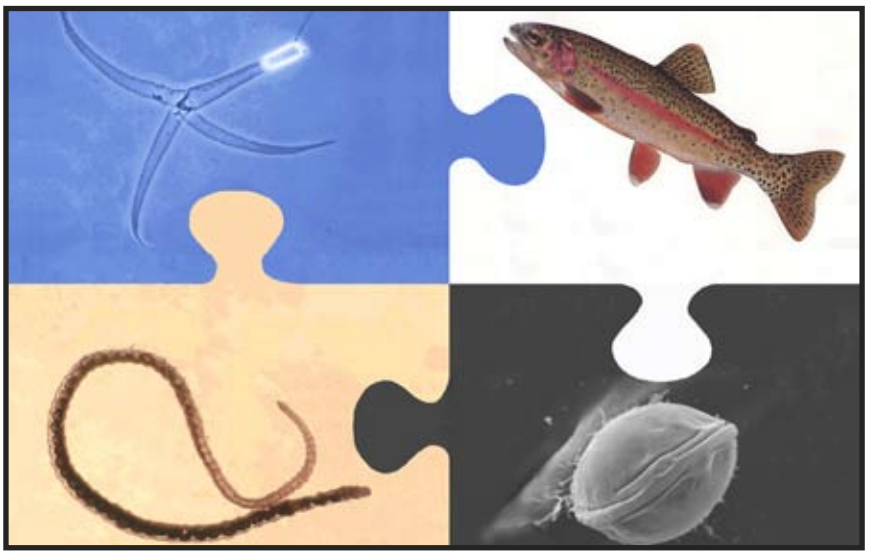
Whirling Disease in the United States

A Summary of Progress in Research and Management
2009



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Collaborations among federal, state and non-governmental organizations have been a key factor in the extensive research and management efforts related to whirling disease. The collaborators include the Whirling Disease Initiative, U.S. Fish and Wildlife Service, state fish and wildlife agencies, Whirling Disease Foundation, National Park Service, U.S. Geological Survey, U.S. Forest Service, Bureau of Land Management, Federation of Fly Fishers, Trout Unlimited, academic institutions, and the American Fisheries Society.

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Contents

Introduction

Whirling disease is widely known in the United States as a serious fish health issue, causing salmonid population level effects, and also associated with economic and ecological impacts. The disease is, however, much more complex than it appears on the surface. Whirling disease is caused by the microscopic parasite *Myxobolus cerebralis*, whose complicated life cycle relies upon two aquatic hosts: an oligochaete worm *Tubifex tubifex* and a salmonid fish. Understanding whirling disease requires comprehensive knowledge of the parasite as well as the ecology of the aquatic environment, invertebrate host, and the fish host. *Myxobolus cerebralis* is an introduced species to North America and was first described in Europe more than one hundred years ago. Its introduction to North America occurred over 50 years ago.

Impacts of the whirling disease parasite are highly variable. The introduction of *M. cerebralis* does not always result in severe wild trout population declines and there are many instances where the parasite has persisted for decades with no observable effect. However, when high numbers of parasites combine with susceptible fish, whirling disease can result in high mortality rates. In some locations, severe wild trout population declines have been attributed to whirling disease. Fish culture has also been significantly impacted by the parasite as its detection may require quarantine of a culture facility, destruction of fish stocks, disinfection, facility renovations, and even closure. Thus, fish culture has had significant economic impacts from preventative actions and mitigation associated with whirling disease.

Myxobolus cerebralis continues to be detected in new locations each year, and thus the key for future management efforts lies in predicting and managing the risks associated with whirling disease. Fortunately, an extensive body of research is available to provide information about whirling disease and a broad suite of management tools and techniques can be employed to prevent the spread of *M. cerebralis* and to mitigate its impacts. *Myxobolus cerebralis* is likely to persist in North America. However, the management options generated through intense and collaborative research will help ensure that popular sport fisheries and valued native trout species will also persist. The purpose of this document is to summarize the current status and knowledge on whirling disease and *M. cerebralis* and to make it easily available for researchers, fish managers and fish culturists. This review builds on earlier reviews, focusing on new research that reveals details of the invertebrate host ecology, epidemiology within salmonids, management efforts, and improved diagnostic techniques.

Life Cycle and Description of *Myxobolus cerebralis*

Whirling disease is caused by the microscopic parasite *Myxobolus cerebralis*, one of the most pathogenic and intensely studied members of a diverse group of parasites known as myxozoans (Lom and Dykova 1992; Hedrick et al. 1998). Myxozoans are multicellular organisms and, though originally considered protozoans, they are now known to be members of the Kingdom Animalia, specifically cnidarians (Jimenez-Guri et al. 2007). Kerans and Zale (2002) and Hedrick and El-Matbouli (2002) provide further review of *M. cerebralis* taxonomy and evolution. Like other myxozoan parasites, *M. cerebralis* takes several unique physical forms and requires both an invertebrate and a vertebrate host to complete its life cycle (Figure 1).

Myxobolus cerebralis has two distinct infective stages: the myxospore and the triactinomyxon. The myxospore infects the invertebrate host, *Tubifex tubifex* worms, and the triactinomyxon infects the vertebrate host, salmonid fish (Markiw and Wolf 1983; Wolf and Markiw 1984). The parasite's two infective stages were formerly thought to represent two separate species because they differ greatly in physical appearance and infect unique hosts. However, in 1983 the *M. cerebralis* myxospore and triactinomyxon were recognized as belonging to a single species (Markiw and Wolf 1983; Wolf and Markiw 1984), and this discovery was later confirmed genetically (Andree et al. 1997). Previous names for the organism included *Myxobolus chondrophagus*

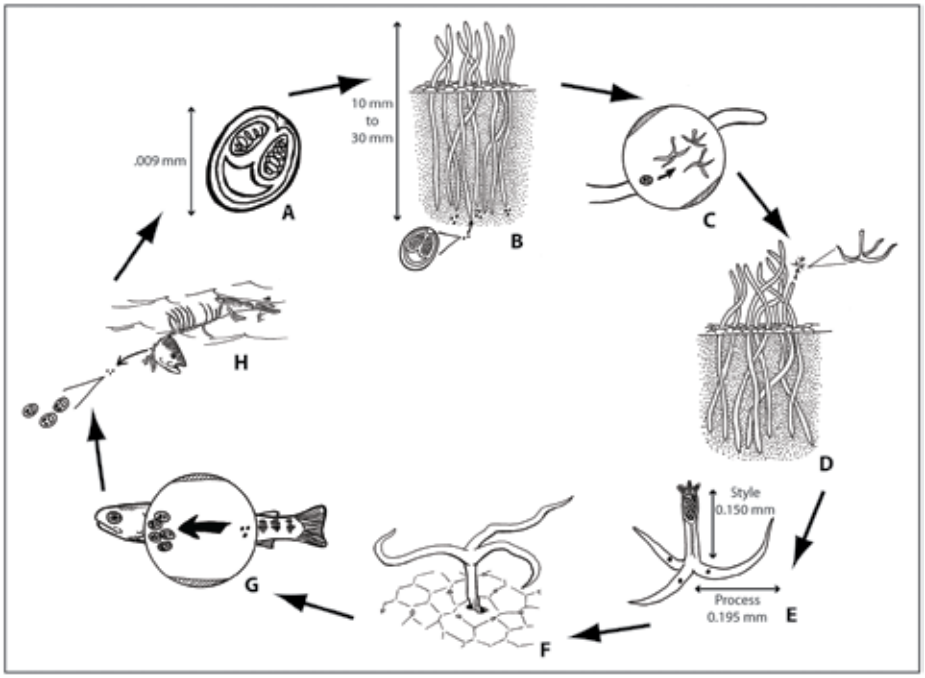


Figure 1. The complex life cycle of *Myxobolus cerebralis* involves multiple stages of the parasite and requires two hosts: an aquatic worm *Tubifex tubifex*, infected by the parasite's myxospore (A), and a salmonid fish, infected by the triactinomyxon (E) (Illustrations by Claire Emery).

(Höfer 1904), *Lentospora cerebralis* (Plehn 1905), *Myxosoma cerebralis* (Kudo 1933), and *Triactinomyxon gyrosalmo* (Wolf and Markiw 1984). The name *Myxobolus cerebralis* was reestablished in 1984 (Lom and Noble 1984).

The *M. cerebralis* myxospore, which infects the worm host, is a sphere approximately 9 micrometers wide (about the size of a red blood cell) (Figure 1A). The outer covering is a complex polysaccharide matrix that provides protection from the environment (Lom and Hoffman 1971). This protective cover allows the myxospore to be highly durable under adverse conditions including freezing, heat, many chemical treatments, and digestion by wildlife (Hoffman and Putz 1969; Myers et al. 1970; Hoffman and Hoffman 1972; Taylor and Lott 1978; Wolf and Markiw 1982; El-Matbouli and Hoffmann 1991). Recent work by Hedrick et al. (2008) examined the infectivity of myxospores following exposure of freezing, drying, UV irradiation, chlorine and quaternary ammonium. Temperatures below 5 °C for 60 days, and UV radiation resulted in inactivation of myxospores; whereas, depending on the concentration of chlorine and quaternary ammonium, myxospores remained viable (Hedrick et al. 2008). The life span of myxospores is somewhat uncertain. Early studies report they remain viable for between 3 and 30 years (Hoffman et al. 1962; Halliday 1976); however, these studies were conducted before the life cycle was known and these results may require reinterpretation.

The triactinomyxon, the parasite form that infects the fish host, is much larger than the myxospore at approximately 150 micrometers in style height and 195 micrometers in process length (Figure 1E) (El-Matbouli and Hoffmann 1998). This is approximately the size of the period at the end of this sentence; however, the parasite is transparent and usually invisible to the naked eye. Triactinomyxons, often referred to as "TAMs," are neutrally buoyant and float freely in the water column while being transported downstream (Gilbert and Granath 2003). The sporoplasm, a mass of protoplasm containing infective germ cells, is contained in the triactinomyxon style before injection into the fish host (Figure 2). The extended, grappling-hook shape of the triactinomyxon is a fragile structure which is consequently short-lived. Viability depends on environmental conditions such as temperature and water turbulence. Triactinomyxons are generally viable at water temperatures of 7 to 15 °C, surviving for 6 to 15 days (Markiw 1992b, El-Matbouli et al. 1999c), and thus may be transported considerable distances (Gilbert 2002).

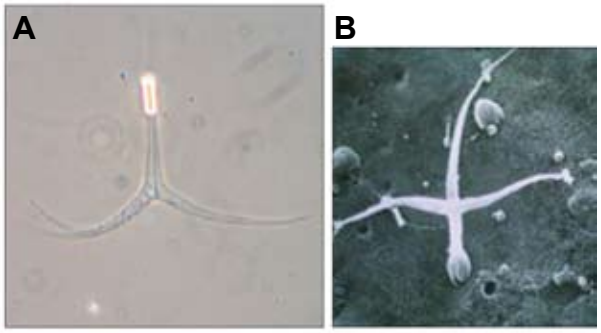


Figure 2. A. Triactinomyxon showing refractile sporoplasm and transparent processes. B. Scanning electron microscopic image of a triactinomyxon penetrating the fish host.

The invertebrate host of *M. cerebralis* is the aquatic oligochaete worm, *Tubifex tubifex* (Figures 1B, 3) (Wolf et al. 1986; Hedrick et al. 1998; Kerans et al. 2004). *Tubifex tubifex* worms average 10 to 30 millimeters in length, are widely distributed around the globe, and are often tolerant of high sediment and nutrient levels (Brinkhurst and Cook 1980; Chapman et al. 1983; Brinkhurst and Kathman 1996). *Tubifex tubifex* ingests *M. cerebralis* myxospores while feeding in benthic sediment (Figure 1B). Once in the worm's intestine, the myxospore opens and the parasite penetrates between cells of the intestinal lining. The parasite replicates, and by a series of cellular divisions, the triactinomyxon is produced (Figure 1C). During its life cycle, *M. cerebralis* multiplies through both sexual and asexual reproduction. Sexual reproduction takes place only within the worm *T. tubifex*, which identifies the worm as the parasite's primary host. For detailed accounts of parasite development, refer to El-Matbouli and Hoffmann (1998), Hedrick et al. (1998), Hedrick and El-Matbouli (2002), and Gilbert and Granath (2003). Although other worm species may ingest

the parasite, *M. cerebralis* is able to replicate and complete development only in *T. tubifex* (Wolf et al. 1986; Kerans et al. 2004; DuBey and Caldwell 2004).

Figure 3. *Tubifex tubifex*.

Development of the triactinomyxon within *T. tubifex* takes between 65 and 120 days, depending on water temperature (Markiw 1986; El-Matbouli and Hoffmann 1998; El-Matbouli et al. 1999c; Gilbert and Granath 2001; Stevens et al. 2001; Blazer et al. 2003; Steinbach 2003; Kerans et al. 2005). Triactinomyxons are expelled with feces from the worm into the water (Figure 1D). The myxospore dose ingested by *T. tubifex* further determines the number of triactinomyxons eventually produced (Markiw 1986; Stevens et al. 2001; Steinbach Elwell et al. 2006). While it appears there is a positive relationship between the numbers of myxospores consumed and triactinomyxons produced, relatively few myxospores are needed to achieve 100% infection within *T. tubifex* (Steinbach Elwell et al. 2009). Infection in *T. tubifex* is persistent and marked by a cyclic release of triactinomyxons over time in the laboratory (Gilbert and Granath 2001). Seasonal and diel fluctuations in triactinomyxon release have also been observed in natural systems (Lukins 2004). *Tubifex tubifex* have reportedly released triactinomyxons for at least one year (El-Matbouli and Hoffmann 1998) and even more than two years (Reno and Lorz 2005) after a single exposure to myxospores. Once triactinomyxons are released, they float suspended in the water column until they encounter a fish host or are destroyed by environmental conditions. Infection by *M. cerebralis* can negatively impact the growth, reproduction and survival of the *T. tubifex* host (El-Matbouli and Hoffmann 1998; Stevens et al. 2001; Hedrick and El-Matbouli 2002; Gilbert and Granath 2003).

The salmonid host becomes infected with *M. cerebralis* through contact with free-floating triactinomyxons (Wolf and Markiw 1984). Once a triactinomyxon attaches to a susceptible fish host, expulsion of the sporoplasms into the fish occurs within ten minutes presumably from both mechanical and chemical stimuli (Figure 1F) (El-Matbouli et al. 1995; El-Matbouli et al. 1999b; Kallert et al. 2005a). Triactinomyxons only release sporoplasms into live salmonids and are unable to infect dead or non-salmonid fish (El-Matbouli et al. 1999b; Adkison et al. 2001; Adkison et al. 2002; Hedrick and El-Matbouli 2002). Parasite dose strongly determines the severity of whirling disease, which generally increases with the number of triactinomyxons the fish encounters (Hoffman 1974; O'Grodnick 1979; Markiw 1991, 1992a, 1992b; Hedrick et al. 1999a; Thompson et al. 1999; Densmore et al. 2001; Ryce et al. 2001; Ryce et al. 2004; Ryce et al. 2005).

Most research on *M. cerebralis* development in fish has been conducted using cultured rainbow trout (*Oncorhynchus mykiss*), and the summary included herein describes an approximate timeline of parasite development in rainbow trout. The timing of developmental stages is highly temperature-dependent and may also vary from one salmonid species to another. During the first 10 days of fish infection, the parasite multiplies through a series of asexual cellular divisions and migrates to the central nervous system. The parasite avoids the fish's immune response by utilizing nerve tissue as a pathway for movement (El-Matbouli et al. 1995; Adkison et al. 2002). Further studies suggest that the location (e.g. epithelium, cartilage or nerve ganglia) of immune response within the fish differs among salmonid species (Adkison et al. 2003). Infection by *M. cerebralis* occurs when the parasite successfully invades the fish host; however, infection does not necessarily result in the onset of whirling disease (Markiw 1992a). The disease state depends on complex interactions among the hosts, pathogen, and the environment (Hedrick 1998), and is characterized by inflammation, tissue damage, and other clinical signs

as described below. The signs of whirling disease and their severity often vary by species. Asymptomatic carriers of the parasite are common and can act as vectors for the parasite.

Approximately 20 days after exposure the parasite concentrates in cartilage, primarily in the cranium and spine. *Myxobolus cerebralis* digests cartilage, causing lesions and inflammation, and potentially damaging the skeletal structure of the fish (El-Matbouli and Hoffmann 1998). MacConnell and Vincent (2002) describe these effects in detail. The parasite subsequently develops into the hardier myxospore (Figure 1G). Myxospore development takes between 52 and 120 days, and is closely linked to temperature (Halliday 1973b; Markiw 1992a; Hedrick and El-Matbouli 2002). Myxospores can become encased in bone as skeletal development replaces cartilage with bony structures. Eventually, death and decomposition of the fish release the mature myxospores back into the environment (Figure 1H) (El-Matbouli et al. 1992). As discussed above, myxospores may even be carried and released by fish that exhibit no signs of whirling disease.

Locations of lesions vary among fish species, and can explain variability in disease severity and survival among different species. Cartilage lesions observed by histology in rainbow trout tend to concentrate in the cranium and base of the brain, but can be found throughout cartilage in the body (Figure 4) (MacConnell and Vincent 2002). In brown trout, lesions concentrate in fin rays and gill arches (Hedrick et al. 1999a; Baldwin et al. 2000). Yellowstone cutthroat trout tend to have lesions located in the cranium and jaws (Murcia et al. 2006). External cysts containing spores are atypical, but have been observed on the opercula and fin rays of infected fish (Taylor and Haber 1974). Impacts upon infected fish are likely to be greater when lesions are located in the cranium compared to fin rays.

The clinical signs of whirling disease can be dramatic (Figure 5) and include:

- ***Whirling behavior.*** Tail-chasing or "whirling" behavior, for which whirling disease is named, results from spinal cord constriction and brain stem compression (Rose et al. 2000). Whirling fish cannot maintain equilibrium and swim erratically, often until exhausted. This behavior likely reduces survival in the wild if fish are unable to seek food or avoid predators.

- ***Blackened tail.*** A blackened tail is caused by pressure on nerves that control pigmentation (Halliday 1976; Schaperclaus 1991).

- ***Skeletal deformities.*** Permanent deformities of the head, spine, and operculum are caused by cartilage damage, associated inflammation, and interference with normal bone

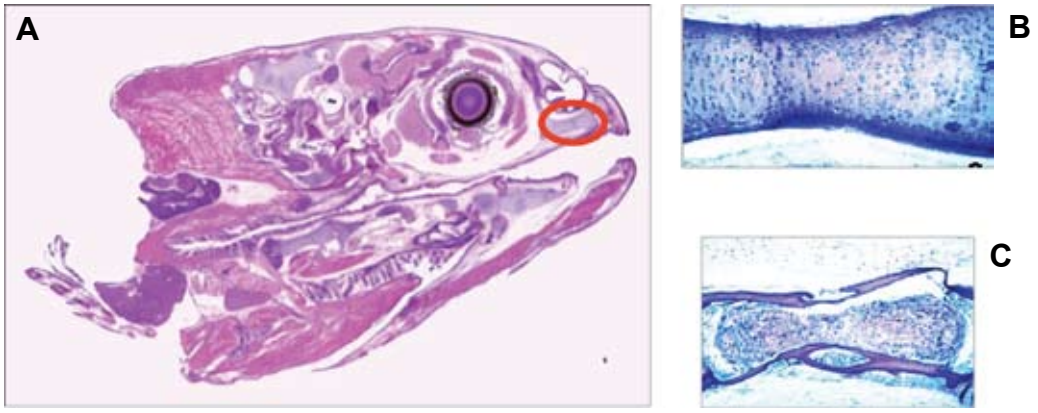


Figure 4. Thinly sliced cross sections of infected fish tissues reveal microscopic damage caused by *Myxobolus cerebralis* to cartilage when compared to healthy fish. A. Cross section through rainbow trout head showing location of cartilage (red circle) in smaller images. B. Cartilage section from an uninfected fish. C. Cartilage section from a heavily infected fish. Images courtesy of Sylvia Murcia, Montana State University and Beth MacConnell, US Fish and Wildlife Service.

development (Wolf 1986; Schaperclaus 1991; Hedrick et al. 1999a MacConnell and Vincent 2002). Commonly observed deformities include shortened operculum, indented skull, crooked spine and shortened nose.

- ***Mortality.*** Whirling disease can result in death of the fish host, particularly among young fish of susceptible species and strains (Hoffman 1974; Markiw 1991). Mortality may be caused by direct physical damage or a resulting inability to feed or evade predators.

These clinical signs are not unique to whirling disease, and can be caused by other conditions. For example, erratic swimming and blackened tail can be caused by bacterial coldwater disease (Margolis et al. 1996; American Fisheries Society Fish Health Section 2007). Therefore, whirling disease cannot be diagnosed based on physical signs alone and must be confirmed by methods that specifically identify the parasite (see section on Detection Techniques).

In addition to the whirling disease signs previously described, *M. cerebralis* infection can cause reduced fitness by slowing growth rate (Uspenskaya 1957; Hoffman 1974; Hedrick et al. 2001a; MacConnell and Vincent 2002) and reducing swimming performance (Ryce et al. 2001; Schisler 2006). This can reduce survival and reproductive success of infected fish, particularly when under additional stress. For instance, rainbow trout exposed to *M. cerebralis* experienced increased mortality at elevated water temperatures compared to non-exposed fish (Schisler et al. 2000).

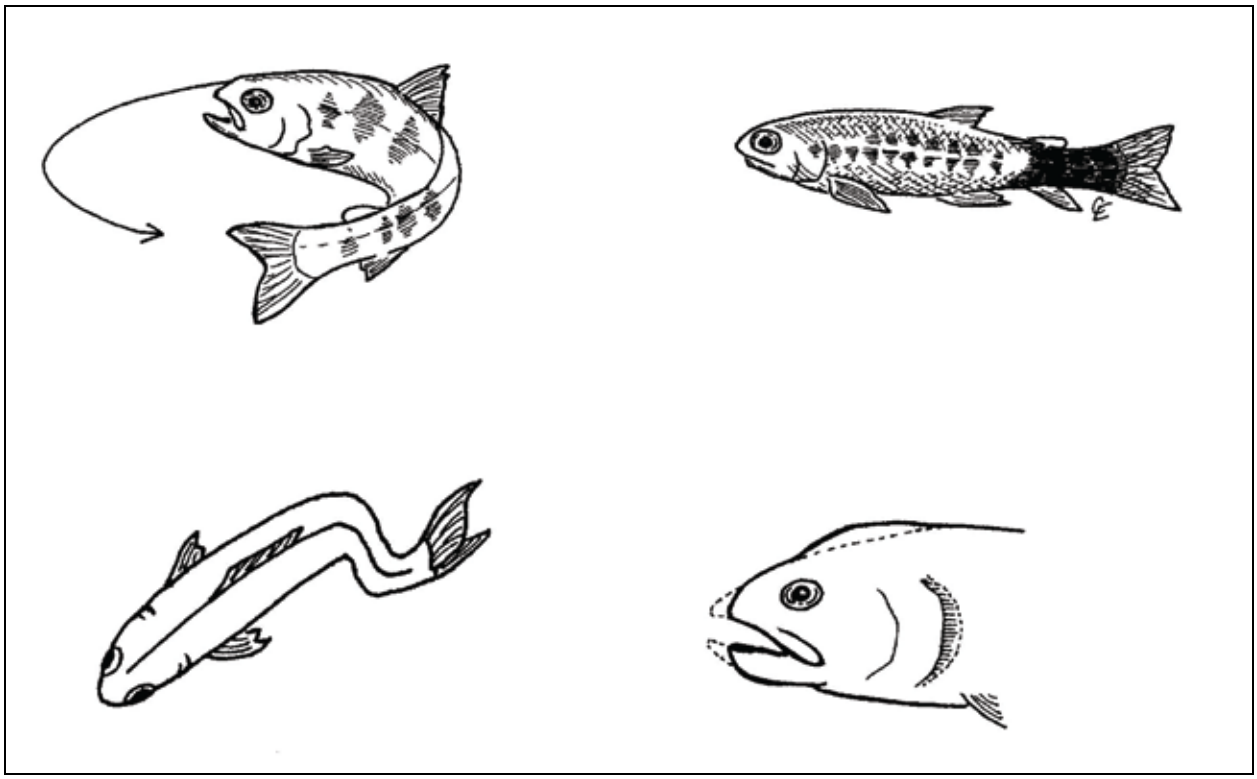


Figure 5. Clinical signs of whirling disease include whirling behavior, blackened tail, skeletal deformities of the spine and head, and mortality.

History of Whirling Disease in the United States

Whirling disease originated in Europe and was first described in 1903 by Bruno Höfer in Germany (Höfer 1903). Evidence suggests *M. cerebralis* originated in Europe as a parasite of brown trout, but the disease was not observed until the more susceptible rainbow trout were imported to Europe from North America about 1900 (Hoffman 1970; Halliday 1976; Nickum 1999). Höfer observed clinical signs of whirling disease among captive-reared rainbow trout and detected microscopic parasitic spores in the craniums of affected fish. The parasite was named *Myxobolus cerebralis*, and Höfer provided the first description of the parasite and resulting disease. Bartholomew and Reno (2002) provide a comprehensive review of the parasite's international spread and the resulting impacts. Between 1900 and 1950, the parasite was detected in several European countries (Bartholomew and Reno 2002). In many of these locations, severe losses of cultured rainbow trout and brook trout were reported. Between 1950 and 2000, the parasite was found across Europe, northern Asia, New Zealand, the United States, and in Morocco, Lebanon, and South Africa.

It is unknown precisely when the first introduction of *M. cerebralis* to North America occurred or how it happened. Exchanges of live fish, fish eggs, and frozen fish were common between Europe and the United States, and the parasite was probably introduced inadvertently through the transfer of infected fish or fish products (Hoffman et al. 1962; Hoffman 1990). Trout showing signs similar to whirling disease were reported in New York during the 1930s, but the presence of *M. cerebralis* was not confirmed at this time (P. Hulbert, New York Department of Environmental Conservation, personal communication). The first confirmed detection of *M. cerebralis* in the United States occurred at the Benner Spring Fish Research Station, Pennsylvania, in 1958, following a disease outbreak that began in 1956 (Hoffman et al. 1962). Around the same time as the discovery in Pennsylvania, the parasite was also present in Nevada. Examination of archived fish cranial tissue samples revealed that *M. cerebralis* was present in 1957 at the Verdi Fish Hatchery on the Truckee River in Nevada (Taylor et al. 1973).

Since the first detection of the whirling disease parasite in the United States, it has been confirmed in hatcheries, ponds or streams in 25 states (Figure 6). By 2009, the parasite was detected in Alaska, Arizona, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, Ohio, Oregon, Pennsylvania, New Mexico, Utah, Vermont, Virginia, Washington, West Virginia, and Wyoming (Table 1). Although the parasite has been detected in 25 states, *M. cerebralis* is not necessarily established or widespread in each state. In some states, the parasite has been detected in most major river systems. In other states, the parasite was confirmed in a single hatchery or pond. For example, the parasite's presence in Alaska was confirmed only by the detection and sequencing of *M. cerebralis* DNA; spores were not observed (Arsan et al. 2007). *Myxobolus cerebralis* may be present without causing disease, and detection of the parasite does not necessarily imply fish developed clinical whirling disease.

Myxobolus cerebralis was detected in many fish culture facilities across the country between 1957 and 1990, but there were few reports of whirling disease in wild fisheries during this time (Yoder 1972; Hoffman 1990; Modin 1998; Bartholomew and Reno 2002). Within hatcheries, detection of the parasite often resulted in the subsequent destruction of fish stocks, disinfec-

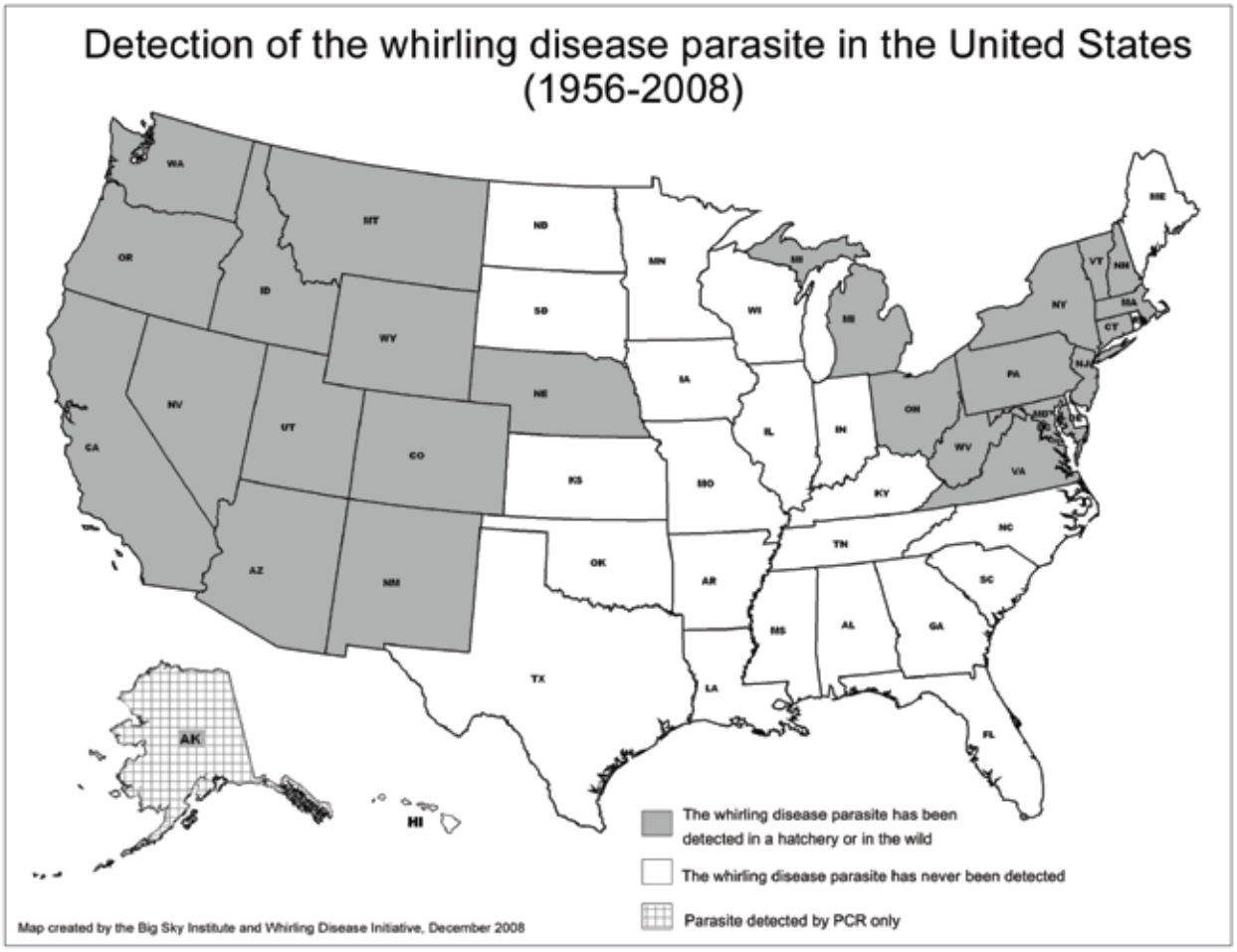


Figure 6. The whirling disease parasite has been detected in 25 states, and its distribution within those states varies widely. The map depicts states where the parasite has been detected one or more times by any method in either fish culture or in the wild. The map does not indicate the continued presence of the parasite or the extent of distribution within the state. For example, the parasite has been detected in Arizona, but there is no evidence that it has become established in that state (Rob Bettaso, Arizona Department of Game and Fish, personal communication). In Alaska, the parasite's DNA was detected among trout at one hatchery, but no stages of the parasite have been observed visually (Arsan et al. 2007).

tion, renovation of facilities, and even hatchery closures. Whirling disease became considered a manageable problem in fish culture, but was not expected to cause widespread disease or population losses among wild salmonids (Modin 1998; Bruneau 2001). In the mid-1990s, dramatic wild trout population declines in Colorado and Montana were attributed to whirling disease (Nehring and Walker 1996; Vincent 1996). This development controverted earlier expectations and generated great concern among resource managers, conservationists, and anglers. The discovery that wild fish were significantly affected resulted in a major increase in time and resources dedicated to whirling disease research and management.

Since the 1990s, coordinated, multi-agency research efforts have yielded important advances in understanding and managing the challenges of whirling disease. *Myxobolus cerebralis* in fish culture remains an expensive and serious problem. Despite improved diagnostic techniques and stringent policies to prevent the parasite's spread, each year the parasite has been detected in additional public and private facilities. For example, in early 2007 new detections of *M. cerebralis* were reported from Alaska (Arsan et al. 2007), Maryland (S. Rivers, Maryland Department of Natural Resources, personal communication), and Utah (C. Wilson, Utah Division of Wildlife Resources, personal communication).

Fortunately, many strategies and technologies can be employed to disinfect hatcheries, exclude the parasite, and produce parasite-free fish. In the wild, patterns of *M. cerebralis* distribution, infection and disease impacts are much more variable and difficult to predict because these patterns are influenced by complex interactions among many environmental and human factors. Developments in wild trout research and management have provided tools and techniques to predict risk, prevent parasite introductions, and mitigate the impacts of *M. cerebralis*.

Table 1. The detection of the whirling disease parasite in the United States.

State	First Detection	Reference
Pennsylvania	1958	The parasite was confirmed in 1958 following an outbreak that began in 1956 (Hoffman et al. 1962).
Nevada	1957	The parasite was first confirmed in 1966 (Yasutake and Wolf 1970). Examination of archived samples reportedly detected *M. cerebralis* from tissues collected in 1957 (Taylor et al. 1973)
Connecticut	1961	Hoffman et al. 1962
Virginia	1965	Hoffman 1990
California	1966	Yasutake and Wolf 1970
Massachusetts	1966	Hoffman 1990
West Virginia	1966	V. Blazer, U.S. Geological Survey, personal communication
New Jersey	1967	V. Blazer, U.S. Geological Survey, personal communication
Michigan	1968	Hnath 1970; Yoder 1972
Ohio	1968	Tidd and Tubb 1970
New Hampshire	1980	Hoffman 1990
New York	1984	Hoffman 1990; P. Hulbert, New York Department of Environmental Conservation, personal communication
Oregon	1986	Holt et al. 1987; Lorz et al. 1989
Colorado	1987	Barney et al. 1988; Walker and Nehring 1995
Idaho	1987	Hauck et al. 1988
New Mexico	1987	Hansen et al. 2002
Wyoming	1988	Records showed infected rainbow trout from Colorado had been stocked in 1986 and 1987, resulting in the parasite being documented in 1988. Wyoming Game and Fish 1989; Mitchum 1995
Utah	1991	Wilson 1991; Heckmann et al. 1992
Montana	1994	Vincent 1996
Maryland	1995	S. Rivers, Maryland Department of Natural Resources, personal communication
Washington	1996	J. Thomas, Washington Department of Fish and Wildlife, personal communication
Arizona	2000	R. Bettaso, Arizona Game and Fish Department, personal communication
Nebraska	2001	D. Rosenthal, Nebraska Game and Parks Commission, personal communication
Vermont	2002	B. Johnston, Vermont Department of Fish and Wildlife, personal communication
Alaska	2006	Arsan et al. 2007

Factors Influencing Infection and Disease in Salmonids

Salmonid Factors

Age and Size. The development and severity of whirling disease depend significantly on the age and size of the salmonid host when exposed to the parasite. It has long been known that very young fish are most vulnerable to *M. cerebralis*, and susceptibility decreases with age and growth as bone replaces cartilage in the developing fish (Hoffman and Byrne 1974; Halliday 1976, O'Grodnick 1979; Markiw 1991, 1992a; El-Matbouli et al. 1992). More recent studies have demonstrated that younger fish are more vulnerable to nerve damage due to the parasite (Rose et al. 2000).

Salmonid eggs cannot be infected by *M. cerebralis* (Putz and Hoffman 1966; Markiw 1991). This is probably because eggs do not provide the physical or chemical cues for the parasite's injection through the egg surface, and eggs do not contain the tissues necessary for parasite development. Upon hatching, young salmonids can be highly susceptible to *M. cerebralis* (Markiw 1991). Later, it appears that fish reach a developmental threshold during which they develop immunity or resistance. For rainbow trout, it was long thought that fish older than 4 to 8 months or longer than 5 to 13 centimeters were resistant to development of whirling disease (Hoffman 1961, 1976; Halliday 1976; Wolf 1986; Lom 1987; Schaperclaus 1991; Garden 1992). Later studies found rainbow trout appear to reach a threshold of greater resistance 9 weeks after hatching and when larger than 4 cm fork length (Ryce et al. 2004, 2005). In more resistant Chinook salmon (*Oncorhynchus tshawyscha*) this resistance threshold developed as early as 3 weeks after hatching (Sollid et al. 2003). However, infection of larger fish can occur under high exposure doses or sustained parasite challenge, conditions that might be encountered in the wild. For example, yearling bull trout (*Salvelinus confluentus*) and adult rainbow trout (*O. mykiss*) (Markiw 1992a) developed myxospores when challenged at a high dose under laboratory conditions (Bartholomew et al. 2003), and steelhead (*O. mykiss*) smolts (90 g) naturally exposed to the parasite became infected (Sollid et al. 2004).

Salmonid responses to *M. cerebralis* infection vary among genera, species, strains, and individuals (Table 2). Most species of the genus *Oncorhynchus* experience high prevalence and severity of disease, and high mortality rates if exposed to a sufficient parasite dose when susceptible (summarized by MacConnell and Vincent 2002; Sollid et al. 2002, 2003; Vincent 2002; Wagner et al. 2002a; Bartholomew et al. 2003; DuBey et al. 2006; Murcia et al. 2006). Highly susceptible species include rainbow trout, cutthroat trout (*O. clarki*), and sockeye salmon (*O. nerka*). However, some species of the genus *Oncorhynchus*, including coho (*O. kisutch*), chum (*O. keta*), and pink salmon (*O. gorbuscha*), do not exhibit clinical signs of whirling disease regardless of dose and develop fewer myxospores than rainbow or cutthroat trout at similar exposure levels (Halliday 1976; O'Grodnick 1979; Hedrick et al. 2001). Species in the genus *Salmo*, such as brown trout (*S. trutta*) and Atlantic salmon (*S. salar*), can become infected; however, clinical signs are limited and fewer myxospores develop than in other trout species when exposed under similar conditions (Hoffman 1990; Hedrick et al. 1998; Thompson et al. 1999; Baldwin et al. 2000). Brown trout have low susceptibility to whirling disease and will develop clinical signs only when exposed to high numbers of parasites. Brown trout and Atlantic salmon are likely less susceptible to the parasite due to co-evolution with *M. cerebralis* in their

Table 2. Susceptibility to whirling disease among species of salmonids by laboratory or natural exposure to *Myxobolus cerebralis* at vulnerable life stages.

Genus	Species/Subspecies	Common Name	Susceptibility
Oncorhynchus	*mykiss*	Rainbow trout	3
	mykiss	Steelhead trout	3
	clarki	Cutthroat trout	2-3
	c. bouvieri	Yellowstone cutthroat	3
	c. lewisi	Westslope cutthroat	2
	c. pleuriticus	Colorado River cutthroat	2
	c. virginalis	Rio Grande cutthroat	2
	c. stomias	Greenback cutthroat	2
	tshawytscha	Chinook salmon	2
	nerka	Sockeye salmon	3
	keta	Chum salmon	1 S
	gorbuscha	Pink salmon	1 S
	masu	Cherry salmon	1 S
	kisutch	Coho salmon	1
Salvelinus	*fontinalis*	Brook trout	2
	malma	Dolly Varden	1 S
	confluentus	Bull trout	1
	namaycush	Lake trout	0
Salmo	*salar*	Atlantic salmon	2 S
	trutta	Brown trout	1
Prosopium	*williamsoni*	Mountain whitefish	2
Thymallus	*thymallus*	European grayling	2 S
	arcticus	Arctic grayling	0
Hucho	*hucho*	Danube salmon	3

0 = Resistant, no spores develop
1 = Partial resistance, clinical disease rare and develops only when exposed to very high parasite doses
2 = Susceptible, clinical disease common at high parasite doses or when very young, but greater resistance to disease at low doses
3 = Highly susceptible, clinical disease common
S = Susceptibility is unclear (conflicting reports or insufficient data)

Sources: MacConnell and Vincent 2002; B. MacConnell, U.S. Fish and Wildlife Service, personal communication.

native Europe (Halliday 1976). Species in the genus *Prosopium*, such as mountain whitefish (*P. williamsoni*), are susceptible to infection (Taylor 1974; Baldwin et al. 1998; MacConnell and Vincent 2002) and fry experience high rates of mortality following exposure to high numbers of the parasite (Schisler et al. 2008). Species of the of the genus *Salvelinus* exhibit variable responses to *M. cerebralis*. Brook trout (*S. fontinalis*) are susceptible to whirling disease when subjected to high doses of the parasite; they can develop moderate clinical signs and myxospore quantities (O'Grodnick 1979; El-Matbouli et al. 1992; Thompson et al. 1999). Bull trout (*S. confluentus*) have shown moderate infection and variable disease signs (Vincent 2002; Bartholomew et al.

2004). Lake trout (*S. namaycush*) exhibits strong resistance to whirling disease and are unlikely to develop myxospores (O'Grodnick 1979; Blazer et al. 2004). Species of the genus *Thymallus*, such as arctic grayling (*T. arcticus*), are resistant to infection and do not develop myxospores (Hedrick et al. 1999b).

Life History. In wild salmonid populations, the impacts of whirling disease are strongly affected by life history. When and where trout spawn, when the fry emerge, where they rear, and the abundance of triactinomyxons all factor into the risk of a population developing whirling disease (Downing et al. 2002, reviewed by Kerans and Zale 2002). Since *M. cerebralis* poses the greatest threat to fish during hatching and emergence, the timing and location of those events critically determine the risk of whirling disease. Fish are most vulnerable if they hatch during the peak release of *M. cerebralis* triactinomyxons, which depends on water temperature and typically occurs from June through September (Thompson and Nehring 2000; Downing et al. 2002). Rainbow trout typically spawn during the spring and hatch in early summer (Behnke 1992). Their emergence coincides with the seasonal peak of triactinomyxon release and the fry are at high risk for whirling disease. In contrast, species that spawn in the fall and hatch during late winter to early spring, before the seasonal peak in triactinomyxon densities, are a larger size when they first encounter the parasite. Consequently these fish are less susceptible to development of whirling disease. Examples of species with less susceptible life histories include Chinook salmon (Sandell et al. 2002), lake trout, and brook trout (Behnke 1992). In spring creek systems, where water temperatures are moderate and constant throughout the year, a different pattern of infection has been observed. High infection rates were observed in the fall suggesting fall spawning species may be vulnerable to infection in spring creek systems (Anderson 2004).

Strain. It was generally considered that within salmonid species susceptibility was similar; however, recent studies have reported substantial intraspecies variation in susceptibility to *M. cerebralis*. Whirling disease resistance among rainbow trout has been of particular interest and has been intensely studied. When exposed to identical doses of *M. cerebralis* triactinomyxons, some strains of rainbow trout develop severe cartilage lesions while other strains experience only a mild infection (Densmore et al. 2001; Vincent 2002; Wagner et al. 2002a; Hedrick et al. 2003; Wagner et al. 2006).

Selection for resistance could be expected to occur in naturally reproducing populations in *M. cerebralis* endemic areas. The resistance of progeny of Colorado River rainbow trout produced from adult fish that were present either before or after establishment of *M. cerebralis* has been compared in two independent studies. The two studies were contradictory and it was not clear whether selection for resistance had occurred (Thompson et al. 1999; Ryce et al. 2001). In contrast, preliminary research on wild rainbow trout of the Madison River, Montana, suggests rainbow trout that survived the severe whirling disease outbreak in the 1990s may have passed along genetic resistance to their offspring (Vincent 2006). In experimental challenges, fry from parents that were hatched since whirling disease was discovered in the Madison River show a decrease in clinical disease compared with fry from parents hatched prior to the appearance of *M. cerebralis*. However, despite these indications of increasing resistance, and partial recovery of younger age classes, numbers of older adult rainbow trout in the Madison River have not increased, thus leaving questions about the trade-offs of developing resistance (E.R. Vincent, Montana Fish, Wildlife & Parks, personal communication).

Differences in resistance have also been reported from domestic trout strains. Two strains of rainbow trout, DeSmet and German (GR; also referred to as Hofer strain), have demonstrated resistance to *M. cerebralis* infection and whirling disease when compared to other rainbow trout strains. DeSmet rainbow trout originated in DeSmet Lake, Wyoming, where eggs were historically gathered for distribution to state and federal hatcheries (Wagner et al.

2006). DeSmet rainbow trout and their descendants, including the Fish Lake-DeSmet, Harrison Lake, and Wounded Man Lake "strains," have been evaluated for resistance, but results have been inconsistent (Densmore et al. 2001; Vincent 2002; Wagner et al. 2002a; Hedrick et al. 2003; Wagner et al. 2006). The GR rainbow trout originated from a private hatchery in Bavaria, Germany (Hedrick et al. 2003). It is likely that this strain has been in contact with the parasite for more than 100 years and developed resistance during this time. Hedrick et al. (2003) reported that the GR rainbow trout had acquired significant resistance to whirling disease when compared to North American rainbow trout. Further research has evaluated the application of this strain in development of *M. cerebralis*-resistant broodstocks. Since the GR rainbow trout are highly domesticated, it may be desirable to develop a breeding program to incorporate both whirling disease resistance and characteristics of wild rainbow trout (Schisler et al. 2006). Experiments conducted by Schisler et al. (2006) showed a significant reduction in spores per fish among the first generation cross (F1 generation) between the GR and the wild Colorado River rainbow (CRR) compared to those in the CRR strain; however, spore counts were not reduced to the levels of the GR strain. A backcross, created by spawning an F1 generation individual with a CRR, also showed a reduction in spore counts compared to the CRR strain; however, spore counts were higher than in the F1 generation (Schisler et al. 2007). Experimental stocking of these crosses into certain Colorado waters is showing early signs of success, with evidence that these fish are reproducing in the wild and showing decreased clinical signs of whirling disease (Kowalski et al. 2008a, b, 2009). Management agencies in other states are carefully considering potential risks and benefits for utilizing selective breeding and stocking of disease-resistant fish in their programs. Resistance in salmonids continues to be an important area of study, as one strategy for managing whirling disease is the utilization of an *M. cerebralis*-resistant fish in stocking and aquaculture programs (see Control and Management section).

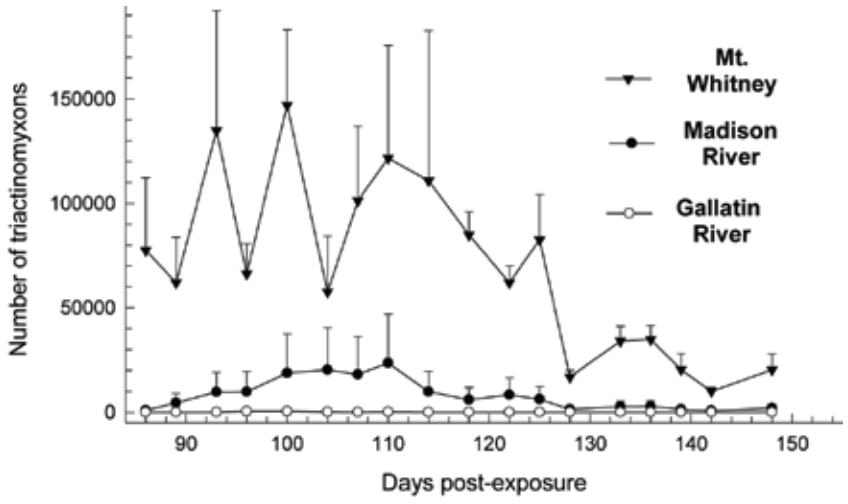
Invertebrate Factors

Benthic Community Composition. *Tubifex tubifex* worms must be present for completion of the *M. cerebralis* life cycle. This species is widely distributed in the United States and occupies habitats that are highly variable in environmental quality, sediment size, and water velocity (Brinkhurst 1964; Brinkhurst and Jaimeson 1971). Densities of *T. tubifex* are typically 6,000 to 12,000 individuals per square meter and may be even higher in areas of rich organic sediment (Lazim and Learner 1986). *Tubifex tubifex* are generally found in the top 5 cm of the sediment within a benthic assemblage that includes other worm species, nematodes, tardigrades, hydra, sphaeriid clams, larval midges and flies (Merritt and Cummins 1996; Mermillod-Blondin et al. 2001). Other worm species may be able to deactivate *M. cerebralis* myxospores, thereby acting as a biological filter to remove myxospores from the environment. However, one laboratory study revealed the presence of *Limnodrilus hoffmeisteri* did not reduce the prevalence of infection within *T. tubifex* when exposed to *M. cerebralis* suggesting the success of the parasite was unaltered by the presence of this non-susceptible species (Steinbach Elwell et al. 2009). Myxospores have reportedly opened within the gut of several worm species (e.g. *Limnodrilus hoffmeisteri*, *Ilyodrilus templetoni* and *Quistadrilus multisetosus*) (M. El-Matbouli, University of Munich, personal communication), but did not complete development into triactinomyxons (M. El-Matbouli, University of Munich, personal communication; Kerans et al. 2004).

Prevalence of Infection. In natural stream systems, the prevalence of infection among *T. tubifex* is variable and typically less than 10% are infected by *M. cerebralis* (Rognlie and Knapp 1998; Zendt and Bergerson 2000; Beauchamp et al. 2002; DuBey and Caldwell 2004). Low infection prevalence suggests that abundance of myxospores is probably low in most systems and that the distribution of triactinomyxons is variable (Nehring and Walker 1996; Rognlie and Knapp 1998; Zendt and Bergerson 2000; Kerans

and Zale 2002). Field observations suggest that only a few infected *T. tubifex* (1 to 6 percent infection prevalence) are needed for infection to occur in wild salmonids (Allen and Bergerson 2002; Krueger et al. 2006). *Tubifex tubifex* often occur in high abundance in small, discrete areas within a stream, which can become point sources of *M. cerebralis* triactinomyxons. These point sources are often called "hot spots" of whirling disease and can increase risk of salmonid infection for miles downstream (Granath et al. 2007). Krueger et al. (2006) identified "hot spots" in side channels of the Madison River, Montana and found that the density of infected *T. tubifex* was the best predictor of site-specific contribution to whirling disease risk in fish. Despite these links, density of *T. tubifex* or the prevalence of infection in *T. tubifex* as solitary variables have not been strongly correlated with whirling disease risk in fish (Hiner and Moffitt 2001).

Lineage and Genetic Variation. Like salmonid fishes, *T. tubifex* vary in their susceptibility to *M. cerebralis* infection, and release widely different numbers of triactinomyxons when exposed to similar doses of myxospores (Figure 7) (Stevens et al. 2001; Kerans et al. 2004; Steinbach Elwell et al. 2006). Attempts to explain this variation have been correlated with unique "lineages", a term that refers to the genetic relatedness among individuals. Using a section of mitochondrial DNA known as 16S, six mitochondrial lineages of *T. tubifex* have been identified (Sturmbauer et al. 1999; Beauchamp et al. 2001). These lineages vary in susceptibility to infection and number of triactinomyxons produced. *Tubifex tubifex* of lineages I and III are the most susceptible to infection by *M. cerebralis* and can release high numbers of triactinomyxon, although production can vary widely between worm populations (Stevens et al. 2001; Beauchamp et al. 2002; Kerans et al. 2004; Steinbach Elwell et al. 2006; Arsan and Bartholomew 2008; Baxa et al. 2008; Rasmussen et al. 2008, Hallett et al. 2009). Not all lineage I worms are susceptible (Arsan et al. 2007; Hallett et al. 2009), and those that are do not produce high numbers of spores (Kerans et al. 2004). *Tubifex tubifex* of lineage V and VI are considered resistant to infection and release of triactinomyxons has not been ob-



▼ = number of triactinomyxons released by worms from Mt. Whitney State Fish Hatchery, CA
● = number of triactinomyxons released by worms from Madison River, MT
○ = number of triactinomyxons released by worms from the Gallatin River, MT

Figure 7. The triactinomyxon release from *Tubifex tubifex* worms of different lineages over time following exposure to mxyospores. Mt. Whitney and Madison River worms are lineage III and Gallatin River worms are lineage I. From Kerans et al. 2004, used with permission from Journal of Parasitology.

served following exposure to myxospores (Beauchamp et al. 2005; DuBey et al. 2005; Steinbach Elwell et al. 2006); lineage II worms have not been documented in the U.S. and have not been assessed for susceptibility. Researchers theorize that the *T. tubifex* lineages considered resistant to *M. cerebralis* may act as a biological filter by taking in myxospores (Beauchamp et al 2005). Researchers are currently examining the effect of introducing resistant *T. tubifex* to streams where the impacts of whirling disease have been observed (Winkelman et al. 2007). Managers hope resistant *T. tubifex* could help reduce whirling disease risk within salmonid populations.

The lineage classification described above is not the only method used to group *T. tubifex* and explain variability in susceptibility to *M. cerebralis*, and the 16S mitochondrial assay is limited in application. The technique can accurately group worms based on genetic relatedness, but the 16S mitochondrial DNA assay does not identify genes responsible for the worms' resistance or susceptibility to *M. cerebralis*. Additionally, the 16S mitochondrial DNA assays may not account for all the genetic diversity within *T. tubifex* (Hallett et al. 2005a). For example, observations of various populations of *T. tubifex* belonging to 16S mitochondrial DNA lineage III produced different numbers of TAMs when exposed to similar numbers of myxospores (DuBey et al. 2005; Rasmussen et al 2007). Furthermore, the assay can misidentify species of worms (Hallett et al. 2009). Another analysis that uses a different region of DNA, the internal transcribed spacer region 1 (ITS1), can differentiate *T. tubifex* from other worm species in North America (Kerans et al. 2004; Hallett et al. 2005a). Additionally, randomly amplified polymorphic DNA (RAPDs) can also be used to differentiate strains of *T. tubifex* that vary in susceptibility (Steinbach Elwell et al. 2006, Baxa et al. 2007, Rasmussen et al. 2008 , Hallett et al. 2009). However at this time, use of 16S mitochondrial DNA assay remains the best tool for predicting susceptible lineages.

Many researchers have speculated that *T. tubifex* may actually represent several species of worms, because of noted variation in growth rates, reproduction and environmental requirements among strains (Bonomi and Di Cola 1980; Poddubnaya 1980; Milbrink 1983a; DuBey et al. 2005). Further, the genetic variation within 16S mitochondrial DNA and allele frequency analysis among strains of *T. tubifex* also suggests speciation within *T. tubifex* (Anlauf 1997; Anlauf and Neumann 1997; Sturmbauer 1999; Ersésus et al. 2000; Beauchamp et al. 2001, 2002). This diversity in *T. tubifex* lineages may help explain the variation observed in salmonid whirling disease. Thus, understanding the genetic composition of worm communities may be valuable for managers in predicting whirling disease risk for salmonids in natural systems.

Environmental Factors

Environmental factors strongly influence infection by *M. cerebralis* and the occurrence of whirling disease among salmonids. Environmental factors including water temperature, substrate, and flow can directly and indirectly influence the impacts of *M. cerebralis* on fish. These factors affect the parasite, its hosts, and the risk of disease, and may account for much of the variability observed in *M. cerebralis* detections and impacts.

Water Temperature. Water temperature strongly affects the development of *M. cerebralis* and has been linked to triactinomyxon production and duration of triactinomyxon release. Triactinomyxon production from *T. tubifex* is generally greatest at temperatures ranging from 10 to 15 °C and is reduced at higher and lower temperatures (El-Matbouli et al. 1999c; Kerans and Zale 2002; Blazer et al. 2003; Kerans et al. 2005). At temperatures above 20 to 25 °C, triactinomyxon production may cease, and the pathogen may lose virulence (El-Matbouli et al. 1999c; Blazer et al. 2003). Infection prevalence and disease severity among fish are highly related to water temperature, and are highest in the 10 to 15 °C window of high triactinomyxon production (Baldwin et al. 2000; Vincent 2002; Hiner and Moffitt 2002; Krueger et al. 2006). Water temperatures also influence health and growth rates of the parasite hosts. Salmonid

morbidity and mortality due to *M. cerebralis* may increase with cumulative stressors, such as when *M. cerebralis*-infected trout are exposed to high water temperatures (Schisler et al. 2000).

Substrate. Fine sediment is associated with increased risk of *M. cerebralis* infection and infection severity in fish. Fine particles are likely to collect in the same areas as the small myxospores, and may hold myxospores attached to their surface (Lemmon and Kerans 2001). *Tubifex tubifex* distribution is strongly influenced by the composition and organic content of the substrate, and they prefer silt and clay substrates (Gilbert and Granath 2003; Krueger et al. 2006). The worms also reproduce faster in silt than in coarse substrate types (Arndt et al. 2002), and produce more triactinomyxons (Arndt et al. 2002; Blazer et al. 2003). Researchers are also currently investigating how substrate influences the various lineages of *T. tubifex*, whether certain lineages are favored in specific habitat types, and how these factors relate to infection severity among fish.

Additional Environmental Factors. Many physical and chemical environmental factors are associated with increased risk of the parasite's establishment and proliferation. These include flow, organic material, conductivity, stream gradient, elevation, impoundments and land use. Flow is an important environmental factor highly related to sediment sizes and distribution, as well as the amount and quality of fish habitat. Although flushing flows may reduce whirling disease impacts by periodically flushing fine substrates and associated oligochaetes (Modin 1998; Hallett and Bartholomew 2008), and low-flow side channels are associated with "hot spots" (Krueger et al. 2006), flow is not consistently related to infection prevalence and severity (Baldwin et al. 2000). The amount of organic material in a stream is positively associated with abundance of *T. tubifex* and could be linked to increased whirling disease risk among fish (Zendt and Bergersen 2000; Allen and Bergersen 2002; Thompson et al. 2002; Kaeser and Sharpe 2006; Kaeser et al. 2006). Conductivity has been associated with increased prevalence of infection among sentinel fry (Sandell et al. 2001). Kaeser et al. (2006) observed similar associations and suggested that conductivity could serve as a gross indicator of *T. tubifex* abundance. Lower stream gradient and elevation have also been linked to increased risk of infection (Schisler and Bergersen 2002). Land uses and management practices like grazing frequency, road density, and riparian area disturbance can also be used to predict whirling disease risk, which may increase with disturbance and abundance of *T. tubifex* (Zendt and Bergersen 2000). Impoundments, both natural and constructed, have also been associated with increased proportions of *T. tubifex* (Zendt and Bergersen 2000) and infection severity (Hiner and Moffitt 2002).

Impacts of *Myxobolus cerebralis* and Whirling Disease

Ecological Impacts

Whirling disease can cause wild trout population declines, changes in fish community composition and, potentially, food chain effects. Wild trout population declines were first observed in North America in the upper Colorado River watershed, Colorado in 1993 (Walker and Nehring 1995). In some waters, declines were severe. In one 3.2 km (2 mi) reach of the Gunnison River, numbers of wild rainbow trout greater than 15 cm (7 in) declined from approximately 11,000 to 86 fish between 1987 and 2003, a loss greater than 99 percent (Nehring 2006). Similar declines in wild rainbow trout were reported in at least five other Colorado rivers during this period (Nehring 2006). Population declines were also observed during this period in wild rainbow trout of the Madison River, Montana. In 1994, wild rainbow trout were reduced nearly 90 percent from historic averages in sections of the upper Madison River (Vincent 1996). These declines were especially marked among wild rainbow trout less than two years old.

More recently, declines among Yellowstone cutthroat trout in Yellowstone National Park, Wyoming, have been linked to whirling disease in combination with other factors, including drought and predation by illegally introduced lake trout (Koel et al. 2005a, b; Koel et al. 2006a, b; Murcia et al. 2006). Yellowstone cutthroat trout in Pelican Creek have been significantly affected by whirling disease. Pelican Creek once supported one of the largest spawning runs of Yellowstone cutthroat trout from Yellowstone Lake, with up to 30,000 upstream migrants per year (Koel et al. 2005a). High infection prevalence and disease severity among Pelican Creek cutthroat trout have contributed to a severe decline in this spawning population. Angling on Pelican Creek was completely closed in 2004, reports have described the spawning population as "essentially lost," and few wild-reared fry were observed between 2001 and 2004 (Koel et al. 2005a, b).

In Utah, declines in wild rainbow and cutthroat trout populations in several streams (e.g., the Beaver and Logan rivers) may be linked to *M. cerebralis* (Wilson 2006). Biologists also speculated there were wild rainbow trout population declines in the Big Lost River, Idaho due to a combination of whirling disease and drought; however, population impacts were not observed in other drainages where the parasite was present (Elle 1997). These and other wild trout population declines in the Intermountain West have led to concerns about how whirling disease could further affect native trout already threatened by habitat loss, invasive species, predation and hybridization.

In addition to causing declines in wild trout populations, whirling disease can potentially lead to changes in fish community composition, as more resistant species such as brown trout replace species more susceptible to the disease. In several Montana drainages (Madison River, Rock Creek and Willow Creek), there were decreases in rainbow trout populations while brown trout remained constant or increased (Baldwin et al. 1998). Researchers found catchable sized brown trout appeared to be replacing rainbow trout in Rock Creek and maintaining an overall high density of trout in the fishery (Granath et al. 2007). Similarly, brown trout numbers have increased in the Beaver River, Utah while wild rainbow have diminished, a pattern potentially linked to whirling disease (Wilson 2006). Reduced abundance of some wild salmonids could also affect food chains for fish-eating predators such as bears, eagles and

other fishes (e.g., Koel et al. 2005b). Mountain whitefish are susceptible to *M. cerebralis* during their early life stages (Schisler et al. 2008), and if the disease causes population declines of this important forage fish, the food chain impacts for predatory fish such as the threatened bull trout could be significant.

Population losses of the magnitude described above have not been consistently observed despite the widespread distribution of *M. cerebralis*. Population-level impacts of whirling disease have not been reported in the eastern and coastal-western United States despite the parasite being widely distributed in these regions. Modin (1998) noted the widespread occurrence of *M. cerebralis* in wild and cultured trout of California. Severe whirling disease outbreaks were noted in fish culture settings, and occasional skeletal anomalies were observed in wild trout; however, wild trout population impacts were not detected. Conspicuous declines in wild trout population of Pennsylvania or the mid-Atlantic region as a result of whirling disease have not occurred, despite the presence of *M. cerebralis*, *T. tubifex*, and susceptible trout species (Kaeser and Sharpe 2006; Kaeser et al. 2006). Surveys in New York also found no evidence of harm to wild trout populations (Schachte and Hulbert 1998; Hulbert 2005).

Thus, the local and regional impacts of whirling disease in the United States remain variable and difficult to predict. Kaeser et al. (2006) speculated that outbreaks of whirling disease might have occurred among wild trout in Pennsylvania, but that there was insufficient data to support this. Without sufficient information about fish abundance and infection dynamics from long-term monitoring programs, it's possible that whirling disease impacts could go unnoticed or unexplained. Additionally, the dynamics of the disease are complicated by the ecology of aquatic ecosystems, the parasite's two hosts, and the difficulty in accurately detecting the parasite. Wild trout declines in the Intermountain West have generated continuing concern about popular sport fisheries and conservation of native salmonids. These losses have persisted in some waters; while in others, fish populations may be recovering to some extent. As the parasite continues to spread, ongoing research regarding risk assessment, ecological factors, host resistance and immunity will improve knowledge and refine predictions about the impacts of whirling disease.

Economic Impacts

Wild and cultured trout represent several important commercial activities that can be affected by *M. cerebralis* and whirling disease: recreational fishing, private fish culture, and government expenditures for research, fisheries management and fish culture. It is difficult to calculate the full economic value associated with recreational trout fishing, but it is clearly an important income for many communities. The 2006 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation estimated 6.8 million freshwater anglers outside of the Great Lakes spent an estimated $2,038,500 on trout fishing trips and equipment that year (USFWS 2006). A 2003 study estimated economic benefits of recreational trout fishing in the Golden Trout Wilderness Area of California at $148,000 to $713,000 a year (Alkire 2003). The U.S. Fish and Wildlife Service calculated net economic benefits of rainbow trout raised in the National Fish Hatchery System. In federal fiscal year 2004, these rainbow trout generated 3.9 million angler days, $173 million in retail sales, and a total economic benefit of $325 million nationally (USFWS 2006). When wild trout population declines were first linked to whirling disease, many suspected that economies would be damaged based on declines in recreational fishing and tourism.

Despite these concerns, a large economic impact on recreational fishing and tourism has not been documented. In an evaluation of recreational fisheries in Montana and Colorado, no negative effects upon angler satisfaction and local fishing economics could be detected five years after whirling disease caused severe wild trout population declines (Duffield et al. 1999a). There are several reasons why economic impacts on the angling economy may be minimal. In many cases, anglers will continue to report sat-

isfaction despite a reduced catch, and will return to the same site (Duffield et al. 1999a). Abundant brown trout may replace rainbow trout and provide ample angling opportunities (Baldwin et al. 1998). Anglers may also simply choose a nearby stream to fish (Duffield et al. 1999b), as in Yellowstone National Park after the closure of the Pelican Creek fishery (T. Koel, National Park Service, personal communication). Additionally, although wild trout abundance may be greatly reduced locally, the number of trout that remain may still be relatively high compared to other waters. For example, the estimated numbers of rainbow trout young-of-the-year per mile on the Madison River, post-whirling disease, are still high compared to many other trout streams in Montana (B. Rich, Montana Fish, Wildlife & Parks, personal communication). While no obvious economic harm to recreational fishing due to whirling disease has been reported, the topic has not been completely investigated.

Aquaculture is a major U.S. industry requiring large investments and generating considerable economic benefits. The total value of aquaculture products in the United States was more than $1 billion in 2005, generating an annual payroll of nearly $170 million (USDA 2006). Salmonids, particularly rainbow trout, are raised for many purposes including food consumption and transplanting into private ponds, lakes and rivers for recreation and conservation. Sales of trout, primarily for food, in the United States totaled nearly $80 million in 2005 (USDA 2006) and increased to $87.5 million in 2007 (USDA 2008). Trout were also distributed in 2007 for restoration, conservation, and recreation, primarily from state and federal hatcheries, for a total value of approximately $100 million (USDA 2008). The combined value of trout sold and distributed was almost $190 million in 2007. Diseases like whirling disease pose large risks to fish culture operations and profits. Of the 34.3 million trout intended for sale that were lost in 2007, 86% were lost due to disease (USDA 2008). This amounted to a lost sales value of approximately $35-60 million. If a similar proportion of trout intended for other distribution were lost due to disease, it could have meant an additional $39 million value lost.

Private and publicly owned fish culture operations have sustained large economic costs because of *M. cerebralis*. The parasite has impacted fish culture by causing fish mortalities and reduced fitness, triggering the destruction of infected fish, requiring disinfection and renovation of facilities, causing the quarantine and closure of facilities, reducing the number of fish available for sale and stocking, and requiring additional preventative measures. In Utah, the economic impacts of the parasite on state and private trout hatcheries have been described as "devastating" (Wilson 2006). After the parasite was detected in several state and private facilities, many tons of fish had to be destroyed and disposed of. Facilities were quarantined while millions of dollars were spent to disinfect and renovate them, and some facilities were forced to close when the costs of parasite removal were too great (Wilson 2006; Utah Department of Agriculture and Food 2006; House 2006). In 2005, the total value of Utah trout sales dropped almost 30% or approximately $220,000 from the previous year for reasons including whirling disease, which had caused six privately owned facilities to close that year (House 2006). In Colorado, the state spent more than $11 million to modernize hatcheries for whirling disease prevention and management between 1987 and 2006 (M. Jones, Colorado Division of Wildlife Resources, personal communication). A multimillion dollar renovation of the federal Leadville National Fish Hatchery in Colorado rid the facility of *M. cerebralis*. In 2007, more trout could be raised at the facility and restrictions were lifted on the locations where those fish could be stocked (Stromberg 2007). Effects were even seen in Maryland following an epizootic in 2007. After *M. cerebralis* was detected in trout from three rearing stations, 130,000 infected rainbow and brown trout were destroyed, two of the stations were disassembled, and the third station is undergoing extensive renovation (Rivers 2008).

The challenges posed by whirling disease have increased the amounts spent to study and manage fish health among wild and cultured populations. State and federal resources have been leveraged for scientific research to study

M. cerebralis and whirling disease, particularly since the wild trout declines of the 1990s. Between 1997 and 2006 congressional funding was provided to the Whirling Disease Foundation and the Whirling Disease Initiative for research associated with whirling disease through a cooperative agreement with the U.S. Fish and Wildlife Service. More than 100 investigations were completed through the Whirling Disease Initiative with approximately $8 million in federal funding between 1997 and 2006 (Galli-Noble and Stromberg 2007). These research efforts usually incorporated academic researchers, state agencies and other partners from across the nation and even around the world. These partnerships generated an additional $5 million in matching contributions for Whirling Disease Initiative research. After merging with Trout Unlimited in 2007, the Whirling Disease Foundation continues to fund education and research on whirling disease. Other federal spending related to whirling disease research has occurred through agencies such as the National Park Service and through programs including the National Wild Fish Health Survey. States have also invested significant funds into whirling disease research. Overall, research efforts have yielded valuable information about diagnostics and detection, risk assessment and modeling, prevention and management to address the risks posed by *M. cerebralis* infection and whirling disease.

Prevention and Management

Detection Techniques

Detection of *Myxobolus cerebralis* in Fish

There are many diagnostic techniques to detect the presence of *M. cerebralis* in its hosts and the environment, and to measure the severity of whirling disease. The techniques vary in sensitivity, purpose, cost, and equipment required, and the advantages and disadvantages of a number of these assays have been reviewed both here (Table 3) and by Andree and Hedrick (2002) and Kelley et al. (2004b). Development of diagnostic methods has been a major research focus because of the importance of detecting *M. cerebralis* when present at low levels, and certain protocols are endorsed by the American Fisheries Society for use in official fish health inspections (see AFS- FHS 2007 Blue Book: *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens*). The additional challenge of detecting the parasite from its invertebrate host and from environmental samples has led to development of a variety of techniques suited for different purposes.

For most diagnostic methods to detect *M. cerebralis* in fish, the fish sampled must be a certain age or reared for a minimum number of degree-days, and the tissue taken from specific locations on the body (AFS-FHS 2007). Most sampling procedures require death of the fish host. The required number of fish sampled varies by circumstance and application. A typical statistical sample is based on an assumed infection prevalence of five percent of the population; for example, a sample of 60 is adequate for assessing the prevalence of infection within a population of 100,000 fish (AFS-FHS 2007).

Plankton Centrifuge Spore Concentration. One of the first protocols developed for detecting *M. cerebralis* myxospores in the fish host was spores by plankton centrifuge. This method continues to be used to quantify myxospores. The head and fins of fish are macerated in a blender, and then myxospores are concentrated using a plankton centrifuge (O'Grodnick 1975b). After concentration, myxospores can then be observed microscopically (40X magnification) and quantified using a hemacytometer. This technique is relatively simple and low in cost, and requires a blender, plankton centrifuge, and a microscope. Only the myxospore stage of the parasite is detected with this method and it may be difficult to distinguish between similar-appearing myxozoan species.

Pepsin-Trypsin Digest. The pepsin-trypsin digestion method (PTD) is a sequential digestion of the cartilaginous tissues (head and spine) of the fish using enzymes and centrifugation (Markiw and Wolf 1974). This method results in a concentrated pellet of myxospores. Myxospore abundance is estimated by diluting the pellet and counting using a hemacytometer. Like the plankton-centrifuge method, PTD detects only myxospores and cannot distinguish between morphologically similar myxozoan species. The enzymatic digestion makes spore identification easier, compared to a sample prepared with plankton centrifuge, by decreasing the amount of tissue in the sample.

Histology. Pathologists employ histology to detect *M. cerebralis* and related tissue damage in fish samples that have been preserved in paraffin wax, sliced into micro-thin sections, and stained. Disease severity can be determined by assigning a categorical score based on the extent of the infection, lesion severity and associated physical damage (e.g. MacConnell-Baldwin scale; Figure 4) (Baldwin et al. 2000; Andree et al. 2002). Histopathology can detect multiple stages of the parasite in addition to mature myxospores, thus allowing earlier infections to be detected; how-

Table 3. A summary of tools for detection of Myxobolus cerebralis. These methods have applications for research, diagnostics and fish health inspections. For diagnostic and inspection purposes, the AFS-FHS Blue Book (2007) provides guidelines for sample collection and accepted protocols. For research, a broader variety of methods are appropriate and the sample type and when it is collected will vary with the research question. The analytical sensitivity and specificity of each method may also vary depending on the sample type and when it is collected.

Sample	Method	Sample Type	Detection Target	Minimum Time Post-Exposure to Collect Sample	Specificity[1]	Sensitivity[2]	Application[3]
FISH	Plankton Centrifugation	Head, vertebrae, skeleton	Myxospore	6 mo or 1800 degree-days	++	++	Diagnostics Research
	Pepsin-Trypsin Digest (PTD)	Head, vertebrae, skeleton	Myxospore	6 mo or 1800 degree-days	++	++	Diagnostics Inspection – screening[4] Research
	Histology	Tissue section (e.g. head, gill arches, vertebrae, fin rays)	Myxospore, developmental stages	6 mo or 1800 degree-days	+++	++	Diagnostics Inspection – confirmation[5] Research
	Polymerase Chain Reaction (PCR)	Half head or cranial punch (inspection) Whole fry or other tissue (research)	DNA	6 mo or 1800 degree-days	+++	+++	Diagnostics Inspection – confirmation[5,6] Research
		Non-lethal (e.g. fin or gill clip)	DNA	Variable, depending on purpose	+++	++	Research
	Quantitative PCR (QPCR)	Half head, cranial punch or whole fry	DNA	Variable	+++	+++	Diagnostics Research
	In Situ Hybridization (ISH)	Tissue section	DNA	Variable	+++	++	Diagnostics Research
	Myxo-Loop-Mediated Isothermal Amplification (LAMP)	Half head or cranial punch, whole fry	DNA	Variable	+++	+++	Research
	Enzyme-linked Immunosorbent Assay (ELISA)	Serum	Antibody	4-6 wks	Unknown	Unknown	Research
	Immunofluoresence	Tissue section	Myxospore, developmental stages	Variable	Unknown	Unknown	Research
WORMS	PCR	Whole or half worm	DNA	Variable	+++	+++	Research
	Release of TAMS	Water sample	Triactinomyxon	65 d	+	++	Research
	Histology	Tissue section - whole worm	Triactinomyxon, developmental stages	Variable	++	++	Research
	ISH	Tissue section - whole worm	DNA	Variable	+++	++	Research
WATER	Filtration	Variable volume	Triactinomyxon	N/A	+	+	Research
	Tamometer	120 liters	Triactinomyxon	N/A	+	++	Research
	Sentinel fish	50 fish per cage	Triactinomyxon[7]	24 h to 6 mo[8]	+++	++	Research
SEDIMENT	Centrifugation	0.1 to 1 gram	Myxospore	N/A	+	+	Research

Table 3. continued.

1 Specificity refers to the ability of the method to differentiate M. cerebralis among other parasites. Criteria for specificity:
+ spores can be differentiated morphologically but sample source doesn't eliminate similar species
++ spores can be differentiated morphologically and sample source eliminates most similar species
+++ assay specific for M. cerebralis
2 Sensitivity refers to the method's ability to detect M. cerebralis at low concentrations.
3 Diagnostic methods are those described in the AFS-FHS Blue Book (2007) Section 1: Diagnostic Procedures for Finfish and Shellfish Pathogens; those specified for inspection purposes are for regulatory purposes and are detailed in Section 2: USFWS/AFS-FHS Standard Procedures for Aquatic Animal Health Inspections. Research methods are either not considered validated or are standard methods used on non-traditional samples (e.g. PCR on worms).
4 Fish health inspection protocols require screening by examination for spores in cranial cartilage processed by PTD.
5 Fish health inspection protocols require confirmation by identification of parasite stages in histological sections or by amplification of parasite DNA by PCR.
6 The PCR assay approved for fish health inspections specifically uses the primers by Andree et al. 1998.
7 Sentinel fish are used to detect presence of the parasite (triactinomyxons stage) in water; however, one of the assays used to detect infection in fish must be used to confirm infection status.
8 Age of host at sampling will depend on assay selected; e.g. PCR may be done after 24 h, myxospore detection after several months.

ever, definitive diagnosis of infections without mature spores must be inferred based on the site of infection (i.e. cartilage). Because only a portion of the fish can be examined by this method, the parasite may be missed at very low parasite concentrations. Despite this disadvantage, histology remains an important diagnostic and research tool.

In-situ hybridization. In-situ hybridization (ISH) labels the target *M. cerebralis* DNA in the host tissue with the use of specific probes that bind parasite DNA (Antonio et al. 1998, 1999). Sample preparation is similar to that for histopathology, and requires micro-thin layers of cartilage and tissues of the head. In-situ hybridization provides a visual indication of infection location in the host tissue, even for very low numbers of parasites (Figure 8). The ISH procedure is specific and can detect all stages of the parasite in tissues of both fish and worm hosts. However, in comparison to histology this method is expensive, time intensive and requires a technician trained in this technique to interpret the results; thus ISH has been used primarily as a research tool.

Polymerase Chain Reaction. The polymerase chain reaction (PCR) is another molecular technique that detects *M. cerebralis* DNA at all stages of parasite development. This method amplifies the target DNA of *M. cerebralis* from a digested tissue sample, producing a unique band on an ultraviolet-sensitive gel medium (Figure 9) (Andree et al. 1998). There are several variations on the PCR, including single-round PCR, nested PCR and real-time or quantitative PCR (QPCR) (Andree et al. 1998; Schisler et al. 2001; Kelley et al. 2004b). Quantitative PCR detects the presence of *M. cerebralis* and determines the quantity of parasite present. These tests may vary not only in methodology, but also in the gene sequence targeted (e.g. 18S ribosomal DNA, heat shock protein gene). Costs vary according to reagents and equipment required, and the tests require trained technicians. The application of QPCR has been limited due to increased costs; however, the technique is favored due to the decreased time for analysis, reduced risk of contamination, and increased analytical sensitivity (Kelley et al. 2004b). When used as a confirmatory assay for inspections, the test uses primers developed by Andree et al. (1998) on a half-head, core or PTD sample (AFS-FHS 2007). PCR techniques are highly sensitive and specifically identify all stages of *M. cerebralis*.

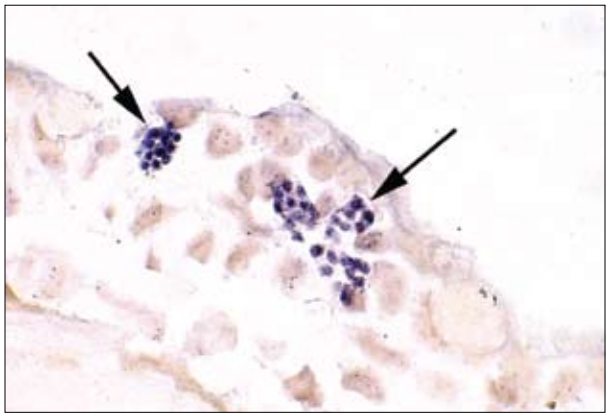


Figure 8. Sporoplasm cells of *Myxobolus cerebralis* in the epidermis of rainbow trout as revealed by in situ hybridization (ISH) using labeled probes to the parasite 18S rRNA gene. Arrows indicate aggregates of the sporoplasm cells 2 h following waterborne exposures to the triactinomyxon stages of the parasite (photo courtesy R. P. Hedrick and D. Baxa, University of California, Davis).

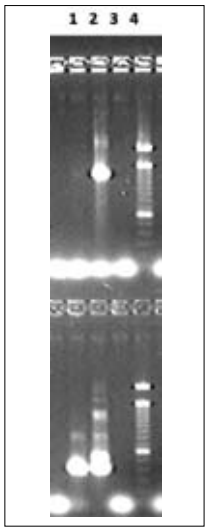


Figure 9. Results of a nested polymerase chain reaction (PCR) on an electrophorectic gel display the first round of the assay on the top panel and the second round on the bottom. Lane 1 - lightly infected fish; Lane 2 - heavily infected fish; Lane 3 - uninfected control; Lane 4 - molecular weight standards. Image courtesy of C. Rasmussen, USGS Western Fisheries Research Laboratory.

Non-Lethal Sampling Techniques for PCR. Many non-lethal sampling techniques for PCR analyses have been attempted using fin tissue, opercula, gill filaments, and skin scrapes (Toner et al. 2004). Examination of gill filaments for *M. cerebralis* by PCR suggests that positive results matched positive histological results, and sampling of that tissue produced no negative effects upon the fish (Schill et al. 1999). PCR analysis of gill filament samples was more sensitive for detection of *M. cerebralis* compared to samples from the caudal fin or operculum, but was less sensitive than lethal methods (Sandell et al. 2000). Although non-lethal sampling locations are preferred over lethal techniques for obvious reasons, PCR results using these sampling techniques have been inconsistent and are not validated by the American Fisheries Society for use in inspections.

Loop-mediated isothermal amplification. Loop-mediated isothermal amplification (Myxo-LAMP) technology detects *M. cerebralis* DNA by amplification of target DNA and visual observation using dyes. Myxo-LAMP is similar to PCR, but takes less time and fewer resources, making the technique suited for small laboratories (El-Matbouli and Soliman 2005).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) determines whether a fish has been exposed to *M. cerebralis* by detecting a specific immune response. The technique measures antibodies to *M. cerebralis* in serum from fish at any age, and determines whether exposure to the parasite has occurred; it does not indicate manifestation of disease. Samples required for ELISA are generally lethal when working with small fish, but are not lethal to large fish (Adkison et al. 2005). Variable antibody responses among individuals and non-specific reactions make the application of this method most appropriate for laboratory populations where history of exposure to other myxozoans is limited. Although promising, at this time antibodies are not widely available and this technique requires further research before broad application is appropriate.

Immunofluorescence assay. In addition to their utility in an ELISA, antibodies specific for *M. cerebralis* have been used to detect the parasite in frozen tissue sections (Wicks and Youssef 2004). This method could be developed for diagnostic or research purposes, however antibodies are not commercially available.

Detection of *Myxobolus cerebralis* in *Tubifex tubifex*

Identification of T. tubifex. Two techniques are used to identify *T. tubifex*: visual identification using a taxonomic key, or genetic identification using PCR. To identify worms using taxonomic key, specimens are mounted on a microscope slide and viewed with a phase-contrast microscope (Kathman and Brinkhurst 1998). Physical identification of *T. tubifex* requires the presence of hair and pectinate chaetae (Figure 10A) and individuals must be sexually mature with developed penis sheaths (Figure 10B) (Kathman and Brinkhurst 1998). Usually, only a small

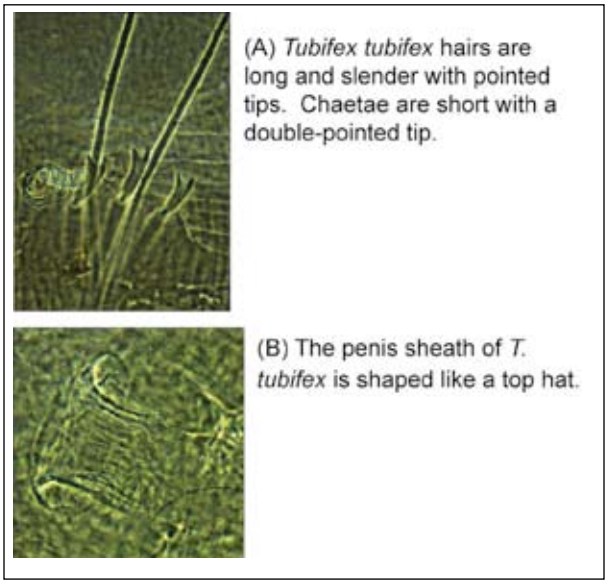


Figure 10. *Tubifex tubifex* can be distinguished from other oligochaete worms using identifying features: hair and pectinate chaetae (A) and the penis sheath (B). Photos courtesy of B. L. Kearns, Montana State University.

percentage of worms can be identified to species when collected from field locations because there are generally few sexually mature worms (Brinkhurst 1996; Kathman and Brinkhurst 1998;

Rognlie and Knapp 1998). Due to the limitations of physical identification and some PCR assays, the reliance on a single identification tool is not sufficient for conclusive identification of *T. tubifex*. However, Hallett et al. (2005a) have made strides in developing a reliable PCR assay for identification of *T. tubifex*.

Visual Observation of Triactinomyxon Release. Diagnosis of *T. tubifex* infection by *M. cerebralis* can be accomplished by visual observation of triactinomyxon release. Additionally, estimates of triactinomyxon production and infection prevalence can be obtained (El-Matbouli et al. 1999c; Stevens et al. 2001; Beauchamp et al. 2002; Blazer et al. 2003; Steinbach 2003; Beauchamp et al. 2006; Steinbach Elwell et al. 2006, Hallett and Bartholomew 2008). Detection of TAM release by groups of worms requires fine sieves (25 micrometer mesh size) to filter triactinomyxons from the water, and a microscope. Detection from individual worms is typically done by separating them into small, shallow well plates from which water can be removed and examined directly for TAMS (Figure 11).

Figure 11. Worms in well plates for microscopic observation of triactinomyxon release (Photo courtesy of L.C. S. Elwell).

Additional T. tubifex Techniques. Histopathology, PCR, ISH, and Myxo-LAMP can all be applied to worm samples to determine the presence of parasite DNA, prevalence of infection, and location of infection. Due to the worm's small size (10 to 30 millimeters), the entire worm is usually used in sample preparation; hence, these techniques are always lethal to the worm. If physical identification is also required, the anterior portion of the worm can be used for PCR and the posterior portion for physical identification. Histopathology has also been used to characterize infection; however, the small size of *T. tubifex* makes this very time-consuming. Although several researchers have noted tissue damage within worm intestines due to *M. cerebralis* infection (El-Matbouli and Hoffmann 1998; Hedrick and El-Matbouli 2002), a rating system for disease severity has not been applied to *T. tubifex*.

Detection of *Myxobolus cerebralis* in the Environment

Detection of Triactinomyxons in Water Samples. Field diagnostic tools give researchers and managers indication of the presence of *M. cerebralis* in a particular body of water. Detection of the parasite in water can be accomplished directly by filtration (Nehring and Thompson 1999), or indirectly, using caged sentinel fish. One filtration method for detecting triactinomyxons in water uses a glass bead filter (tamometer) that processes large volumes of water to concentrate triactinomyxons, thus providing a quantitative estimate (Lukins 2004). The tamometer can be transported to the site by trailer, or water can be filtered on site and triactinomyxons further concentrated in the laboratory using the glass bead filter (Lukins et al. 2003). This method requires specialized equipment and trained personnel. In areas where other myxozoans are prevalent, it may be difficult to distinguish *M. cerebralis* triactinomyxons from those of other species. Methods developed for molecular detection of other myxozoans in water samples are quantitative and more specific (Hallett and Bartholomew 2006; Bartholomew et al. 2007a) and could also be applied to *M. cerebralis*.

Sentinel Fish Exposures. An indirect method for assessing the presence of *M. cerebralis* triactinomyxons in a river is by exposure of susceptible fish. Fish must be of a known uninfected source and an age when susceptible to infection. They are held in cages that allow water to pass

through, thus exposing them to the same environmental conditions as wild fish in the same location (Figure 12) (e.g., Hiner and Moffitt 2001; Sandell et al. 2002; Krueger et al. 2006). After an exposure period of 8 to 15 days, the fish are removed from the cage. Small fish may be sacrificed and assayed whole using PCR or reared in an *M. cerebralis*-free environment to allow complete parasite development. After approximately 4 months (or 1,800 degree days C), the fish are then sacrificed, and heads or other cartilaginous locations are examined by histology, PCR, or PTD. Because triactinomyxons can disperse over a long distance, sentinel fish are most useful for assessing *M. cerebralis* presence or absence rather than precise locations of parasite concentration. However, because fish are exposed for a longer period they can provide information that can be useful in predicting population effects on resident fish.

Figure 12. Sentinel cages are used in field experiments to assess *M. cerebralis* infection risks under natural conditions. The cages are anchored in the stream with test subject fish contained inside.

Detection of Myxospores in Sediment. Extraction methods for *M. cerebralis* myxospores from sediment samples have used centrifugation in combination with detergent action and visual examination (Lemmon and Kerans 2001; Gates and Guy 2006). These methods for recovering the parasite are useful for laboratory studies and could provide some insights into myxospore abundance in the wild; however, the inherent patchy distribution of the parasite and the low sensitivity of this assay make it impractical for most uses.

Risk Assessment and Modeling

Risk assessment and modeling techniques are being applied to predict the risk of *M. cerebralis* introduction, establishment, and potential whirling disease impacts under various circumstances. These methods take into account the numerous factors that must coexist for successful introduction of *M. cerebralis* to a new location, including susceptible hosts, optimal temperatures for parasite development, and appropriate habitat. Bartholomew et al. (2005) provide a working model to estimate the risks associated with whirling disease and identify measures to minimize these risks. This model and others (Bruneau 2001; Engelking 2002; Schisler and Bergersen 2002) help to identify pathways for introduction and the likelihood for *M. cerebralis* to become established and affect fish populations.

For example, Schisler and Bergersen found that in high-elevation Colorado streams, the risk of establishment of *M. cerebralis* was low due to the lack of ideal *T. tubifex* habitat, the long distances to other infected waters, and the termination of fish stocking into high elevation areas (Schisler and Bergersen 2002). Similarly, Arsan and Bartholomew (2008) predicted that the risk for establishment of *M. cerebralis* in Alaska was low in southeast Alaska, where *T. tubifex* were not encountered and where stream characteristics may limit availability of suitable habitat. In southcentral Alaska, *T. tubifex* was abundant at many sites and risk of dissemina-

tion from a point source was increased, but the predominance of non-susceptible lineages of *T. tubifex* may reduce the risk of establishment. These and other studies that attempt to understand why the parasite spreads rapidly in some locations while not in others are critical for predicting future spread and allocating resources to areas most at risk.

Other models to explain and predict whirling disease risk reveal the fundamental processes of invasion and spread of *M. cerebralis* and illustrate gaps in our knowledge (Kerans and Zale 2002). Modeling by Hiner and Moffitt (2002) examined the roles of temperature and the composition of the invertebrate community for infection risk and disease severity in rainbow trout. In that study, the mean water temperatures during exposure to triactinomyxons, and the density of oligochaetes (worms) and chironomids (midges), were both correlated to higher disease severity in trout. Other modeling approaches include a dynamic model of *M. cerebralis* infection that addressed the importance of juvenile trout emergence timing on infection and the distribution and abundance of *T. tubifex* on persistence of the parasite (Reno 2003, Reno and Lorz 2005), and a life cycle model that contributes to our understanding of the evolutionary and ecological relationships between *M. cerebralis* and its hosts (Kerans and Zale 2002).

Research has demonstrated that some characteristics of host biology (e.g. innate susceptibility, early life history) and the environment (e.g., temperature) are strong predictors of successful establishment, and therefore are helpful in formulating risk assessments for establishment. However, recent work on land use assessment suggests specific land uses in a watershed are also important in the establishment of *M. cerebralis* and may potentially be used as predictors as well. A preliminary assessment of large-scale land use and its association with whirling disease severity suggests that land uses that can increase sediment input into streams, such as roads and agriculture, are positively correlated with infection in rainbow trout (McGinnis 2007).

Vectors and the Spread of Myxobolus cerebralis

The movement of *M. cerebralis*-infected fish is thought to be the primary vector by which the parasite has spread in the United States (Hoffman 1970, 1990; Hedrick et al. 1998; Bartholomew and Reno 2002). Because a single fish infected by *M. cerebralis* can carry hundreds of thousands of myxospores, the movement of these fish may easily transmit the parasite (Reno 2003; Hallett and Bartholomew 2008). Salmonid eggs cannot serve as vectors for *M. cerebralis* since the parasite cannot be transmitted vertically from infected parental fish to eggs (O'Grodnick 1975a), and eggs do not become infected if exposed to triactinomyxons (Markiw 1991). Although eggs cannot be infected, eggs transported in contaminated water could transmit the parasite; therefore, disinfection of eggs and transport water is recommended. There is limited experimental evidence that living, infected fish may shed viable *M. cerebralis* myxospores (Taylor and Haber 1974; Nehring et al. 2002). At one time, it was suggested that this occurred through excretion of fish fecal material; however, it is unlikely that mature myxospores enter the digestive system. It is more likely that small numbers of myxospores could be released through surface abrasion of granulomous lesions near the surface of the fish's skin, on areas like the operculum or fin rays (Taylor and Haber 1974; E. MacConnell, U.S. Fish and Wildlife Service, personal communication). It is generally accepted that *M. cerebralis* is primarily spread once an infected fish dies and cartilage degrades, releasing myxospores (Reno 2003; Hallett and Bartholomew 2008).

The movement of *M. cerebralis*-infected fish can occur naturally or through human activities. The range of salmonid fishes can vary widely and cover thousands of miles in their lifetime (Behnke 1992). For example, Pacific salmon migrate from inland waters of the Snake and Columbia rivers to the Pacific Ocean and then return to spawn. Though generally returning to natal streams, salmonids can also stray and pioneer new territories. A single infected

stray fish can be a potential source of introduction for the parasite into new areas, as observed in the Deschutes River, Oregon (Engelking 2002, Zielinski 2008). Legal transfers of infected fish have primarily occurred as a result of fish stocking activities by state and federal agencies. States have widely varying policies regarding supplementation of salmonid stocks and some policies have permitted stocking of *M. cerebralis*-infected trout and salmon in waters where the parasite is already established. In Colorado, managers and biologists now suspect that stocking infected trout continued to add myxospores to rivers until whirling disease resulted in large population declines (Nehring 2006). This additive effect virtually eliminated wild trout reproduction and recruitment in some Colorado waters. Difficulty in accurately detecting *M. cerebralis* infection has also led to accidental introductions of the parasite. Improvements in diagnostic tools have made fish health screening easier and more effective, which has reduced the risk of accidental parasite introduction. In addition, stringent regulation and increased knowledge of the risks has reduced the number of infected fish being stocked. Most states no longer stock *M. cerebralis*-infected trout intentionally, and among states that do, the practice is largely limited to waters not connected to any streams that can support wild salmonids.

Illegal transfers of *M. cerebralis*-infected fish are now perhaps the highest-risk human activity spreading the parasite. The construction of ponds on private property has become extremely common, and individuals may stock their ponds by purchase of fish through the private aquaculture industry. Most fish producers in the industry follow fish health regulations set by their state, and take proactive steps to ensure the fish they sell are free of specific pathogens such as *M. cerebralis*. Fish health monitoring and certification programs conducted by each state strive to ensure compliance and enforce regulations in order to protect consumers and the environment. However, these processes are not foolproof, and *M. cerebralis*-infected fish have been sold, transferred, and stocked in many areas. Such activity can introduce the parasite to previously uncontaminated waters, potentially leading to establishment of *M. cerebralis* in private ponds and adjacent watersheds. Most states require a permit before allowing pond construction, and an additional permit before stocking fish. For the best protection against spreading pathogens, individuals should follow the appropriate laws and regulations determined by each state, and also request proof of fish health certification before purchasing fish from a private grower. The penalties for sales of infected trout can be significant. In Colorado, a private trout grower prosecuted for knowingly selling *M. cerebralis*-infected trout that were then planted in Colorado, New Mexico and Utah was fined nearly $30,000 (Rodebaugh 2005; Stromberg 2006). With private pond construction on the rise in many states, this problem could continue to increase. Individuals may also illegally plant fish in public waters, a practice often called "bucket biology." This practice has been responsible for the introduction of a variety aquatic species that can negatively impact aquatic ecosystems (McMahon and Bennett 1996; Knapp et al. 2001; Cohen and Moyle 2004). Enforcement and education about responsible and legal fish stocking could reduce further introduction by these routes.

Myxobolus cerebralis may also be spread through the movement of water or sediments containing the parasite. Parasite spores are dispersed naturally in the water column and can spread downstream, even through interdrainage canals and underground springs (Wilson 2006). Anglers, boaters, and other recreational enthusiasts can also act as vectors by transporting sediment, plant materials, and even small animals among bodies of water. Laboratory experiments confirmed that, if not properly cleaned, wading boots can transport viable myxospores (P. Reno, Oregon State University, personal communication). In the field, one investigation determined that an individual wearing felt-soled wading boots could transport approximately 17 grams of sediment potentially containing myxospores (Figure 13; Gates 2007). Given the highly mobile character of today's anglers, it appears that *M. cerebralis* could be transported great distances if gear is not properly cleaned and dried. Wa-

tercraft, equipment, and trailers can transport *M. cerebralis* and other aquatic invasive species (e.g. zebra mussels: Johnson et al. 2001). People who move watercraft among bodies of water can prevent the spread of these organisms by carefully cleaning all surfaces and allowing the gear

Figure 13. The spores of *M. cerebralis* can be attached to felt soles. The electron micrograph shows myxospore adhered to a rubber boot (photo courtesy of K. Gates)

to dry between trips. Cleaning and disinfection techniques are further discussed in the management section of this publication.

Wildlife can also spread *M. cerebralis*. Piscivorous wildlife, including fish, birds and mammals, which ingest *M. cerebralis*-infected fish, can spread the parasite between drainages. Passage of viable myxospores through the digestive system of piscivorous birds has been demonstrated (Taylor and Lott 1978; El-Matbouli and Hoffmann 1991. Given the rate of passage through the birds' digestive system (Brugger 1993; Barrows et al. 1999; Hilton et al. 2000), and wide ranges often overlapping incidences of *M. cerebralis* infection (e.g. American white pelicans: Koel et al. 2006a), it is possible that these animals can transport *M. cerebralis* (Taylor and Lott 1978; Kerans et al. 2007; Arsan and Bartholomew 2008). Piscivorous fish may also transport the parasite by traveling long distances and passing viable myxospores in their feces (El-Matbouli and Hoffmann 1991; Arsan and Bartholomew 2008). Mammals are also suspected of transporting the parasite after eating infected fish; however, experimental evidence suggests mammalian digestion deactivates myxospores (El-Matbouli et al. 2005). Myxospores may also be transported in mud on the feet and fur of wildlife. Ongoing research investigations are examining the role of wildlife as vectors to spread *M. cerebralis*.

Other potential vectors include the release of infected *T. tubifex* from the aquarium trade (Lowers and Bartholomew 2003; Hallett et al. 2005b), improper disposal of infected fish parts, use of infected fish parts as bait, and effluent from commercial fish processing (Arsan and Bartholomew 2008). Understanding vectors for the spread of *M. cerebralis* informs risk assessment, management and policy decisions. Risk assessments have utilized this information to estimate risk and rank the importance of various vectors (Figure 14; Bruneau 2001; Engelking 2002; Schisler and Bergersen 2002; Bartholomew et al. 2005; Arsan and Bartholomew 2008). Many uncertainties still remain about the quantitative importance of each type of vector. Perhaps more importantly, many questions remain regarding how to most effectively manage human behavior and aquatic resources given the knowledge we have about the spread of this pathogen.

Control and Mitigation

Once *M. cerebralis* is present in natural habitats where susceptible hosts co-exist and environmental conditions are appropriate, it is generally considered impossible to eradicate. However, it is possible to control or slow further spread of the parasite and to mitigate its impacts. In fish culture facilities, there are many strategies to prevent the introduction of *M. cerebralis*,

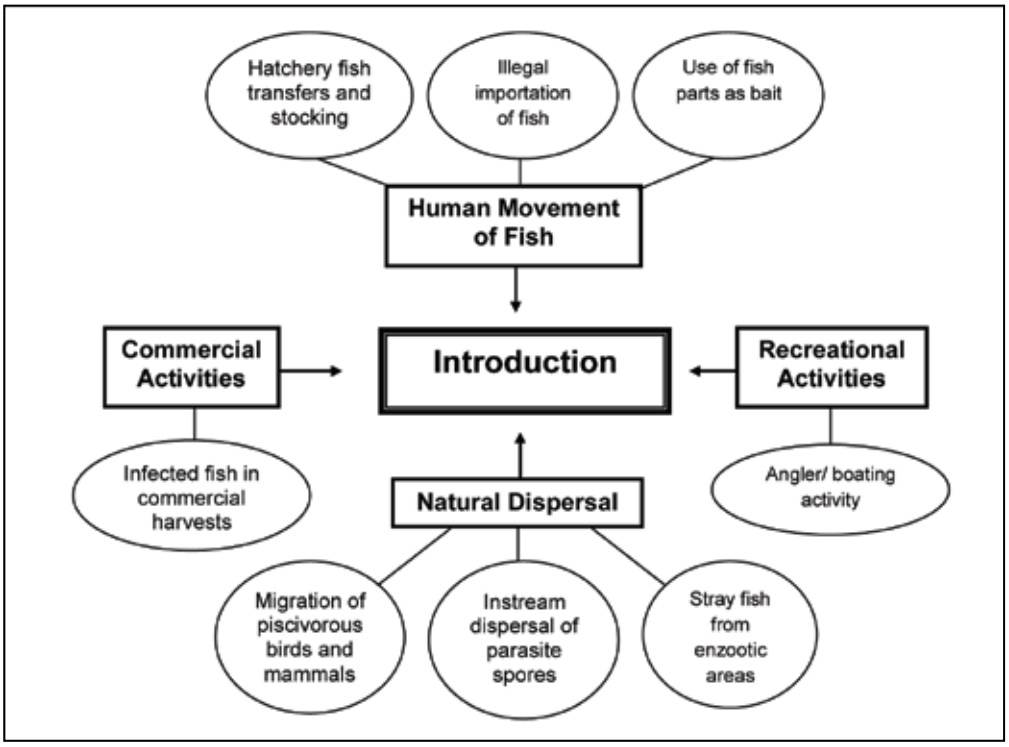


Figure 14. Introduction of *Myxobolus cerebralis* can occur through a variety of pathways, with examples illustrated here. Risk assessments can be used to identify these pathways and provide a qualitative or quantitative probability of these events occurring.

to disinfect the facility, and to control its spread. One example of successfully preventing spread of *M. cerebralis* from an infected hatchery to wild salmonid populations in Oregon is summarized by Bartholomew et al. (2007b), who monitored infection in the adjacent creek before and following closure of earthen ponds on the facility. In that case, although the hatchery provided ample habitat for *T. tubifex*, the oligochaete was not abundant in the creek and partial closure of the hatchery removed the source of infection. In Maryland, a recent detection of *M. cerebralis* in hatchery fish prompted aggressive action by the Department of Natural Resources to prevent the spread of the parasite. This included testing all fish production facilities and destroying infected fish, the development of new bio-security protocols, testing resident fish and surface waters regularly for the parasite and a new angler education program. These efforts suggest the parasite has remained localized in the area of original detection (Rivers 2008).

Fish culture strategies to deal with *M. cerebralis* include infrastructure alterations, application of chemicals for disinfection, and careful regulation and inspections. Wagner (2002) provides an excellent review of physical, chemical, and medical treatments tested for management of *M. cerebralis*. Physical modifications of facilities by converting earthen-bottom ponds and raceways to concrete in order to remove *T. tubifex* worm habitat have been very effective in reducing disease severity. Markiw (1992c) revealed that earth-bottom raceways contained worm populations, whereas concrete raceways could be cleaned to remove organic wastes and worms. While these modifications are important, they will not result in eradication of the parasite unless a secure, pathogen-free water supply is available. Unfiltered surface water sources may be vulnerable to *M. cerebralis* introductions by humans and wildlife. Using groundwater minimizes the potential for water to contain triactinomyxons or infected fish. Many facilities are converting from surface water sources to groundwater sources to minimize

exposure to water containing *M. cerebralis*.

Incoming hatchery water sources can be treated by ozonation, chlorination and ultraviolet light treatment to deactivate triactinomyxons (Markiw 1992c; Hedrick et al. 1998; Hedrick et al. 2000). The use of filters to reduce infection among hatchery fish has been less effective. Sand-charcoal filters and 10 to 20 micrometers (µm) nitex cloth sieves have been more effective than commercial 25 µm filters at removing triactinomyxons (Hoffman 1962; Hoffman 1974; Wagner 2002; Arndt and Wagner 2003). Recent unpublished studies in Utah indicate that certain commercial filtration systems that use a combination of three-dimensional filtration media and ultraviolet light are effective at removing triactinomyxons from hatchery water supplies (Arndt 2005). However, these options can be expensive ($450,000 to $900,000) to implement. If water sources cannot be effectively secured or treated, and the facility cannot be disinfected, outdated hatcheries may be decommissioned.

Several drugs have been tested in an effort to reduce or avoid infection in fish, but with little success (Hoffman et al. 1962; Taylor et al. 1973; Alderman 1986; El-Matbouli and Hoffmann 1991b; Doyle et al. 2003; Clarkson et al. 2004). Although experimentation with Fumagillin has shown some minor success (El-Matbouli and Hoffmann 1991b; Staton et al. 2002), the utility of drugs in reducing infection prevalence in fish is limited, due to the complexity in Federal Drug Administration drug registration and approval, and difficulty for use with wild fish.

Regulations regarding fish health inspections and stocking of *M. cerebralis*-infected fish vary from state to state. For example, Montana and New York do not allow stocking of any infected fish into public waters; conversely, California and Colorado may allow stocking of infected fish into waters where the parasite has previously been detected. As described in the Vectors section of this document, this practice is largely limited to waters not connected to any streams that can support wild salmonids, and the highest level of protection for wild salmonids is provided when there is no stocking or transfers of *M. cerebralis*-infected fish.

Control of *M. cerebralis* in the wild is more difficult than in culture facilities, but there are opportunities for decreasing disease impacts through fish stocking practices and stream modifications. In systems where fish are stocked, larger fish (> 40 mm, fork length) that are less susceptible to infection result in decreased spore numbers, although larger fish can still become infected (Ryce et al. 2004). Further information on the influence of fish age and size on whirling disease risk may be found in the Salmonid Factors section of this document. Stocking larger fish is one of the most simple and most effective management strategies available.

Stream habitat modification has been proposed as a means to reduce the impacts of whirling disease, but their benefits have been difficult to assess. Small-scale habitat changes (100 meter reaches) have been evaluated in hopes of promoting habitat less favorable for *T. tubifex*, and reducing the number of triactinomyxons in a stream. For example, berms have been used to isolate preferred *T. tubifex* habitat and triactinomyxon "hot-spots" from the main stream channel (Waddle et al. 2006). Another investigation found that while worm populations can be reduced by small-scale alterations, these localized effects may not diminish the impacts of the parasite within the surrounding stream reach (Thompson and Nehring 2003, 2004). Sediment deposition is difficult to control in natural situations, and engineered habitats may be subjected to unpredictable natural forces that may affect the longevity of a structure. Additionally, the effort and dollars spent on the implementation of stream modification must be balanced with the expected results in reducing the parasite load. In other words, the effects of a well-designed small-scale habitat alteration may not outweigh the total input of triactinomyxons from the entire stream system. Also, reducing sediment loads in one location may cause transport downstream, thereby moving infection sources to a new location.

An alternative to engineered stream modifications is passive stream restoration. Evaluation of the exclusion of livestock grazing is underway in Utah and could lower stream

temperatures by increasing riparian shading, and reducing the quantity of fine sediment and nutrients entering the stream. This could improve both fish health and reduce the risk of infection from *M. cerebralis* (Hansen et al. 2006). Finally, in regulated rivers, stream flow modifications may increase flushing flows that could serve to reduce infection severity by removing fine sediments and reducing *T. tubifex* habitat (Milhous 2005).

Taking advantage of the variations in natural resistance between species of salmon and strains of trout can also be an effective management tool. As described in the Salmonid Factors section, salmonids are highly variable in susceptibility to *M. cerebralis* and this resistance may be an innate characteristic of the species (e.g. coho salmon), or it may have evolved through natural selection in the presence of the parasite (e.g. brown trout, certain strains of rainbow trout). Preliminary research by E. R. Vincent (Montana Fish, Wildlife & Parks, personal communication) on the Madison River, Montana, suggests that progeny of wild rainbow trout that have survived the brunt of infection may have genetic resistance to infection. This resistance may be the result of natural variability, strong directional selection or genetic mutation. Fostering wild fish populations with high genetic diversity may increase the likelihood of population persistence and resilience despite the presence of pathogens. The application of this knowledge as a management tool is uncertain but promising.

Additionally, the domestic GR (German or Hofer) strain of rainbow trout, which has reduced susceptibility to *M. cerebralis*, is being evaluated to determine the mechanisms of resistance and their potential to survive and reduce parasite levels in *M. cerebralis*-positive waters (Hedrick et al. 2003; Bartholomew et al. 2004; Wagner et al. 2006; Schisler et al. 2006; also see Strain section under Salmonid Factors). It is expected that *M. cerebralis*-resistant trout would have higher survival rates, successful reproduction, and could lower the number of parasites in the ecosystem by reducing the number of myxospores produced. By crossing this strain with locally adapted strains of rainbow trout, managers hope to develop a fish for stocking that would have resistance to whirling disease and retain genetic traits important for survival in the wild. The risks and benefits of this approach have been carefully considered and currently these fish are being used experimentally in three state hatchery programs (Colorado, Utah, and California). Initial results of stocking of crosses between GR trout and locally adapted Colorado River rainbow trout are promising, with survival and reproduction in the Gunnison River, one of the most heavily impacted rivers in Colorado.

Using a similar approach, lineages of *T. tubifex* associated with resistance to *M. cerebralis* may be used to reduce whirling disease in salmonids. An experimental introduction of resistant *T. tubifex* lineages into a Colorado stream is being conducted by the US Geological Survey Colorado Cooperative Fish and Wildlife Research Unit and the Colorado Division of Wildlife. The stream naturally contains high numbers of susceptible lineage III *T. tubifex*. Researchers will examine the effects of introduced resistant-lineage worms on the prevalence of infection and production of triactinomyxons (Thompson et al. 2008, Winkelman et al. 2007). Managers hope resistant *T. tubifex* could lower infection prevalence among susceptible *T. tubifex* through competitive interactions, and by de-activation of myxospores by resistant *T. tubifex*. The extensive distribution and densities of aquatic worm populations and the complexity of natural ecosystems suggest that this could be a difficult method for altering the effects of whirling disease; however, it may have some application for newly created water systems, such as private ponds. As with resistant fish, the benefits and risks of introducing organisms into the wild will be carefully considered.

An important method for limiting the spread of *M. cerebralis* and minimizing its impacts is providing information to the public and engaging the public in resource management. Anglers, boaters and others must be aware of the potential role they play in the transport of *M. cerebralis*.

Several recommended precautions exist to prevent the spread of *M. cerebralis* and other aquatic invasive species:

- Never transport live fish from one water body to another.
- Rinse all mud and debris from equipment and wading gear with clean water. Drain water before leaving the river or lake. Allow boats and gear to dry between trips.
- Do not use trout, whitefish or salmon parts as bait.
- Dispose of fish entrails and skeletal parts away from streams or rivers. Dispose of salmonid fish parts in the garbage rather than a kitchen disposal.

Individuals with private fish ponds should be aware of fish health regulations, and management choices for their pond that could pose risks to nearby streams. Pond owners should obtain any necessary permits before stocking fish into a pond, and ask to see a fish provider's fish health certification before purchasing fish. Hobbyists with home freshwater aquaria should consider using an alternative food source to live *T. tubifex* worms, and never release worms or live fish into the wild.

Conclusion

Whirling disease presents an informative case study in a variety of ways – as an exotic species, a myxozoan parasite, a fish pathogen, a perceived natural catastrophe, a perceived non-event, a threat to wild and native salmonids, a risk to fish culture, and collaborative research and management. The work of researchers continues to contribute to a greater understanding of *M. cerebralis* disease ecology and to a better understanding of host-parasite interactions and invasive species ecology. Current research is focused on understanding the dynamics of *M. cerebralis* ecology at a landscape level, characterizing the mechanisms of resistance in both hosts, refining risk assessments, and improving management tools. The breadth of research that has taken place since the discovery of *M. cerebralis* has expanded our understanding of this parasite, but also revealed how much there is still to understand. As researchers continue to examine the complexities of *M. cerebralis*, a better understanding of this parasite will emerge and management strategies will become increasingly effective at protecting and enhancing salmonid populations.

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