

1999 REPORT TO CONGRESS

Whirling Disease Initiative

submitted by the

**Whirling Disease Steering Committee
of the
National Partnership
for the Management of Wild and Native Coldwater Fisheries**

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BACKGROUND

Over the past decade, the microscopic parasite *Myxobolus cerebralis*, which causes whirling disease in many salmonid fish species, has been spreading and infecting hundreds of river and stream reaches in the Western United States. The impacts of this parasite on susceptible trout can be dramatic: darkening of the tail, skeletal deformities, frenzied tail chasing (thus the name “whirling” disease), and death. This microbe is extremely hardy and long-lived, with a life cycle that employs both a fish host and an aquatic worm host known as *Tubifex tubifex*.

A Eurasian native, *M. cerebralis* made its way to North America in the 1950s. It was once believed to be relatively harmless to wild fish, but research in the mid-1990s found that it was decimating rainbow trout populations in some of the Rocky Mountain region’s finest river fisheries, from Montana’s Madison River to the Gunnison River in Colorado. Native cutthroat trout and whitefish are susceptible, and school is still out on bull trout and anadromous populations. Whirling disease is therefore a major threat both to biodiversity and to the multi-million dollar fishing and tourism economy.

The whirling disease parasite has now been reported in 23 states -- from New York to California, and most recently, New Mexico -- and has generated great concern among anglers, scientists, and fisheries managers.

THE WHIRLING DISEASE INITIATIVE

In 1997, the Whirling Disease Initiative was established under the National Partnership for the Management of Wild and Native Coldwater Fisheries. The Initiative’s purpose is to promote, prioritize, and help fund cooperative research with direct implications for whirling disease afflicting wild trout populations. The program is administered from Montana State University-Bozeman, where a Whirling Disease Steering Committee made up of representatives from multiple states and agencies convenes in person or by conference phone. The Committee prepares an annual research plan, issues Requests for Proposals based on identified priorities, selects and approves projects for funding following scientific peer review, and makes available the research results to all partners and cooperators via mail and an expansive web site. This competitive grants program began in 1997 and since then has continued to fund new research projects in every year.

Because so little was known about whirling disease, the Steering Committee has chosen to support a mix of projects, from basic research (providing the building blocks for management solutions) to applied research geared more directly at rapid response management solutions. Embracing both sets of research needs enhances the opportunities for finding both short-term and long-term solutions to the whirling disease problem.

For the 1998-99 cycle, 17 different research projects and 3 program grants were funded with a total of \$593,225 in federal dollars, and leveraging an additional \$507,605 in match. Research teams include 31 investigators from six states. These projects are characterized by excellent cooperation from multiple agencies and a high level of communication, due in part to annual symposia and field ecology workshops and current whirling disease web site.

HIGHLIGHTS OF THE 1998-1999 RESEARCH RESULTS

Susceptible worms

Some *Tubifex* worms show resistance to the parasite. Other non-*Tubifex* species of worm can also ingest *M. cerebralis* spores, but are “dead-end” hosts where the parasite cannot develop further. *This may open up possibilities for biological control* of the disease using non-susceptible worms. Production of whirling disease spores in worms is also significantly influenced by water temperature.

Trout life history can influence the course of whirling disease

The numbers of susceptible *Tubifex* worms and severity of infection can vary throughout watersheds. This finding, coupled with the knowledge that different fish can have different life histories – e.g., have different times for spawning, or spawn in different tributaries vs. mainstem rivers, etc. – indicates that trout life history patterns can have an important effect on the severity of whirling disease within different fish populations. *By seeking fish with life histories that promote survival against whirling disease, it may be possible to restore depleted fisheries.*

Mountain whitefish can be infected

Mountain whitefish exposed to *M. cerebralis* at different ages and at different doses became infected with whirling disease, showing clinical signs of disease and evidence of infection in examination of fish tissues. *Like trout, whitefish are susceptible to whirling disease.*

Wild rainbow trout in the Colorado River do not appear to be developing resistance

Earlier experiments suggested that more recent generations of wild rainbow trout in the Colorado River were showing increased resistance to whirling disease when compared to earlier generations in the same river – an example of natural selection at work. However, controlled laboratory tests of the fish *did not provide any conclusive evidence that offspring from the more recent generations had any greater resistance to whirling disease.*

SUMMARY

While little hope exists for the eradication of whirling disease, research has helped highlight management approaches that offer promise for containing and decreasing the impact, and eliminating approaches that appear ineffective. In the coming year, the Initiative will increase its focus on experimental management efforts, to begin testing some of the more promising strategies, while continuing to support vital basic research to enhance our overall understanding of this disease.

The Steering Committee particularly thanks Montana's Congressional delegation – Senator Max Baucus, Senator Conrad Burns, and Congressman Rick Hill – for their advocacy of this research; and the Representatives of the National Partnership for their time and guidance.

SPAWNING AND REARING ECOLOGY OF THE MADISON RIVER RAINBOW TROUT IN RELATION TO WHIRLING DISEASE INFECTION RISK

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Introduction

Whirling disease has severely reduced Madison River rainbow trout populations. By 1994, population monitoring sections showed 90% decline of rainbow trout populations compared with historic averages. Madison River rainbow trout populations have since stabilized near 10% of their previous abundance. This suggests some fish have life history characteristics that enable them to elude high whirling disease infection.

Whirling disease infection risk is not uniform throughout the upper Madison River system. Temporal variability of infection rate exists. Vincent (personal communication) suggests that infection rate is highly correlated with water temperature, with the highest infection rates occurring near 13 °C. Some rainbow trout may emerge early enough to allow sufficient ossification to protect them from the highest infection risk. In this scenario, possibly only the earliest emergent fry from the earliest spawners are surviving. Preliminary studies, using sentinel cages and juvenile rainbow trout indicate great spatial variation in whirling disease infection rates in the upper Madison River as well (Vincent, personal communication). Therefore, rearing location during the time of greatest vulnerability influences the risk of whirling disease infection.

The variation in rainbow trout population responses among whirling disease infected river systems may be better explained with more complete understanding of the role of life history variations. Because young trout are most susceptible, examining when and where trout spawn and rear may reveal life history traits that limit exposure to whirling disease, even in highly infected river systems. The goal of this study is to identify life history characteristics maintaining this population in the presence of whirling disease. Recognizing the various life history strategies used by rainbow trout in the Madison River is an important step in mitigating for severe population declines. Specifically, timing and location of spawning and rearing of rainbow trout in the upper Madison River have been investigated. This information is compared to risk of infection determined by sentinel fish cages and water temperature. Managers can use this information to study how each life history strategy influences population response to the disease. Understanding how spatial and temporal variation in spawning and rearing influences rainbow trout population risks in whirling disease infected rivers is essential to developing disease management strategies.

Methods

Spawning timing and location were determined using redd surveys and radio telemetry. Weekly totals of spawning monitoring areas were calculated to determine the peak and duration of the spawning season.

Twenty-eight adult rainbow trout were implanted with radio transmitters in September and October 1998. Relocating tagged fish revealed migration patterns and previously unidentified spawning areas.

Redd caps trapped emerging rainbow trout fry to determine emergence timing. Emergent fry, when found in the traps, were identified, measured, and released. The first of several successive days with fry present in the trap indicated fry emergence.

Seven sites between Quake Lake and McAtee Bridge were electrofished periodically to detect rearing location and migration of young-of-year (YOY) rainbow trout. Sampling took place once in June, July, and August in 1998, and mid-July, early August, late August, and mid-September in 1999.

Results

In 1998, a total of 853 redds were counted. Fifty percent were found above Reynolds Pass Bridge, 38 percent were found above the confluence of the West Fork Madison River, and the rest were found above McAtee Bridge. The number of redds found and the relative distribution were similar in 1998 and 1999. Weekly totals indicate peak spawning occurred over the last 3 weeks of April and tapered off in May in 1998. The 1999 data indicate the spawning season was underway from early April until mid May.

Despite some pre-spawning mortality, migration and spawning data were collected for 21 of the radio tagged fish. Twelve migrated upstream and spawned in mainstem side channels, 4 migrated to spawn in Madison River tributaries, while 5 showed no movement throughout the spawning season. It is unknown whether the stationary fish spawned or not.

In general, mainstem spawners migrated and spawned earlier than tributary spawners. The mean initial migration date for mainstem spawners was March 23, and the mean spawning date was April 30. In contrast, tributary spawners averaged May 2 as an initial migration date and May 27 as a spawning date. Tributary spawners spawned between May 21 and May 31. Alternatively, mainstem spawners spawned from March 12 to June 1.

In 1998, 12 of 20 capped redds captured emerging fry. Fry emerged in middle to late May in two redds, in the first week of June in one redd, and between the last week of June and the third week of July in nine redds. In 1999, emergence data was collected from 31 redds. Fry emerged from 22 redds between June 28 to July 11. Emerging fry were captured from the remaining capped redds between June 3 and August 3.

In 1998, the June and July YOY sampling took place before most rainbow trout fry emerged. Subsequently, all fry monitoring sites showed an increase in rainbow YOY relative abundance in August. The 1999 results show steady declines in rainbow trout YOY abundance in all sites throughout the summer.

Discussion

The results of this study reveal two Madison River rainbow trout spawning strategies. A substantial portion of the rainbow trout population migrates to and spawns in side channels of the mainstem Madison River. A second spawning strategy is that of the tributary spawners. The tagged fish spawning in tributaries revealed a spawning strategy that was overlooked using redd surveys alone.

Rainbow trout emergence is concentrated in the end of June and early July. The YOY monitoring in 1998 supports the emergence timing data collected. Because juvenile trout are most susceptible, their location during the period immediately following emergence is crucial in evaluating infection risk. The distribution of rainbow trout YOY in the monitoring sites resemble the distribution of redds in the area. This data suggests no large-scale migrations of YOY rainbow trout during their first summer.

Given that spawning and rearing is concentrated in the upper portion of the study section and infection risk is spatially variable in the upper Madison River, focusing management actions in low infection risk areas would be logical. Therefore, by encouraging spawning in low infection risk areas (e.g., tributaries and side channels of lower sections) fewer fish will be exposed to high levels of whirling disease.

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**AN ASSESSMENT OF RESISTANCE TO WHIRLING DISEASE IN COLORADO RIVER
RAINBOW TROUT AND SNAKE RIVER CUTTHROAT TROUT EXPOSED TO *M.*
CEREBRALIS IN THE COLORADO RIVER**

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In 1996-97, we conducted a sentinel fish study that included Snake River cutthroat trout *Oncorhynchus clarki bouvieri* obtained from spawn from the South Platte River in Colorado, a site where *M. cerebralis* was enzootic for about eight years at the time of egg collection, and two groups of rainbow trout *O. mykiss* (Thompson et al. 1999). One of the rainbow trout groups was from wild Colorado River parents aged 4 and 5 years (determined by scale analysis), which recruited to adulthood during the first years of *M. cerebralis* infestation in the Colorado River. The other group was from the general population of parents; the vast majority of these parents recruited before whirling disease was manifested in the upper Colorado River. Results from that study showed that the Snake River cutthroat trout developed lower prevalence and individual concentrations of *M. cerebralis* spores than most other groups. Also, on two separate occasions, rainbow trout originating from young parents exhibited much lower concentrations of *M. cerebralis* spores than rainbow trout originating from the general population of parents, although survivorship differences between these two groups were insignificant. These findings lead to the hypothesis that the Snake River Cutthroat trout and the rainbow trout from younger parents might be exhibiting early adaptive responses to the presence of *M. cerebralis*. Another study was designed to address this hypothesis.

The 1998-99 sentinel fish experiment again included two groups of rainbows (designated Rbp6 and Rbp8), but this time all parents were aged by scale analysis. Parents that could not be reliably aged were excluded from parenting experimental fish. By 1998, "young" parents were a maximum of 6 or 7 years old, and "old" parents were a minimum of 8 years old. Two groups of Snake River cutthroat trout were also studied. One group came from the South Platte River (Srnc), and the other originated from the broodstock maintained by the Wyoming Game and Fish Department (Srnw), which was not previously exposed to *M. cerebralis*. Two replicates of each of the four groups were exposed as naïve fish at four locations in the upper Colorado River for 36 d beginning July 14, 1998. Locations were chosen to represent widely varying levels of exposure, which was estimated by filtering *M. cerebralis* triactinomyxons at each site at least weekly during the exposure period. At the end of the exposure period, all groups were moved to the lowermost site at Breeze Bridge, and held there until July 14, 1999. All replicates were monitored monthly for survival and external signs of whirling disease. Each replicate was sampled for myxospore concentration analysis by pepsin-trypsin digest (Markiw and Wolf 1974), survival permitting, each month from April through July 1999.

Estimated exposure over 36 days, modeled assuming 60-mm fish and a current velocity of 15 cm per second, ranged from 180 triactinomyxons at Breeze Bridge to 401,000 in the North outlet of Windy Gap Reservoir. The estimated exposure was 58,000 at the Hitching Post Bridge and 3,900 above Windy Gap Reservoir. Actual sizes of our study fish were generally smaller than modeled. At the end of the 36-d exposure, Srnw averaged 64 mm, Srmc averaged 44 mm, Rbp6 averaged 40mm, and Rbp8 averaged 41 mm.

Early mortality exceeded expectations, especially at Hitching Post Bridge, and complicated analysis of survival. However, analysis without the Hitching Post location indicated that survival among the Rbp6 (77%) was significantly worse than among the Rbp8 (86%) by nine weeks post exposure ($P = 0.037$). This result is opposite of that reported at this meeting last year (Ryce et al. 1999, unpublished work) for fish from the same cohorts 20 weeks post exposure in a laboratory. This fails to support the hypothesis of early adaptive response to *M. cerebralis* infection among the progeny of rainbow trout that recruited after the parasite became established in the Colorado River.

There were significant differences in mean spore concentrations among fish groups by April 1999 (Table 1). However, there was not a significant difference in spore concentration between the two rainbow trout groups ($P = 0.38$), which fails to support the hypothesis of early adaptive response of rainbow trout. The large differences in spore concentration between the rainbow and cutthroat groups suggests that using Snake River cutthroat trout in some recreational fisheries would result in fewer spores being introduced to the environment.

Table 1. Mean *M. cerebralis* spore concentrations in April 1999 for each fish group and exposure location.

Location or group	Rbp6	Rbp8	Srmc	Srnw
Above Windy Gap	1,825,000	1,575,000	217,000	27,000
North Outlet	1,651,000	1,589,000	804,000	225,000
Hitching Post	1,125,000	910,000	294,000	37,000
Breeze Bridge	231,000	220,000	21,000	6,000

The very high spore concentrations observed among rainbow trout exposed above Windy Gap Reservoir were surprising. This location yielded the next to lowest estimated exposure, yet was significantly higher in mean spore concentration than the Hitching Post site ($P = 0.0001$). Apart from this location for the rainbow trout, and for all locations among the cutthroat trout, April spore concentrations fell in the same order as the estimated exposure to triactinomyxons. This pattern was generally maintained during later samples for all groups. Fish at the Breeze Bridge in particular consistently showed lower spore concentrations than at other locations.

This study failed to support the hypothesis that rainbow trout were experiencing a rapid adaptive response to the presence of *M. cerebralis* in the Colorado River. Results for the cutthroat trout are less conclusive, because initial size differences between the two cutthroat groups complicate the analysis and interpretation of the data. The better performance of cutthroat trout originating from the Wyoming broodstock may simply reflect their larger size at exposure. However, it is clear that Snake River cutthroat trout exposed at a size similar to the rainbow trout developed fewer *M. cerebralis* spores. The study supplies additional confirmation that Windy Gap Reservoir is a point source of *M. cerebralis* infection, despite the fact that it is not stocked water and there is no fishing allowed in the reservoir.

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ULTRASTRUCTURE OF THE TRIACTINOMYXON OF *MYXOBOLUS CEREBRALIS*

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Although the general ultrastructural features have been reported for the triactinomyxon of *M. cerebralis* (El-Matbouli and Hoffmann, 1998; El-Matbouli, Hoffmann and Mandok, 1995; El-Matbouli et al., 1999), many of the ultrastructural details of the TAM are still not known. The purposes of this investigation were to define in greater detail the ultrastructural features of the TAMs of *M. cerebralis* as well as to study the localization of monoclonal antibodies (mabs) directed against various TAM components.

The TAM was covered by three longitudinal plates that trifurcated posteriorly to form the three rays. Each plate consisted of a membrane and an underlying electron-dense layer which was folded back on itself to form a double layer. The plates were held together by sutures that consisted of a thin electron-dense interposed strip that extended from the tips of the rays to the tips of the polar capsules. A thick layer of moderately electron-dense material was situated between the two membranes at the anterior end of the TAM immediately above each polar capsule. The polar capsules consisted of a moderately electron-dense filament embedded in an extremely electron-dense medium, whereas the contents of polar capsules with everted filaments consisted of electron-dense granules scattered in an electron-lucent medium. The anterior 1/3 of the polar filament consisted of a covering membrane with numerous external blebs, and underlying electron-dense layer and fibrous core. The posterior 2/3 was covered by a membrane that lacked blebs, and had a thin electron-dense layer and a electron-lucent core filled with electron-dense granules. As the polar filament everted, its anterior 1/3 was derived from the filament encased within the polar capsule, whereas its posterior 2/3 was derived from the contents and inner surface of the polar capsule.

We have subcloned more than 30 hybridomas that produce mabs against the TAMs of *M. cerebralis*. The mabs were selected for subcloning and culture expansion based on their fluorescent staining patterns with freshly harvested TAMs. Fluorescent microscopy and immunoelectron microscopy were then used to study in further detail the localization of mabs on the surfaces of the TAMs, polar capsules, polar filaments and sporoplasms. Mabs 987 and 988 reacted with the entire surface of the TAM, the sporoplasm and the polar capsules; mab9833A reacted with polar capsules and the sporoplasm; and mab 9813 exhibited a uniform fluorescent staining of the sporoplasm and reacted with everted filaments. Mabs 9833A, 988816, 985, 984B reacted with the surfaces of polar capsules, whereas mab 983B reacted with the entire polar capsule. Mabs 989A and 9832 stained the sporoplasm but did not react against the polar capsules. Mab 988 reacted against the surface of the TAM, and faintly stained everted polar filaments and the sporoplasm. Receptors against which mab 9813 reacted were found to be more numerous in the distal 1/3 of everted filaments than in the proximal 2/3. Receptors for mab 9813 were uniformly distributed on the surface of the polar capsules and the sporoplasm but were lacking on the surface of the TAM. Certain mabs are currently being evaluated for their ability to inhibit the attachment of the polar filament and sporoplasms to the surfaces of salmonids and to cultured salmonid cells.

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**DISTRIBUTION OF *MYXOBOLUS CEREBRALIS* IN YELLOWSTONE CUTTHROAT TROUT
ONCORHYNCHUS CLARKI BOUVIERI IN YELLOWSTONE LAKE AND ITS TRIBUTARIES.**

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Introduction

Since the confirmation of whirling disease in rainbow trout *Oncorhynchus mykiss* in the Madison River in 1994, fishery management biologists in Yellowstone National Park have been testing resident fish populations for the presence of *Myxobolus cerebralis*. Juvenile rainbow trout, Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri*, brown trout *Salmo trutta*, or mountain whitefish *Prosopium williamsoni* captured during electrofishing surveys of "boundary area" study streams were examined for the presence of cranial spores. No positive fish were detected in any of eight study streams between 1995 and 1998.

In fall of 1998, a sample of 41 cutthroat trout captured during an annual gill netting survey of Yellowstone Lake was similarly analyzed. Initial results indicated a positive *M.cerebralis* infection rate of approximately 10-25%. Length-age relationships suggested that the positive cutthroat trout ranged from two to five years old. Because Yellowstone cutthroat trout and rainbow trout appear to have similar susceptibility to whirling disease (MacConnell, *et al.* 1997, Elle 1998), concern about the long-term abundance of the Yellowstone Lake cutthroat trout increased considerably. The Yellowstone Lake cutthroat trout population is valued as one of the most important inland cutthroat trout populations in the inter-mountain west, not only because it is the world's largest native cutthroat trout population, but these fish reside in the most extensive intact area of cutthroat trout lake habitat (Gresswell 1995). Thus, in addition to uncertainties about how where and when *M. cerebralis* was introduced into Yellowstone Lake, there is no information about the lakewide distribution of the parasite and its effects on individual cutthroat trout there. In 1999, the National Park Service (NPS) and the USFWS Bozeman Fish Health Lab (FHL) initiated a preliminary study to obtain some of this unknown information that is necessary for appropriate management. Here we describe some of our results from this initial survey.

Methods

Three different groups of Yellowstone cutthroat trout were selected for *M. cerebralis* testing:

- ◆ Various size and age classes captured during the fall cutthroat trout monitoring survey. Shallow water sets at several standardized long-term sites throughout the lake.
- ◆ Large cutthroat trout incidentally killed during the NPS season-long lake trout gillnetting effort (Mahony and Ruzycski 1997).

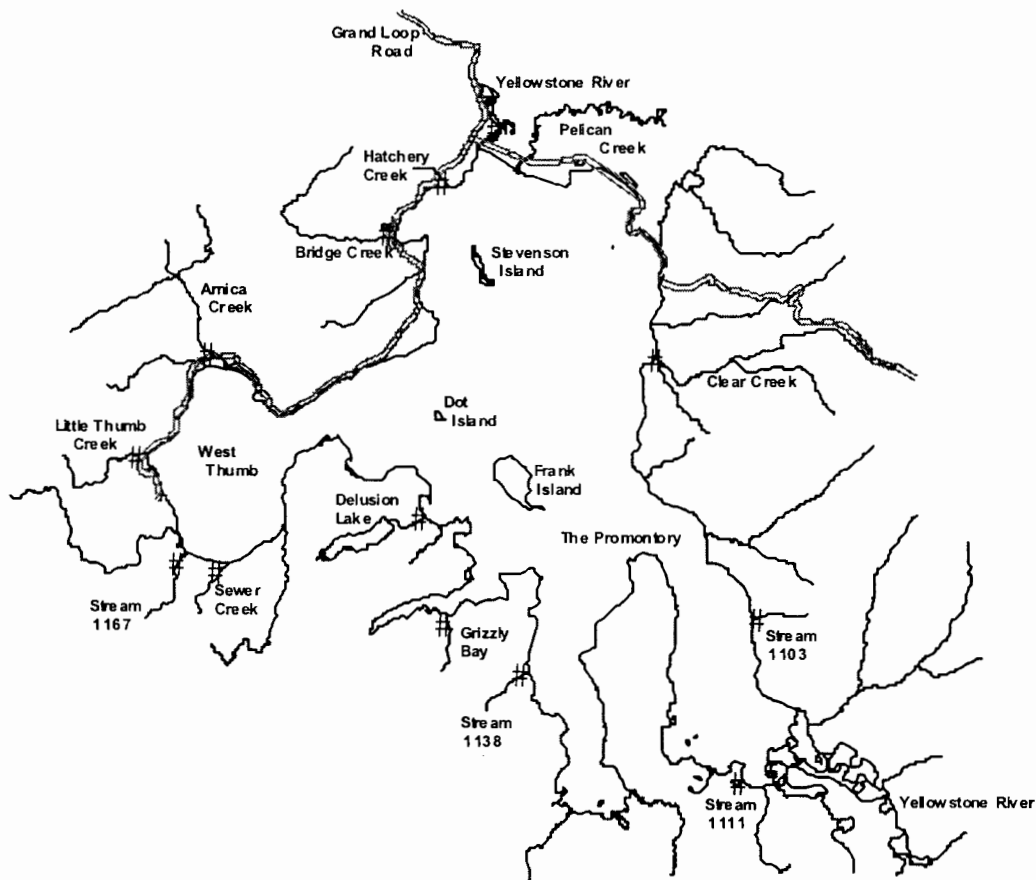


Figure 1. Whirling disease sentinel cage sites on tributaries of Yellowstone Lake, 1999.

- ◆ Young of the year cutthroat trout placed in sentinel cages in twelve known cutthroat trout spawning tributaries of the lake (Fig 1). Additional sentinel testing occurred near Fishing Bridge (primary spawning area immediately downstream of the lake outlet) and at Buffalo Ford, a heavily used angling area of the Yellowstone River several miles downstream from Fishing Bridge.

All fish in the first two groups were individually measured to the nearest mm, weighed, sexed, and classified with respect to sexual maturity. Scales were collected from a length stratified subsample. This information will be used to estimate annual growth rates and population age-structure. Gillnet catch location for each cutthroat trout was recorded with boat-mounted GPS equipment. Heads from these cutthroat trout were subsequently transferred to FHL for histological analyses. Each fish was initially screened with the pepsin/trypsin digest method. Positive results were subsequently corroborated with additional histological examination, PCR confirmation, or both.

In order to anticipate optimum exposure time at the sentinel cage testing sites, Onset temperature loggers were installed in early June at each of the test sites. Eggs were collected from approximately six pairs of spawning cutthroat trout captured from a tributary to Yellowstone Lake. These were transferred to a State of Wyoming isolation facility and reared to about 7-8 weeks old. The cutthroat fry were then exposed (100 fish/cage) in cages for 10 to 15 days in late August. At the end of the exposure period, the fry were reared at the Montana FWP isolation facility prior to analyses for whirling disease parasite presence and severity. Histological/PCR confirmation proceeded as described above.

Results

Each of the three sample groups of Yellowstone cutthroat trout described above contains several hundred individuals. Because many of cutthroat trout were not sampled until September 1999, much of our analyses and data interpretation (including disease severity indices) has not yet been completed. Consequently, data presented here relates primarily to specific sites from the first two groups of fish and may not necessarily be representative of the entire population.

At the Pelican Creek site, nine of 25 cutthroat trout were infected with *M. cerebralis* spores. Total length of positive fish ranged from 375 to 475 mm. One third of the infected fish were mature females that were classified as prespawners (eggs developed sufficiently for fish to spawn in late spring of 2000). Near Arnica Creek, a sample of 25 similarly-sized cutthroat trout yielded no confirmed parasite infection. Initial screening of 27 fish from the central portion of the lake and an additional sample of 40 cutthroat trout from the Southeast Arm of the lake indicates that some fish are positive for *M. cerebralis*. Histological or PCR confirmation has not been conducted for these suspected positive, however.

In order to minimize cutthroat mortality, gill nets used in the lake trout survey consist of large meshes designed to allow most of cutthroat trout to escape. As a result, most of the fish from this second survey consist of small sample sizes ($N < 5$) of larger cutthroat trout from a few restricted areas of lake. A large majority of these trout were captured in the West Thumb area of Yellowstone Lake. Fourteen of 75 cutthroat trout collected during 22 separate gillnet sets were confirmed positive, which represents an infection rate of slightly less than 20%. Similar to results from the fall survey, most of the positive fish were mature adults. Seasonal infection rates showed minor differences. No external signs of disease were apparent in any of the sampled cutthroat trout.

Discussion

Because the cutthroat trout in Yellowstone Lake are mobile and may reside in different parts of the lake during different portions of their life history (Gresswell *et al.* 1994), conclusions related to initial locations where adult cutthroat trout acquired the infection are exceedingly difficult to substantiate. Preliminary testing of fry from sentinel cages suggests that Arnica Creek is whirling disease free, which would appear to correlate well with the gill net data. This would appear to contradict the results from Clear Creek, where sentinel fish also tested negative, but fish caught during the 1998 gill net survey showed the presence of the disease.

Assuming that the positive fish identified in this study were infected as fry suggests that some cutthroat trout in Yellowstone Lake are able to survive *M. cerebralis* infection without any obvious effects. Initial examination of stream temperature data suggests that in some streams, water temperatures increased dramatically in a short period of time. When the cutthroat fry emerged, ambient water temperature was considerably greater than that of peak TAM emergence. Our pilot survey will continue for several years to examine more closely this possibility. If the greatest probability of infection occurs in the late fall during the descending portion of the annual temperature profile, then cutthroat trout in Yellowstone Lake may be less vulnerable because most have already emigrated into the lake (Benson 1960, Varley and Gresswell 1988). Continued examination of the distribution of this parasite with a focus on densities, age classes, and habitat should assist NPS in preserving this valuable native fish species.

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SUSCEPTIBILITY OF MOUNTAIN WHITEFISH TO *MYXOBOLUS CEREBRALIS*

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Whirling disease of salmonids is caused by the myxosporean parasite *Myxobolus cerebralis*. Although endemic to Eurasia and present in North America since the 1950s, the disease garnered renewed interest and significance when it was implicated as the causative agent for major declines in wild trout populations in recreationally and economically important fisheries in Colorado and Montana in the 1990s. Subsequently, research needs concerning the parasite, disease, and relationships to fish and tubificid hosts were discussed and identified in a variety of national and regional forums. Considered to be among the highest priorities to make fisheries management decisions was the completion of comparative salmonid susceptibility tests to *M. cerebralis* in standardized, replicated, and controlled laboratory experiments.

M. cerebralis has a broad specificity for salmonids with rainbow trout being highly susceptible to infection. Susceptibilities of salmonid fishes have been subjectively inferred from experiments in which fish have been exposed to unknown quantities of *M. cerebralis* triactinomyxons in open waters or contaminated hatcheries. Whereas these investigations have offered important insights into species-specific susceptibilities, confounding variables have affected the interpretation of and confidence in some results. Triactinomyxon densities and exposures are unknown, challenged fish ages, sizes, and physiological conditions vary, exposure conditions are not standardized, and controls and replication may be absent. Only standardized, controlled, and replicated exposures to known doses of triactinomyxons under laboratory conditions allow cause-and-effect relationships to be elucidated, such that any differences can be more fully investigated to reveal underlying causes.

Our focus was on the comparative testing of susceptibility of different ages of mountain whitefish, *Prosopium williamsoni*, and rainbow trout, *Oncorhynchus mykiss*, exposed to different doses of *M. cerebralis* triactinomyxons under standardized laboratory conditions. By assessing how pathogenesis and parasite development differs in fish of different species and ages under similar conditions, our study was designed to gain a better understanding of this parasite and its impact on wild fish populations, thereby remedying critical management information deficiencies.

The specific objectives of our research were to:

1. Determine the susceptibility of mountain whitefish to *M. cerebralis*; and
2. Determine the effects of age and parasite dose on the development of *M. cerebralis* in mountain whitefish.

Methods

Eggs from wild mountain whitefish were collected during late autumn 1998, fertilized, and water hardened on site. Fertilized eggs were disinfected and then transferred to the Bozeman Fish Technology Center (BFTC), where they were incubated and reared at 10 EC until exposed. Rainbow trout were obtained as eyed eggs from a hatchery in Montana, and also incubated and reared at 10 EC at the BFTC. Mountain whitefish and rainbow trout at each exposure age were matched in degree days post-hatch.

Exposures were conducted at the Wild Trout Research Laboratory (WTRL) at Montana State University using *M. cerebralis* triactinomyxons produced on-site. Three replicate groups of 25-30 mountain whitefish each were exposed to one of two doses of triactinomyxons (1,000 or 10,000 triactinomyxons per fish) or a sham control exposure (0 triactinomyxons) at 3, 7, or 11 weeks post-hatch. Parallel groups of rainbow trout of the same ages were exposed simultaneously to the same doses of triactinomyxons. Each lot of fish was exposed together in a small exposure chambers for 2 hours.

Following exposure, lots were maintained separately in isolated 38-L glass aquaria supplied with filtered and oxygenated 15EC water by a recirculating process system at the WTRL. Fish were fed daily with a standard trout feed. Mortalities were counted and removed daily. Fish were examined visually weekly for clinical signs of whirling disease (blacktail, skeletal and cranial deformities, whirling behavior, etc.).

Fish from each replicate were sampled at 2 hours and 2 and 5 months post-exposure and preserved for histological assessments of infection and severity of pathology. The MacConnell-Baldwin (M-B) scale, which grades number of parasites, damage to cartilage, and host inflammatory response on a 5-point scale, was used to evaluate infection. Effects of age-at-exposure and dose on survival, clinical signs, M-B grades, and spore production were compared.

Results

Exposure to doses of 10,000 triactinomyxons/fish caused direct and rapid mortality of 3 and 7 week post-hatch mountain whitefish. Mortality in these groups occurred during the 2-h exposure or within 2 h post-exposure. Histological examination of these fish showed that numerous triactinomyxons had penetrated the epithelium. Rainbow trout concurrently exposed to this dose at the same ages did not suffer similar direct mortality.

Few mountain whitefish, including unexposed controls, survived to 5 months post-exposure. Survival was less than 15% in all replicates. Survival rates of rainbow trout were higher and compared favorably with rates observed in our previous studies. Failure of proper gas bladder inflation during early development appeared to be the most probable cause of high mortality in our mountain whitefish (F. T. Barrows, BFTC, personal communication).

Clinical signs of whirling disease (blacktail, whirling behavior, and skeletal deformities) were observed in both exposed rainbow trout and mountain whitefish, but not in controls. Clinical signs tended to appear a few weeks later post-exposure in mountain whitefish than in rainbow trout. Also, inflammatory response in mountain whitefish (as judged by histological examination) at 2 months post-exposure tended to be less severe than in analogous rainbow trout, although such responses were at an initial stage in both species at that time. By 5 months post-exposure, responses in both species were similar, and included severe cartilaginous lesions, presence of vegetative and sporogonic stages of *M. cerebralis*, and considerable granulomatous inflammation. Caudal lesions of the spine were especially prevalent in mountain whitefish compared to rainbow trout, and closely resembled those found in wild juvenile mountain whitefish collected from the Madison River in September 1999.

Conclusions

Mountain whitefish are susceptible to infection with *M. cerebralis*, develop clinical whirling disease, and serve as hosts for development of what appear to be viable myxospores of *M. cerebralis*. High mortality among all treatment groups of mountain whitefish precluded inference concerning effects of *M. cerebralis* on survival. Nevertheless, implications of these findings for wild populations of mountain whitefish and sympatric salmonids may be consequential.

FISH HOST SPECIFICITY OF THE TRIACTINOMYXON SPORES OF *MYXOBOLUS CEREBRALIS*

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Since the first report from Griffin and Davis (1978) it has been known that salmonids offer some immune response to infection with *Myxobolus cerebralis*. More recently, Hoffmann and El-Matbouli (1996) reported that starting five days after rainbow trout were exposed to the triactinomyxon stages of *M. cerebralis*, parasitic stages in the subcutis were surrounded by macrophages. The authors found that fish, which have been previously infected with *M. cerebralis*, appear to be resistant to reinfection.

Several species of salmonids have been reported as susceptible to *M. cerebralis* infection and subsequent development of whirling disease (O'Grodnick 1979, El-Matbouli et al. 1992). The fish host range for *M. cerebralis* is derived principally from observations of epizootics in captivity and more recently of wild salmonids or from experimental life-box exposure studies (O'Grodnick 1979). These studies indicate a spectrum of susceptibility ranging from high among rainbow trout, Danubian salmon (*Hucho huchu*), and sockeye salmon (*Oncorhynchus nerka*) to intermediate among chinook salmon (*O. tshawytscha*), cutthroat trout (*O. clarki*) and Atlantic salmon (*Salmo salar*), to more resistant brown trout (*S. rutta*) and coho salmon (*O. kisuth*).

In the fish health lab at the University of California in Davis, we investigated the interaction between the triactinomyxon stages of *M. cerebralis* and the penetration of the sporoplasm on juvenile rainbow trout, brown trout and coho salmon by scanning electron microscopy. The results have shown differences in the general appearance of the epidermis as well as the number and apparent efficiency of attachment and penetration of the triactinomyxon spores.

All three fish species, of approximately the same size and age, were exposed to equal numbers of triactinomyxons (50,000 per fish) from a single triactinomyxon production unit and examined 2 min post exposure. Both coho salmon and brown trout epidermis had fewer and less distinct mucus cells respectively mucus secretion. Triactinomyxons attaching to both coho salmon and brown trout epidermis were fewer in number and often possessed extruded filaments that failed to penetrate the skin to securely hold the triactinomyxon spore for delivery of the sporoplasm cells. In contrast, abundant triactinomyxons, fully penetrated filaments and many penetrating sporoplasm cells were seen entering at mucus cell openings. These preliminary results suggest inherent differences in the initial contact and penetration of the triactinomyxon between species that may be related to both structural and secretory products that differ between the host fish species.

At Institute of Zoology, Fish Biology and Fish Disease in Munich, we have been studying the comparative susceptibility of different strains of rainbow trout in Germany and one rainbow trout strain delivered to Germany as eyed eggs from the Trout Lodge in USA, to the infection with whirling disease. The preliminary results from our field exposure experiments demonstrate that the trout strain derived from USA is highly susceptible for whirling disease infection compared with those from Germany. More studies are underway to explore the factors causing such differences in the susceptibility of different strains from the same species to whirling disease.

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