Research Findings 1998-1999
Whirling Disease Initiative

National Partnership on the Management of Wild and Native Cold Water Fisheries
Montana University System Water Center, MSU-Bozeman

Category	Title	Principal Investigator	Other Partners	Award / Match
1. Diagnostic Study	**Determination of the Sensitivity of a PCR Assay for *Myxobolus cerebralis***	**Thomas J. Baldwin,** Veterinary Pathologist Washington Animal Disease Diagnostic Laboratory Department of Veterinary Microbiology & Pathology Washington State University Pullman, WA 99164 ph. 509.335.6010 fax: 509.335.7424 e-mail: tjb@vetmed.wsu.edu	Gayle C. McGhee, Associate in Research Department of Veterinary Microbiology & Pathology, WSU	$24,104/$17,246
Research Findings: Large numbers of *M. cerebralis* myxospores, and occasional myxospores from non-salmonid fish (not *M. cerebralis*), were obtained and stored -80° C. The purpose was to determine the sensitivity and specifically of a PCR assay modified from that described by Andree and Hedrick, utilizing these myxospores as a known study set. The PCR assay has been optimized for use with *M. cerebralis* myxospores obtained by pepsin-trypsin digest, and the minimum concentration of myxospores detectable, but inconsistently. Detection was 100% using 2,500 spores/reaction tube. In an effort to unify results and decrease myxospores, filtration in combination with additional enzymatic digestion and density separation are being utilized to remove contaminant DNA. When relatively pure myxospores samples are obtained, large numbers of known *M. cerebralis* and non-*M. cerebralis* myxospores will be tested by the PCR assay, and the sensitivity and specificity of the assay determined.				
2. Economic Study	**Economic Consequences of Whirling Disease in Montana Stream Fisheries**	**John W. Duffield,** Research Professor Department of Economics University of Montana 3699 Larch Camp Road Missoula, MT 59803 ph. 406.728.9510 fax: 406.728.9510 e-mail: bioecon@marsweb.com	(1) David A. Patterson Associate Professor Department of Mathematics, UM (2) John Loomis, Professor Department of Agricultural and Resource Economics, Colorado State University, Fort Collins, CO (3) Chris Nehar, Bioeconomics, Inc., 721.2265	$60,000/$22,668 (+ $2,000 private funding)
Research Findings: This study, (including Colorado information, but with particular focus on the Missouri and Madison Rivers), analyzed how reduction in trout populations affects angler use, and how reduction in angler expenditures impacts local economies. The basic finding is that the angler response is considerable, although not proportional. For example, a 10% reduction in trout population might cause a 4-6% reduction in angler use. Economic impacts vary with the site. While the Madison is more of a destination fishery, with multi-day visits, guides, and average expenditure of $222, the Missouri draws local, one-day anglers who spend $48 a day. Time-series data on the Madison showed that a 75% reduction of larger rainbow trout from 7 years in the '90s compared to 7 years in the '80s; while angler use has been fairly consistent since the mid-80s.				

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3. Laboratory Study	**Efficacy of Fumagillin to Prevent Experimentally Induced Whirling Disease in Rainbow Trout *Oncorhynchus mykiss***	**Dave Erdahl,** Fisheries Biologist and Chief National INAD Office US Fish & Wildlife Service Bozeman Fish Technology Center/NIO 4050 Bridger Canyon Road Bozeman, MT 59715 ph. 406.587.9265 ext. 125 fax: 406.582.0242 e-mail: dave_erdahl@fws.gov	(1) Crystal Hudson, Fish Health Biologist and Director Bozeman FHC, USFWS (2) Jim Bowker, Fisheries Biologist and Asst INAD Coordinator USFWS, BFTC (3) C.A. Speer, Professor Veterinary Molecular Biology, MSU-Bozeman (4) Robert G. White, Director, MT State Cooperative Fisheries Program, MSU-Bozeman	$30,000/$55,000
Research Findings: Five trials were conducted evaluating the efficacy of fumagillin to control/prevent whirling disease in salmonids. Four trials involved rainbow trout; one trial involved steelhead trout and Chinook salmon. Based on spore count data and histological evaluation of fish, 180 and 240 days post-exposure, fumagillin treatment did not appear to be efficacious. In all trials, spores count data and histological score were similar in both positive control and treated fish; and, with the exception of Chinook salmon, exhibited clinical signs of whirling disease to at least some degree.				
4. Laboratory Studies	**Parameters that Determine Development and Production of *Myxobolus cerebralis* in *Tubifex tubifex***	**Willard O. Granath, Jr.,** Pathologist University of Montana Division of Biological Sciences Missoula, MT 59812-1002 ph. 406.243.2975 fax: 406.243.4184 e-mail: snail@selway.umt.edu	none	$47,366/$23,372
Research Findings: Progress was made towards defining parameters that determine the development of parasite in its worm host. Regarding temperature effects on development, TAMs survive longer in cold water (4EC) than warm (12EC); but fewer TAMs are produced from worms at lower temperatures. TAMs may survive twice as long in a fecal packet than if water-borne. At least one strain of *T. tubifex* is resistant. Additionally, some worm infected in areas remain "clean," but become infected in laboratory experiments, indicating that other factors besides resistance play a role in nature. No *oligochaetes* besides *T. tubifex* are found to be hosts. ***Significance:*** Since there is variability in the susceptibility of *T. tubifex* worms, their mere presence in a drainage does not indicate that whirling disease will be established.				

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5. Laboratory Study	**Production of *Myxobolus cerebralis* Triactinomyxons: Potential Alternative Hosts and Effects of Tubificid Assemblage Structure**	**Billie L. Kerans,** Invertebrate Biologist Montana State University Biology Department Bozeman, MT 59717 ph. 406.994.3725 fax: 406.994.3190 e-mail: ubibk@montana.edu	none	$28,956/$10,000
Research Findings: Genetically distinguishable, geographic variants of *T. tubifex* (worms) produced numbers of *M. cerebralis* TAMs when given similar doses of *M. cerebralis* spores under similar lab conditions. California worms consistently produced an order of magnitude greater number of TAMs than worms from the Madison River. Gallatin River worms produced only a few TAMs when given a spore dose of 1,000 spores/worm, and no TAMs when given a dose of 250 spores/worm. When worms were given doses of 50 to 1,000 spores/worm, overall TAM production was not related to spore dose. However, timing of peak TAM release was influenced by spore dose. TAM production of worms was higher at 15° C than at 8° C. However, TAMs were produced approximately 7 weeks later when worms were held at 8° C than at 15° C. Thus, the differential in TAM production could be related to the differing lengths of time that worms were producing TAMs. Reproduction of worms was impaired by *M. cerebralis* infections. *L. hoffmeisteri* and *L. templetoni* did not produce TAMs.				
6. Laboratory Study	**Effects of Age, Dose, and Environmental Stress on Development of Whirling Disease in Rainbow Trout**	**Elizabeth MacConnell,** Fish Health Biologist US Fish and Wildlife Service Bozeman Fish Technology Center 4050 Bridger Canyon Road Bozeman, MT 59715 ph. 406.587.9265 ext. 129 fax: 406.582.5942 e-mail: bmacconnell@montana.campus.mci.net	Alexander V. Zale, Asst Unit Leader and Affiliate Associate Professor MT Cooperative Fishery Research Unit; MSU-Bozeman	$12,196 ($28,000 rollover)/$31,766
Research Findings: The development of whirling disease in rainbow trout was affected by both age at first exposure and parasite dose. The older the fish were when first exposed to *M. cerebralis*, the longer it took for clinical signs to develop. No clinical signs of whirling disease were recorded for fish exposed after 13 weeks post-hatch. Age of fish at first exposure and parasite dose rate significantly affected survival. During the first 2 months of age (at 13° C) there was greater survival with decreased parasite dose. The number of *M. cerebralis* spores significantly increased with decreasing age at first exposure. Severity of infection increased with parasite dose if first exposed at ages younger than 9 weeks post-hatch. Swimming performance at 17 weeks post-exposure decreased with increasing severity of infection.				

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7. Laboratory Study	**Laboratory Investigations of Mountain Whitefish *Prosopium Willliamsoni* Susceptibility to *Myxobolus cerebralis***	**Elizabeth MacConnell,** Fish Health Biologist US Fish and Wildlife Service Bozeman Fish Technology Center 4050 Bridger Canyon Road Bozeman, MT 59715 ph. 406.587.9265 ext. 129 fax: 406.582.5942 e-mail: bmacconnell@montana.campus.mci.net	Alexander V. Zale, Asst Unit Leader and Affiliate Associate Professor MT Cooperative Fishery Research Unit; MSU-Bozeman	$26,870/$23,700
Research Findings: This study tested the susceptibility of different ages of mountain whitefish to doses of 1,000 or 10,000 parasites per fish. Mortality was observed in 3 and 7 weeks post-hatch mountain whitefish during exposure to doses of 10,000 triactinomyxons/fish. Histological examination showed that numerous parasites had penetrated the epithellum. Few whitefish, including unexposed controls, survived to the end of the study. Clinical signs of whirling disease were first observed 9 weeks post-exposure, compared to 5 weeks in rainbow trout. Vegetative and sporogonic stages of *M. cerebralis* and inflammatory lesions were seen in cartilaginous tissues (primarily spine) of whitefish collected 5 months post-exposure. Based on the limited number of survivors in this study, whitefish are susceptible to infection and the development of whirling disease.				
8. Laboratory Study	**Identify and Characterize the Adhesion Molecules Involved in Infection, Migration and Propagation of *Myxobolus cerebralis* in Salmonid Hosts**	**C.A. Speer,** Professor Montana State University Veterinary Molecular Biology Bozeman, MT 59717-3610 ph. 406.994.6389 fax: 406.994.6389 e-mail: uvscs@gemini.oscs.montana.edu	(1) Robert F. Bargatze, Vice President and Senior Research Scientist, Montana Immuno Tech, Inc. (2) Crystal Hudson, Director, Bozeman Fish Health Center, USFWS	$35,000/$39,492
Research Findings: This project has developed an in vito cultivation system for the presporogonic stages of *Myxobolus cerebralis*. Cultures of epidermal, neural and cartilaginous cells from salmonids were infected with sporoplasms derived from the TAMs. Several monoclonal antibodies were generated that showed different reaction patterns on the TAMs as determined by immunofluorescence microscopy. These monoclonal antibodies and their vitro cultivation system will be used in future experiments to study the adhesion molecules involved in parasite attachment, migration and development in salmonid hosts.				

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9. Field Study	**Distribution and Seasonal Occurrence of *Myxobolus cerebralis* in the Lostine River, Oregon**	**Jerri L. Bartholomew,** Microbiologist Oregon State University Department of Microbiology 220 Nash Hall Corvallis, OR 97331 ph. 541.737.1856 fax: 541.737.0496 e-mail: bartholj@bcc.orst.edu	John L. Fryer, Professor Emeritus, Department of Microbiology, Oregon State University	$33,037/$34,541
Research Findings: This continuing research on the ecology of the parasite in the northeastern Oregon Lostine River may be particularly significant because six salmonid species are present, two of which are anadromous. The high incidence of infection in sentinel fry receiving a short exposure between July and April suggests that the fry of rainbow and steelhead trout, and Chinook salmon, are likely to experience a significant level of infection. However, the population effects may be minimal in an infected area. This may be the result of less worm habitat and lower fish density than in other affected rivers. The loss of the parasite stages to the Pacific Ocean in the anadromous hosts may also play a role.				
10. Field Study	**Dynamics of Whirling Disease on the Cache La Poudre River**	**Eric P. Bergersen,** Fisheries Biologist Colorado Cooperative Fish & Wildlife Research Unit Colorado State University 201 Wagar Building Fort Collins, Colorado 80523 ph. 970.491.5396 fax: 970.491.1413 e-mail: ericb@cnr.colostate.edu	(1) R. Barry Nehring, Life Sciences Researcher IV, Colorado Division of Wildlife (2) Brady Allen, GRA, Fish Pathologist, Colorado Division of Wildlife	$33,348/$22,000
Research Findings: This study examined the role of a whirling disease infected rainbow trout rearing unit, and headwater impoundment stocked with infected fish on the distribution of *M. cerebralis* in the Cache La Poudre River. Denisities of *T. tubifex* were 163,000/m2 in the rearing unit, while river sites averaged less than 1/m2, although desities in alcoves reached 50/m2. While worm densities in the river were lower than in the rearing unit, species diversity was higher. T. tubifex comprised about 98% of the worms in the rearing unit, and about 50% in the river. More worms were infected below the rearing unit (6%) than above (1%) or in the unit (2%). Elevated infection rates in young-of-the-year trout below the rearing unit were attributed to the high numbers of TAMs entering the river from the unit. TAMs were low below the impoundment, but all tested trout were infected, suggesting that low TAM rates can infect an entire fish population.				

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11. Field Study	**Relationship of *Myxobolus cerebralis* Infected *Tubifex* to Infection Rates and Severity of Whirling Disease in Trout: An Integrated Study of Rock Creek, Montana**	**Willard O. Granath, Jr.,** Pathologist University of Montana Division of Biological Sciences Missoula, MT 59812-1002 ph. 406.243.2975 fax: 406.243.4184 e-mail: snail@selway.umt.edu	(1) E. Richard Vincent, Coordinator Whirling Disease Program, MTFWP (2) Billie L. Kerans, Assistant Professor, Biology Department, MSU-Bozeman (3) James R. Winton, Chief Fish Section, Western Fisheries Research Center, Seattle (4) James E. Gannon, Assoc Professor, Division of Biological Sciences, UM	$27,013/$6,945
Research Findings: Progress was made in relating the level of infection of the worm host to trout. Significance: While fish may be infected throughout a drainage, the worm host may not be, indicating that the fish are probably infected by drifting TAMs.				
12. Field Study	**Tubificid Ecology and *Myxobolus cerebralis* Infections in the Madison River Drainage: Year Two**	**Billie L. Kerans,** Invertebrate Biologist Montana State University Biology Department Bozeman, MT 59717 ph. 406.994.3725 fax: 406.994.3190 e-mail: ubibk@montana.edu	(1) E. Richard Vincent, Coordinator Whirling Disease Program, MTFWP (2) Michael M. Gangloff, Research Associate, Biology Department, MSU-Bozeman	$28,324/$22,439
Research Findings: Two management strategies that seem promising to combat whirling disease are enhancement of whirling disease-resistant fish life history types, and modification of the environment to reduce its favorability to *T. tubifex*. These studies were undertaken to assist these strategies, and revealed: (1) Whirling disease severity is spatially and temporally variable even within one stream. There may be locations and times where fish may be able to escape severe infections because infection rates are low. (2) *T. tubifex* and/or other tubficids were found in locations regardless of whether sentinel fish tested positive or negative for whirling disease. However, worm density was one factor that was associated with high disease severity in sentinel fish. (3) Highest tubificid densities were in soft sediments, and core samples in soft sediments give adequate information about tubificid assemblages and densities.				

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13. Field Study	**Maintaining Wild Trout in Whirling Disease Infected Rivers: Mitigating Trout Declines by Enhancing Habitat and Life History Types of Survivors in the Upper Madison River**	**Thomas E. McMahon,** Fisheries Biologist Montana State University Biology Department Bozeman, MT 59717 ph. 406.994.2492 fax: 406.994.7479 e-mail: ubitm@msu.oscs.montana.edu	(1) Bradley B. Shepard, Fishery Biologist, MT Fish, Wildlife, and Parks (2) E. Richard Vincent, Coordinator Whirling Disease Program, MTFWP	$11.343/$33,100
Research Findings: The survey results show that the Madison River rainbow trout are primarily late mainstream spawners. (In contrast, Missouri River rainbows are early spawners emergers.) Other observations showed that rainbow spawn primarily in the upper section of the river, that a key feature in spawning success is the presence of shallow side channels, and that infection risk varies substantially. Eventually the spawning and rearing information will be overlaid with "hot spot" data, to identify when and where vulnerable fry are most in danger of infection.				
14. Field Study	**Relation of Life History Type to Whirling Disease Susceptibility in Missouri River Rainbow Trout**	**Thomas E. McMahon,** Fisheries Biologist Montana State University Biology Department Bozeman, MT 59717 ph. 406.994.2492 fax: 406.994.7479 e-mail: ubitm@msu.oscs.montana.edu	(1) Alexander V. Zale, Asst Unit Leader, MT Cooperative Fishery Research Unit; MSU-Bozeman (2) Michael M. Gangloff, Research Associate, Biology Department, MSU-Bozeman (2) E. Richard Vincent, Coordinator Whirling Disease Program, MTFWP	$60,304/$118,866
Research Findings: Whirling disease was discovered in the Little Prickly Pear Creek, a major spawning tributary of the Missouri River, in 1996. Research documented a substantial increase in the disease, and its spreading to other major spawning tributaries. Further: (1) Missouri rainbow are primarily tributary spawners; (2) post-spawning fish sometimes move long distances downstream, which can spread the disease; (3) a majority of the juveniles rear in the tributaries past their vulnerable period; and (4) the worm host populations and severity of infection vary throughout the watershed. These findings support the theory that trout life history can strongly affect the response of a rainbow population to whirling disease.				

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15. Field Study	**An Assessment of Possible Resistance to Whirling Disease among Rainbow Trout and Snake River Cutthroat Trout after Exposure to *Myxobolus cerebralis* Infection in the Upper Colorado River in Middle Park, Colorado**	**R. Barry Nehring,** Life Sciences Researcher IV Colorado Division of Wildlife 2300 S. Townsend Montrose, CO 81401 ph. 970.249.3431 fax: 970.249.2857 e-mail: barry.nehring@state.co.us	none	$53,000/$30,000
Research Findings: In July of 1998, two sizes of Snake River cutthroat (SRC) and two groups of wild-strain rainbow (R) of different parentage were exposed for 35 days to four levels of TAMs in the upper CO River. The R were progeny of parental cohorts recruited before and after *M. cerebralis* became enzootic in the river. The larger SRC were 55 mm; the smaller SRC and R were 35 mm. After 35 days, all were moved to one location where low-level exposure continued 11 months. After nine to 12 months, cranial *M. cerebralis* burdens among R groups were 10-100 times greater than those in the larger SRC groups, and 2-10 times greater than the smaller SRC group. Survival of the larger SRC was much better than the three smaller groups. At a given exposure site, there was no difference in survivorship or burden.				
16. Lab Component	**Laboratory Assessment of Possible Selection for Resistance to Whirling Disease Among Progeny of Colorado River Rainbow Trout *(Oncorhynchus mykiss)***	**Alexander V. Zale,** Assistant Unit Leader and Affiliate Associate Professor Montana Cooperative Fishery Research Unit Montana State University Biology Department Bozeman, MT 59717 ph. 406.994.2380 fax: 406.994.7479 e-mail: zale@montana.edu	Eileen K. N. Ryce Graduate Research Asst Montana Cooperative Fishery Research Unit Biology Department MSU-Bozeman	$22,000/$16,270
Research Findings: A sentinel fish experiment, conducted in Colorado, suggested that progeny of wild rainbow trout known to have recruited during the early years of *M. cerebralis* infestation in the Colorado River developed lower myxospore loads than fish of the same cohort but which were progeny of older fish that recruited prior to the presence of whirling disease. The inference was made that selection for resistance to whirling disease may have been responsible. This lab study was to determine whether rainbow trout produced from these two parent lines show different levels of resistance or infectivity to *M. cerebralis* over a range of TAM dosages. A domestic strain of rainbow trout was used to serve as a reference against the Colorado rainbow trout. Results from the study did not provide conclusive evidence that offspring from parents who survived the early years of *M. cerebralis* infection in the upper Colorado River display any degree of resistance to infection by this parasite. There was also no evidence found in spore counts, level of infection, or in swimming performance that there had been a selection for resistance to whirling disease among the two groups of Colorado River rainbow trout.				

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17. Contract	**Aquatic Oligochaete Workshop**	**R. Deedee Kathman,** Director Aquatic Resources Center PO Box 345 6604 Third Street College Grove, TN 37046 ph. 615.368.7592 fax: 615.368.7657 e-mail: aquatres@ix.netcom.com	none	$10,072.11/$
Results: Obligations have been met.				
18. Contract	**Standard Field and Laboratory Protocols for Oligochaete Analysis (in cooperation with the Fish Health Database)**	**R. Deedee Kathman,** Director Aquatic Resources Center PO Box 345 6604 Third Street College Grove, TN 37046 ph. 615.368.7592 fax: 615.368.7657 e-mail: aquatres@ix.netcom.com	none	$10,292.24/$
Results: Obligations have been met; protocols are available.				
19. Contract	**Fish Health Database**	**Daniel Goodman,** Professor of Ecology Biology Department Cooley Lab 7 Montana State University Bozeman, MT 59717 ph. 406.994.3231 fax: 406.994.2490 e-mail: goodman@rivers.oscs.montana.edu	none	$20,000/$
Results: Initially co-funded in 1997 through the USFWS and National Partnership, this database obtains and analyzes fish health data from locations across the country. A GIS interface exhibiting watershed boundaries was developed so that data can be accessed by queries that are specific to a location. The 1998 support emphasized the integration of additional fields into the database to accommodate environmental variables that may correlate with fish health, and enable whirling disease epidemiological analyses.				

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20. Contract	**Finding Solutions to Whirling Disease in Wild Trout: Interactions Among the Parasite and its Hosts**	**Mansour El-Matbouli** Department of Medicine and Epidemiology School of Veterinary Medicine University of California Davis, CA 95616 ph. 530.752.9318 fax: 530.752.0414 e-mail: melmatbouli@ucdavis.edu	none	$20,000/$

Research Findings:

This research showed the significant relationship between the release of infective TAMs from the worm, and water temperature—information that can be used to manage the disease. (1) 15° C is the optimal temperature for TAM development and release from the *Tubifex* worm host. (2) A significant number of TAMs developed at these temperatures survived up to 15 days in water. (3) Non-susceptible *tubificid* worms are able to ingest *M. cerebralis*, but unable to develop and release the fish-infective form of the parasite, leading to possibilities for biological control of the disease through interbreeding of susceptible and non-susceptible worm host populations.