attempted. Likewise, if *M. cerebralis* is isolated from imported product then prevention of spread and elimination of the pathogen may be possible (see Figure 3). Compared with the other two response options, eradication can have the highest short-term economic costs. However, if *M. cerebralis* were successfully eradicated, long-term economic benefits could outweigh those short-term costs.

Identifying either of the following will decrease the likelihood of successfully eradicating this parasite in the short term:

- Parasite confirmed in wild fish stocks that are not in a land-locked lake system.

- Parasite confirmed in fish farm/hatchery where effluent water returns to a stream or lake system in which there is the possibility of wild salmonids and susceptible *Tubifex* hosts.

Figure 4- - Identification of *M. cerebralis* in imported product

The purpose of this manual is not to describe all the possible scenarios in which *M. cerebralis* may be detected in Australia. However, the detection of *M. cerebralis* in imported product (e.g. imported salmonid products) deserves mention. Were such detection to be made, it is agreed that such product should be destroyed with the ultimate objective of preventing establishment of infection in Australia. It is acceptable to hold such product in storage prior to destruction to allow confirmatory testing to be conducted if required[31]. Other actions to be taken are:

- Tracing of any product that may have already been released into the Australian market
- Determining where the product came from, and how much product has already entered Australia prior to the infected shipment.
- Investigating how *M. cerebralis* could have been in such product.
- Increased monitoring of salmonid stocks in areas where such product may have been shipped.
- Education of public, aquaculturalists and government agencies on the possibilities of establishment of infection in Australia through product release in areas of *Tubifex* habitat.

3.3.1.1 Quarantine and movement controls

The objective of quarantine and movement control is to limit further spread of the parasite (see section 3.2.2). Further spread will potentially jeopardize the ability to eradicate *M. cerebralis*.
When eradication is the option of choice, it is essential that the exposure status of fish be known. If there is uncertainty, then fish should be treated as exposed.

3.3.1.2 Unexposed fish

Young unexposed fish may be allowed to grow out provided there is no risk of future exposure to the infective triactinomyxon (TAM) stage of the parasite. This potentially can be achieved through strict farm biosecurity and hygiene practices or by transferring these fish to saltwater (if fish are tolerant of this change in salinity). Harvest size unexposed fish should be harvested out and slaughtered for human consumption. It will take over 50 days post exposure to TAMs for development of infective myxospores in these fish if infection by TAMs is successful. Exposure within 50 days of slaughter will not result in an increased risk of spread.
Destruction of unexposed fish should only be treated as a last resort, even when the preferred option is eradication.

3.3.1.3 Clinically diseased and dead fish

Removal, destruction and disposal of all diseased and dead fish is essential to eradicate *M. cerebralis*. These fish, along with infectious wastes, are the main source of myxospores in the environment. Burial sites should be chosen carefully to ensure

[31] For example, if the initial screening test is done using a Polymerase Chain Reaction (PCR) method, further histological testing to identify myxospore in cartilage may be desirable.

there is no contact with waterways or predators/scavengers. However, delays may occur before a population can be destroyed and disposed of due to the limitations of destocking entire premises. Such delays may allow further spread of the parasite, and should be considered when determining whether or not to attempt eradication.
All destruction should be conducted in a humane manner.

3.3.1.4 Eggs and reproductive material

Thorough washing in sterile water and ensuring the water in which eggs incubate does not contact susceptible *Tubifex* worms will prevent establishment of infection. TAMs will not survive the incubation period of the eggs, and hence will not be present when susceptible fish hatch.

3.3.1.5 *Tubifex* worms and potential *Tubifex* habitat

All *Tubifex* worms that could potentially be infected must be destroyed or the TAMs released from these worms prevented from coming in contact with any susceptible salmonid host. Eliminating the habitat (e.g. replacing earthen pond bottoms with concrete) will effectively stop TAM production from these areas. When infected worms are located in streams, rivers or lake systems, habitats can be modified but not sufficiently enough to eliminate all worms. In such cases, eradication in the short term is unlikely to be successful. Eradication in the long term may be possible through the prevention of repopulation of such areas with susceptible salmonids, thereby again breaking the lifecycle of the parasite.

3.3.1.6 Treatment of infected fish

There are no treatments for whirling disease that effectively eliminate the parasite from the fish and worms so this is not an option for eradication.

3.3.1.7 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.

When eradication is the chosen option, all product that could potentially spread the parasite must either be disposed of or treated to ensure parasite inactivation.
Treatment of fish products to ensure inactivation of the parasite is outlined in Section 2.2.7.

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

3.3.1.8 Decontamination

All tanks, materials and equipment including vehicles and buildings that may be contaminated must be cleaned and disinfected for successful eradication. The critical stage is the myxospore, which is highly resistant (see section 2.2.9).
If disinfection cannot be achieved effectively, then contaminated materials and equipment should be destroyed. At all stages of decontamination, steps must be taken to prevent any spread of infection via water, wastes or materials, especially into natural waterways.

3.3.1.9 Restocking measures

It is possible that native Australian finfish species are not susceptible to whirling disease. Restocking with such species is possible in an eradication program if the resistance of these species is confirmed.

3.3.1.10 Criteria for Proof of Freedom

Proof of freedom from whirling disease will be important for trade. It is also important for Australia's salmonid industries. Currently, there is no criteria that outlines what is required to declare a country free from whirling disease. The World Animal Health Organisation, or OIE (Office Internationale des Epizooties) in its Aquatic Animal Health Code (OIE 2003) outlines a number of other finfish diseases and what is required for declaration of freedom for a country or zone with respect to these diseases.

3.3.2 Option 2 - CONTAINMENT, CONTROL AND ZONING

If eradication is discounted then containment and prevention of further spread is the preferred strategy in order to protect and maintain uninfected areas.

Section 3.2 outlines the initial response. Additional measures to be taken under this control option are:

- *Implementation of a zoning program*

  Care must be exercised in the development of such a program for whirling disease. Factors such as the poor sensitivity and specificity of the diagnostic procedures available, case definitions and bias in sampling procedures must be considered prior to implementation to ensure such a program would achieve the desired objectives.

  Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN **Zoning Policy Guidelines**.[32] This document is based on terrestrial diseases, though many of the principles still apply.

- *Management options to reduce the severity and incidence of infection*

  Farms in the affected areas would need to consider options to reduce exposure to *M. cerebralis* – see Chapter 2.

- *Restocking with less susceptible fish species*

  Farms in control areas may elect to restock with less susceptible fish species (e.g. Australian natives) if this is deemed economically viable. Alternatively, preventing exposure of young (< 7 weeks) salmonids to TAMs will significantly reduce the incidence of clinical disease, though such fish may still be infected.

3.3.3 Option 3 – CONTROL AND MITIGATION OF DISEASE

If this control option is chosen, measures taken will be aimed at managing the disease in affected areas. Such measures are detailed in Chapter 2.

3.4 Funding and compensation

Currently, there is no requirement for compensation to be paid to farms where destruction of fish is ordered. This is being reviewed.

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[32] http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717.

APPENDIX A: PROCEDURES FOR TRANSMISSION OF DIAGNOSTIC SPECIMENS TO OR FROM AAHL

VETERINARY COMMITTEE PROTOCOLS

Submission of Specimens to AAHL

1. All primary or index case diagnostic testing for emergency disease incidents will be done at AAHL as far as is practical. This aligns with AAHL's national role and obligations to lead the development and improvement of testing for exotic and emergency diseases.

2. 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 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.

3. 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.

4. 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 / delegated Health Officer will be advised prior to notifying the relevant State Chief Veterinary Officer.

5. 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 despatch to an appropriate overseas laboratory. The State CVO or State Veterinary Laboratory personnel may wish to discuss the appropriate laboratory to send the material.

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

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

8. 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

9. There are 3 categories of specimens

   Category 1: Routine submission: no suspicion of exotic or emerging diseases, e.g. 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.

10. In terms of the Control Centre Management Manual, specimens in Category 2 align with the ***Investigation Phase*** while specimens in Category 3 align with the ***Alert Phase.***

Despatch of Specimens

11. ***Category 1*** specimens: The submitter to contact the AAHL Duty Veterinarian (or, in the case of aquatic animal specimens, the OIC AAHL Fish Diseases Laboratory [AFDL]) and advise by phone or fax the details of the despatch and testing required

12. ***Category 2*** specimens arising from the investigation phase: The submitter will:

    - inform the Director of the Laboratory making the submission who will advise State CVO; and
    - inform the AAHL Duty Veterinarian (or OIC AFDL) as for ***Category 1.***

13. ***Category 3*** specimens arising from the alert phase: The submitter will:

    - obtain the approval of the State CVO prior to despatch; and
    - advise the AAHL Duty Veterinarian (or OIC AFDL), the Victorian CVO, and Victorian AQIS VO by phone or fax of full details of despatch to facilitate passage of the specimens through (usually) Melbourne airport, collection by AAHL personnel and quick passage to AAHL
    - Ensure the State CVO notifies the Director of AAHL or the Director's delegate.

14. All specimens be accompanied by a completed specimen advice note (SAN). These are available from the CSIRO web site or can be sent on request.

15. The Victorian AQIS VO 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 VO only needs to be notified when the specimens are being flown through Tullamarine.

Summary

16. This protocol sets down the need for specimens to be sent to AAHL at the earliest opportunity for the exclusion of exotic or emerging diseases.

17. It is essential that AAHL staff be informed of **Category 2** specimens being forwarded to AAHL.

18. Formal notification of the submission of **Category 3 specimens** should be from the State CVO to the Director or Director's delegate and the duty vet in the affected State should notify the AAHL Duty Veterinarian (or OIC AFDL). The State CVO needs to approve such submissions.

19. All specimens be accompanied by a completed specimen advice note.

APPENDIX B IDENTIFICATION OF *MYXOBOLUS CEREBRALIS* (WHIRLING DISEASE)

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

Spore Detection Method

This method detects the microscopic spores of *Myxobolus cerebralis* in the cartilaginous tissues of the heads of salmonid fishes. 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 *Myxobolus cerebralis* spores.

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

PCR Method

This 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 bone, cartilage and gills from older fish.

Briefly, the samples are boiled, 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 DNeasy Tissue Kit (Cat: 69504). The preparation is incubated at 55^{O}C, with occasional vortexing, for 1 hour or until digestion is complete. Following centrifugation, the aqueous supernatant is used for DNA extraction using the commercial kit.

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GLOSSARY

AQUAVETPLAN	A series of documents that describe the Australian response to exotic aquatic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
AUSVETPLAN	A series of documents that describe the Australian response to exotic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
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.
Declared area	An area that has been subjected to a legal declaration and includes both a restricted area and a control area.
Decontamination	Includes all stages of cleaning and disinfection.
Disinfectant	An agent used to destroy microorganisms outside a living animal.
Disposal	Sanitary removal of fish carcases and things by burial, burning or some other process so as to prevent the spread of disease.
ELISA	Enzyme linked immunosorbent assay — a serological test designed to detect and measure the presence of antibody or antigen in a sample. The test uses an enzyme reaction with a substrate to produce a colour change when antigen-antibody binding occurs.
Exophthalmia	Protrusion of the eyeball from the orbit, caused by disease or injury.
Fish byproducts	Products of fish origin destined for industrial use (e.g. fishmeal).
Fish products	Fish meat products and products of fish origin (e.g. eggs) for human consumption or use in animal feeding.
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 lease.

Mitigation	Reduction in severity, eg mitigation of the impact of disease is to decrease the severity of the impact of the disease.
Movement control	Restrictions placed on movement of fish, people and things to prevent spread of disease.
PCR	A diagnostic technique involving the production of millions of copies of a specific target DNA segment *in vitro*.
Premises or area	Production sites that may range from an aquarium to an aquaculture lease in the open ocean.
Quarantine	Legal restrictions imposed on a place, fish, vehicle, 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.
Sentinel fish	Fish of known health status monitored for the purpose of detecting the presence of a specific exotic disease agent.
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 species	Fish that can be infected with the disease.
Suspect premises or area	Where the emergency disease is suspected but not yet confirmed. The reason for the suspicion varies with the agent, however it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, people or things that may be implicated in the spread of disease.
Vector	A living organism that transmits an infection 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 zones.
Zoonotic disease	Disease transmissible from animals to humans.

ABBREVIATIONS

AAHL	Australian Animal Health Laboratory
AFDL	AAHL Fish Diseases Laboratory (formerly AFHRL)
AFHRL	Australian Fish Health Reference Laboratory
AQIS	Australian Quarantine and Inspection Service
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	Chief Veterinary Officer
ELISA	enzyme linked immunosorbent assay
IFAT	indirect fluorescent antibody test
OIE	Office International des Épizooties (World Organisation for Animal Health)
PCR	polymerase chain reaction
SCFA	Standing Committee on Fisheries and Aquaculture

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