

Project Findings 2002-2003

Whirling Disease Initiative

Contract Dates: May 1, 2002 - September 30, 2003

Total Awarded: \$534,416; Total Match: \$442,292

Category	Title	P.I.	Other Partners	Award/Match
Immunology and Fish Pathology	Temporal Analysis of Immunity to <i>M. cerebralis</i> in Resistant and Susceptible Species Using Real-Time Taqman Quantitative PCR to Track Replication of the Parasite in the Fish	Mark A. Adkison , Research Associate University of California, Davis Department of Veterinary Medicine and Epidemiology One Shields Avenue Davis, CA 95616 Phone: 530/752-9318; Fax: 530/752-0414 maadkison@ucdavis.edu	(1) Dr. Ronald P. Hedrick Professor of Fish Pathology, University of California-Davis; rphedrick@ucdavis.edu	\$49,731 \$12,242

Abstract: This project used TaqMan PCR technology to detect differences in parasite replication in the skin, nerves, and cartilage of resistant (brown trout, coho salmon, some strains of cutthroat trout, and the German Hofer strain of rainbow) and susceptible species (other rainbow strains). The investigators tracked the rate of replication of the parasite in the resistant and susceptible species over the first two months of infection, as it traveled through the three major sites of replication, the skin, the nerves and the cartilage, in the resistant species and compared it to the rate of replication in the susceptible domestic rainbow trout. They found that *M. cerebralis* replication followed a similar pattern in the rainbow trout, cutthroat trout and coho salmon from 1-hour post exposure to 56 days post exposure. Therefore it appears that the prevention of the initial sporoplasm migration into the epithelium is a major point of immunity in West Slope cutthroat trout and coho salmon in the first two months post-infection. In the brown trout, parasite numbers decreased at the later time points as parasite numbers increased in the rainbow, cutthroat and coho. This indicates the mechanism(s) of immunity to *M. cerebralis* in brown trout is different from West Slope cutthroat trout and coho salmon.

Category	Title	P.I.	Other Partners	Award/Match
Epidemiology	Effects of <i>Myxobolus cerebralis</i> Infection on Chinook Salmon and Steelhead Trout in Northeastern Oregon	Jerri L. Bartholomew , Senior Researcher Oregon State University Department of Microbiology 220 Nash Hall Corvallis, OR 97331-3804 Phone: 541/737-1856; Fax: 541/737-0496 bartholj@orst.edu	(1) Paul W. Reno, Assoc. Professor, Hatfield Marine Science Center, Comes 2030 SE Marine Science Dr., Newport, OR 97365-5296; 541/867-0147 (p); 541/867-0105 (f); paul.reno@hmsc.orst.edu	\$35,063 \$85,230
<p>Abstract: 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 <i>M. cerebralis</i> infection on anadromous salmon at acclimation and during the critical phase where they enter salt water. Investigations on <i>M. cerebralis</i> 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. Further, PCR analysis of ossified cranial elements from steelhead juveniles held at the Wallowa facility for 6 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 <i>M. cerebralis</i> can compromise survival of juvenile salmonids during saltwater adaptation. In addition to the direct effects of <i>M. cerebralis</i> 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.</p>				
Habitat Restoration & Management / Hydrology	Effects of Spring Creek Rehabilitation on Infection Rates of Whirling Disease in Trout	Patrick Byorth , Fisheries Biologist Montana Fish, Wildlife and Parks 1400 South 19th Avenue Bozeman, MT 59718 Phone: 406/994-6938; Fax: 406/994-4090		\$4,000 \$8,600
<p>Abstract: This project generated baseline data to examine the effects of rehabilitating two Montana spring creeks on whirling disease severity. Presence or absence of whirling disease and infection rates before habitat enhancement were determined. Rainbow, brown, and brook trout fry were placed in sentinel cages in each stream, reared, and sacrificed for analysis of infection rates by <i>M. cerebralis</i>. The investigators monitored water temperatures and discharges of the creeks and documented basic habitat conditions. Trout abundance was determined by electrofishing. Three Dollar Spring Creek supported juvenile brown trout, while East Gallatin Spring Creek supported few fish of any size or species. Sentinel cage experiments indicated that <i>M. cerebralis</i> was present in both streams, although only one test fish in 700 was infected in East Gallatin Spring Creek. Infection rates were much higher in Three Dollar Spring Creek, although results were inconsistent. Severe infection rates documented in early experiments were not duplicated. Possibly restoration efforts affected conditions in a short time to decrease infection rates. Data collected in this study should be compared with similar measurements at least a year after restoration is completed to better illustrate the efficacy of spring creek restoration in waters infected with <i>M. cerebralis</i>.</p>				

Category	Title	P.I.	Other Partners	Award/Match
Lab Methods	A Quantitative PCR (QPCR) Approach to Rapidly Distinguish Between <i>Myxobolus</i> Species and Assess Infection Severity in Fish	Ken Cain , Assistant Professor Aquaculture & Fish Health University of Idaho Department of Fish & Wildlife Resources P. O. Box 441136 Moscow, ID 83844-1136 Phone: 208/885-7608; Fax: 208/885-9080 kcain@uidaho.edu	(1) Dr. Keith Johnson, ID Dept. of Fish & Game (2) Dr. Matt Powel and Dr. Ken Overturf, UI Hagerman Fish Culture Experiment Station; and Dr. John Wood, Pisces Molecular, Inc.	\$35,167 \$17,496
<p>Abstract: The objectives of this study were to: 1) design primer/probe combinations specific for detecting <i>M. cerebralis</i> based on the heat shock protein 70 (Hsp 70) and 18s ribosomal DNA (rDNA) gene sequences, both of which are highly conserved and species specific, 2) determine sensitivity of QPCR analysis in relation to current diagnostic methods and 3) identify potential relationships between the parasite's genetic copy number and infection severity. In this study primer/probe combinations developed for the Hsp 70 and 18s sequences were specific for <i>M. cerebralis</i>. It was shown that QPCR analysis was as sensitive as current PCR techniques and both were more sensitive than histopathology. In addition, detection capabilities using both primer/probe combinations correlated with infection severity within infected juvenile rainbow trout. These results suggest QPCR may be useful as a diagnostic method to simultaneously detect <i>M. cerebralis</i> in infected samples and give a relative indication of infection severity. Such an assay could reduce time and labor when compared to screening large numbers of samples with traditional diagnostic methods.</p>				
Invertebrate Ecology & Taxonomy	Ecological Differentiation and Survivability of <i>Tubifex tubifex</i> Infested with <i>Myxobolus cerebralis</i> in the San Juan River Tailwater New Mexico	Colleen Caldwell , Research Fishery Biologist, Assistant Leader New Mexico Cooperative Fish & Wildlife Research Unit Box 30003 - MSC 4901 Las Cruces, NM 88003-8003 Phone: 505/646-8126; Fax: 505/646-1281 ccaldwel@nmsu.edu	(1) Robert DuBey, M.S., Fishery Specialist, Whirling Disease Project Coordinator, NM State University, Department of Fishery and Wildlife Sciences, Box 30003, MSC 4901, Las Cruces, NM 88003-8003; 505/646-1544 (p); 505/646-1281 (f); rdubey@nmsu.edu	\$25,000 \$37,402

Abstract: This research related *T. tubifex* lineage genetic variation to trophic conditions using cloned monocultures of lineage-specific *T. tubifex* from different habitats in the San Juan tailwater. Progeny of *T. tubifex* from the tailwater were used to establish two single-lineage laboratory stock cultures. Experimental tests of monocultures III and VI were replicated with infection challenges (0 and 500 spores/worm), water temperatures of 5, 17, and 27° C and diurnal photoperiods of 12:12, 14:10 and 16:8 (dark:light) to simulate a range of seasonal temperatures and photoperiods. Lineage III exhibited greater adult mortality than lineage VI regardless of treatment. Earlier field study findings were supported by the experimental study, as lineage VI exhibited a wide tolerance to environmental conditions and resistance to *M. cerebralis* infection whereas lineage III exhibited intolerance to variation and susceptibility to *M. cerebralis* infection up to 4%. The life histories of *T. tubifex* lineages and the effects that regulated flows may have on the species can be used to affect *T. tubifex* abundance and distribution in tailwater systems. These options may include altering regulated tailwater temperatures through different reservoir penstock releases at times when specific *T. tubifex* lineage communities are vulnerable, and providing scouring flow regimes to reduce organic loading and *T. tubifex* densities, both of which might reduce infection levels.

Category	Title	P.I.	Other Partners	Award/Match
Epidemiology	Epidemiology of Whirling Disease: An Integrated Study of the Rock Creek Drainage, Montana	Willard O. Granath, Jr. Professor of Microbiology University of Montana Division of Biological Sciences 32 Campus Drive #4824 Missoula, MT 59812-4824 Phone: 406/243-2975; Fax: 406/243-4184 snail@selway.umt.edu	(1) Michael Gilbert, Postdoctoral Research Associate, Div. Biological Sciences, UM, Missoula, MT 59812 (2) Billie L. Kerans, Associate Professor, Department of Biology, MSU, Bozeman 59717; Eric Reiland, Fisheries Biologist, MT Dept. of Fish, Wildlife & Parks, 3201 Spurgin Rd., Missoula, MT 59801	\$83,432 \$54,436

Abstract: This study continued a long-term project that began in 1998. The main objectives are to assess the overall epidemiology of whirling disease throughout this ecosystem including the relationship of *M. cerebralis*-infected *T. tubifex* to infection rates and severity of whirling disease in trout. The effect of water flow and habitat conditions on the transmission of the parasite were also examined and work was continued to genetically-characterize *T. tubifex* recovered from this drainage. Overall, the results indicated that infected *T. tubifex* are found throughout the drainage, but they are present in much greater numbers and are more widely dispersed in areas with degraded riparian habitat. Also, trout become infected at many locations where infected *T. tubifex* were not recovered, and it is possible that the fish at some of these sites were being infected by parasites originating several hundred or even thousands of meters upstream. Results also indicated that infection rates in sentinel fish have increased throughout the course of this study, with one exception, when a less susceptible test strain of rainbow trout was used for the exposures. It also appears that the range of *M. cerebralis* within the Rock Creek drainage is still expanding, more than 5 years after its initial detection. Heretofore no timeline concerning the spread of whirling disease, after the introduction of the parasite into a river drainage, has been established. Preliminary data do not reveal a correlation between localized streamflow and the severity of infection in sentinel fish; however, total water flow appears to affect the severity of disease as an apparent dilution effect on TAMs was observed in the upper portion of the drainage. All three *T. tubifex* genotypes (as determined using RAPD's) that are present in the study reaches are susceptible to infection.

Epidemiology	Relating <i>M. cerebralis</i> Infection in Native Yellowstone Cutthroat Trout and <i>T. tubifex</i> with Environmental Gradients at Multiple Spatial Scales	Billie L. Kerans , Associate Professor Montana State University Ecology Department 311 B Lewis Hall Bozeman, MT 59717 Phone: 406/994-3725; Fax: 406/994-3190 bkerans@montana.edu	(1) Dr. Todd M. Koel, Supervisory Fisheries Biologist, National Park Service, Fisheries and Aquatic Sciences Section, P.O. Box 168, YNP, WY 82190; 307/344-2281 (p); 307/344-2211 (f); todd_koel@nps.gov (2) Charlotte Rasmussen, Researcher, Western Fisheries Research Station, ISGS-BRD, 6505 NE 6th St., Seattle, WA 98115; 206/526-6282, ext. 322; 206/526-6654 (f); charlotte_rasmussen@usgs.gov, and Ecology Dept., MSU-Bozeman	\$112,973 \$ 99,650
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Abstract: This was the beginning of a three-year project to examine Yellowstone Cutthroat trout populations in Yellowstone River, Pelican Creek, and Clear Creek within Yellowstone Park. The investigators conducted detailed examinations of whirling disease infection risk as it relates to tubificid assemblages, prevalence of infection in tubificids, spore loading in adult spawning trout, physical and chemical features of the watercourses chosen, and life history patterns of trout. Data collection and interpretation has not been concluded.

Category	Title	P.I.	Other Partners	Award/Match
Fish Culture and other	Demonstration and Evaluation of Wetlands Treatment for <i>Myxobolus cerebralis</i> actinospore attenuation	Eric Krch , Program Engineer Buckhorn Geotech, Inc. 222 South Park Avenue Montrose, CO 81401 Phone: 970/248-6828, ext. 13; Fax: eric@buckhorngeo.com		\$10,000 \$25,500

Abstract: This project took the results from previous filtration research and developed a vertical flow pond filter sized to accommodate the range of pond inflow rates. The site was pond #6 at the Cap K Ranch near Basalt, Colorado, which discharges to the Frying Pan River. Influent water passed by gravity through a coarse metal plate, graded gravel and a sand filter. The filter was covered with mulch and planted with rushes. The filter achieved complete TAM removal during summer 2003. However, it suffered serious, non-reversible clogging and operated at 15% efficiency for most of the summer. The investigators, who will continue with additional configurations and trials during 2004, concluded that masonry sand in a large-scale demonstration has proven its target removal effectiveness in regards to *M. cerebralis* TAMS. Its hydraulic capability is, however, limited (size based on 0.015 m³/min/m²) requiring frequent monitoring and maintenance efforts.

Ecological Modeling & Statistics	Development of Empirical Models of <i>Myxobolus cerebralis</i> to Predict Risks for Populations of Fish Across River Drainages	Christine M. Moffitt , Research Scientist University of Idaho Department of Fish and Wildlife Resources College of Natural Resources P. O. Box 441136 Moscow, ID 83844-1136 Phone: 208/885-7047 (o); Fax: 208/885-9080 cmoffitt@uidaho.edu	(1) Keith Johnson, ID Fish & Game, 1800 Trout Road, Eagle, ID; 208/334-3791 (p); 208/334-2114 (f); kjohnson@idfg.state.id.us (2) Bruce Rieman, USFS, Rocky Mountain Research Station, Boise, ID	\$35,000 \$42,236
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Abstract: The investigators explored the efficacy of using landscape level tools in GIS to develop a model to predict the intensity of infection of *M. cerebralis* in fish and *T. tubifex* worm populations. They developed models to explain variation in infection using empirical data gathered from several locations, and refined these by testing within the Pahsimeroi River basin during the summer of 2003. The final analysis is not completed.

Category	Title	P.I.	Other Partners	Award/Match
Ecological Modeling & Statistics	Determination of β , the Infection Efficiency, of Myxospores and TAM Stages of <i>M. cerebralis</i> Infection and a Laboratory Model of the Entire Infectious Cycle	Paul W. Reno , Associate Professor Oregon State University Hatfield Marine Science Center 2030 S. Marine Science Dr. Newport, OR 97365-5296 Phone: 541/867-0147 (o); Fax: 541/867-0105 paul.reno@hmsc.orst.edu		\$49,922 \$39,500
Abstract: A laboratory simulation of the parasite life cycle was established, beginning with infected fish carcasses. Naturally decaying fish were able to infect <i>T. tubifex</i> present at densities of approximately 8,000/m ² and the worms in turn produced high levels of TAMs in the water. Sentinel fish held downstream from the worms contracted <i>M. cerebralis</i> 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.				
Habitat Restoration & Management / Hydrology	Evaluating the Efficacy of Physical Habitat Modification to Reduce the Impacts of <i>M. cerebralis</i> Infection in Streams	Kevin G. Thompson , Aquatic Wildlife Researcher Colorado Division of Wildlife 2300 S. Townsend Avenue Montrose, CO 81401 Phone: 970/252-6037; Fax: 970/252-6053 kevin.thompson@state.co.us		\$39,000 \$0
Abstract: 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 one treated site there was a reduction in floating parasite spore numbers in flowing water for a year, but the aquatic worm population came back quickly in the good worm habitat that remained after construction. At the other site, there was a reduction in aquatic worm numbers for at least a year, but there was no decrease in the number of floating parasite spores in flowing water downstream of the site. 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.				

Category	Title	P.I.	Other Partners	Award / Match
Epidemiology, Parasitology	Temporal, Spatial, and Discharge, Mediated Dynamics of Triactinomyxon Abundances and Infection Risk Estimated Directly by Packed-Bed Filtration	Alexander V. Zale , Co-op Unit Leader; Research Fishery Biologist; Affiliate Associate Professor Montana State University Department of Ecology Bozeman, MT 59717 Phone: 406/994-2380; Fax: 406/994-7479 zale@montana.edu	(1) Frederic T. Barrows, Research Physiologist, Bozeman Fish Technology Center, USFWS, 4050 Bridger Canyon Rd., Bozeman 59715; 406/586-9265, ext. 130 (p); 406/586-5942 (f); rbarrows@mcn.net	\$55,128 \$20,000

Abstract: 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, except in one low-temperature sample when fish did not become infected despite presence of TAMs. Packed-bed filtration proved to be a rapid, efficient, and effective method for assessing whirling disease infection risk among wild trout.