Project Findings 2001-2002
Whirling Disease Initiative

National Partnership for the Management of Wild and Native Cold Water Fisheries
Water Center, MSU-Bozeman

Category	Title	Principal Investigator	Other Partners	Award/Match
1. **(1)** **Fish Pathology**	**Effects of *Myxobolus cerebralis* 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 ph. 541-737-1856 fax: 541-737-0496 e-mail: bartholj@bcc.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	$52,022/$67,013

Project Findings:

This project assessed the susceptibility of chinook salmon to *M. cerebralis* and examined current management practices for anadromous salmon that may lead to parasite exposure. Laboratory challenges at different ages demonstrated infection severity is lower and disease resistance develops earlier in chinook salmon than in the rainbow trout. Chinook salmon were also more resistant to repeated challenge, suggesting greater resistance under natural conditions. In addition to the potential direct effects of clinical disease, exposure of anadromous salmonids to *M. cerebralis* may contribute to the dissemination of the parasite. Exposures of sentinel rainbow trout demonstrated presence of the parasite at all acclimation sites. Detection of infection in steelhead smolts demonstrates that infection occurs even when exposure is delayed until one year of age. Further, infected fish from eastern Oregon have been detected as adults straying into lower Columbia River tributaries, suggesting that these fish may interfere with management efforts to prevent the spread of *M. cerebralis*. Finally, transfer of naturally-exposed and experimentally-challenged steelhead to salt water demonstrated a decreased ability to survive, suggesting that *M. cerebralis* infection may contribute to mortality during saltwater adaptation.

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2. **(12)** **Invertebrate Ecology and Taxonomy**	**Application of DNA-based Genetic Markers to Determine Differences in Susceptible and Non-susceptible *Tubifex tubifex* Populations to *Myxobolus cerebralis* from the Upper Colorado River and Windy Gap Reservoir**	**Katherine A. Beauchamp,** Post-doctoral Research Associate University of California, Davis Department of Medicine and Epidemiology School of Veterinary Medicine One Shields Avenue Davis, CA 95616 ph. 530/752-9318 fax: 530/752-0414 e-mail: kabeauchamp@ucdavis.edu	(1) Ronald P. Hedrick, Professor, rphedrick@ucdavis.edu (2) R. Barry Nehring, Research Biologist, CO Division of Wildlife, 2300 S. Townsend, Montrose, CO 81401; 970-252-6008 (p) 970/252-6053 (f) barry.nehring@state.co.us	$55,000/$0
Project Findings: The investigators examined the geographic distribution of *Tubifex tubifex* by genetic screening at 20 sites above, below and within the Windy Gap Reservoir on the upper Colorado River. The sites varied with respect to land and water use practices and represented habitats that were presumed to be more or less conducive to oligochaete abundance and diversity and where impacts of whirling disease on rainbow trout were considered high, moderate or low. The results suggest that the distribution of various genotypes of *T. tubifex* are one important factor in determining whether rainbow trout in specific aquatic environments will be more apt to experience severe effects of whirling disease. Sites with a greater abundance of *T. tubifex* from *M. cerebralis* resistant lineages may prevent the parasite from reaching concentrations where rainbow trout population impacts occur.				
3. **(2)** **Fish Culture**	**The Effect of Chemical Control of *Tubifex tubifex* on the Incidence of Whirling Disease in Colorado Hatcheries**	**Eric P. Bergersen,** Assistant Leader Colorado State University Colorado Cooperative Fish and Wildlife Research Unit 201 Wagar Building Fort Collins, Colorado 80523 ph. 970-491-1415; fax: 970-491-1413 e-mail: ericb@cnr.colostate.edu	(1) Dan Kowalski, Graduate Research Assistant, Colorado State University, Fort Collins, CO 80523 970-491-1416 (p) 970-491-1413 (f)	$15,390/$4,185
Project Findings: The objectives of this study were to assess two common pesticides, Bayluscide and TFM, as chemical controls of *T. tubifex*, examine intraspecific differences in toxicity, and make management recommendations on the possibility of chemical worm control. Worms susceptible to *M. cerebralis* infection were more sensitive to both chemicals than worms resistant to the parasite in the toxicity tests. The toxicity differences of exposed and unexposed worms within a lineage were not as great as between lineages. Due to the rapid degradation, relatively selective toxicity, cost, and regulatory status, Bayluscide appears to be the best candidate for further study as a chemical control of the oligochaete host of the parasite causing whirling disease in salmonid fishes.				

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4. **(6)** **Invertebrate Ecology and Taxonomy**	**Ecological Differ-entiation and Survivability of *Tubifex tubifex* Infested with *Myxobolus cerebralis* in the San Juan River, New Mexico Tailwater "Blue-Ribbon Trout Fishery"**	**Colleen Caldwell,** Research Fishery Biologist, Assistant Leader New Mexico State University US Geological Society New Mexico Cooperative Fish and Wildlife Research Unit MSC 4901, Box 30003 Las Cruces, NM 88003-8003 ph. 505-646-8126 fax: 505-646-1281 e-mail: 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 505-646-1544 (p) 505-646-1281 (f) rdubey@nmsu.edu	$25,000/$26,491
Project Findings: This was the first part of a two-year study to establish and characterize the distribution and environmental constraints of genetic variants of *T. tubifex* within the San Juan River tailwater in the vicinity of the Navajo Dam, New Mexico. Sixty benthic and sediment samples were collected in summer and winter 2001. The benthic samples were processed to establish tubificid density, community structure, and the percentage of *T. tubifex* within each population. The percentage of *T. tubifex* within the enumerated samples ranged from 1 % to 96 %. TAM screening for whirling disease showed infection rates ranging from 0% to 15% within the sample reach with higher percentages in samples from deep habitat. Three genetic lineages of *T. tubifex* were identified from study samples. *T. tubifex* lineage monocultures are now being established to experimentally explore the survivability of encysted *T. tubifex* and the post-treatment viability of *M. cerebralis* spores under controlled temporal and thermal regimes.				
5. **(9)** **Epidemiology**	**Epidemiology of Whirling Disease: An Integrated Study of the Rock Creek Drainage, Montana**	**Willard O. Granath,** Professor of Microbiology University of Montana Division of Biological Sciences 32 Campus Drive #4824 Missoula, MT 59812-4824 ph. 406-243-2975 fax: 406-243-4184 e-mail: snail@selway.umt.edu	(1) Eric Reiland, Fisheries Biologist, MT Dept. of Fish, Wildlife & Parks, Missoula (2) Billie L. Kerans, Asst. Professor, Biology Department, MSU, Bozeman (3) Charlotte Rasmussen, Molecular Biologist, Western Fisheries Research Center, Seattle	$49,932/$54,624
Project Findings: In Rock Creek, western Montana, results of a four-year project indicate that infected *T. tubifex* are present in much greater numbers and are more widely dispersed in areas with degraded riparian habitat. However, trout become infected at many locations where infected *T. tubifex* have not been recovered, and it is possible that fish are infected by parasites originating hundreds or thousands of meters upstream. It also appears that the range of whirling disease within the Rock Creek drainage is still expanding, more than four years after its initial detection. Total water flow appears to affect the severity of disease; an apparent dilution effect on TAMs was observed in the upper portion of the drainage. Genetic analysis using randomly amplified polymorphic DNA methods indicated the presence of at least three distinct genotypes of *T. tubifex* within the Rock Creek drainage; all three are susceptible to infection.				

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6. **(10)** **Fish Pathology**	**Mechanisms of Resistance to *Myxobolus cerebralis* Infection in Brown Trout, Cutthroat Trout and Coho Salmon in Comparison to the Highly Susceptible Rainbow Trout**	**Ronald P. Hedrick,** Professor of Fish Pathology University of California, Davis Department of Medicine and Epidemiology School of Veterinary Medicine One Shields Avenue Davis, CA 95616-8737 ph. 530-752-9318 fax: 530-752-0414 e-mail: rphedrick@ucdavis.edu	(1) Mark A. Adkison, Research Associate, Dept. of Medicine and Epidemiology, School of Veterinary Medicine, UC-Davis 95616 530-752-9318 (p) 530-752-0414 (f) maadkison@ucdavis.edu	$25,536/$0

Project Findings:

The investigators used scanning electron microscopy (SEM) to compare the number of triactinomyxons (TAMs) that attached in the first 10 minutes of exposure to rainbow trout, westslope cutthroat trout, brown trout and coho salmon. They also used histology and light microscopy to determine the number of sporoplasm somatic cells that had successfully migrated into the epithelium one hour after attachment of the TAMs. The data indicate that the resistance of coho salmon and brown trout to whirling disease is conferred by different immune mechanisms. Coho salmon prevent infection by inhibiting the migration of most of the sporoplasm somatic cells into the epithelium, ultimately resulting in a light infection. In brown trout the parasite successfully invades and becomes established in the epithelium; at some subsequent point between the epithelium and the cartilage, possibly in the cranial nerve ganglia and nerve roots, the immune response engages and significantly reduces parasite numbers. Resistance in westslope cutthroat, although much less strong, may be due to similar mechanisms as seen in the coho salmon.

7. **(14)** **Invert Ecology and Taxonomy**	**Competitive Effects of Tubificid Assemblages on Triactinomyxon Production of *Tubifex tubifex***	**Billie L. Kerans,** Associate Professor Montana State University Ecology Department Bozeman, MT 59717 ph. 406-994-3725 fax: 406-994-3190 e-mail: bkerans@montana.edu	(1) Charlotte Rasmussen, Researcher, Western Fisheries Research Station, ISGS-BRD, 6505 NE 6th St, Seattle, WA 98115 206-526-6282, ext. 322 (p) 206-526-6654 (f)	$47,909/$0

Project Findings:

The overall goal of this project is to determine the mechanisms underlying spatial variability in salmonid whirling disease risk that relate to tubificid assemblages, in order to develop potential management strategies that can be used to ameliorate the disease. A preliminary conclusion from one year of work is that the prevalence of infection in *T. tubifex* is density dependent: the higher the abundance of *T. tubifex*, the lower the prevalence of infection. The investigators observed that as myxospore dose increases from 10 to 100 per worm, the prevalence of infection in *T. tubifex* increases. Prevalence of infection in *T. tubifex* and numbers of TAMs produced by individuals appears to be minimally affected by the presence of the aquatic oligochaete *Limnodrilus hoffmeisteri*, which does not sustain the whirling disease infection. Finally, under laboratory conditions the presence of resistant *T. tubifex* may decrease the numbers of TAMs produced by susceptible *T. tubifex* early in the release process. However, as release proceeds, the treatments with both susceptible and resistant strains produce similar numbers of TAMs.

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8. **(15)** **Habitat Management**	**Prevalence and Severity of *Myxobolus cerebralis* Infection Related to Water Temperature and flow Regimes of Native Cutthroat Trout, *Onchorynchus clarki bouvieri*, Spawning Tributaries of Yellowstone Lake**	**Todd Koel,** Chief Fisheries and Aquatic Sciences Section National Park Service PO Box 168 Yellowstone National Park, WY 82190 ph. 307-344-2281 fax: 307-344-2211 e-mail: todd_koel@nps.gov	(1) Crystal Hudson, Director, Bozeman Fish Health Center, USFWS, 920 Technology Blvd, Suite G, Bozeman, MT 59718 406-582-8656 406-587-3998 (f) crystal_hudson@fws.gov (2) Daniel Mahony, Senior Fishery Biologist, National Park Service, YNP, WY 307-344-2280 (p) 307-344-2323 (f) dan_mahony@nps.gov	$52,000/$80,000

Project Findings:

The overall goal of this project was to clearly define the relationship between temperature and stream flow regimes in the Yellowstone cutthroat trout spawning tributaries to Yellowstone Lake and the rate and intensity of whirling disease infections. The three principal activities were to characterize physical habitat in 13 diverse streams, characterize the distribution of *T. tubifex* in the streams, and expose cutthroat trout there o ascertain whirling disease virulence. Moderate to high infections by *M. cerebralis* were found in Pelican Creek and the Yellowstone River below Fishing Bridge. No other tributaries tested positive for the disease. Laboratory testing of adult cutthroat trout that are incidentally killed during lake trout gillnetting operations continues to indicate the presence of M. cerebralis lakewide, with the greatest numbers (19%) infected in the northern region of the lake. There is a high risk of infection in additional tributaries to Yellowstone Lake. Analyses of landscape-scale environmental attributes suggest that Beaverdam Creek, Trail Creek, and Chipmunk Creek are likely candidates for supporting the spread of this parasite.

9. **(18)** **Habitat Management**	**A Proposal for Demon-stration and Evaluation of Alternative Methods of Triactinomy-xons of *Myxobolus cerebralis* for Control of Whirling Disease**	**Eric Krch,** Program Engineer Colorado Division of Wildlife Buckhorn Geotech 222 S. Park Ave. Montrose, CO 81401 ph. 970-249-6828 fax: 970-249-0945 e-mail: eric@buckhorngeo.com	(1) R. Barry Nehring, Research Biologist, CO Division of Wildlife, 2300 S. Townsend, Montrose, CO 81401 970-252-6008 (p) 970-252-6053 (f) barry.nehring@state.co.us	$49,260/$15,470

Project Findings:

The goal of this research project was to determine the feasibility of direct filtration methods for removal of *Myxobolus cerebralis* TAMs in various filtration media. Bench testing and field demonstrations were conducted using domestic and natural water sources to develop a scope of operational considerations and evaluate filtration media types. The results of the fieldwork suggest that direct passive filtration is possible; however, operation and maintenance are crucial elements in full-scale operations. The results of the research provide design considerations, filtration media selection, and application rates of infected influent to achieve desired rates of *M. cerebralis* TAM removal.

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10. **(16)** **Fishery Management**	**Interaction of Life History, Fish Size, and Infection Risk on Population-Level Effects of Whirling Disease on Wild Rainbow Trout**	**Thomas E. McMahon** Associate Professor Montana State University Ecology Department Bozeman, MT 59717 ph. 406-994-2492 fax: 406-994-7479 e-mail: ubitm@montana.edu	(1) Andrew Munro, GRA, Ecology Dept., MSU-Bozeman amunro@montana.edu (2) Alexander V. Zale, Acting Leader, MT CFRU, Ecology Dept., MSU-Bozeman zale@montana.edu (3) Stephen A. Leathe, Regional Fisheries Manager, MFWP, Gr Falls sleathe@state.mt; (4) George Liknes, Fishery Biologist, MFWP, Gr Falls r4fish@mcn.net	$49,864/$77,150
Project Findings: This long-term study on the Missouri River in Montana combines monitoring of the spread of infection with detailed measures of population response to the disease. The investigators are testing whether spawning and rearing in disease-free areas is a viable management option for maintaining and enhancing trout populations in infected systems. During this study cycle rainbow trout populations remained high and did not drop as a result of whirling disease. However, there are signs of impending population decline as the remaining adults are nearing the end of their life span and the size structure of the adult population shows a much-reduced number of younger fish than in pre-whirling disease years. Expansion of whirling disease into lower tributaries, while not observed to a great degree thus far, could have catastrophic consequences since it appears that most of the adult population is supported at present by recruitment from these tributaries.				
11. **(17)** **Ecological Modeling**	**Development of Empirical Models of *Myxobolus cerebralis* 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 PO Box 441136 Moscow, ID 83844-1136 ph. 208-885-7047 fax: 208-885-9080 e-mail: 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	$27,587/$19,680
Project Findings: The investigators developed empirical models that describe both population dynamics and landscape-level factors associated with the prevalence, intensity of infection, and likelihood of risk to fish populations of *Myxobolus cerebralis*. They created a simple dynamic disease model for *M. cerebralis,* a discrete compartmental model to explain the course of disease in both fish and worms. They drafted a landscape-level model that incorporate GIS-based information extracted from digital maps. This model uses three landscape attributes: channel slope, catchment area, and elevation. All these factors can indirectly affect the intensity of infections. Through it surrogate, elevation, temperature is correlated with tractinomyxon releases, and overall fish production, growth and reproduction. This project is continuing into another funding cycle to refine the empirical models, to adding other landscape level metrics, and improve the manner that the geospatial data are collected from digital maps.				

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12. **(19)** **Invert Ecology and Taxonomy**	**Characterization of the Response of Genetically Distinct *Tubifex tubifex* Populations to *M. cerebralis* Infection in Laboratory and Natural Systems**	**Charlotte Rasmussen,** Research Associate Western Fisheries Research Center, USGS-BRD, 6505 NE 65th St Seattle, WA 98115 ph. 206-526-6282, ext 322 fax: 206-526-6654 e-mail: charlotte_rasmussen@usgs.gov	(1) Billie L. Kerans, Assoc. Professor, Ecology Dept, MSU-Bozeman (2) James R. Winton, Chief, Fish Disease Section, Western Fisheries Research Center, Seattle (3) Alison E. L. Colwell, Microbiologist, Western Fisheries Research Center, Seattle	$50,685/$42,060
Project Findings: This project entailed a series of experiments to test previous information indicating that genetic differences among *T. tubifex* contribute to the differences in distribution and severity of whirling disease seen among wild fish populations. The results suggest there are four genetically-distinct clades. The two groups of worms showing high and moderate TAM production may comprise one large related groups, while the remaining two distinct clades consist of low producers and non-producers. Field studies to determine the genetic composition of naturally-occurring *T. tubifex* populations in order to compare the genetic and biological results obtained from the laboratory experiments were expanded. Preliminary genetic analysis from several streams in Montana indicates that these populations consist of individuals with moderate to high TAM production, which is consistent with the disease epidemiology seen on these river systems.				
13. **(20)** **Lab Methods**	**Rapid Identification of Immature and Mature *Tubifex tubifex* by Monoclonal Antibodies**	**Donald W. Roberts,** Invertebrate Pathologist Utah State University Department of Biology 5305 Old Main Hill Logan, UT 84322-5305 ph. 435-797-0049 fax: 435-797-1575 e-mail: dwroberts@biology.usu.edu	Nabil N. Youssel, Department of Biology, Utah State University, Logan, UT 435-797-2513 nabil@biology.usu.edu	$35,123/$0
Project Findings: The only worm species known to replicate *M. cerebralis* is *Tubifex tubifex*, and most of the year it is impossible to identify an aquatic worm as *T. Tubifex* using conventional microscopy. In this project the investigators developed monoclonal antibodies (MAbs) to identify *T. tubifex.* Each MAb is produced by a tissue culture cell line derived from a single spleen cell from mice that have been injected with the proteins from the worm of interest. The investigators have succeeded in isolating hybridoma cell lines which produce antibodies specific for *T. tubifex* and *Rhyacodrilus* , another aquatic worm found in similar habitats. MAbs made to one geographical isolate of *T. tubifex* recognize *T. tubifex* ranging from Ontario to California. Contingent on further funding, twelve anti *T. tubifex* MAbs will be evaluated to determine which will function most effectively for worm identification in the laboratory and in the field. This research will provide tools to improve and facilitate studies on the ecology and pathology of the worm phase of whirling disease.				