



Whirling Disease in the United States

A Summary of Progress in Research and Management

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Foreword

Four years ago the term “whirling disease” entered the lexicon of trout fishers throughout the United States. Although it had been in a number of fish hatcheries around the country since at least the 1970s, whirling disease had not troubled most fish managers, who took solace in the absence of proof that the disease had the same deadly effects in the wild that it had demonstrated in a captive fish breeding environment. But then, beginning in 1994, came disturbing reports from Colorado and Montana suggesting that whirling disease was responsible for the decline of such blue-ribbon fisheries as the Madison, the Colorado, the South Platte, and the Gunnison. In short order, whirling disease became a specter haunting the future of American trout fishing.

Having sounded the alarm about whirling disease—and especially about fish management practices that ignored the disease’s threat to fish in the wild—Trout Unlimited, through the Coldwater Conservation Fund, quickly sought to gauge the disease’s potential impact on wild trout and salmon resources. “Whirling Disease in the United States,” issued in 1996, was the first-ever assessment of scientific knowledge and management practices related to whirling disease. The 1996 report, which was peer-reviewed by a distinguished panel of experts, also set forth a blueprint for future research projects and management practices that would improve our ability to contain the disease and overcome the challenges it poses.

Following the report’s issuance, Trout Unlimited successfully urged Congress to appropriate new research funds, launched public education projects in partnership with government agencies and the private sector, and aggressively advocated for reforms in state and federal fish stocking practices. The Coldwater Conservation Fund also helped fund several important research initiatives. Now, several years after whirling disease’s emergence as a recognized threat to coldwater fisheries, we offer a follow-up report, whose purpose is to evaluate progress in implementing the 1996 report’s research and management recommendations.

This report attempts to answer a basic question: What have scientists and fishery managers learned about whirling disease? To start with, it is important to note that no one as yet has been able to explain why whirling disease has had such deadly effects on wild fish in Colorado and in some Montana waters, but has not demonstrated pronounced adverse effects on fish in such places as California and New York. Yet scientists have gained new insight into the environmental factors that seem to influence the intensity of infection. Adding strength to the role of environmental factors, there has been no evidence of genetic differences in *M.cerebralis* (the whirling disease parasite) in samples from a broad geographic range. In addition to the varying influence of environmental factors, researchers have confirmed varying responses to infection among salmonid species. To aid in the disease’s detection, thanks to work done at the University of California-Davis, we now have a quick, cost-effective diagnostic test. These are all solid achievements, made possible by public-private sector cooperation in meeting the disease’s threat.

In the final analysis, success in controlling whirling disease will depend on the willingness of fishery managers to use the information and tools that science provides. We remain far from finding a “cure”; indeed, we may never find one. That by itself conveys a valuable, cautionary lesson. Whirling disease demonstrates that, in addition to documented genetic risks, the use of artificially propagated fish for supplementation can entail significant pathological risks to wild populations and should be undertaken with far greater care and deliberation than has been the norm. It also tells us that our ability and willingness to protect and restore our streams and rivers may prove the best long-term protection to whirling disease and other pathogens.

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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 hatcheries and/or in the wild in a total of 22 states: Alabama, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nevada, New Hampshire, New Jersey, New Mexico, New York, Ohio, Oregon, Pennsylvania, Utah, Virginia, Washington, West Virginia, and Wyoming.

Early reports of whirling disease were often accompanied by forceful actions, for example, destruction and burial of fish. By the 1980s, improvements in fish culture practices had minimized the impacts of whirling disease in hatcheries and many fish health professionals viewed *M. cerebralis* as an undesirable but manageable parasite. In 1994, this conventional wisdom was turned on its head when studies on the Colorado and Madison Rivers suggested that whirling disease was responsible for dramatic declines in wild rainbow trout populations. Since that time, major new efforts have been directed towards research and management to address the threat posed to trout and salmon by the parasite.

Life Cycle

M. cerebralis has a complex life cycle, requiring two different hosts (salmonid fish and *Tubifex* worms) and producing two very different spores. The spores that develop in infected fish are tiny — less than 10 µm across (1 µm = 0.001mm). The spores are released when an infected fish dies and decomposes, or when they are 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 highly 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 *Tubifex tubifex*, a small aquatic oligochaete worm related to common earthworm. The parasite appears to be very specific to *T. tubifex* as a host –

efforts to infect other aquatic worms have failed. Once in the worm, the parasite penetrates between cells in the gut epithelium and undergoes multiple divisions before ultimately forming triactinomyxons, the spore form that is infective to fish. Triactinomyxons are shaped like a grappling hook and are much larger than the spore form that infects *Tubifex* (the “arms” of the hook are about 170 µm long). They begin to be released from infected worms 90 days after exposure, and can continue to be released for as long as one year after infection.

Salmonid fish become infected through contact with waterborne triactinomyxons or through ingestion of infected *T. tubifex* worms. The triactinomyxons attach to the trout and release infective sporoplasms into the trout. Once in the fish, the parasite multiplies and begins to migrate through the epidermis, the subcutis, and to the nerves. Once in the nerves, the parasite is effectively “shielded” from the fish's immune response. The parasite then migrates to the cartilage, where it does its real damage, digesting the cartilage matrix. In the cartilage, the parasite ultimately transforms into the tiny spores that go on to infect *Tubifex* worms and continue the life cycle.

Impacts on Individual Fish and Populations

The impacts of whirling disease on susceptible fish can be dramatic: darkening of the tail (“blacktail”); frenzied tail chasing (“whirling”) by fish when they are feeding or are alarmed; skeletal deformities (sometimes of the spine, primarily 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. In addition to these direct impacts, whirling disease may result in reduced performance among infected fish and increased vulnerability to other pathogens.

In the wild, experience with whirling disease has been varied. While research from Colorado and Montana clearly demonstrates that whirling disease can have major effects on wild populations, other states have a long record of experience where healthy wild trout populations coexist with *M. cerebralis*. In the Colorado River,

recent year classes of rainbow trout have virtually disappeared while brown trout have been less affected. Scientists from the Colorado Division of Wildlife have concluded that whirling disease is implicated in wild trout population declines (though it is not necessarily the only cause). In Montana, field sampling revealed dramatic population declines (as much as 90%) in wild rainbow trout in sections of the Madison River contaminated with *M. cerebralis*. There is also evidence suggesting that whirling disease may be involved in population declines in Idaho's Big Lost River and Utah's Beaver River. In contrast, several other rivers in Idaho (e.g., South Fork Boise), New York (e.g., Wiscoy Creek), and other states have *M. cerebralis* present, but have not experienced associated population declines.

Factors Influencing Disease

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

- **Environmental stress** – environmental stressors such as pollution, crowding, or abnormal temperatures generally make fish more susceptible to disease.
- **Infective dose** – as fish are exposed to increasing doses of triactinomyxons, the severity of disease increases.
- **Fish age** – older fish are less susceptible to disease than younger fish, since the cartilage that is affected by the parasite is converted to bone as fish age.
- **Fish species** – different species of fish differ in their susceptibility to whirling disease; generally speaking, rainbow trout are considered among the most susceptible species while brown trout are highly resistant. Most species of cutthroat trout also appear to be vulnerable, while grayling seem to be highly resistant.

In addition to the factors that affect the susceptibility to disease of individual fish, it appears that ecological factors may play a significant role in determining the severity of effects on wild populations. Whirling disease infectivity appears to be greater in high-productivity streams. Water with more sediment and organic matter may also have greater disease problems, as it provides more favorable habitat for *Tubifex* worms. Water temperature can

have pronounced effects on the release of triactinomyxons; it appears that 15°C may be optimal for production of triactinomyxons, while releases are slower at lower temperatures and may be eliminated at temperatures of 20°C or more. There also appear to be infection “point sources” — locations where production of triactinomyxons is especially high and disease is especially severe.

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. Among states that report *M. cerebralis*, there have been two main approaches used to address the spread of disease through fish stocking. Under one approach, no stocking of infected fish is allowed; under the other, infected fish are stocked only in waters where *M. cerebralis* is already found or where infected fish have been stocked previously. Some states have used a hybrid of these approaches.

Once established in a natural system, whirling disease can spread as infected fish move up or down stream and as triactinomyxons are carried downstream. The parasite may also be carried by predators that eat infected fish and then shed spores in their feces. Given the resistance of spores, they may also be transferred through mud on waders, boots, boats, or other items moved between infected and uninfected waters. Whirling disease could also be spread through shipments of fresh, frozen, or brined food fish infected with *M. cerebralis* (in fact, it is believed that whirling disease first came to the United States in frozen food fish from Europe). Fish eggs do not become infected, so properly 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 ways 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. Drugs (such as fumagillin) may also be effective in combating infection and disease. For wild populations, it is generally thought that the parasite cannot be eliminated once it is established; however, California has witnessed the decline of *M. cerebralis* in some formerly infected habitats.

The Whirling Disease Research Agenda: A Summary of Progress

When Trout Unlimited's Coldwater Conservation Fund first issued "Whirling Disease in the United States" in 1996, it included a series of priority research recommendations. Researchers from state and federal agencies and academic institutions have tackled many of the priorities identified in the 1996 report, with funding support from the Coldwater Conservation Fund, the Whirling Disease Foundation, the U.S. Fish and Wildlife Service, and state fishery agencies. Following is a brief summary of progress that has been made in addressing the 1996 agenda.

Highest priority.

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

A polymerase chain reaction (PCR) test has been developed at the University of California-Davis and is currently being validated by several researchers in the western United States. In situ hybridization (ISH) has also been used to detect nucleic acid sequences from *M. cerebralis* in fish hosts.

- ▶ **Improving understanding of the host-parasite relationship and dissemination of the parasite.**
- **Biological and genetic assessment of *M. cerebralis*.** Analysis of ribosomal DNA sequences in *M. cerebralis* from Germany, California, Montana, and Colorado detected no strain difference, and parasites from California, Montana, and Colorado were also found to all possess virulence for rainbow trout.
- **Research on infection and disease in different species or strains of salmonids.** Researchers have found most cutthroat trout quite susceptible, though Snake River cutthroat may be somewhat more resistant. Bull trout and mountain whitefish can also be infected. Arctic grayling can be infected, but appear to be quite resistant. Trout hybrids (including brownbows, splake, brake trout, and tiger trout) all proved capable of becoming infected with *M. cerebralis*.
- **Studies on the oligochaete worm host.** Genetic analysis has found significant differences between

"Tubifex" from the Great Lakes and "Tubifex" from California, suggesting that they may be different strains, sibling species, or different species. New research has also detailed the progression of infection in *T. tubifex* from initial exposure through production of triactinomyxons.

- **Studies on early infection.** Researchers have better identified the points of initial infection in fish and have found that triactinomyxons have little specificity in recognizing fish hosts, though infection does not progress in nonsalmonids.
- **Field studies on the dynamics of infection and disease in wild fish populations.** Field studies in several states have found varied results, from New York – where no clinical signs and no population effects have been observed – to Colorado, where significant impacts on rainbow trout have been seen in the Colorado, Gunnison, Poudre, Rio Grande, and South Platte Rivers. Recent studies in Montana have suggested that stream temperature may influence the levels of infectivity in streams, producing seasonal peaks of infection.
- **Field studies on the dynamics of infection in oligochaete worms.** *T. tubifex* worms in Montana were primarily found in polluted sites where normal benthic community diversity had been reduced. In Colorado *T. tubifex* were found in a wide range of lakes and streams, though the number of populations decreased as elevation increased. Researchers also developed a technique for filtering and quantifying triactinomyxons in flowing waters, finding that triactinomyxons appear in far greater quantities around the end of springtime runoff, when stream temperatures reached about 10°C or more.

High priority.

- ▶ **Host resistance and immunity to *M. cerebralis*.** Researchers studying early host reaction to infection found that starting five days after trout were exposed, parasitic stages in the subcutis were surrounded by round cells and macrophages. Apparently, parasites that have not yet reached nerve cells within five days are removed by immune cells. Other studies suggest that fish that have been previously infected are resistant to re-infection.

Whirling Disease and Fish Management: A Summary of Progress

In the years since whirling disease was identified as the cause of rainbow trout declines in the Madison and Colorado Rivers, fish managers have stepped up their efforts at controlling the parasite — especially in the intermountain west, where whirling disease has been best documented as a problem for wild trout fisheries.

Colorado. Shifts in stocking policy (cutting back on the stocking of infected fish in areas where they could impact wild trout) have resulted in dramatically reduced stocking of many Colorado waters, especially on the western slope. With cutbacks in stocking and impaired wild trout populations resulting in fewer fish available for harvest in western Colorado, the Colorado Division of Wildlife (CDOW) also adopted emergency reductions in bag limits. The CDOW has made a major investment (\$7.9 million) in modernizing fish hatcheries in an effort to eradicate *M. cerebralis* from those hatcheries, and has maintained a wide-ranging program for whirling disease research.

Idaho. The Idaho Department of Fish and Game (IDFG) does not stock any *resident* trout that test positive for whirling disease, but some hatchery-reared *anadromous* fish are stocked after being exposed to the parasite. IDFG conducted fish disease inventories during 1994-95, and since 1996 IDFG has monitored wild populations where *M. cerebralis* was found during those inventories. Studies have also examined the timing and severity of infection in the South Fork Boise River, evaluated in-situ hybridization as a means of determining infection rates in fish, and tested the efficacy of the drug fumagillin in reducing infection in cultured fish.

Montana. When whirling disease emerged as a threat to wild trout in Montana, Governor Marc Racicot convened a multi-interest task force to develop strategies for addressing the disease. The task force has developed not only recommendations, but also tools for education and outreach. Since 1994, the Montana Department of Fish, Wildlife and Parks (MFWP) has conducted state-wide surveys of approximately 300 waters and detected the parasite in over 60 sites. MFWP remains dedicated to a wild trout philosophy and has launched a wide-ranging

research program to develop strategies for managing wild trout in the presence of *M. cerebralis*. In addition to MFWP efforts, Montana State University houses a wild trout laboratory and a competitive grants program through the National Partnership on the Management of Wild and Native Cold Water Fisheries.

Utah. From before the time *M. cerebralis* was first found in Utah, the state has treated the parasite as a prohibited pathogen, barring the transport and release of infected fish. Thus far, whirling disease has not been found in Division of Wildlife Resources (DWR) hatcheries. The private sector has not been so fortunate, with several facilities testing positive for *M. cerebralis*. In response to the threat of whirling disease, DWR has increased the frequency of fish-health inspections at some hatcheries and has shifted to greater use of resistant brown trout in streams that test positive for the parasite. The DWR has also been active in whirling disease research, conducting fish health surveys of wild population and testing the susceptibility of trout hybrids.

U.S. Fish and Wildlife Service. The U.S. Fish and Wildlife Service (USFWS) has supported research at several top laboratories and has also supported a competitive grants program through the National Partnership on the Management of Wild and Native Cold Water Fisheries. The National Partnership has provided over \$920,000 in competitive grants to support whirling disease research, leveraging over \$880,000 in matching funds. The USFWS has also launched a national wild fish health survey, analyzing fish samples from wild populations for disease. The USFWS operates numerous hatcheries, one of which tests positive for whirling disease – the Leadville National Fish Hatchery in Colorado. The USFWS is currently considering options for its future operations. Under the Lacey Act's provisions for control of injurious wildlife, the USFWS is also responsible for establishing and enforcing the list of prohibited pathogens for import into the United States. *M. cerebralis* was removed from the list of prohibited pathogens in 1993 and remains unregulated.

U.S. Forest Service and Bureau of Land Management. The Bureau of Land Management (BLM) and the U.S. Forest Service together manage more than 500 million

acres of federal lands. In general, the agencies take the position that they manage the lands and defer to the appropriate state to manage fish and wildlife on the federal lands unless otherwise required by law, regulation or policy. In 1988, the Forest Service prepared an Environmental Assessment that permitted the stocking of fish

infected with whirling disease on Forest Service and BLM lands. It is the current policy of the Forest Service and the BLM that if a state wishes to stock fish exposed to whirling disease on public lands, it may do so without additional environmental review under NEPA.

Whirling Disease in the United States

Preface

In 1996, TU issued its first national assessment of whirling disease, providing a summary of what was then known about the biology of *Myxobolus cerebralis*, its distribution in the United States, and inspection and control programs that were in place in different states. The intense research effort surrounding whirling disease has generated so much new information that after less than three years we are updating the report in order to reflect this new knowledge. The new assessment provides an updated summary on the state-of-the-science, as well as information about recent and ongoing management efforts. A list of major references is also included (see Appendix). It is our hope that the report will be a useful reference for managers and the interested public.

Whirling disease is a parasitic disorder of salmonids caused by the microscopic parasite *M. 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, improvements in culture practices minimized the impacts of whirling disease under aquaculture conditions. Because there were no reports of serious problems with the parasite in natural settings, many fish health professionals viewed *M. cerebralis* as an undesirable but manageable parasite.

The conventional wisdom about whirling disease was turned on its head in 1994, when studies from rivers in Colorado and Montana suggested that whirling disease was associated with dramatic declines in wild rainbow trout populations. Since that time, widespread concern over the disease has led to a nationwide fish health research effort of unprecedented scope. Whirling disease

research has drawn support from the federal government, from state governments across the country, and from private-sector groups such as Trout Unlimited (TU) and the Whirling Disease Foundation. Many of the best minds in fisheries science have been drawn into the effort to understand and combat *M. cerebralis*. At the same time, managers in several states have taken extraordinary steps in an effort to contain and control the parasite – restricting the stocking of infected fish and in some cases destroying them; implementing regulation changes to protect affected fish populations; and engaging in a widespread campaign of public education to inform anglers about the disease.

A Brief History of Whirling Disease in the United States

Whirling disease was discovered in 1893, when Dr. Bruno Höfer reported the disease from rainbow trout in Germany and proposed the name *Myxobolus cerebralis* for the parasite (Höfer 1903). Subsequent reports of the parasite initially came from Europe; this, coupled with the relative resistance to whirling disease observed in brown trout, suggested that the parasite originated in Europe as a parasite of brown trout (Halliday 1976). Only when susceptible rainbow trout were imported from North America and 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 either hatcheries or the wild in a total of 22 states: Ala-

cruitment 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 (Vincent 1996). These reports renewed concern about whirling disease among fisheries managers and the public, with emphasis shifting from concern about impacts on fish culture to concern about impacts on wild populations of trout – an issue which had previously received only minimal attention. In the wake of documented impacts of whirling disease on wild trout, several states adopted more aggressive control policies. For example, New York prohibits stocking of fish from lots tested positive for *M. cerebralis*. In Colorado, where a large portion of state hatchery capacity has been found positive for *M. cerebralis*, the stocking of infected fish in streams was reduced from 125 streams in 1994 to 6 streams in 1997.

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, which in turn initiated an infection in salmonids that ultimately produced myxospores. Antigenic homology of the myxospore and triactinomyxon 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 genetic confirmation of the relatedness of the triactinomyxon and myxosporean forms (Andree et al. 1997a). Several other myxosporean species have also been shown to require aquatic oligochaetes as alternate hosts (see summary in El-Matbouli et al. 1995). The oligochaete host for *M. cerebralis* is apparently restricted to *T. tubifex*, as researchers have been unable to infect other worms, including *Limnodrilus*, *Quistadrilus*, *Ilyodrilus*, *Dero*, *Stylaria*, and *Aeolosoma* (Wolf and Markiw 1984, Hedrick et al. 1996).

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 8.7µm long by 8.2µm wide by 6.3µm thick (1µm=0.001 mm). A mucous envelope is found around the posterior end of the spore. Spores contain two ovoid polar capsules measuring approximately 5µm by 3µm 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 by other investigators. 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

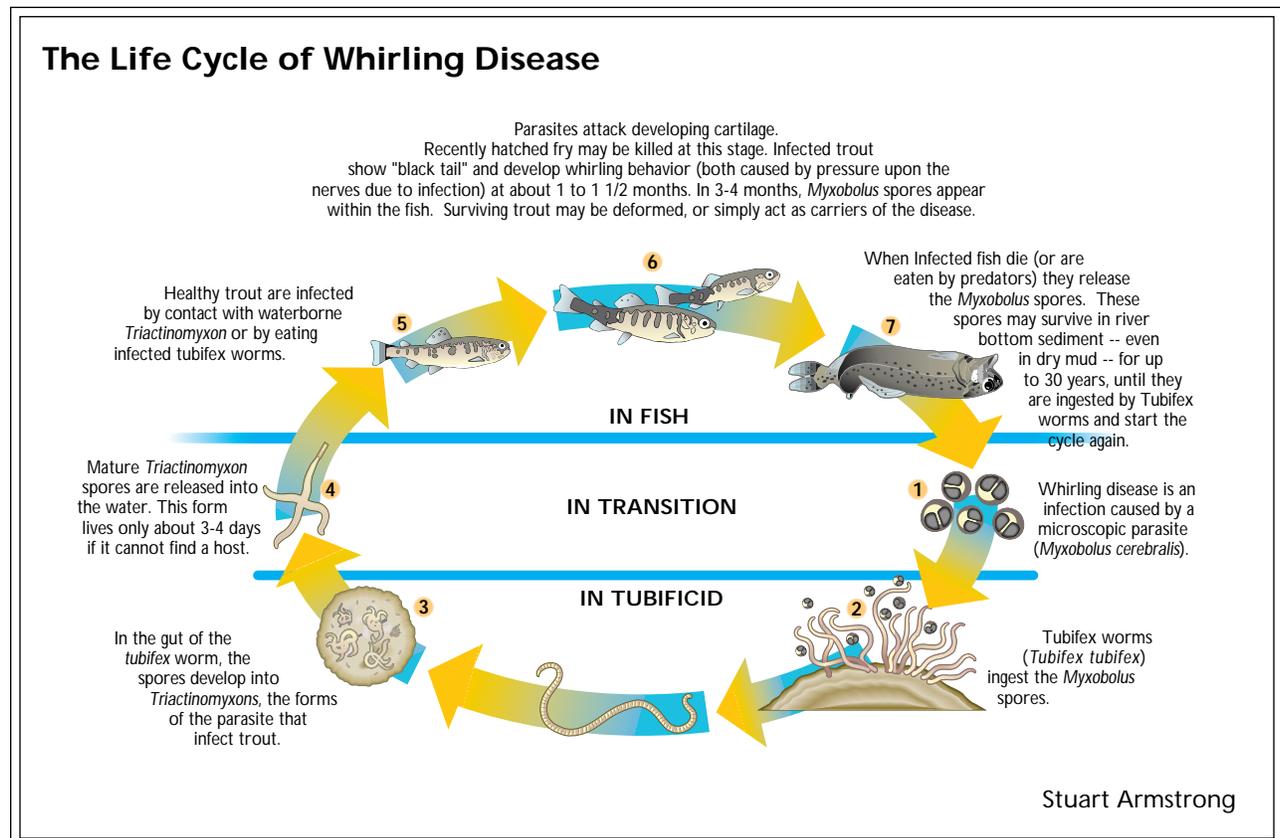
the operculum of infected cutthroat trout; they speculated that rupture of these cysts could also lead to transmission of spores from live fish.

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 (Hoffman 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 small aquatic oligochaete worm which is fairly closely related to common earthworms. In the gut lumen of a

tubificid worm, the myxospores extrude their polar capsules and attach to the gut epithelium by polar filaments. The valves of the myxospore shell then open along the suture line, and the sporoplasm enters the worm host between the gut epithelial cells. Myxospores were found in the gut lumen of *T. tubifex* five days after exposure to the worms (El-Matbouli and Hoffmann 1998). Interestingly, El-Matbouli et al. (1998a) observed that myxospores are also ingested and sporoplasms released in the gut of non-susceptible worms just as in *T. tubifex*, but further development of the parasite failed.

After the sporoplasm penetrates between the *Tubifex* worm's gut epithelial cells, its two nuclei undergo multiple divisions, forming multinucleate cells. These stages then divide to form numerous uninucleate cells between cells of the gut epithelium. Some of these stages undergo further nuclear and cellular divisions, forming additional multinucleate and uninucleate cells. Others fuse together to provide binucleate stages. These stages begin to appear 10 days (uninucleate) to 25 days (binucleate) post-exposure, and may persist for more than a year (El-Matbouli and Hoffmann 1998).



At 35 days post-exposure, the nuclei in the binucleate stage divide to form a four-nuclei stage, which then divides to form a four-cell stage — two enveloping somatic cells and two generative cells (α and β). Three mitotic divisions followed by one meiotic division produce 16 haploid gametocytes. Each gametocyte from the α line unites with one from the β line to produce eight zygotes. This is the only phase of the *M. cerebralis* life cycle in which sexual stages occur. Meanwhile, the somatic cells divide twice to produce eight enveloping cells (El-Matbouli and Hoffmann 1998).

Each zygote then undergoes two mitotic divisions to produce a four-cell stage. Three cells are located peripherally and divide to form three capsulogenic and three valvogenic cells, while the fourth cell is located centrally and ultimately undergoes numerous mitotic divisions to form the sporoplasm of the triactinomyxon. Subsequently, the capsulogenic cells and sporoplasm are enclosed within a shell composed of three valves. Behind the sporoplasm, the valvogenic cells extend in folded membranes that ultimately turn into the cell walls of the stylus and three projections of the triactinomyxon spore. This final stage, with pansporocysts containing eight folded triactinomyxon spores, begins to appear 90 days post-exposure (El-Matbouli and Hoffmann 1998). Free floating triactinomyxons appear shortly afterward (Wolf and Markiw 1984; El-Matbouli and Hoffman 1989). Triactinomyxons may also be released from *Tubifex* worms in fecal packets (Gilbert and Granath 1998). The release of fecal packets may correspond to rapid environmental changes or stress (unpublished data, UC-Davis).

The triactinomyxon is much larger than the myxospore and shaped like a grappling hook. It is topped by an episporium about 36 μ m long, containing 64 spherical sporoplasms, and capped with three polar capsules. Below the episporium the style extends another 90 μ m, then attaches to three tapering, tail-like appendages that are approximately 170 μ m 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 infection of the worm by the 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 nine 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 three to four 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 *T. tubifex* worms (Wolf and Markiw 1984). Within five 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 ten 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 apparently have little specificity in recognizing fish hosts, provided the fish are alive. Sporoplasms were detected in salmonids and non-salmonids alike two hours after exposure, though in nonsalmonids the parasites degenerated and infection did not progress further (Hedrick et al. 1997). More recent work suggests that the triactinomyxons may have somewhat more specificity in recognizing hosts than initially thought, though penetration was still observed in nonsalmonids (Hedrick, personal communication).

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 two to four hours post-exposure; after 24 hours only a small number of single-cell stages were recognizable (Markiw 1989a; El-Matbouli et al. 1995).

By two 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 four 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 begin 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 classic form of *M. cerebralis* that is infective to *T. tubifex*.

Taxonomy of *M. cerebralis*

Myxobolus cerebralis (formerly "*Myxosoma*" *cebralis*) 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.

Diagnosis of Whirling Disease

The American Fisheries Society Fish Health Section diagnostic procedures use detection of *M. cerebralis* spores for diagnosis of whirling disease (Lorz and Amandi 1994). Two primary methods have been used for spore detection: use of a continuous plankton centrifuge as described by O'Grodnick (1975b) and an enzymatic digest method ("pepsin-trypsin digest") described by Markiw and Wolf (1974a). Markiw and Wolf (1978) also discussed fluorescent antibody techniques for identification of *M. cerebralis*.

More recently, a polymerase chain reaction (PCR) test has been developed for detection of *M. cerebralis* (Andree et al. 1997b). As this test relies not on the presence of spores, but rather on the presence of parasite DNA, it has the advantage of being able to detect all life stages of the parasite in either fish or worm hosts. The PCR test also offers greater sensitivity than existing methods. Several researchers have been involved in validating the test both in terms of sensitivity and specificity (Andree et al. 1998; Ellis et al. 1997; Ellis et al. 1998), and continuing studies are planned.



Skeletal deformities, such as spinal curvature and misaligned jaws, can be indicators of diseased fish.

In situ hybridization (ISH), another DNA-based test for detecting *M. cerebralis*, was described by Antonio et al. (1997). The ISH procedure can recognize all stages of the parasite in tissue, and allows researchers to visualize the exact location of the parasite in target tissues. It offers great promise as a research tool.

Impacts of Whirling Disease on Individual Fish

The impacts of whirling disease on susceptible fish can be spectacular (Uspenskaya 1957; Bogdanova 1960; Hoffman et al. 1962; Hoffman 1962, 1966; Elson 1969; Roberts and Elson 1970; Markiw 1992c). Some readily noted signs of severe disease include:

- frenetic tail chasing (“whirling”) by fish when feeding or alarmed, often to the point of exhaustion; originally believed to be caused by damage to the cartilage and a granulomatous response around the organs of equilibrium, though research by Rose et al. (1998) suggests that the cause may be damage to the spinal cord and to the axons in the neural pathway that transmits swimming control signals; dissipates in older fish
- darkening of the tail in fish; believed to be 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 (Wolf et al. 1981). Similarly, fish may be infected subclinically and show no external signs of disease (Halliday 1974a).

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). Researchers at UC-Davis attempted to replicate these results and did not observe such heavy, immediate mortality. However, fish were severely crippled and presumably would not have survived in the wild (Hedrick, personal communication).

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).

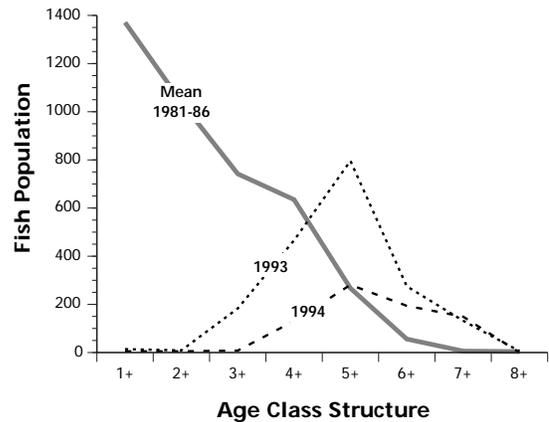
Impacts of Whirling Disease on Wild Populations

Most early research on whirling disease focused on laboratory or fish culture settings. It had been widely thought that impacts on wild populations were minimal, but detailed studies on susceptible young fish in contaminated waters were lacking (Anonymous 1988). Over the past three years, however, there has been increased interest in this topic and new research has been conducted to look at whirling disease in the wild.

Research from Colorado and Montana clearly demonstrates that whirling disease can have major effects on wild populations of salmonid fish. Evidence from Utah and Idaho suggests that whirling disease may be affecting some wild populations. On the other hand, some states have a long record of experience suggesting that healthy wild trout populations can coexist with *M. cerebralis*.

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 almost entirely absent. 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. They 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). Some factors other than whirling

disease were also implicated, including gas bubble disease, bacteria, external fungi, and ectoparasites, but appeared to be of importance primarily as exacerbating stressors (Walker and Nehring 1995).

While the Colorado River has been the most intensively studied for whirling disease, data from other rivers also indicate significant population level effects from whirling disease. Biologists have observed dramatic declines in rainbow trout density and biomass in the South Platte River near Deckers, as well as an irregular year-class distribution similar to that observed in the Colorado River (Nehring et al. 1997). Rainbow trout populations in the Gunnison River Gorge have also suffered significant declines in both numbers and biomass from 1994 through 1997 (Hebein et al. 1998). Interestingly, data from Hebein et al. (1998) indicates that brown trout numbers and biomass in the Gunnison have increased as the rainbow trout numbers have decreased, resulting in relatively stable levels of total trout biomass. Rainbow trout population declines have also been documented in the Cache la Poudre and Rio Grande Rivers (Nehring 1996).

Montana. Field sampling in Montana 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 remained relatively stable. Prior to the discovery of *M. cerebralis* in the Madison, other possible factors in the decline were evaluated and discarded by the Montana Department of Fish, Wildlife, and Parks, including flows, water quality, water temperatures, fish habitat changes, and fishing pressure (Vincent 1996). Histology demonstrated severe disease (cartilage destruction, granulomatous response) in wild fish from the river (MacConnell and Lere 1996), lending further support to the hypothesis that whirling disease is primarily responsible for the declines in rainbow trout populations.

Brown trout population declines were also observed in Poindexter Slough and Ruby River, leading to some speculation that whirling disease may have population-level effects on brown trout as well as rainbow trout. Opitz and Zale (1998) investigated this possibility, looking at abundance of young-of-year brown trout, exposing sentinel fish, and conducting histological examinations. Initial results suggest that whirling disease is not

a major factor in the brown trout population declines in these two rivers. No precipitous declines in young-of-year abundance were observed, and histological examinations found most fish to be negative for whirling disease while those that were positive had only minimal or mild infections.

Idaho. *M. cerebralis* has been found in numerous Idaho waters, including the Coeur d'Alene River, upper Salmon River, South Fork Boise River, Big Lost River, South Fork Snake River, and Teton River. Population data from a study of several Idaho streams indicated no strong association between the presence of the parasite and recruitment failure in the South Fork Boise, Big Wood River, and Silver Creek. In part of the Big Lost River, however, rainbow trout populations had declined and few age 0 or age 1+ fish were present. Not all sections of the Big Lost River exhibited population losses, and other factors (including an extended drought) may be contributing to the declines (Elle 1997a).

Utah. Utah's Beaver River is a popular trout fishery for which population data "pre-whirling-disease" were available. Population surveys from 1997 found that rainbow trout numbers and biomass were down significantly from 1988 levels. However, total trout biomass was not significantly different, with increases in brown trout offsetting losses or rainbow trout (Wagner et al. 1998).

New York. Studies in New York have found no significant population effects associated with whirling disease in streams. Where *M. cerebralis* has been found in sampling of wild populations, no clinical signs have been observed. Current and historic fish population data from two infected streams, Wiscoy Creek and Clear Creek, suggests that the presence of *M. cerebralis* has not resulted in population declines in these streams (Schachte and Hulbert 1998).

Factors Influencing Infection and Disease in Trout

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

Environmental stress. Halliday (1973b) found that the parasite developed more rapidly and that disease signs were more common in fish held at higher water tem-

peratures. In general, environmental stressors such as pollution, crowding, or abnormal temperatures will make fish more susceptible to disease (Goede 1986). Ecological variables are discussed further in the next section.

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. In exposure tests with 2-day old rainbow trout, increasing doses of triactinomyxons resulted in increasing levels of mortality – from 68% mortality at 10 triactinomyxons/fish to 100% mortality at 1000 triactinomyxons/fish; 4% of uninfected control fish perished (Markiw 1991).

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. Numerous laboratory and sentinel fish experiments have looked at the susceptibility of different salmonids. Generally, rainbow trout are considered among the most susceptible species while brown trout are highly resistant. Other species found in the United States which can become infected include: Snake River, greenback, Colorado River, and Rio Grande cutthroat trout (Thompson et al. 1998), Yellowstone and westslope cutthroat trout (Vincent 1997), bull trout (McDowell et al. 1997), steelhead (Horsch 1987), arctic grayling (MacConnell et al. 1997), Atlantic salmon (Hoffman 1990), golden trout (Anonymous 1988), sockeye, coho, and chinook salmon (O'Grodnick 1978a, 1979), and mountain whitefish (Baldwin et al. 1997).

O'Grodnick (1978a, 1979) observed clinical signs of whirling disease in experimentally infected rainbow

trout, brook trout, sockeye salmon, and chinook salmon. No clinical signs were found 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).

Sentinel fish studies in Colorado (Thompson et al. 1998) found evidence of infection in brown trout, rainbow trout, and four subspecies of cutthroat trout (Colorado River, Greenback, Rio Grande, and Snake River). Whirling behavior, a clinical sign thought to indicate more severe infection, was observed in rainbow trout and in Colorado River, Greenback, and Rio Grande cutthroat trout. Snake River cutthroats appeared to be somewhat more resistant to disease, while brown trout were the most resistant species (based on spore counts and clinical signs observed). Previous studies had found brook trout to be highly vulnerable to infection, as well (Thompson et al. 1997).

Sentinel fish studies in Idaho found that rainbow trout and Yellowstone cutthroat trout were both vulnerable to infection with *M. cerebralis*. Based on spore counts, clinical signs observed, and histology, it appeared that the cutthroat trout were somewhat less affected by the parasite than the rainbow trout (Elle 1997b). In contrast, studies in Montana found Yellowstone cutthroat trout to be highly affected by whirling disease, as were westslope cutthroat trout and three strains of rainbow trout (DeSmet, Deschutes, Eagle Lake). Brown trout were relatively unaffected (Vincent 1997). Other studies in Montana (MacConnell et al. 1997) and at the University of California-Davis (McDowell et al. 1997) found grayling to be quite resistant to whirling disease, though the Montana studies indicated that they could be infected with the parasite.

Studies in Utah evaluated the susceptibility of several salmonid hybrids. Brownbows (rainbow female x brown male), splake (lake female x brook male), brake (brown female x lake male) and tiger trout (brook female x brown male) were exposed in two reservoirs. Each type of hybrid proved capable of becoming infected with *M. cerebralis*, though a hierarchy of resistance has not yet been developed (Wilson et al. 1997b).

Ecological Factors and Whirling Disease in Wild Trout

Why is whirling disease a serious problem in some waters harboring *M. cerebralis* and not in others? Clearly, part of the answer is that different species of salmonids have different vulnerability. However, many scientists have also begun to look at ecological variables that may influence the course of infection in wild populations. The cumulative affects of environmental stresses (flow alteration, temperature stress, pollution, biological alteration, etc.) likely play an important role in influencing infection and disease. Some specific factors that have been examined include:

Stream productivity. 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).

Sediment/organic material. Waters with more sediment and organic matter may provide more favorable habitat for *Tubifex* worms, potentially leading to greater disease problems. For example, Modin (1998) 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. Gustafson (1997) found that *T. tubifex* worms in Montana were generally found in greatest abundance in polluted sites where normal benthic community diversity had been reduced.

Seasonality/water temperature. Water temperature can have pronounced effects on the development of *M. cerebralis* in *Tubifex* worms and on the release of triactinomyxon spores. Most parasite developmental stages in the gut epithelium of *Tubifex* worms were destroyed after 24 hours when held at 30°C. At 25°C most were destroyed after three days, and at 20°C most were destroyed after ten days. In contrast, complete development of triactinomyxon spores was observed in worms

held at 5, 10, and 15°C (El-Matbouli et al. 1998b). In worms already producing triactinomyxons, release of these spores ceased within four days when worms were held at 25 and 30°C, and within 15 days at 20°C. It appears that 15°C may be optimal for production of triactinomyxons, with release continuing but at a slower rate when water temperatures are lower (El-Matbouli et al. 1998b).

Field studies have also indicated that water temperature may be an important factor. In young-of-the-year rainbow trout exposed in sentinel cages at different times of year, infection rates showed a seasonal pattern with significant correlation to the average water temperatures when fish were exposed. Fish began to exhibit more severe infections when they were exposed at water temperatures of 9°C, with infection peaking at about 14°C and declining when water temperatures exceeded 17°C (Vincent 1998).

Infection "point sources." In studies on the Colorado River, Schisler et al. (1997) found that the percentage of trout fry displaying clinical signs of whirling disease decreased as distance downstream from Windy Gap Reservoir increased. They suggested that disease in wild populations may be influenced by specific "point sources" for infectivity, such as Windy Gap Reservoir.

Immune Response to *M. cerebralis* in Fish

For many years, it has been known that trout offer some immune response to infection by *M. cerebralis*. For example, Griffin and Davis (1978) detected circulating antibodies in infected rainbow trout. More recent studies have shed further light on the immune response mounted against *M. cerebralis*.

Hoffman and El-Matbouli (1996) observed that, starting five days after trout were exposed, parasitic stages in the subcutis were surrounded by round cells and macrophages. Apparently, parasites that have not yet reached nerve cells within five days are removed by immune-defense cells. For those that reach nervous tissue, 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. Once in the nerve tissue, the parasite is effectively shielded from attack by the immune system.

However, fish that have been previously infected ap-

pear to be resistant to re-infection. Hoffman and El-Matbouli (1996) found that when rainbow trout fingerlings previously exposed to *M. cerebralis* were re-exposed eight weeks later, no triactinomyxons had released their sporoplasms after one hour and no penetrating parasite cells were observed in the epidermis of the re-exposed trout. Hedrick et al. (1997) also observed evidence of some acquired immunity, finding that fish exposed to a high dose of triactinomyxons (1,350 per fish) developed resistance to re-infection between 24 and 36 days after initial exposure (at 15°C). Fish exposed to a lighter dose (200 triactinomyxons per fish) did not display resistance to reinfection.

In sentinel fish studies, Thompson et al. (1998) exposed two groups of rainbow trout – one spawned from wild trout in the Colorado River that recruited prior to population effects of whirling disease, and the other spawned from trout that recruited after whirling disease effects began to appear. The offspring of the pre-whirling-disease parents had significantly higher spore loads than the progeny of post-whirling-disease parents. Although survival rates were similar for the two groups, the lower spore loads in post-whirling-disease trout may indicate that some level of resistance has been developed in these surviving fish. More studies are underway to explore this possibility.

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*. States have primary authority over fish and wildlife management, and have taken a variety of approaches to addressing the spread of disease through transfer or stocking of fish from both public and private facilities. Among those states that currently report the presence of *M. cerebralis*, two major approaches have been taken to addressing this problem. 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. In some areas, a hybrid of these two approaches has been used.

Once established in a natural system, whirling disease can spread as infected fish move up or down stream and as 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 United States 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 means 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 three 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'Grudnick 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 and Eradication of Whirling Disease

A great deal of research has been directed at developing ways to control whirling disease in fish culture settings. The approaches 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. Hoffman and Putz (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) 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 pathogen-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 become carriers of *M. cerebralis*.

For wild populations, the conventional wisdom is that *M. cerebralis* cannot be eliminated once it is established (e.g., see Hoffmann and El-Matbouli 1996). Failed eradication efforts have been documented in the literature. Michigan took extraordinary measures, using fish toxicants and even chlorinating a stream in an unsuccessful effort to eliminate the pathogen (Hnath 1988). Utah used rotenone to keep a river "trout-free" for several consecutive years in an effort to break the life cycle of *M. cerebralis* and eliminate it, but these efforts at eradication have apparently failed (Wilson et al. 1997a). However, California has witnessed the decline of *M. cerebralis* in formerly infected habitats. Following closure of a rearing pond near Garrapata Creek, *M. cerebralis* has no longer been found in wild rainbow trout in the creek. Similarly, the parasite is no longer found in the Carmel River and at Coleman Fish Hatchery (or in wild fish in the hatchery's water supply). In these waters, at least, it appears that eliminating the source of infection resulted in parasite numbers dropping below detectable levels (Modin 1998).

The Whirling Disease Research Agenda: A Summary of Progress

When “Whirling Disease in the United States” was first issued in 1996, it included a series of priority research recommendations. As reflected throughout this updated report, significant progress has been made on many of these fronts. Researchers from state and federal agencies and academic institutions have tackled many of the priorities identified in the 1996 report, with funding support from TU, the Whirling Disease Foundation, the U.S. Fish and Wildlife Service, and state fishery agencies. Following is a brief summary of progress that has been made in addressing the 1996 agenda.

Highest Priority.

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

A polymerase chain reaction (PCR) test has been developed at the University of California-Davis (Andree et al. 1997b) and is currently being validated by several researchers in the western United States (Andree et al. 1998; Ellis et al. 1997; Ellis et al. 1998). If the test proves to be both sensitive (able to detect light infections) and specific (not yielding “false positives”), it may become the accepted standard for detection of *M. cerebralis*. Already, the PCR test is proving to be a valuable research tool and the technology has been transferred to state and federal fish health workers. In situ hybridization (ISH) has also been used to detect nucleic acid sequences from *M. cerebralis* in fish hosts. The ISH test has the added advantage of allowing researchers to see visually where within a fish the parasites were detected (Antonio et al. 1997).

► Improving understanding of the host-parasite relationship and dissemination of the parasite.

- **Biological and genetic assessment of *M. cerebralis*.** Researchers have looked at ribosomal DNA sequences in *M. cerebralis* from Germany, California, Montana, and Colorado in an effort to deter-

mine whether there are different geographic strains. No differences were detected. Parasites from California, Montana, and Colorado were also found to all possess virulence for rainbow trout when exposed to controlled dosages in a laboratory setting (McDowell et al. 1998). Further research on genetic diversity among *M. cerebralis* is currently underway using DNA microsatellite markers (Colwell et al. 1998).

- **Research on infection and disease in different species or strains of salmonids.** Numerous studies have looked at the susceptibility of different species and strains of salmonids. Researchers have found most cutthroat trout quite susceptible (greenback, Colorado River, Rio Grande – Thompson et al. 1998; Yellowstone, westslope – Vincent 1997), though Snake River cutthroat may be somewhat more resistant (Thompson et al. 1998). Bull trout (McDowell et al. 1997) and mountain whitefish (Baldwin et al. 1997) can also be infected. Arctic grayling can be infected but appear to be quite resistant (MacConnell et al. 1997). Trout hybrids (including brownbows, splake, brake trout, and tiger trout) all proved capable of becoming infected with *M. cerebralis* (Wilson et al. 1997b).
- **Studies on the oligochaete worm host.** Identifying *T. tubifex* has proven to be difficult, and it appears that early reports of disease-resistant *T. tubifex* may have resulted from misidentification. Genetic analysis has found significant differences between “Tubifex” from the Great Lakes and “Tubifex” from California, suggesting that they may be different strains, sibling species, or different species (Beauchamp et al. 1998). Analysis using randomly amplified polymorphic DNA (RAPDs) markers found some significant differences between *T. tubifex* from different locations (including the Great Lakes, Spain, California, Utah, Montana, Maryland, and Washington), but also

noted no variation between individuals within a given population, suggesting that local populations were closely related or clonal (Rasmussen et al. 1998). New work has also detailed the progression of infection in *T. tubifex* from initial exposure through production of triactinomyxons (El-Matbouli and Hoffmann 1998).

- **Studies on early infection.** Researchers have better identified the points of initial infection in fish and have found that triactinomyxons have little specificity in recognizing fish hosts, though infection does not progress in nonsalmonids (Hedrick et al. 1997).
- **Field studies on the dynamics of infection and disease in wild fish populations.** Field studies in several states have looked at wild populations of trout where *M. cerebralis* has become established. In New York, no clinical signs and no population effects have been observed (Schachte and Hulbert 1998). In contrast, significant impacts on rainbow trout have been seen in the Colorado and South Platte Rivers (Nehring et al. 1997), the Gunnison River (Hebein et al. 1998), the Cache la Poudre and Rio Grande Rivers (Nehring 1996), and the Madison River (Vincent 1996, MacConnell and Lere 1996). Field studies in Idaho have had varied results, with no population effects observed in the South Fork Boise River, Big Wood River, and Silver Creek, while rainbow populations may be affected in the Big Lost River (Elle 1997). Recent studies have suggested that stream temperature may influence the levels of infectivity in streams, producing seasonal peaks of infection (Vincent 1998). “Point sources” of infection may also be an important factor in determining the severity of *M. cerebralis* infections in wild populations (Schisler et al. 1997).
- **Field studies on the dynamics of infection in oligochaete worms.** There is much yet to be learned about Tubifex worms, but scientists are rapidly gaining a better understanding of these creatures. Gustafson (1997) found that *T. tubifex* worms in Montana were primarily found in polluted sites where normal benthic community diversity had been reduced. However, Gustafson (1997) also noted that *T. tubifex* could also be found in highly

oligotrophic waters where macroinvertebrate diversity was also low. McAfee (1998) found *T. tubifex* in a wide range of lakes and streams in Colorado, even at elevations over 10,000 feet, though the number of populations decreased as elevation increased. Thompson and Nehring (1998) developed a technique for filtering and quantifying triactinomyxons in flowing waters. They noted some seasonal patterns in spore numbers, with triactinomyxons appearing in far greater quantities around the end of runoff, when stream temperatures reached about 10°C or more.

High priority.

► Host resistance and immunity to *M. cerebralis*.

Hoffman and El-Matbouli (1996) assessed early host reaction to infection, observing that starting five days after trout were exposed, parasitic stages in the subcutis were surrounded by round cells and macrophages. Apparently, parasites that have not yet reached nerve cells within five days are removed by immune cells. Once in the nerve tissue, the parasite is effectively shielded from the immune system. Other studies suggest that fish that have been previously infected are resistant to re-infection (Hoffman and El-Matbouli 1996, Hedrick et al. 1997).

While much has been accomplished in addressing these research priorities, a great deal of work remains. PCR and ISH techniques must be validated and (if necessary) adjusted. We must improve our knowledge of the impacts *M. cerebralis* has on specific strains of trout, especially strains of native fish that may already be at risk. Far more must be learned about *T. tubifex* populations and ecological factors influencing those populations. Our understanding of infection dynamics in wild trout (and worm) populations is still in its infancy – further study should shed more light on questions of how whirling disease progresses in wild populations and why it proves disastrous in some settings while having little effect in others. The challenge is great, and continued support from state agencies, the federal government, and private sector partners like TU and the Whirling Disease Foundation will be critical in addressing these important unanswered questions.

Whirling Disease and Fish Management: A Summary of Progress

In the years since whirling disease was identified as the cause of rainbow trout declines in the Madison and Colorado Rivers, fish managers have stepped up their efforts at controlling the parasite – especially in the intermountain west, where whirling disease has been best documented as a problem for wild trout fisheries. While many states have been working to address whirling disease using a variety of different strategies, this report will discuss four key states that reflect a range of different approaches and salmonid resources: Colorado, Idaho, Montana, and Utah. It will also describe the federal-level response of the U.S. Fish and Wildlife Service, the U.S. Forest Service and the Bureau of Land Management.

This management summary focuses on the four above-mentioned states for several reasons. First, in Colorado and Montana (and to a lesser extent, Utah), there is scientific evidence that whirling disease is a factor in the decline of infected wild fish populations. Second, the four states are all found in the Intermountain Region where it appears that fish populations are most susceptible to heavy mortality from whirling disease. The states share many habitat characteristics - topography, seasonal stream flows, water temperatures, etc. - that scientists are studying as possible factors influencing the parasite's ability to impair salmonid recruitment. Finally, while the states all have important wild trout resources that may be jeopardized by whirling disease, they also have very different approaches to stocking programs and whirling disease management. Looking at these four states therefore gives a good cross-section on how whirling disease is being addressed in the portion of the country that appears to be most at risk.

Colorado. Whirling disease has led to dramatic changes in Colorado's fish management programs, including shifts in stocking programs, large investments in hatchery cleanup, changes in fishing regulations, and increased emphasis on research. Many of these changes have been necessitated by the unique problems the Colorado Divi-

sion of Wildlife (CDOW) has had with whirling disease in state hatcheries; of the 15 trout hatcheries operated by the CDOW, only five test negative for *M. cerebralis* (and one of those is considered suspect).

In Colorado, stocking programs have been shifted dramatically in response to concerns about whirling disease impacts on trout. In 1994, before whirling disease was recognized by the CDOW as a threat to wild trout, 125 rivers/streams in the state were stocked with trout exposed to *M. cerebralis*. By 1997, that number had been reduced to six rivers (portions of the Arkansas, Cache la Poudre, Colorado, East, Gunnison, and South Platte Rivers). Under current stocking policy, waters in the state have been classified into three categories. "Protected" habitats are those where fish exposed to *M. cerebralis* cannot be stocked, and make up most of the lakes and streams in western Colorado. "Restricted" habitats are those where "lightly infected" trout may be stocked and include the aforementioned rivers and several high-use reservoirs where *M. cerebralis* is already found. "Unrestricted" habitats – basically, low elevation waters where wild trout populations are not found – may be stocked with trout regardless of their status for *M. cerebralis*. These shifts in stocking policy have resulted in dramatically reduced stocking of many waters, especially on the western slope, and somewhat increased stocking in some waters (primarily reservoirs along the urban Front Range). This represents a dramatic shift in stocking policy relative to whirling disease from the late 1980s – early 1990s, when exposed fish were stocked broadly within the state in drainages where *M. cerebralis* had already been found (except for native cutthroat trout waters, where stocking of exposed fish was not allowed).

The CDOW has also adopted new policy for disease testing. General testing of hatcheries (including private facilities) will be done using the standard pepsin-trypsin digest method. However, fish in CDOW hatcheries that are destined for especially sensitive waters (e.g., cutthroat recovery waters) must also be tested using the experi-

mental polymerase chain reaction test, to provide greater certainty that infected fish are not accidentally introduced into these habitats.

With cutbacks in stocking and impaired wild trout populations resulting in reduced fish available for harvest in western Colorado, the CDOW also adopted emergency reductions in bag limits. From the standard bag limit of eight trout per day, bag limits west of the Continental Divide (where stocking reductions have been most significant) were reduced to four trout for lakes and two trout for streams.

Because of the shortage of unexposed trout for stocking in protected habitats, the CDOW has also made a major investment in modernizing fish hatcheries in an effort to eradicate *M. cerebralis* from those hatcheries. The basic strategy is to shift production from surface water supplies (which may be contaminated with *M. cerebralis*) to secure well and spring water supplies. This represents a trade-off of up-front cost and reduced total production for greater security against disease. In total, the CDOW has committed \$7.9 million to this hatchery modernization program. Along with this funding for its hatchery system, the CDOW will also spend more than \$330,000 in license revenues and Sport Fish Restoration funds on whirling disease research. In partnership with Trout Unlimited and Coors Brewing Company, CDOW has also developed signs discussing whirling disease that will be used for angler education.

Idaho. The Idaho Department of Fish and Game (IDFG) does not stock any resident trout that test positive for whirling disease. This has led to changes at the state's Hayspur Fish Hatchery, where the surface water source tests positive for *M. cerebralis*. As a result, IDFG has not reared fish on that surface water since 1995.

While infected *resident* trout are not stocked, some hatchery-reared *anadromous* fish do become exposed to the parasite before stocking. In the upper Salmon River drainage, the surface water above the Pahsimeroi and Sawtooth hatcheries tests positive for *M. cerebralis*. Steelhead eggs taken at these facilities are hatched and reared to smolt size in the Hagerman Valley in parasite-free waters. However, they must be returned to the Salmon River facilities for acclimation and imprinting before outmigration. Either during the acclimation period or during outmigration, steelhead do come in contact with

the parasite and may become infected. Chinook salmon at both facilities are also reared on well water during initial rearing periods, but are exposed to the parasite later in the rearing cycle before release.

IDFG conducted fish disease inventories during 1994-95; since 1996 IDFG has monitored wild populations where *M. cerebralis* was found during those inventories. In most of the waters monitored, no severe population losses have been found. However, trout populations have declined in the upper Teton and Big Lost Rivers. Sentinel fish exposures in these rivers resulted in high levels of infection and suggest the whirling disease is probably limiting recruitment. Other systems which have tested positive for the parasite include the South Fork Boise and Big Wood rivers. In these systems, sentinel fry exposures indicate low to moderate levels of infection consistent with samples of wild trout. Population monitoring does not indicate any major declines in the South Fork Boise and Big Wood rivers despite the presence of the parasite since the late 1980s.

Research has been another key element of Idaho's response to whirling disease. During the past year, studies by IDFG and the University of Idaho have looked at the timing and severity of infection in the South Fork Boise River, using sentinel fry exposures to compare infection levels from April to September. In other studies, the University has tested in-situ hybridization analysis as a means of determining infections rates of sentinel fish. IDFG also joined with the U.S. Fish and Wildlife Service's Bozeman Fish Technology Center to test the efficacy of the drug fumagillin to reduce infection of fry reared in waters positive for the parasite.

Montana. Montana has long been known as a mecca for wild trout fishing, and the state's outstanding fisheries support a thriving tourism industry. As a result, when whirling disease emerged as a threat to wild trout, combating this parasite became a high priority throughout the state. Concern was not limited to the Montana Department of Fish, Wildlife, and Parks (MFWP), but went up to the highest levels of state government, where Governor Marc Racicot convened a task force to develop strategies for addressing whirling disease in Montana. The task force included scientists, anglers, businesspeople, government officials, and others concerned about the state's trout resources. It has devel-

oped not only recommendations, but also tools for education and outreach to schools and the angling public. In addition, all members of the Montana Congressional delegation became personally involved as advocates for wild and native trout.

Before whirling disease was first found in the Madison River in December 1994, *M. cerebralis* had never been detected in any fish in Montana. Since that time, MFWP has conducted state-wide surveys of approximately 300 waters and detected the parasite in over 60 sites. Infected fish have been found in most of Montana's best-known trout drainages. However, the impacts to date have generally not been as severe as those seen on the Madison and fishing remains superb even in affected streams.

Montana has a well-deserved reputation for its wild trout fisheries; the state pioneered wild trout conservation and manages its rivers and streams for self-sustaining populations. MFWP remains dedicated to this wild trout philosophy and has launched a wide-ranging research program to help develop strategies for managing wild trout in the presence of *M. cerebralis*. Much of this management-oriented research has focused on trout life histories. Researchers with MFWP hope that trout with life histories that separate them (either spatially or temporally) from the most intense areas of infection may be able to survive and thrive despite whirling disease. For example, research suggests that triactinomyxon (TAM) production is highly dependent on water temperature, falling off dramatically if temperatures vary above or below a narrow range. Thus, earlier-spawning rainbows might hatch and grow while TAM numbers are lower, and be less vulnerable to disease when the "flurry" of TAMs arrives. Alternatively, tributary-spawning strains might spend the most vulnerable portions of their lives in uninfected or less-infected tributaries before moving into the mainstem rivers.

In addition to research on trout life histories relative to whirling disease, MFWP has also been involved in a wide range of studies on the susceptibility of different salmonid species, on seasonal patterns of infection in rivers, and on the possibilities for light initial infections to produce acquired immunity in different strains of trout. The MFWP budget includes a \$207,000 line item for such whirling disease activities, but when all whirling disease related work is included that figure increases substantially.

In addition to MFWP efforts, Montana State University now houses a wild trout laboratory and the National Partnership on the Management of Wild and Native Cold Water Fisheries. The Partnership, supported by federal funds, is involved in managing data from national fish health surveys and provides research grants to scientists nationwide on a competitive basis.

While Montana rivers and streams are managed almost exclusively for wild trout, trout stocking is used to support lake and reservoir fisheries. To date, no Montana hatcheries (public or private) have tested positive for *M. cerebralis*. State policy does not allow stocking of fish from infected hatcheries.

Utah. From before the time *M. cerebralis* was first found in Utah, the state has treated the parasite as a prohibited pathogen, barring the transport and release of infected fish. Thus far, the Division of Wildlife Resources (DWR) has maintained a clean bill of health – whirling disease has yet to appear in state hatcheries. The private sector has not been so fortunate, with several facilities testing positive for *M. cerebralis*.

The DWR has taken several steps in response to the whirling disease threat. State hatcheries located close to waters known to harbor the parasite are now being inspected twice per year (previously, inspections took place once yearly). Fish from the Loa hatchery continue to test negative, but because of its location (in the midst of a wild trout epizootic) it stocks only into waters that test positive for *M. cerebralis* or in dead-end drainages with no wild, reproducing salmonids. In streams that have tested positive, managers have tried to avoid stocking rainbow trout and instead use larger-sized brown trout where feasible. The DWR has also been active in whirling disease research, conducting fish health surveys of wild population and performing the only susceptibility work to date on a range of salmonid hybrids (splake, brake trout, tiger trout, brownbows). Researchers have also conducted studies on cutthroat susceptibility, on vital staining techniques that will help in discovering effective ways to kill *M. cerebralis* spores and triactinomyxons, and on pathogenesis of the parasite in tubificid worms. In partnership with Utah State University, the DWR has worked to develop a monoclonal antibody against the parasite for use in detection. Finally, a substantial education initiative (partially funded by Trout

Unlimited) is producing brochures, bumper stickers, and signs to inform the public about whirling disease.

Under recently adopted legislation, fish health policy will be established by a state fish health board. This board is balanced between wildlife/fishing interests and aquaculture interests, with two representatives from the DWR, two representatives from the Utah Department of Agriculture, one angling representative (who is a member of the TU National Resource Board), and one private aquaculture representative. The board is chaired by a non-voting representative from the Utah State University faculty. This board will shape future policy decisions for whirling disease and other fish health issues.

U.S. Fish and Wildlife Service. While fish management is generally a state-level issue, the national significance of whirling disease has also led to a federal response through the U.S. Fish and Wildlife Service (USFWS). Federal efforts have focused on participating in and funding research. Researchers at the USFWS Bozeman Fish Technology Center – as well as federal researchers in Washington and West Virginia with the U.S. Geological Survey's Biological Resources Division (BRD) – have been involved in a great deal of research discussed in this report, including work on fish susceptibility, genetic probes, and the biology and genetics of *Tubifex* worms. USFWS funds have supported work at the University of California-Davis and at BRD labs in Washington and West Virginia. Federal dollars have also been provided to the “National Partnership on the Management of Wild and Native Cold Water Fisheries,” housed at Montana State University. The National Partnership has provided over \$920,000 in competitive grants to support whirling disease research, leveraging over \$880,000 in matching funds. The Partnership has also supported a unique Wild Trout Research Laboratory in Bozeman where aquaria are available for controlled fish disease studies (and where specialized waste treatment ensures that disease is not spread from the facility into the wild).

The USFWS has also launched a national wild fish health survey, providing funding to analyze fish samples from wild populations for disease. The survey will provide information on the presence of fish pathogens and parasites – including *M. cerebralis* – in the wild.

In addition to its support of research and the fish health survey, the USFWS operates many fish hatcher-

ies that produce trout and other cold water species for stocking under state management programs. Currently, only one of those hatcheries tests positive for whirling disease – the Leadville National Fish Hatchery in Colorado. The USFWS has continued to produce fish and stock them from Leadville into waters where *M. cerebralis* has already been found, consistent with Colorado Division of Wildlife policies. The USFWS is currently preparing an environmental assessment for the hatchery, considering options for its future operations (including possible efforts to eradicate the parasite from the hatchery).

As a regulatory agency, the USFWS has a limited role with whirling disease. However, under the Lacey Act provisions for control of injurious wildlife, the USFWS is responsible for establishing and enforcing the list of prohibited pathogens for import into the United States. *M. cerebralis* was removed from the list of prohibited pathogens in 1993 based on the conventional wisdom of the time – that whirling disease did not pose a serious threat to trout. While the conventional wisdom about *M. cerebralis* has changed, its import remains unregulated (due primarily to limitations under international trade agreements). However, some protection against transferring the parasite is provided because the importation of live salmonids has been prohibited except with a case-by-case approval from the Director of the USFWS.

U.S. Forest Service and Bureau of Land Management. The Bureau of Land Management (BLM) and the U.S. Forest Service together manage more than 500 million acres of federal lands, including many of the remaining strongholds of native salmonids in the Western U.S. Neither agency operates fish hatcheries or stocks fish in waters under its jurisdiction. In general, the agencies take the position that they manage the lands and defer to the appropriate state to manage fish and wildlife on the federal lands unless otherwise required by law, regulation or policy (such as the Endangered Species Act). State stocking on federal lands is covered by Memoranda of Understanding between the agencies and the individual states, and there is a categorical exclusion from NEPA for fish stocking.

In 1988, the Forest Service prepared an Environmental Assessment that permitted the stocking of fish infected with whirling disease on Forest Service and BLM

lands. In 1995, after population declines associated with whirling disease had been documented in Colorado and Montana, Trout Unlimited requested that the agencies reconsider the 1988 EA so that the stocking of infected fish on the federal lands would require NEPA review. The agencies asked the Department of the Interior's Regional Solicitor if the stocking of known WD infected fish would require any additional environmental review beyond the categorical exclusion. The solicitor responded that no further NEPA documentation is needed to allow such practices to occur and/or continue. Therefore, it is the current policy of the Forest Service and the

BLM that if a state wishes to stock fish exposed to whirling disease on public lands, they may do so without additional environmental review under NEPA.

In response to further concerns voiced by TU about the stocking of infected fish on federal lands, Forest Service Chief Mike Dombeck (in September 1997) ordered an internal review of the agency's Memoranda of Understanding with the states to "... ensure that Federal interests are not being adversely affected by State stocking programs." Results of this review were not available at the time of this printing.

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