

2003 ANNUAL REPORT

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 spread and infected 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. The whirling disease parasite is extremely hardy and long-lived. Like the malaria parasite, it infects two very different hosts alternately. In the case of whirling disease the life cycle employs a fish host and an aquatic worm host (*Tubifex tubifex*), and so reaching an understanding of the parasite has required defining the biology of infected fish, infected worms, parasite spores and the so-called “TAM” life stage.

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, steelhead and whitefish have been found to be susceptible. Whirling disease is therefore a major threat both to biological diversity and to the Nation’s multi-million-dollar fishing and tourism economy. The whirling disease parasite has been reported in 23 states, from New York to California, and has generated great concern among anglers, scientists, and fisheries managers.

THE WHIRLING DISEASE INITIATIVE

The Whirling Disease Initiative was established by Act of Congress in 1997. Its purpose is to conduct research that develops practical management solutions to maintain viable, self-sustaining wild trout fisheries in the presence of the whirling disease parasite. The Initiative’s ultimate clients are state, tribal and federal fisheries-management agencies.

General oversight of the Initiative is provided by the National Partnership for the Management of Wild and Native Coldwater Fisheries. The National Partnership is a consortium of organizations concerned with the status of wild and native fisheries in the United States – Federal and state agencies, professional associations and private advocacy organizations (Appendix A). Besides providing long-term direction to the Whirling Disease Initiative, the National Partnership has also periodically assessed the overall status of coldwater fisheries to identify areas where similar research initiatives could be warranted (Appendix B). To do this, the Partnership’s Board of Representatives convenes annually for a detailed briefing by whirling disease researchers, and for discussions of fisheries health and research needs.

In-depth scientific direction is given to the Whirling Disease Initiative by its Steering Committee. The committee is made up of representatives from state fish and wildlife agencies, Federal natural resource agencies, and the Whirling Disease Foundation (Appendix C). The Committee prepares an annual research plan, issues Requests for Proposals based on its topical priorities, selects and approves projects for funding following scientific peer review, and distributes the research results within the scientific and fishery management communities and to other stakeholders. The Montana Water Center is the administrative entity that manages the program.

Each year, Federal funding earmarked in the Interior Appropriations Bill comes to the Initiative through the Division of Hatcheries, U.S. Fish and Wildlife Service. Projects are chosen for funding by the Steering Committee, following peer review by three independent reviewers. During the Initiative's six-year history, the Steering Committee has chosen to support a mix of projects, ranging from basic biological research to applied research directly testing potential management solutions. Early projects were principally aimed at explicating the biology of whirling disease. In 2000, the Steering Committee began deliberately shifting the priority toward field research more closely tied to possible management strategies. This "applied" focus has become ever stronger, with the encouragement of large-scale field projects addressing the ecology of whirling disease and the testing of potential methods for controlling its effects or spread.

The Initiative sponsors up to 15 research projects in each cycle. A research cycle generally runs from May of one year through December of the following year, including two field seasons. Typically two to four investigators are involved in each project, and they bring to the project cash or in-kind match of 25 to 150% of the amount of the Federal grant (Appendix D). Students are involved in most projects, either as technicians or, more often, as graduate research assistants. Summaries of all past research projects are available on the Initiative website: <http://water.montana.edu/mwc/programs/fisheries/whirling>

2003 ACTIVITIES

Projects Concluded in 2003

During the 2002-2003 cycle, 12 research projects were funded with a total of \$538,762 in Federal dollars, leveraging an additional \$400,106 in match from state, private and Federal research collaborators. Research teams included 24 investigators from six states. Appendix E gives brief summaries of the twelve projects that were completed in December 2003. Highlights from four projects are given below.

Temporal, spatial, and discharge-mediated dynamics of TAM abundance and infection risk estimated by field filtration: This project tested the Tam-o-meter TAM collection/quantification device and methodology on natural rivers using sentinel-fish cages to demonstrate its efficacy. The investigators examined spatial, temporal, and discharge-mediated dynamics of triactinomyxon concentrations directly using packed-bed filtration at five mainstem sites on the upper Madison River and at Willow Creek, a tributary to the Jefferson River. They also compared findings inferred from indirect sentinel-cage trials and direct measurements. TAM concentrations were consistently higher at Willow Creek than at the five sites on the Madison River, at which concentrations did not

differ spatially. Concentrations at all sites peaked in spring and autumn, partially in response to seasonal changes in water temperature. Variation in concentrations among consecutive days was absent, but strong diel variation was evident; at Willow Creek, concentrations were elevated during periods of low light intensity. No relationship existed between manipulated side-channel discharge rates and TAM concentrations, likely because concentrations were too low during the experiment to detect differences. Conclusions derived from indirect sentinel-cage sampling and simultaneous, direct packed-bed filtration largely corresponded. Packed-bed filtration proved to be a rapid, efficient, and effective method for assessing whirling disease infection risk among wild trout.

Effects of *M. cerebralis* infection on Chinook salmon and steelhead trout in northeastern Oregon: The goal of this research was to provide information relevant to the management of both cultured and wild salmonids in northeastern Oregon by examining the effects of *M. cerebralis* infection on anadromous salmon at acclimation and during the critical phase where they enter salt water. Investigations on *M. cerebralis* at juvenile acclimation sites in the region demonstrated a significantly higher prevalence of infection (65%) in sentinel rainbow trout fry held at the Wallowa facility. PCR analysis of ossified cranial elements from steelhead juveniles held at the Wallowa facility for six weeks demonstrated a comparable prevalence of infection of 52%. When steelhead naturally infected at the Wallowa facility were transferred to salt water, mortality was significantly increased over that of control fish from the same cohort that had not been exposed to the parasite during acclimation. This indicates that *M. cerebralis* can compromise survival of juvenile salmonids during saltwater adaptation. In addition to the direct effects of *M. cerebralis* on survival, this study suggests that infection of anadromous salmonids may contribute to the dissemination of the parasite during migration and thus complicate management efforts to contain the parasite within northeastern Oregon.

Physical habitat modification to reduce the impacts of *M. cerebralis* infection in streams (Colorado): Areas on two streams were physically modified to reduce aquatic worm habitat. Another area was chosen as an unmodified control site for comparison. The investigators looked for before-versus-after changes in the density of aquatic worms, parasite spores produced from aquatic worm samples, numbers of floating parasite spores in water samples downstream of the changed sites, and the percentage of brown trout infected with the parasite. At both sites, aquatic worm samples collected after the habitat changes and held in buckets overnight in a lab produced the floating parasite spores, indicating that infected aquatic worms were still there. Not enough time has passed to test brown trout of the proper age for changes in the percentage of infection. More exploratory sampling upstream and downstream of both treated sites for aquatic worms showed that there were other, smaller worm habitats that contained infected worms. Therefore, although monitoring of the brown trout population will continue to see if changes occur in the percentage of fish infected, initial indications are that small-scale habitat manipulations such as used in this study might not be very successful in changing the percentage of fish infected over an entire stretch of a stream.

Determination of β , the infection efficiency, of myxospores and TAM stages of *Myxobolus cerebralis* infection and a laboratory model of the entire infectious cycle (Oregon):

Experiments were conducted to determine minimum time/dose conditions necessary to establish *M.*

cerebralis infection in the vertebrate and invertebrate hosts. In a range of dosages from 0.01 to 10 TAMs per fish at exposure times ranging from 1 - 600 sec, no clinical signs of whirling disease were noted in exposed trout. Histopathological examination and PCR analysis of the fish are currently underway. A laboratory simulation of the parasite life cycle was established starting with infected fish carcasses. Naturally decaying fish were able to infect *T. tubifex* present at densities of approximately 8,000/m² and the worms in turn produced high levels of TAMs in the water. Sentinel fish held downstream from the worms contracted *M. cerebralis* infection and exhibited high levels of clinical whirling disease. Reproduction of the worm population and re-infection of juveniles likely occurred. This laboratory model of the disease process in moving water can be further used to determine the importance of various factors necessary to establish infection by the parasite in its vertebrate and invertebrate hosts.

Projects Funded Through 2004

In spring 2003, the Steering Committee selected eight new projects for funding in the 2003-2004 research cycle. These range in subject from immunology to epidemiology to the effects of engineering projects on disease severity. A total of 36 investigators from six states are involved. The grant awards total \$557,585 and match funding amounts to \$516,270. These projects got underway May 1, 2003 and will conclude December 31, 2004.

Papers Published or Presented

Between August 2002 and August 2003, six papers stemming from Initiative-sponsored research were published in peer-reviewed literature. Abstracts of papers that were not cited in last year's report are shown in Appendix F. Sixteen papers based partly or wholly on Initiative-funded research were presented at the Whirling Disease Symposium in February 2003 in Seattle.

Annual Meeting of the Board of Representatives of the National Partnership

The Partnership Board of Representatives convened October 21-23, 2003 in Bozeman, Montana (Appendix G), along with the Whirling Disease Steering Committee and several fisheries researchers. The chief topics of deliberation were the extent and severity of whirling disease in the United States, the five-year plan for the WD Initiative (see below), and the addition of an outreach program alongside the ongoing research and testing program. Fishery managers from five states and Yellowstone National Park made presentations on the status of the disease and the official response in their jurisdictions. The general research topics chosen by the Steering Committee for sponsorship during the next five years were discussed by the group at large. The Board directed that there be active outreach to state, tribal and Federal fisheries managers, in addition to research and testing. The principal subject of the outreach is to be Initiative research findings, but related topics that can help fishery managers assess risks and control the effects of the disease will also be subjects for technology transfer. In the near term, the outreach program will comprise up to 20% of the total

program funding.

Development of the Five-Year Plan

As the National Partnership Board directed in its autumn 2002 meeting, during 2003 the Steering Committee developed a rudimentary five-year plan for research and outreach. This was refined following the October 2003 Partnership meeting, and is attached as Appendix H. The cornerstone of the plan is an evaluation and ranking of the utility of potential research topics for development of management tools. Some topics that had been supported strongly in the past, such as oligochaete biology, were judged of lesser use as a management tool, and eliminated from project funding for the next five years.

Tools deemed most vital for advancing management of the disease over the next five years (and, thus, those meriting further research) include those that:

- ◆ assess watershed management actions (flow augmentation, channel modification, riparian habitat improvements, etc.) that could have a marked effect on spread of the disease
- ◆ study the effects of filtration techniques to remove TAMs from the water column
- ◆ refine our understanding of genetic and life history traits of trout strains that affect their resistance to the disease
- ◆ refine methods that diagnose the parasite in worms, fish and water
- ◆ build the knowledge base on vectors for spread of the disease
- ◆ prioritize and quantify risk factors.

The Board also determined research topics not ideal for further funding at this juncture, including:

- ◆ development of advanced mathematical models which predict severity of disease
- ◆ hatchery manipulation techniques
- ◆ prophylactics for control of the disease
- ◆ fish passage barriers as a means to control disease spread
- ◆ investigation of worm strain resistance/susceptibility to the disease.

These research decisions are further articulated in the RFP for the current research year, and are described in more detail in Appendix H.

As part of its Five-Year Plan, the Partnership Board also developed elements of an outreach plan that will provide fisheries managers with research information needed for managing the disease in the field. Much of this plan was developed from recommendations presented in “Findings and Recommendations: Fish Professional Communications Survey and Outreach for the Whirling Disease Initiative” by Strategicom, a Bozeman, Montana marketing firm. This group was contracted by the Partnership to survey a national target group of fish professionals, and recommend outreach tools most appropriate for reaching the target group. The consultants found that:

- ◆ fish professionals are interested in receiving more information on whirling disease;
- ◆ whirling disease management is an important part of the job for most fish professionals, and
- ◆ currently, general whirling disease information is not adequately reaching this target group

Over the next five years, the consultants recommend that the Partnership:

- ◆ establish a current electronic database of fishery management professionals in each of the fifty states and four territories
- ◆ assess the whirling disease and fish transfer regulations in each state, including current information on fish transfer laws
- ◆ develop and circulate an annual national “state of whirling disease” report for managers and educators
- ◆ build a national interactive whirling disease website that is marketed and recognized as “the hub” for fact-finding and communication about the disease
- ◆ develop training CD’s and workshop curricula and take them to professional fisheries meetings
- ◆ further build strategic alliances to leverage resources.

The annual range of financial expenditure considered appropriate for the outreach program is \$50,000 minimum, up to 20 percent of the full budget if the Initiative is funded at \$1 million (which it has been, for 2004-2005). The Water Center will create a new position for a Whirling Disease Outreach Coordinator who will build and run the outreach program. The Center will also involve students, and a marketing firm may be engaged to develop information products under contract. All activities will be coordinated with the Whirling Disease Foundation.

PLANS FOR 2004

2003-2004 Ongoing Research Projects

As noted above, eight projects that received funding in May 2003 are underway (Appendix I). These will conclude on or before December 31, 2004. Highlights from five of these ongoing projects are given below.

Effect of riparian zone and associated stream substrata on *T. tubifex*, northeastern U.S.: The objectives of this project are to determine if leaf litter and hence, riparian zone characteristics will affect *T. tubifex* density and/or the ability of resident worms to become infected with *M. cerebralis* spores and to release infective spores. *T. tubifex* have been collected from 11 sites in New York and Pennsylvania, some infected with WD and some not infected, with brown trout, rainbow trout or both. They will be examined for genotype and parasite load. Laboratory exposure of worms to various types and concentrations of leaf litter have begun. These worms will be infected with WD to trace the effect of leaf litter on disease susceptibility.

Development of molecular markers linked to whirling disease resistance in rainbow trout, Montana and Utah: The objective of this project is to develop molecular markers associated with disease resistance in rainbow trout in an effort to develop rainbow trout brood stocks resistant to WD. Work over the past several months has involved developing standard protocols for analysis of the major histocompatibility complex that may play a role in disease resistance in trout. Also, four promising strains of rainbow trout from Utah and three locations in Montana have been

exposed to the disease and sacrificed, and are being tested histologically. Preliminary data for two of the strains suggest that one is highly susceptible to the disease and the other is resistant.

Testing impacts of channel modifications to reduce *T. tubifex* habitat, Colorado: This project is using information from a channel bed survey and hydraulic simulation model completed in Willow Creek to predict changes in *T. tubifex* habitat at a Poudre River site. Tubifex worms have been collected from both streams and examined for genotype. Currently benthic samples are being characterized to determine the nature of any relationship between worm biomass and sediment composition. Stream cross-sections were surveyed and mapped to find areas of sediment accumulation. The channel modifications - filling of a backwater area and conversion to an emergent-plant wetland, and stabilization of eroding banks - has been completed. Modeling of potential additional modifications is being conducted.

Epidemiology of whirling disease in Rock Creek, Montana: This long-term study is assessing the overall epidemiology of whirling disease in a drainage, including the relationship of infected worms to WD infection rates and severity in trout. Worm infection rates of 0-3% were observed during recent field seasons. No obvious correlation between TAM release and either temperature or photoperiod has been observed in the field. Correlation between streamflow and infection severity is being examined with data from several years, most recently June 2003. Sentinel fish were deployed in several places to locate an apparent point source of whirling disease infection in the drainage.

Development and testing of risk assessment tools for *M. cerebralis* in Yellowstone Park: The investigators are examining the spatial and temporal variation in whirling disease risk to cutthroat trout populations, the biological and physiochemical factors that correlate with infection risk, and the life histories of Yellowstone cutthroat trout that possibly could allow some subpopulations to have low risk of infection. They collected stream physical and water-quality data during summer 2003. They monitored disease risk using *in situ* enclosures of sentinel Yellowstone cutthroat trout in three reaches each in the Yellowstone River, Pelican Creek and Clear Creek during three time periods in July, August and September 2003. Most fish have been removed and are now being prepared for disease diagnosis. They have also collected wild-reared, age-zero Yellowstone cutthroat trout in these three streams to estimate infection prevalence in young, wild fish. They had planned to collect oligochaetes for assessment of tubificid assemblages and prevalence of infection during the spring and summer of 2003, but found existing methods for identifying diseased worms not sufficiently sensitive, and so are developing new methods. The data will be used to develop a risk-assessment model for whirling disease transmission in Yellowstone Park.

Solicitation for 2004-2005 Research Projects

The next projects will begin in late spring 2004 and conclude at the end of 2005. A November 2003 Request for Preproposals (Appendix J) yielded 25 preproposals. In December 2003 the Steering Committee considered these and solicited full research proposals from 14 of the project teams (Appendix K). These will be evaluated in late winter, and the chosen projects will get underway in May 2004. The emphases of the 2004-2005 research/demonstration program will be collaborations among scientists and fishery-management agencies to test and monitor the effects of environmental manipulations, projects to quantify the risk of disease transmission by various vectors, further development of diagnostic techniques, and defining disease susceptibility of different strains of rainbow and cutthroat trout.

Outreach to Fishery Professionals

In 2004, the Montana Water Center will conduct a search for a Whirling Disease Outreach coordinator who will initiate all contracted services and manage the outreach program. During the first year, the coordinator will complete the following:

- ◆ compile updates contact list for target audiences
- ◆ update the Whirling Disease Foundation's bibliography of research papers and mount a searchable version that includes theses and dissertations on the web
- ◆ create presentations and technical handouts for fishery professionals and pilot them on the road at selected professional state and national meetings
- ◆ initiate a monthly electronic newsletter and web-based communications forum
- ◆ compile state-by-state information on policies and regulations for website access.

SUMMARY

It is unlikely that fishery managers will be able to eradicate whirling disease from infected drainages. The goal of the Whirling Disease Initiative and other research enterprises is to develop a clear understanding of the disease cycle, the predisposing environmental factors, and methods for maintaining fisheries despite its presence. Initiative research has made great strides in the first two areas, and its work now focuses on the last phase of the effort: assembling, testing and publicizing a toolbox of management methods to support fishery managers as they strive to maintain fisheries in the presence of whirling disease.

The Steering Committee members particularly thank Montana's Congressional delegation – Senator Conrad Burns, Senator Max Baucus, and Congressman Dennis Rehberg – for their advocacy of this research. They also thank the representatives of the National Partnership member organizations for their time and guidance.