

Whirling Disease in the United States

Overview and Guidance for Research and Management

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Foreword

Although it was detected in the United States almost 40 years ago, whirling disease only began to receive national attention early in 1995, due largely to reports from Wyoming that the disease was likely responsible for a 90 percent decline in rainbow trout numbers in the Klondike River. Since then, investigators have confirmed the presence of the disease in several other Montana rivers and in 13 of Colorado's 15 river systems. Although whirling disease has been found in hatcheries and/or in the wild in 21 states, to date investigators in only three states in the Intermountain West (Utah, Colorado, and Montana) have established that the disease has had appreciable negative effects on wild trout populations. Indeed, that fact presents one of the most interesting challenges to advancing our understanding of the disease: namely, isolating the factors that have caused the disease to devastate wild fish in some places, but not in others.

To gather scientific information about whirling disease and to analyze it in a manner that assists scientists and managers in understanding and controlling the disease, TU commenced its national whirling disease assessment in the spring of 1995. With the assistance of a distinguished panel of scientists and resource managers, we prepared for the panel's review a draft report on whirling disease in the United States, summarizing the available scientific literature from around the world. We asked the panel to provide suggestions for research priorities and management strategies. We also surveyed all 50 states regarding the spread of whirling disease and state regulations regarding fish health inspections and transportation of diseased fish. This report is the final product of those efforts.

It is important to note that the views expressed in this report are not necessarily those of Trout Unlimited. The suggestions offered on management and research were developed by the advisory panel, to provide sound scientific guidance for managers and researchers as they develop strategies to address whirling disease.

The report is proof that fishery managers and scientists have come a long way in the past year, increasing our knowledge of whirling disease and adopting a more cautious approach in the face of the uncertainty that remains about the disease's behavior in wild populations. However, there are many states where fish health policies make containing and controlling whirling disease more difficult. For instance, while most states regularly inspect their hatcheries, only one third require inspections for private hatcheries. Of the 21 states where the whirling disease parasite is currently known to occur, seven still stock infected fish.

As the report indicates, much remains to be done. To implement the report's findings and recommendations, TU will focus in the coming months on securing federal, state, and private funding of whirling disease research and on building public awareness of the threat that the disease poses for native and wild salmonids. Unfortunately, whirling disease is a problem that will be with us for the foreseeable future. The report represents an important step in understanding the disease, one that we hope will guide science and management toward responsible solutions.

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Executive Summary

Whirling disease is a disorder of trout and salmon caused by the microscopic parasite *Myxobolus cerebralis*. It was first reported in the United States in 1958, in Pennsylvania. Since that time, *M. cerebralis* has been found in 21 states: Alabama, California*, Colorado*, Connecticut*, Idaho*, Maryland*, Massachusetts, Michigan*, Montana*, Nevada*, New Hampshire, New Jersey, New Mexico, New York*, Ohio, Oregon*, Pennsylvania*, Utah*, Virginia*, West Virginia, and Wyoming* (* indicates states which currently report the parasite). Early reports of whirling disease were often accompanied by forceful actions, for example, destruction and burial of infected fish. More recently, several states have allowed infected fish to be used for food purposes or for stocking in areas where the parasite is already found.

Impacts

The parasite attacks the cartilage of infected trout and its impacts on susceptible trout can be dramatic: darkening of the tail; frenzied tail chasing (“whirling”) by fish when they are feeding or are alarmed; skeletal deformities (especially of the head); and heavy mortalities in young fish. The parasite literally digests the cartilage of infected fish, and is often associated with a severe inflammatory response.

Different species vary in how susceptible they are to whirling disease; rainbow trout are highly susceptible, brown trout are highly resistant (but are often carriers), and brook trout fall in between. Other species that can be infected include golden trout, cutthroat trout, grayling, Atlantic salmon, sockeye salmon, chinook salmon, and whitefish. Coho salmon and lake trout become infected only rarely or not at all.

Other factors also influence the severity of infection. Younger fish are more vulnerable to disease than older fish. Severity of infection also correlates to the number of parasites to which a fish is exposed. Poor environmental conditions and other stressors make a fish more susceptible to disease.

In fish hatcheries infected with *M. cerebralis*, severe disease can be avoided by taking precautionary measures

(such as keeping young, highly susceptible fish in disease-free water until they are larger and more resistant). While fish may still become infected and act as carriers of disease, they will not show clinical signs. In the wild, it has generally been assumed that whirling disease is not a problem. Recent reports from Colorado and Montana have led many to question that assumption.

In the Middle Park section of the Colorado River, recent year classes of rainbow trout have virtually disappeared, while brown trout populations have been less affected. After extensive research, scientists from the Colorado Division of Wildlife concluded that whirling disease was a factor in the loss of rainbow trout (though not necessarily the only cause). In Montana, scientists with the Department of Fish, Wildlife and Parks believe whirling disease is responsible for dramatic declines (up to 90%) in rainbow trout numbers in the Madison River. However, in many other areas there is a long record of professional experience suggesting that whirling disease has not been a problem in wild populations. It is not known why whirling disease appears to be a severe problem in some areas, while it seems relatively harmless in others.

Life cycle

M. cerebralis has a complex life cycle, requiring two different hosts and producing two very different spores. The spores that develop in infected fish are tiny — less than 10 μm across (1 μm =0.001 mm). These spores are released when an infected fish dies and decomposes, or passed through the digestive tract of a predator that eats the fish. The spores are extremely durable and can remain viable for years. Spores are also extremely resistant and can withstand freezing, a variety of chemical treatments, and passage through the digestive tract of predators. Spores are less resistant to extreme heat.

The parasite's life cycle continues when one of these spores is ingested by a small, pollution-tolerant aquatic worm, *Tubifex tubifex*. The parasite infects the gut of the worm, and after approximately 3 months begins to produce a different spore, the triactinomyxon. This is the

stage that infects trout; fish do not infect other fish. The triactinomyxon is shaped like a grappling hook, and is much larger than the other spore (the "arms" of the hook are approximately 170 μm long). It is also much less durable; triactinomyxons only survive for 3-4 days. However, they can be released from infected worms as long as one year after infection.

Trout become infected when they encounter water-borne triactinomyxons or when they eat an infected worm. The triactinomyxon attaches to the fish and releases its infective sporoplasms. Once in the fish, the parasite begins to divide and increase its numbers while moving through the epidermis, the subcutis, the nerves of the fish, and finally into the cartilage. It is in the cartilage that the parasite does its damage and ultimately produces the tiny spores that go on to infect *Tubifex* worms and continue the life cycle.

Spread of whirling disease

One major means by which whirling disease is spread is the movement of live, infected fish in association with fish culture and stocking activities. Most states (80%) regularly inspect their hatcheries for disease. Among those states that currently have whirling disease, some stock infected fish in waters that have already been exposed to the disease, while others allow no stocking of infected fish. However, regulation of private aquaculture is much looser. Only one third of states require inspections for private hatcheries, and most have no regulations on movement of fish within the state (though imports are regulated by nearly all states). The lack of pathogen inspections for facilities and fish transports makes it difficult to control the spread of *M. cerebralis*.

Once in a stream, whirling disease will spread naturally as infected fish swim up or down stream, and water-borne triactinomyxons are carried downstream. The parasite may also be carried by predators that eat infected fish, then shed spores in their feces into uninfected waters. Given the resistance of spores, they could be transferred through mud on waders, boots, boats, or other items moved between infected and uninfected waters. Another possible vector for spreading the disease are shipments of fresh and frozen food fish (spores remain viable despite freezing). Similarly, shipments of infected *Tubifex* worms could spread the pathogen. Fish eggs do not become infected, so prop-

erly disinfected shipments of eggs should not contribute to the spread of disease.

Control of whirling disease

A great deal of research has been directed at developing measures to control whirling disease in fish culture settings. Treatments that are effective in eliminating spores include ultraviolet irradiation, certain concentrations of chemicals (such as calcium oxide and chlorine), and heat. Some medicated feeds appear to be effective in minimizing infection in fish. Finally, basic changes in operations can be used to minimize disease (though fish may still be carriers): converting to concrete raceways, rearing fry in spore-free water until they are more resistant, and using more resistant species of fish.

Management Considerations

Whirling disease raises many difficult management issues. Innovative, fresh ideas are warranted. Maximal effect will be achieved through collaborative efforts with resource managers, users, and the aquaculture industry. In order to assist managers and the interested public, the following management considerations have been developed by the TU Whirling Disease Advisory Panel. These are not definite rules, but considerations that should be weighed in developing specific management strategies. Several of the Panel's considerations appear below; a complete list can be found on page 21.

- ▶ Public and private hatcheries involved in stocking fish should have regular fish health inspections so as to detect subclinical as well as clinical infections of *Myxobolus cerebralis* and other pathogens of concern.
- ▶ All reasonable measures should be used to ensure that *M. cerebralis* is not spread into waters that are currently free of the parasite.
- ▶ Stocking infected fish, even in waters that already hold *M. cerebralis*, increases the pathogen load in the system and should be discouraged.
- ▶ Any restrictions on stocking developed to address whirling disease should provide equal treatment for public and private hatcheries.

- ▶ Management entities should be encouraged to increase monitoring of public waters for fish pathogens of concern, especially in waters with self-sustaining fish populations.
- ▶ Where hatcheries have become contaminated, efforts should be made to eliminate infection from those hatcheries.
- ▶ Hatcheries where efforts to eliminate *M. cerebralis* repeatedly fail should be considered for closing.
- ▶ Incentives (such as low-interest loans) should be used to make hatchery clean-up more palatable to private facilities.
- ▶ As practicable, states should implement education programs to inform the public about whirling disease, responsible angling, the dangers of bait-bucket biology, and related issues.

Research recommendations

A review of current knowledge on whirling disease demonstrates one fact above all: we still know far too little. The TU Whirling Disease Advisory Panel discussed a variety of research topics and highlighted two major areas of research as highest priority and a third as high priority. These topics are outlined below and in greater detail on page 19.

Highest Priority

- ▶ Development of an enhanced, rapid, and cost-effective diagnostic test for *Myxobolus cerebralis*, such as a DNA-based test

The current diagnostic procedures for *M. cerebralis* are time consuming, expensive, and suffer from a lack of sensitivity. Development of a DNA-based test, with the ability to amplify (e.g. polymerase chain reaction) a parasite specific sequence, would solve several of these problems. A DNA-based approach could detect all life stages of the parasite.

- ▶ Improving understanding of the host-parasite relationship and dissemination of the parasite
- Little is known about the host-parasite relationship of *M. cerebralis*. Quantitative data is needed on several elements of the life cycle of *M. cerebralis* in order to predict the consequences of infection in a given aquatic system.

Among the topics on which research is needed are:

- Biological and genetic assessment of *M. cerebralis*. Parasites from different geographic regions should be studied to assess whether there are multiple strains of differing virulence.
- Research on infection and disease, looking at variables including species or strains of salmonids, fish age, water temperature, parasite dose, and density of fish and oligochaete hosts.
- Studies on the oligochaete worm host. Studies are needed to determine whether there are additional alternate hosts beyond *Tubifex tubifex*, and on the dynamics of triactinomyxon spore production in the worm host.
- Studies on early infection. Research is needed to determine how the parasite recognizes its host, whether fish can be reinfected, and what role environmental stressors may have on infection.
- Field studies on the dynamics of infection and disease in wild fish populations. Studies should examine rates of decline in young-of-the-year trout, incidence of infection, severity of disease, and numbers of spores in fish. The role of various environmental variables in infection/disease should also be investigated.
- Field studies on the dynamics of infection in oligochaete worms. The microhabitats, density, and seasonality of *T. tubifex* and other oligochaete populations beg for description. More must be learned of spatial, seasonal, and temporal patterns of concentration of triactinomyxons in natural waters.

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High priority

- ▶ Host resistance and immunity to *M. cerebralis*
- The basis of the resistance of certain species of salmonids to whirling disease is unknown. Understanding the host mechanisms for defense in these species should provide insights into characteristics required for resistance. Further characterization of host responses (cellular and humoral) is needed. Identification of key parasite antigens that may contribute to immunity, and how the response to these antigens may differ between susceptible and resistant species of salmonids, may provide the basis for future vaccination of hatchery stocks.

Whirling Disease in the United States

Introduction

Whirling disease is a parasitic disorder of salmonids caused by *Myxobolus cerebralis*. The parasite attacks the cartilage of infected trout, and can cause skeletal deformities, particularly of the head; darkening of the tail; a loss of equilibrium and frenetic tail-chasing (from which the disease takes its name); and mortality. Because of its sometimes dramatic impacts on fish culture, *M. cerebralis* was considered a serious pathogen of salmonids until about 1980. By the late 1980s, accepted culture practices minimized the impacts of whirling disease under aquaculture conditions. Since there were no reports of serious problems with the parasite in natural settings, many fish health professionals viewed *M. cerebralis* as an undesirable parasite, but one which could be managed around. This prevailing attitude was reflected in the decision of the Colorado River Wildlife Council in 1988 to downlist *M. cerebralis* from prohibitive to notifiable status. The U.S. Fish and Wildlife Service also removed *M. cerebralis* from its list of injurious wildlife (used in regulating imports) in 1993.

New attention has been focused on whirling disease since 1994, when studies from rivers in Colorado and Montana suggested that whirling disease was associated with dramatic declines in wild rainbow trout populations. This paper was developed in response to this renewed interest in whirling disease. It provides a summary of current information on the biology of whirling disease, an outline of the known distribution of the parasite in the United States, a review of inspection and control protocols in place in different states, recommendations for research, and interim management considerations. A listing of major references, organized by subject, is also included (Appendix C). The summary paper was developed by Trout Unlimited and reviewed by an advisory panel of 13 scientists from the U.S. and Canada (see inside

front cover). It is hoped that this report will provide useful information and guidance for fishery managers and interested members of the public.

A Brief History of Whirling Disease in the United States

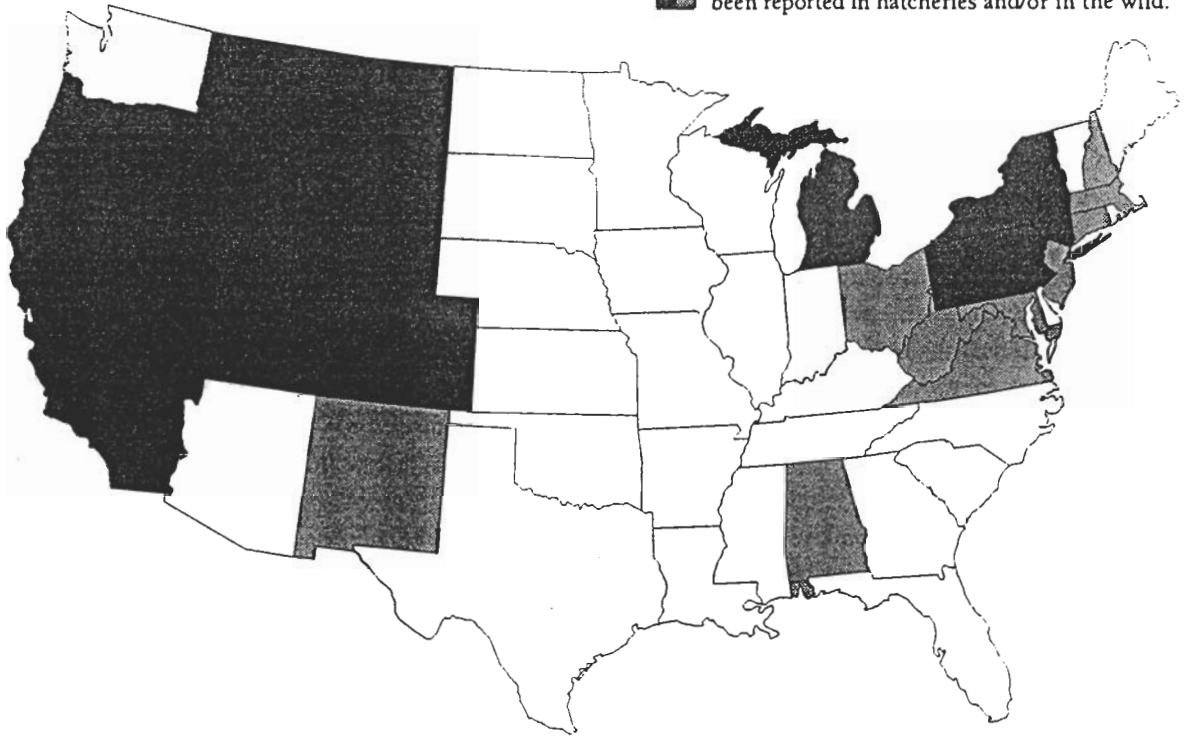
Whirling disease was discovered in 1893, when Hofer reported the disease from rainbow trout in Germany and proposed the name *Myxobolus cerebralis* for the parasite (Hoffman 1990). Subsequent reports of the parasite were initially focused in Europe; this, coupled with the relative resistance to whirling disease observed in brown trout, suggested that the parasite originated in Europe as a non-pathogenic parasite of brown trout (Halliday 1976). Only when susceptible rainbow trout imported from North America were exposed to the parasite was it discovered.

Whirling disease was first diagnosed in the United States in 1958 at the Benner Spring Fish Research Station in Pennsylvania; it has been speculated that *M. cerebralis* arrived at the facility in 1956 through frozen trout imported from Europe (Hoffman et al. 1962). Around the same time, the parasite was found in Nevada, then in Connecticut (1961), Virginia (1965), California (1966), and Massachusetts (1966) (Hoffman 1990). Since that time, *M. cerebralis* has been found in a total of 21 states: Alabama, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nevada, New Hampshire, New Jersey, New Mexico, New York, Ohio, Oregon, Pennsylvania, Utah, Virginia, West Virginia, and Wyoming. A list of known contaminated hatcheries and watersheds from these states is included in Appendix A.

The response to new reports of whirling disease was sometimes very forceful. Over a 22 year period, the state of California destroyed more than 165 tons of fish from contaminated facilities (Manzer 1988). The state of Michi-

Whirling Disease in America

- 11 states that currently report *Myxobolus cerebralis* in wild fish.
- 21 states in which *Myxobolus cerebralis* has been reported in hatcheries and/or in the wild.



gan imposed a quarantine on contaminated facilities and treated the North Branch of the Tobacco River with chlorine in the early 1970s in an effort to disinfect the river system (Hnath 1988). In an early report, Hoffman et al. (1962) recommended destruction of all fish from contaminated facilities; use of a non-contaminated water supply for the hatching house; chemical disinfection of hatchery facilities, ponds, and raceways; and no transfer or stocking of fish from affected facilities.

More recently, fish managers have generally favored milder approaches with an emphasis on avoiding spread of the pathogen to new waters while allowing for management with the parasite in contaminated areas. Hoffman (1990) recommended management measures for contaminated facilities to minimize disease, including the use of concrete raceways, disinfection of earthen ponds, rearing of fry in spore-free water, and use of resistant strains of fish. In a 1988 meeting convened by the Colorado River Wildlife Council Fish Disease Subcommittee, the majority of attendees recommended that

M. cerebralis be reclassified from prohibited to notifiable status and that fish with the parasite be used only where the pathogen is already established or in locations where it was unlikely to be established (Anonymous 1988). The U.S. Fish and Wildlife Service also removed *M. cerebralis* from its list of injurious wildlife (used in regulating imports) in 1993.

In 1993, whirling disease was reported in Colorado in part of the Colorado River that was experiencing recruitment failure in wild rainbow trout, while brown trout populations appeared less affected (Walker and Nehring 1995). In 1994, Montana reported whirling disease in sections of the Madison River which had suffered a 90% decline in estimated rainbow trout numbers (D. Vincent, personal communication). These reports have renewed concern about whirling disease among fish managers and the public, though the emphasis has shifted from concern about impacts on fish culture to concern about impacts on wild populations of trout — an issue which had been only minimally investigated previously.

Life Cycle and Description of *M. Cerebralis*

While the pathogenic effects of whirling disease have been known for almost a century, an understanding of the life cycle of *M. cerebralis* proved elusive. Successful infection of fish in the laboratory depended on exposing fish to tanks holding mud in which *M. cerebralis* spores had been "aged" for approximately four months (Hoffman and Putz, 1969). The mechanism of infection remained unknown until Markiw and Wolf (1983) found that tubificid worms were a necessary alternate host for the parasite; experimental infections were achieved only in the presence of tubificids. Later work showed that the infective unit for fish was a triactinomyxon spore (Wolf and Markiw 1984) and that the alternate host was *Tubifex tubifex* (Wolf et al. 1986). In brief, it was proposed that *M. cerebralis* spores from fish (hereafter referred to as "spores" or "myxospores") initiated an infection in *T. tubifex* that culminated in the production of triactinomyxons (or "actinospores"), which in turn initiated an infection in salmonids that ultimately produced myxospores. Antigenic homology of the myxospore and actinospore forms was demonstrated, lending further support for this hypothesis (Markiw 1989b). The proposed life cycle was initially disputed (Hamilton and Canning 1987), but later confirmed by El-Matbouli and Hoffman (1989) and Hedrick (1990). Molecular studies offered further confirmation of the relatedness of the triactinomyxon and myxosporean forms (Andree and Hedrick 1995). Several other myxosporean species have also been shown to require aquatic oligochaetes as alternate hosts (see summary in El-Matbouli et al. 1995).

Myxospores of *M. cerebralis* are nearly circular in front view. In side view, the spores are lenticular in shape, with two vaulted shell valves joined along a suture line. The spores are often asymmetrical. A prominent furrow is visible in both valves parallel to the suture. The myxospore is extremely small; dimensions are on average 8.7 μ m long by 8.2 μ m wide by 6.3 μ m thick (1 μ m=0.001 mm). A mucous envelope is found around the posterior end of the spore. Spores contain two ovoid polar capsules measuring approximately 5 μ m by 3 μ m and containing coiled polar filaments. The sporoplasm with two nuclei fills the rest of the spore (Lom and Hoffman 1971). Further discussion of spore morphology (based on thin-section electron microscopy) can be

found in Lunger et al. (1975), who generally confirmed the description of Lom and Hoffman except that no mucous envelope was observed. Hedrick et al. (1991) found occasional mucous envelopes.

Myxospores of *M. cerebralis* are found in the cartilage of infected fish and become imbedded in the fish's skeleton as bone deposition replaces the cartilage. Uspenskaya (1957) reported *M. cerebralis* spores in other organs and tissues (including brains; muscles; liver; gall bladder), but her findings have not been confirmed. Location of spores in the cartilage/skeletal tissues continues to be regarded as a critical element in verifying that spores are *M. cerebralis* (Hoffman 1990). Myxospores are released into the environment by decomposition of dead fish or after passing through the digestive tract of predators (Markiw 1992c). Spores may also be released from live fish. Researchers in the Soviet Union (Uspenskaya 1957; Bogdanova 1960) indicated that spores are released from infected fish in the feces, though more recent studies have been unable to confirm this. Taylor and Haber (1974) noted the presence of *M. cerebralis* myxospores in external cysts on the operculum of infected cutthroat trout; they speculated that rupture of these cysts could also lead to transmission of spores from live fish.

Myxospores are extremely persistent. Hoffman et al. (1962) suggested that spores remain viable for up to three years; others have suggested that spores maintain their viability for as many as 12 or even 30 years (Halliday 1976). In addition to a relatively long survival time (by any account), spores of *M. cerebralis* are extremely resistant to a variety of environmental factors. Spores maintain viability when frozen at -20°C (El-Matbouli and Hoffmann 1991b; Hoffman and Putz 1971; Putz 1969), exposed to a variety of chemical treatments (Hoffmann and Hoffman 1972), and after passage through the digestive tract of predators (El-Matbouli and Hoffmann 1991b; Meyers et al. 1970; Taylor and Lott 1978). Spores are less resistant to extreme heat (Hoffman and Putz 1969; Wolf and Markiw 1982).

The next stage of development occurs when spores released from the fish host are ingested by *T. tubifex*, a tiny organic-pollution-tolerant aquatic oligochaete worm which is fairly closely related to common earthworms. Upon contact with epithelial cells in the lining of the worm's gut, the myxospore extrudes its polar filaments

into the cell and the sporoplasm is expelled from the spore and penetrates the host cell. Within the gut epithelium of *T. tubifex*, the parasite develops into the triactinomyxon phase which is infective to trout. Triactinomyxon spores and pansporocysts (spore-forming stages) can first be found in worms approximately 3 months after exposure to the myxospores, and free floating actinospores appear shortly afterward (Wolf and Markiw 1984; El-Matbouli and Hoffman 1989).

The triactinomyxon is much larger than the myxospore and shaped like a grappling hook. It is topped by an epispore about 36µm long, containing 64 spherical sporoplasms, and capped with three polar capsules. Below the epispore the style extends another 90µm, then attaches to three tapering, tail-like appendages that are approximately 170 µm long. These tapering arms provide buoyancy and possibly a means for the triactinomyxon to lodge into the host fish (Wolf and Markiw 1984).

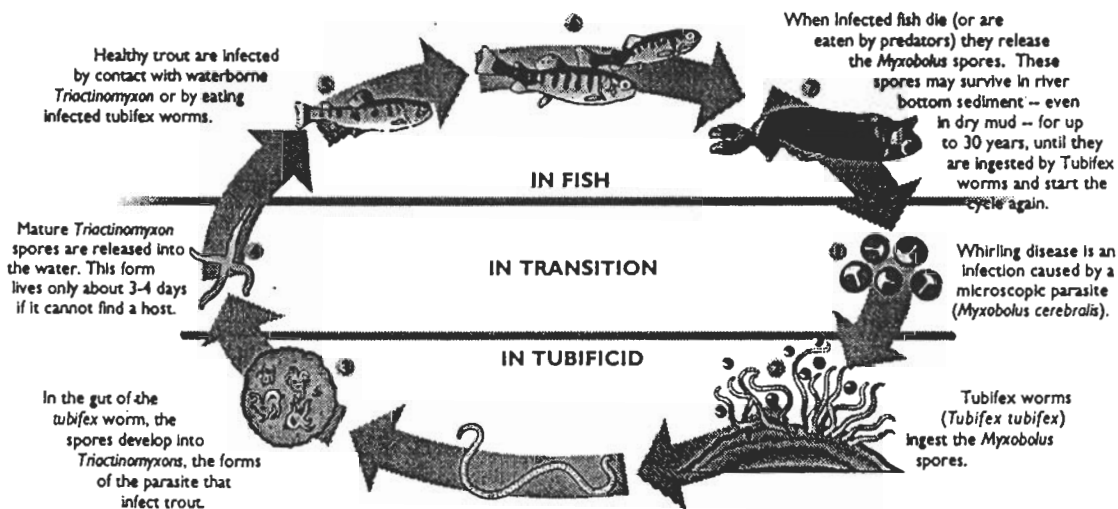
At 12.5°C, release of the waterborne triactinomyxon stage begins 104-113 days after worm exposure to myxospores. Release is greatest during the next 15-60

days, then declines rapidly, but continues at low levels for several months. While triactinomyxons are no longer detected 9 months after worm exposure, sentinel fish placed in tanks with the worms become infected as long as one year after exposure (Markiw 1986). While triactinomyxon release continues over a long period of time, the triactinomyxons themselves are relatively fragile. The triactinomyxon form remains viable for 3-4 days after release at 12.5°C and for less time at warmer temperatures (Markiw 1992b).

Salmonid fish are exposed to the parasite either through contact with waterborne triactinomyxons or through ingestion of infected *Tubifex* worms (Wolf and Markiw 1984). Within 5 minutes post-exposure, triactinomyxons begin to attach to the epidermis of trout and extrude their polar filaments into the epidermis. This anchors the triactinomyxon to the host cell while the sporoplasms penetrate into the epidermis of trout at 10 minutes post exposure (El-Matbouli et al. 1995). This initial penetration takes place in the epithelium of fins, skin, gills, and the digestive tract (Markiw 1989a). The triactinomyxon spores recognize specifically their salmo-

The Life Cycle of Whirling Disease

Parasites attack developing cartilage.
Recently hatched fry may be killed at this stage. Infected trout show "black tail" and develop whirling behavior (both caused by pressure upon the nerves due to infection) at about 1 to 1 1/2 months. In 3-4 months, *Myxobolus* spores appear within the fish. Surviving trout may be deformed, or simply act as carriers of the disease.



Stuart Armstrong

nid hosts and do not extrude polar filaments when they come in contact with other species; how they recognize their host is unknown (El-Matbouli et al. 1995).

During the first 60 minutes following penetration, the sporoplasm aggregates remain compact and migrate intercellularly in the epidermis and gill epithelium. After 60 minutes, the cell enveloping the sporoplasms disintegrates and each sporoplasm penetrates a host epidermal or gill epithelial cell. The sporoplasm cells then undergo an endogenous cleavage producing an inner secondary cell within an enveloping primary cell (El-Matbouli et al. 1995).

Secondary cells then proliferate through rapid, synchronous mitosis, and the host cell nucleus is compressed between the large parasitic aggregate and the host cell plasmalemma (El-Matbouli et al. 1995; Daniels et al. 1976). The secondary cells then undergo endogenous divisions to produce new cell-doublets with an enveloping cell and inner cell. These cell-doublets rupture the membrane of the original primary cell and enter the host cell cytoplasm (El-Matbouli et al. 1995). At this point, some cell-doublets seem to be destroyed within the cytoplasm of the host cell (El-Matbouli et al. 1995; Daniels et al. 1976).

Cell doublets then penetrate the host cell membrane and move into the intercellular space. The extracellular doublets either migrate deeper into the dermis and subcutis or penetrate neighboring epithelial cells where they start the cycle anew (El-Matbouli et al. 1995). The peak of infection in the outer epithelium cells is 2 to 4 hours post-exposure; after 24 hours only a small number of single stages were recognizable (Markiw 1989a; El-Matbouli et al. 1995).

By 2 days post-exposure, aggregates of cell-doublets can be found intercellularly in the subcutis. These stages continue the proliferative cycle of secondary cell mitosis, followed by endogenous divisions to form cell doublets. The parasites then migrate intercellularly in nervous tissue (around 4 days post exposure), with proliferation of cell-doublets continuing while the parasite migrates into the central nervous system. From day 6-14 most parasitic stages are found in the spinal cord; from day 16-24 most are found in the brain. The use of the central nervous system for migration by the parasite may shield it from any host immune reaction during this stage (El-Matbouli et al. 1995).

At 20 days post exposure, parasitic cell-doublets be-

gin to move from nervous tissue into the cartilage. In the cartilage, the primary cell nucleus divides to form many vegetative nuclei, while the inner secondary cell divides to produce generative cells. This plasmodium (or "trophozoite") stage digests the surrounding cartilage matrix (El-Matbouli et al. 1995).

When a plasmodium disintegrates, the released secondary cells repeat the cycle, forming new plasmodia. Around 80 days post-exposure, some free secondary cells, instead of continuing this cycle, will join with one cell enveloping the other and the enclosed cell dividing to form a pansporoblast. Each pansporoblast ultimately produces two spores. For each, two valvogenic cells (which become the spore valves) enclose two capsulogenic cells (which become the polar capsules) and a binucleate sporoplasm (El-Matbouli et al. 1995). When mature, these spores are the form of *M. cerebralis* that is infective to *T. tubifex*.

Taxonomy of *M. cerebralis*

Myxobolus cerebralis (formerly "*Myxosoma*" *cerebralis*) is a member of the Class Myxosporea in the protist Phylum Myxozoa. In light of the recently described two-host life cycle for *M. cerebralis* and several other Myxosporeans, dramatic taxonomic and nomenclatural changes have been proposed for the Myxozoa, which includes the classes Myxosporea and Actinosporea. Since myxosporeans and actinosporeans now appear to be not different organisms but simply alternating life stages, Kent et al. (1994) have proposed suppressing the newer class Actinosporea in favor of the more senior name Myxosporea.

While myxozoans have long been considered protozoans, they exhibit multicellularity and cell differentiation to a greater degree than other protozoans. Recent phylogenetic analysis of ribosomal RNA from myxozoans (Smothers et al. 1994) suggested that myxozoans are closely related to bilateral animals, and that they should be considered a metazoan phylum rather than protozoan. Siddall et al. (1995) used additional molecular and morphological data to suggest that myxozoans are in fact parasitic cnidarians.

Impacts of Whirling Disease

The impacts of whirling disease on susceptible fish can be spectacular. Some readily noted signs of severe dis-

ease include (Uspenskaya 1957; Bogdanova 1960; Hoffman et al. 1962; Hoffman 1962, 1966; Elson 1969; Roberts and Elson 1970; Markiw 1992c):

- ▶ frenetic tail chasing (“whirling”) by fish when feeding or alarmed, often to the point of exhaustion; caused by damage to the cartilage and a granulomatous response around the organs of equilibrium; dissipates in older fish
- ▶ darkening of the tail in fish; caused by pressure on the nerves which control the caudal pigment cells; dissipates in older fish
- ▶ permanent skeletal deformities (sometimes of the spine, primarily of the head); caused by cartilage damage and inflammatory response interfering with normal bone formation; examples include misshapen cranium, shortened operculum, misaligned jaws, and spinal curvature
- ▶ death of young highly susceptible fish

The presence of these signs does not in itself confirm whirling disease; other factors can lead to many of the same signs in trout (e.g., Wolf et al. 1981). Similarly, fish may be infected subclinically and show no external signs of disease (e.g., Halliday 1974a). The American Fisheries Society Fish Health Section diagnostic procedures use detection of *M. cerebralis* spores for diagnosis of whirling disease (Lorz and Amandi, 1994).

Histological inspection of infected fish reveals further pathological impacts. Cartilage tissue is visibly eroded by the trophozoite forms of the parasite; granulomatous lesions (the host inflammatory response) are found in association with the cartilage destruction (Lucky 1970; Taylor and Haber 1974; Hedrick et al. 1991). Uspenskaya (1982) reported that destruction of cartilage is accomplished both through extracellular digestion (lysis) of the cartilage matrix and by phagocytosis of cartilage cells. External cysts containing spores of *M. cerebralis* have also been found along the operculum of infected fish (Taylor and Haber 1974).

Severe cases of whirling disease can lead to death in young fish. Early reports of whirling disease frequently referred to (sometimes heavy) mortalities associated with the disease (Uspenskaya 1957; Hoffman 1962, 1966, 1974; Hoffman et al. 1962; Elson 1969; Havelka

and Volf 1970; Roberts and Elson 1970; Hastein 1971). More recently, Markiw (1991) found heavy mortalities among 2-day old rainbow trout sac-fry exposed to different concentrations of triactinomyxons (mortality ranged from 68% at 10 triactinomyxons/fish to 100% at 1000 triactinomyxons/fish; 4% of uninfected control fish perished).

Whirling disease can also result in compromised performance among infected fish. Some researchers have noted lower growth rates in infected fish than in uninfected controls (Uspenskaya 1957; Havelka and Volf 1970; Hastein 1971; Hoffman 1974), though infected fish were not necessarily stunted (Roberts and Elson 1970). Infected fish may also be more vulnerable to other factors such as parasites, bacterial or viral diseases, and malnutrition (Hoffman et al. 1962). In general, the introduction of any disease-causing organisms leads to reduced performance and increased sensitivity to other stressors (Goede 1986).

Fish do offer some immune response to infection by *M. cerebralis*; Griffin and Davis (1978) detected circulating antibodies in infected rainbow trout. The immune response is likely compromised, however, by the migration of the parasite through the central nervous system, an immunologically privileged area. In their study of the parasite's life cycle, El-Matbouli et al. (1995) found no evidence for contact of the parasite with blood or immunocompetent cells (which could trigger an immune response) during its migration. Interestingly, El-Matbouli et al. (1995) mentioned observing, but did not elaborate on, an acquired immunity among older fish.

The intensity of infection in parasitized trout depends on a variety of factors.

Environmental conditions. Halliday (1973b) found that the parasite developed more rapidly and that disease signs were more common in fish held at higher water temperatures. In general, environmental stressors such as pollution, crowding, or abnormal temperatures will make fish more susceptible to disease (Goede 1986).

Infective dose. As fish were exposed to increasing dosages of triactinomyxons, parasitism (as measured by spore numbers) became more severe (Markiw 1992a). The myxospore burden appeared to plateau at doses of 10,000-100,000 triactinomyxons/fish.

Fish age. The severity of infection decreases with increased age of fish (Markiw 1992a). In older fish, much

of the cartilage susceptible to infection has been converted to bone, making fish more resistant to disease (Halliday 1976). Other reasons for the increased resistance of older fish to disease may include physiological changes in the skin (Markiw 1992a) and acquired immunity (El-Matbouli et al. 1995). While younger fish are generally more vulnerable to disease, eggs and newly hatched sac-fry exposed to infective units do not develop infection (Putz and Hoffman 1966; Markiw 1991). Either those infected with initial forms of whirling disease did not survive, or their underdeveloped organs did not provide conditions that lead to infection (Markiw 1991).

Fish species. Different species (and perhaps strains) of fish differ in their susceptibility to whirling disease. After experimental infections, O'Grodnick (1978a, 1979) observed clinical signs of whirling disease in rainbow trout, brook trout, sockeye salmon, and chinook salmon. No clinical signs were present in brown trout, lake trout, and coho salmon, and no spores were found in lake trout. Rainbow trout were most susceptible to disease. Brook trout, sockeye salmon, and chinook salmon were intermediate in susceptibility. Coho salmon were usually refractory to infection (but occasional spores were found), while lake trout were always refractory (O'Grodnick 1979). In earlier work, however, lake trout were infected with *M. cerebralis* (Hoffman and Putz 1969). While brown trout are usually regarded as resistant carrier fish, they can suffer from clinical whirling disease (Walker and Nehring 1995). Other species found in the United States which can become infected include cutthroat trout (Yasutake and Wolf 1970), steelhead (Horsch 1987), grayling (Havelka and Volf 1970), Atlantic salmon (Hoffman 1990), golden trout (Anonymous 1968), and whitefish (B. Hutchinson, personal communication).

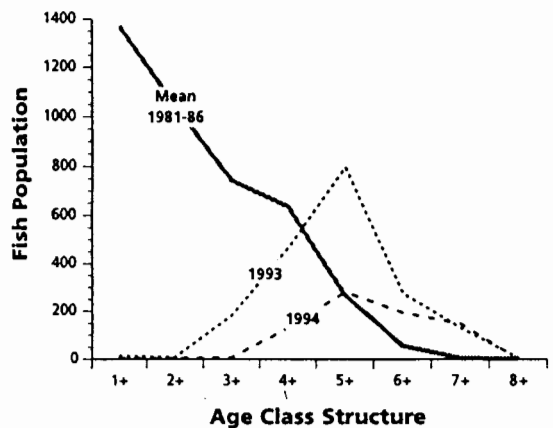
Whirling Disease in Wild Populations

Most research on whirling disease has focused on laboratory or fish culture settings. It has been widely thought that impacts on wild populations are minimal, but detailed studies on susceptible young fish in contaminated waters have been lacking (Anonymous 1988). Over the last year, however, there has been increased interest in this topic and new research has been conducted (refer to the section on ongoing research for more information).

The most extensive work reported thus far comes from

Whirling Disease and Trout Populations

Colorado River Rainbow trout population age structure within the Kemp/Breeze Wildlife Area, Young fish are notably absent.



Source: Walker & Nehring 1995

Colorado. Through routine population sampling, a highly unusual age structure had been found among rainbow trout in the Middle Park section of the Colorado River. The most recent year classes were virtually unrepresented. Brown trout populations had a more normal year-class distribution. Walker and Nehring (1995) conducted studies to assess possible causes for the disappearance of rainbow trout year classes.

Walker and Nehring (1995) reported that whirling disease was implicated as a factor, though not necessarily the sole cause, contributing to the observed decline in rainbow trout recruitment. In examinations of free ranging trout, clinical signs of whirling disease were observed; histology confirmed the presence of *M. cerebralis* and associated pathology. Clinical signs of disease were observed in brown trout as well as rainbow trout, but signs were more severe in rainbow trout. Post-winter survivorship among wild rainbow trout fry was dramatically lower than among brown trout fry (3.2% compared to 33.5%). Sentinel fish held in cages in the river also exhibited clinical whirling disease. Among sentinel fish, overwintering mortality was much higher in exposed rainbow trout (73%) than in exposed brown trout (7%) (Walker and Nehring 1995).

Factors other than whirling disease were also impli-

cated. Exophthalmia was observed among free-ranging rainbow trout, and virtually all fingerling trout examined displayed high amounts of gas in the eye sockets. Light to moderate levels of gas supersaturation were found in the river. It was suggested that gas bubble disease could be an additional factor in the decline of recruitment, perhaps acting synergistically with whirling disease (Walker and Nehring 1995). Bacteria, external fungi, and ectoparasites were also detected, but appeared to be opportunistic and of importance primarily as exacerbating stressors (Walker and Nehring 1995).

Field sampling in Montana has also revealed dramatic population declines in wild rainbow trout (as much as 90% from historic levels) in sections of the Madison River contaminated with *M. cerebralis*, while brown trout populations have remained stable (D. Vincent, personal communication). Histology has demonstrated severe disease (cartilage destruction, granulomatous response) in wild fish from the river (B. MacConnell, personal communication). Whirling disease is believed to be the cause of the decline, as other factors (such as water temperature, flows, water quality) have apparently remained well within normal levels (D. Vincent, personal communication).

Reports from Montana and Colorado have associated whirling disease with population impacts in wild trout. In other areas, there is a long record of professional experience suggesting that whirling disease has not been a problem in free-ranging fish (Anonymous 1988). Clinical signs have only rarely been observed in wild fish, and no population effects have been observed. It remains unknown why whirling disease appears to be a problem in some settings and not in others.

One possible reason is habitat factors. O'Grodnick (1978b) noted that whirling disease infectivity appeared to be greater in high-productivity streams. Despite the stocking of infected fish, whirling disease did not become established in wild populations of rainbow, brown, or brook trout in several relatively infertile mountain streams with low trout numbers. In contrast, infection became established among brown trout in a highly productive limestone stream to which the parasite had been introduced (O'Grodnick 1978b). Modin (personal communication) noted a serious outbreak of whirling disease in a California hatchery that uses a contaminated high-gradient stream as a water supply. Infection was barely detectable in fish from the stream; however, fish

in the hatchery, reared in water that had passed through sediment-laden settling pools, suffered from severe clinical disease. In light of current knowledge about the parasitic life cycle, these differences in infection may be related to the differential availability of suitable *T. tubifex* habitat. However, further research is needed to determine what factors may be involved in the dramatically different experiences being reported in connection with whirling disease in wild populations.

Vectors for the Spread of Whirling Disease

One major vector for the spread of disease is the movement of live fish that carry *M. cerebralis* in association with fish culture and stocking activities. States have primary authority over fish and wildlife management, and have taken a variety of approaches to addressing the spread of disease through fish transfer with both public and private fish culture. A survey was conducted of states to determine what policies were in place with regard to fish culture. Responses were returned from 44 states, of which 4 reported having no salmonid culture. A table summarizing the responses appears as Appendix B.

Among the 14 states that currently report whirling disease, two different approaches have been taken to addressing it. One focuses on minimizing the occurrence of pathogens in general, the other on preventing the spread of pathogens into new waters. Under the first approach, states allow no new *M. cerebralis* to be added to any aquatic systems through the stocking of fish. The second approach is to allow use of infected fish only in waters where whirling disease is enzootic or where infected fish have been stocked previously. States with known *M. cerebralis* contamination have split evenly in choosing between these approaches (see Appendix A).

Among all states, the vast majority (80%) conduct regular fish health inspections for public facilities, though 8 states reported that fish health work was done only on an as-needed basis in response to observed disease problems. Private facilities are subject to much less scrutiny; only one in three states (13/39) requires regular fish health inspections for private fish culture operations. A lack of specific pathogen inspections makes management of pathogen spread difficult.

Survey responses suggest that the most common fish health management strategy for states is to focus on

pathogens crossing the state border. Nearly all states (85%) have some regulation of fish imports into the state, usually with fish health inspection as a requirement. Only 6 states reported no import restrictions. Intrastate movement of fish, on the other hand, is regulated by only a handful of states. Only 28% of states (11/39) reported regulating movement of fish between facilities within the state. It appears that in many states, once a pathogen makes it across the border, it could spread widely. It was assumed that all (or nearly all) states would require stocking permits for release of fish into public waters. However, insofar as escaped fish and effluent from contaminated facilities can spread infection into natural systems, the lack of regulation on intrastate transfer of fish could contribute to the spread of pathogen in the wild. Similarly, infected fish found in public waters could spread the pathogen to aquaculture facilities.

Once established in a natural system, whirling disease can spread as infected fish move up or down stream and water-borne triactinomyxon forms are carried downstream. Yoder (1972) reported that over a three year period, whirling disease spread six miles downstream and 1,500 feet upstream from a point of initial infection at a Michigan hatchery.

While whirling disease in the USA is likely to have spread primarily through the transfer of live fish and by movement of infected fish within streams (Hoffman 1990), the extreme persistence of *M. cerebralis* spores allows for many other vectors of transmission.

Whirling disease could be spread through shipments of fresh, frozen, or brined food fish infected with *M. cerebralis*. Spores remain viable when frozen at -20°C for at least 3 months (El-Matbouli and Hoffmann 1991b). Brined fish also retain viable spores, though hot-smoking at 66°C inactivates spores (Wolf and Markiw 1982). Imports of frozen rainbow trout from Europe are believed to have initially brought *M. cerebralis* to the United States (Hoffman 1990), though this has not been confirmed.

Predators may also spread the parasite to new waters. Spores of *M. cerebralis* survive passage through the alimentary canal of avian predators (Meyers et al. 1970; Taylor and Lott 1978; El-Matbouli and Hoffmann 1991b). Fish-eating birds could eat infected fish, then shed viable spores in their feces into uncontaminated waters. It is not known how significant this vector may be in spreading *M. cerebralis*.

Transfer of fish eggs is not a likely means for transfer of the parasite. Whirling disease is not transmitted vertically, from infected brood fish to eggs (O'Grodnick 1975a). Markiw (1991) also found that eyed eggs exposed to triactinomyxons do not become infected. The parasite could be spread, however, through contamination of egg shipments (Hoffman 1990). Proper disinfection would eliminate this possibility.

Movement of infected *Tubifex* worms (used in association with the aquarium industry) may be another vector for the transfer of *M. cerebralis*. The persistence of the myxospores also makes it possible that infection could be spread by anglers through mud carried on boats, trailers, boots, or other items moved between contaminated and uncontaminated waters. Anglers may also transfer *M. cerebralis* through movement of bait and game fish (or through the water in which the fish are held). It is not known how significant these vectors may be in spreading *M. cerebralis*.

Control of Whirling Disease

A great deal of research has been directed at developing ways to control whirling disease in fish culture settings. The approaches considered range from ultraviolet irradiation of water supplies to administering drugs to exposed fish.

Hoffman (1974) found that irradiation of contaminated water with 2537 Ångstrom wavelength ultraviolet light at a dosage of 35,000 microwatt sec/cm² was effective in preventing infection. Lower dosages reduced but did not completely eliminate infection (Hoffman 1975).

A variety of chemicals have been tested for effectiveness in disinfecting contaminated facilities. Hoffman and Hoffman (1972) found that 1.0%, 0.5% and 0.25% calcium oxide and 1.0% and 0.5% potassium hydroxide killed spores of *M. cerebralis*. Quicklime (CaO) was also effective in simulated pond settings. Putz and Hoffman (1969) found the following effective in killing spores: 0.5% and 2% calcium hydroxide; 1600 ppm available chlorine (as sodium hypochlorite); and 200 and 800 ppm (active ingredient) Roccal.

Heat is also effective in deactivating spores. Hot-smoking of fish at 66°C rendered spores nonviable (Wolf and Markiw 1982), as did heating at 90°C for 10 minutes or 70°C for 100 minutes (Hoffman and Markiw 1977).

The use of medicated feed has also been proposed for

controlling whirling disease. Taylor et al. (1973) found furazolidone to be effective in reducing spore counts in fish. Fumagillin dicyclohexylamine was also found to be effective in combatting infection and disease (El-Matbouli and Hoffman 1991a). Alderman (1986) also reported encouraging preliminary results on the use of Clamoxiquin and Proguanil hydrochloride.

Hoffman (1990) offered more basic recommendations for minimizing disease at contaminated facilities: convert to concrete raceways; raise fry in spore-free water for as long as possible (but at least until they are 6 cm long); disinfect any earthen rearing ponds yearly; and use resistant species of fish. While these measures will help to prevent disease, fish may still be carriers of *M. cerebralis*.

Ongoing Research

Results from research conducted during the 1995 field season should become available before Spring 1996. The research which has taken place includes:

California. Researchers at the University of California-Davis are working to develop a polymerase chain reaction probe (DNA test) that can be used to identify *M. cerebralis* in any of its stages, both in worms and in fish (R. Hedrick, personal communication). In addition to value in diagnosis, the test would be useful in determining whether there are different (perhaps more pathogenic) strains of *M. cerebralis*.

Colorado. Research continues on the issues identified by Walker and Nehring (1995), both in the Colorado River and in other rivers. As in the Colorado, declines in recruitment have been observed in several other rivers contaminated with whirling disease: Cache la Poudre, South Platte, Rio Grande, and the Gunnison. Sentinel fish observations are also underway with cages held in the Colorado River. At several different times during the summer, different ages of brook, brown, cutthroat, and rainbow trout fry were placed in sentinel cages and have been monitored for impacts of disease. Further investigations into the role of gas supersaturation are also being conducted (B. Nehring, personal communication).

Idaho. Work on the molecular biology of Myxozoans continues, with phylogenetic analyses planned with dif-

ferent genes to further test the proposal advanced by Smothers et al. (1994) that myxozoans are metazoans. Additional streams have been surveyed for the possible presence of *M. cerebralis* infections in wild trout. Sentinel fish are also being used to monitor for infectivity in streams (D. Spall, personal communication).

Montana. In addition to extensive testing through the state to identify contaminated waters, a range of research has been conducted in 1995. Studies using sentinel fish are being used to assess the susceptibility of westslope cutthroat trout, arctic grayling, and Eagle Lake rainbow trout. Rainbow trout from six weeks and ten weeks post-hatch have been exposed to contaminated streams and monitored to see what effects age has on the development of disease. Sentinel fish have been exposed to contaminated water for different lengths of time and monitored for differences in disease development. Oligochaete worm samples have been taken to monitor species composition. Research on the rate at which fish pass through the digestive tract of predator birds should shed additional light on one possible vector for transmission of the disease. Work is also underway to determine mineralization rates in different species, which may be a factor in the severity of disease. Extensive population monitoring continues on the Madison River. A portion of the river has been closed to fishing and is being monitored to see what impact this has on survival of adult fish (B. MacConnell, personal communication).

Utah. A variety of trout hybrids are being tested for resistance to whirling disease using both sentinel cages and the stocking of sterile fish. Among the hybrids tested are splake (lake/brook), brake (brown/lake) trout, and tiger (brown/brook) trout. Over recent years, trout have been eradicated from sections of the Fremont River drainage using Rotenone. It is hoped that if these waters are kept salmonid-free for a number of years, all spores remaining in the system will be processed by tubifex worms. Since short-lived triactinomyxons will find no host, infection could thereby be eliminated. Initial results have been mixed, and work will continue to determine whether this proposed method of eradication can be effective (C. Wilson, personal communication).

Research Recommendations

A review of current knowledge on whirling disease demonstrates one fact above all: we still know far too little. The TU Whirling Disease Advisory Panel discussed a variety of research topics and highlighted two major areas of research as highest priority and a third as high priority. These topics are outlined in greater detail below.

Highest Priority

- ▶ Development of an enhanced, rapid, and cost-effective diagnostic test for *Myxobolus cerebralis*, such as a DNA-based test

The current diagnostic procedures for *M. cerebralis* rely on direct observation of the parasite in tissue sections or as extracted from tissues by mechanical and enzymatic treatments. These approaches are time consuming, expensive, and suffer from a lack of sensitivity. In early infections, the spore of the parasite may not be present, therefore confirmation of infection, particularly in the absence of microscopic pathology, may be difficult or impossible. In older fish, microscopic lesions may be absent and spore numbers may be low, particularly in lightly-infected fish. Development of a DNA-based test, with the ability to amplify (e.g. polymerase chain reaction) a parasite specific sequence, would solve several of these problems. Such a test could be applied for detection of the parasite in the oligochaete host as well. A DNA-based approach has advantages over serological or antigen detection because it can detect all life stages of the parasite, even when parasite antigens may change significantly during development.

- ▶ Improving understanding of the host-parasite relationship and dissemination of the parasite

Little is known about the host-parasite relationship of *M. cerebralis*. For example, in some situations infection can result in severe disease while in others the parasite does not produce disease. Quantitative data is needed on several elements of the life cycle of *M. cerebralis* in

order to predict the consequences of infection in a given aquatic system. Several lines of investigation should be pursued at both laboratory experimental and field ecological levels. Work should be done using standardized methods so that results from different regions will be comparable. Because of the interactive aspects of this work, each of these research directions has equal and highest priority:

- Biological and genetic assessment of *M. cerebralis*. Parasites from different geographic regions should be studied to assess whether there are multiple strains of differing virulence (measured by disease induced and spores produced in a standardized fish strain). If there are different strains, those from different waters could be identified, helping to determine how the parasite has been vectored into and through the United States.
- Research on infection and disease in different species or strains of salmonids. This work could have important applications, as the use of disease-resistant stocks may offer potential for restoring wild populations. Studies would also help determine the risks posed by whirling disease to already threatened native species. Beyond species or strain differences, variables that should be examined in relation to patterns of disease and infection include fish age, water temperature, parasite dose, and density of fish and oligochaete hosts.
- Studies on the oligochaete worm host. Studies are needed to determine whether there are additional alternate hosts beyond *Tubifex tubifex*, whether *T. tubifex* from different regions differ in infectibility and numbers of parasites produced. Additional research is also needed on the dynamics of triactinomyxon spore production in the worm host.
- Studies on early infection. Research is needed to determine how the parasite recognizes its host,

whether fish can be reinfected, and what role environmental stressors may have on infection.

- Field studies on the dynamics of infection and disease in wild fish populations. Standard, reproducible population work needs to be supplemented with study of rates of decline in young-of-the-year trout, and these fish should be tested for incidence of infection, severity of disease, and numbers of spores. The role of environmental variables on patterns of infection/disease should also be investigated.
- Field studies on the dynamics of infection in oligochaete worms. Virtually nothing is known of this topic. The microhabitats, density, and seasonality of *T. tubifex* and other oligochaete populations beg for description. These, in turn, lead to the critical question of spatial, seasonal, and temporal patterns of concentration of triactinomyxons in natural waters. A technique to quantify triactinomyxons in free flowing streams would be very useful in elucidating the dynamics of actinospore release.

High Priority

► Host resistance and immunity to *M. cerebralis*

The basis of the resistance of certain species of salmonids to whirling disease is unknown. Similarly, little is known about the immune response of both susceptible and resistant species of salmonids to the causative agent, *M. cerebralis*. Although most salmonids are susceptible to the parasite, coho salmon, lake trout, and splake (brook trout x lake trout) are noticeably more resistant or refractory. Understanding the host mechanisms for defense in these species should provide insights into characteristics required for resistance. Among susceptible species, a strong cellular response is observed to early invading and certain sporulating stages. Serum antibodies indicating a humoral response have been detected to spores in chronically infected fish. These trout may also resist reinfection. Further characterization of these responses (cellular and humoral) are needed. Identification of key parasite antigens that may contribute to immunity and how the response to these antigens may differ between susceptible and resistant species of salmonids may provide the basis for future vaccination of hatchery stocks. Similar research is needed on mechanisms of host immunity in oligochaete worms. Virtually nothing is known of this topic currently.

Management Considerations

Whirling disease raises many difficult management issues. Previous efforts to control whirling disease impacts have failed in some areas. Innovative, fresh ideas are warranted. Maximal effect will be achieved through collaborative effort with resource managers, users, and the aquaculture industry.

In order to assist managers and the interested public, the following management considerations have been developed by the TU Whirling Disease Advisory Panel, a group of 13 leading scientists and fish managers from throughout North America. These are interim suggestions and may need modification as new research increases our understanding of whirling disease. Both consensus and majority considerations are listed. Where there are differing minority views, they are noted. The Advisory Panel encourages the development of additional innovative management strategies.

These are not definite rules, but considerations that should be weighed in developing specific management strategies. The Advisory Panel recognizes that strategies will differ between areas based on watershed conditions, management objectives, agency structure, and for other situation-specific reasons. We hope that these considerations prove helpful as those specific strategies are developed.

Unanimous Agreement:

Inspection

- ▶ Public and private hatcheries involved in stocking fish should have regular fish health inspections so as to detect subclinical as well as clinical infections of *Myxobolus cerebralis* and other pathogens of concern.

Management and stocking issues

- ▶ All reasonable measures should be used to ensure that *M. cerebralis* is not spread into waters that are currently free of the parasite.

- ▶ Stocking infected fish, even in waters that already hold *M. cerebralis*, increases the pathogen load in the system and should be discouraged.
- ▶ Heavily-infected, clinically-diseased fish should not be stocked.
- ▶ Any restrictions on stocking developed to address whirling disease should provide equal treatment for public and private hatcheries.
- ▶ Wherever possible, control efforts should be designed as experiments (with appropriate controls), so that results can be more broadly used.

Monitoring

- ▶ Management entities should be encouraged to increase monitoring of public waters for fish pathogens of concern, especially in waters with self-sustaining fish populations.
- ▶ Field biologists should be educated about whirling disease so that they can incorporate basic monitoring for the disease into their regular stream surveys.

Hatchery management

- ▶ Where hatcheries have become contaminated, efforts should be made to eliminate infection from those hatcheries.
- ▶ Hatcheries where efforts to eliminate *M. cerebralis* repeatedly fail should be considered for closing.
- ▶ Incentives (such as low-interest loans) should be used to make hatchery clean-up more palatable to private facilities.

Education

- ▶ As practicable, states should implement education programs to inform the public about whirling dis-

ease, responsible angling, the dangers of bait-bucket biology, and related issues.

Majority Agreement:

Inspection and fish transport

- ▶ All public and private hatcheries (including those not directly involved in stocking fish) should be encouraged to have regular fish health inspections for *M. cerebralis* and other pathogens of concern.

A minority of the Advisory Panel felt that hatcheries not involved in stocking fish posed sufficient

risk of spreading *M. cerebralis* that inspection should be required, not merely encouraged.

- ▶ In order to minimize the risk of spreading pathogens of concern, live fish being transported should be accompanied by inspection certificates.

A minority of the Advisory Panel disagreed. Two different reasons for disagreement were cited by two different segments of the minority: (1) such a process would be onerous, and have little value in some states; and (2) the requirement for inspection certificates should extend to dead fish as well as live fish.

Appendix A: Incidence and Control of Whirling Disease

Hatcheries and waters listed are those that have tested positive within the past three years; some past infections are noted. (SFH = state fish hatchery)

State	Infected hatcheries	Infected waters	Control policies
Alabama	No response; independent reports show past infection in fish from one private hatchery	No response	No response
California	Mt. Whitney SFH; Black Rock SFH; private hatchery in Mokelumne River watershed	Found in the South Fork American River, Trinity River, Wolf Creek, Battle Creek, Deer Creek, Garrapata Creek, Big Creek, Carmel River, Aptos Creek, La Honda Creek, and multiple points in these river drainages: Owens River, Lahontan (including the Truckee, Carson and Walker Rivers), Stanislaus River, Calaveras River, Mokelumne River, Feather River, and San Lorenzo River	State stocks no infected fish; some limited private stocking has been allowed in waters where whirling disease is enzootic
Colorado	Mt. Shavano SFH; Poudre Canyon SFH; Chalk Cliffs SFH; Mt. Ouray SFH; Roaring Judy SFH; Finger Rock SFH; Rifle Falls SFH; Watson Lake SFH; Leadville NFH; private hatcheries in Larimer, Chaffee, Douglas, and Park Counties	Found in parts of all major coldwater drainages except for the Animas and North Republican Rivers	Final policy is being developed; currently, stocking is allowed of lightly infected fish only (<10,000 spores/ head) in some wild trout waters where <i>M. cerebralis</i> is already found
Connecticut	Burlington SFH; Kensington SFH	None reported	Exposed fish are stocked only in waters previously stocked from the positive state facilities
Idaho	Hayspur SFH; Sawtooth hatchery; Pahsimeroi SFH; private hatchery in Lost River watershed	Found in the Upper and Lower Salmon River, North Fork Coeur d'Alene, St. Joe River, South Fork Boise River, Big Wood River, Salmon Falls Creek, Warm Springs, Pahsimeroi, Hawley Creek, Lemhi River, Lawson Creek, and Silver Creek	State has limited stocking of anadromous fish exposed to <i>M. cerebralis</i> ; state trout hatcheries do not stock infected fish; private hatcheries cannot stock infected fish in public waters
Maryland	None reported	Found in fish from net pens on the North Branch of the Potomac	No stocking of infected fish is allowed
Massachusetts	Past infection at one private hatchery	None reported	Not applicable (parasite not currently found in state)

State	Infected hatcheries	Infected waters	Control policies
Michigan	Past infections at Sturgeon River SFH and five private hatcheries	Found in Tobacco River	No stocking of infected fish allowed
Montana	None reported	Found in Madison River; Jefferson River; Ruby River; Red Rock Creek; Clark Fork River; Racetrack Creek; Flint Creek; Clark Canyon Reservoir; Willow Creek; Poindexter Slough; Ruby Reservoir; Swan River; Boulder River; Stuart Mill Creek; Blaine Spring Creek; Warm Spring Creek	No stocking of infected fish allowed
Nevada	Gallagher SFH	Some infected fish stocked in Humboldt and Truckee River; in past, infected fish were stocked in Carson River; East Fork Owyhee River; and East Walker River, but state has not sampled wild fish to determine if infection is present	No stocking of infected fish interstate waters; stocking of infected fish limited to waters previously stocked with infected fish
New Hampshire	Past infection in private hatchery near Pelham	None reported	Not applicable (parasite not currently found in state)
New Jersey	Past infection at Hackettstown SFH	None reported	Not applicable (parasite not currently found in state)
New Mexico	None reported	In past, found only in ponds planted with infected fish from out-of-state	Not applicable (parasite not currently found in state)
New York	Caledonia SFH; Rome SFH; Salmon River SFH; Chateaugay SFH; private hatcheries in the Delaware River, Lake Erie, and Mohawk River watersheds	Found in Cattaraugus Creek tributaries; Genesee River tributaries; tributary to Willowemoc Creek; tributary to Mohawk River; Marble River; Keuka Lake inlet (Lake Ontario)	No stocking of infected fish allowed
Ohio	Past infection in private hatchery near Castalia	None reported	Not applicable (parasite not currently found in state)
Oregon	Wallowa SFH	Grande Ronde River; Imnaha River	No transfer of live fish from infected watersheds (Grande Ronde, Imnaha) into other watersheds
Pennsylvania	Tylersville SFH; Benner Spring SFH; Bellefonte SFH; Reynoldsdate SFH; Pleasant Gap SFH; two private hatcheries	Falling Spring Creek, Spring Creek, Fishing Creek; Bear Run; suspected in other unassessed waters	No stocking of infected fish the Lake Erie basin

State	Infected hatcheries	Infected waters	Control policies
Utah	Private hatcheries in the Fremont R. drainage (2); Sevier R. drainage (2); and Bear R. drainage (3)	Found in the Fremont River (and Forsyth and Mill Meadow Reservoirs); UM Creek; Otter Creek; Little Bear River (and Porcupine and Hiram Reservoirs); Blacksmith Fork of the Logan River; South Fork of the Ogden River (and Causay Reservoir)	No stocking of infected fish is allowed
Virginia	Marion SFH; Montebello SFH	None reported	Stocking of infected fish limited to waters previously stocked with infected fish
West Virginia	Past infections at Edray SFH and in private facilities in Preston and Randolph Counties	None reported	Not applicable (parasite not currently found in state); in past, infected fish stocked in waters with no wild trout
Wyoming	None reported	North Platte River; Laramie River	No stocking of infected fish is allowed

Appendix B: Fish Health Inspection and Regulation

State	Inspection requirements	Regulations on fish import and movement between hatcheries
Alabama	No response	No response
Alaska	No response	No response
Arizona	State hatcheries inspected annually; state may inspect any private facility or shipment	Inspection required for live fish imported into the state or transported between stations of different ownership
Arkansas	Periodic inspection of state facility; no inspection required for private hatcheries	Inspection required for imports
California	Wild broodstocks are tested annually; state hatcheries monitored on an ongoing basis; no inspection required for private hatcheries	Inspection required for imports; transfer of fish from infected facilities is not permitted to areas where whirling disease is not enzootic
Colorado	State hatcheries inspected annually at least; annual inspection required for private hatcheries	Annual inspections required for facilities before movement of fish; inspection required for imports
Connecticut	State hatcheries inspected annually at least; no inspection required for private hatcheries	Permits required for import of live fish or fish eggs
Delaware	No salmonid culture	No salmonid culture
Florida	No salmonid culture	No salmonid culture
Georgia	State hatcheries inspected annually (approx.); no inspection required for private hatcheries	State restricts imports from facilities/regions with pathogens of concern
Hawaii	No response	No response
Idaho	State hatcheries inspected annually at least; private hatcheries producing fish for release in public waters must be inspected for stocking permits; no inspection required for private hatcheries in food fish production	State regulates both import and movement of fish between hatcheries
Illinois	No response	No response
Indiana	State hatcheries inspected annually; no inspection required for private hatcheries	Inspection required for imports
Iowa	No regular testing conducted	Inspection required for imports
Kansas	No regular testing conducted	No restrictions
Kentucky	No response	No response

State	Inspection requirements	Regulations on fish import and movement between hatcheries
Louisiana	No salmonid culture	No salmonid culture
Maine	State and private hatcheries inspected annually	Inspection required for imports
Maryland	No regular testing conducted	Permit required for imports
Massachusetts	State hatcheries inspected annually at least; no inspection required for private hatcheries	Inspection required for imports
Michigan	State hatcheries inspected annually; no inspection required for private hatcheries	Inspection required for imports
Minnesota	State and private hatcheries inspected annually	State restricts import and intrastate movement of fish with certifiable diseases
Mississippi	No salmonid culture	No salmonid culture
Missouri	State hatcheries inspected periodically; no inspection required for private hatcheries	Inspection required for imports
Montana	State and private hatcheries inspected annually	Permit required for imports
Nebraska	State and private hatcheries inspected annually	Inspection required for imports; infected facilities quarantined
Nevada	State hatcheries inspected several times a year; no inspection required for private hatcheries	Imports require permit and must be from an approved source
New Hampshire	State hatcheries monitored on ongoing basis; no inspection required for private hatcheries	Inspection required for imports
New Jersey	State hatcheries inspected annually; no inspection required for private hatcheries	No restrictions
New Mexico	State hatcheries inspected annually; no inspection required for private hatcheries	Inspection required for imports
New York	State and private hatcheries inspected annually	Fish imported into state for stocking purposes must be inspected for whirling disease
North Carolina	No regular testing conducted	Permit required for imports
North Dakota	Public hatcheries inspected twice a year; no inspection required for private hatcheries	Inspection required for imports
Ohio	State hatcheries inspected annually; no inspection required for private hatcheries	No restrictions
Oklahoma	No regular testing conducted	Permit required for imports

State	Inspection requirements	Regulations on fish import and movement between hatcheries
Oregon	Most state hatcheries tested annually, some every five years; state may require inspection for private hatcheries	Transport permits required for fish movement; inspection required for imports
Pennsylvania	State hatcheries tested annually; no inspection required for private hatcheries	Transfers between (and imports into) state hatcheries are reviewed
Rhode Island	No regular testing conducted for state facilities; no private trout farms in state	Inspection required for imports; no private trout farms in the state
South Carolina	Federal hatchery tested annually; no inspection required for private hatcheries	No restrictions
South Dakota	Public hatcheries inspected annually; no inspection required for private hatcheries	Inspection required for imports
Tennessee	No regular testing conducted	Inspection required for imports
Texas	No response	No response
Utah	State hatcheries inspected annually at least; annual inspection required for private hatcheries selling live fish	Fish cannot be transferred from infected facilities; inspection required for imports
Vermont	State and private hatcheries inspected annually	Imports and in-state breeder's licenses both require inspections
Virginia	Broodstock hatchery inspected; no regular testing program otherwise	No restrictions
Washington	State hatcheries tested every three years; private hatcheries tested annually	Transport and import of fish require permit; inspection
West Virginia	State hatcheries tested annually; no inspection required for private hatcheries	Inspection required for imports
Wisconsin	State hatcheries tested annually; no inspection required for private hatcheries	Inspection required for imports
Wyoming	State and private hatcheries tested annually; wild broodstock also tested	Fish cannot be transferred from infected facilities; inspection required for imports

Appendix C: Readings on Whirling Disease

Control

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