

Project Summaries 2005 – 2006/07 Whirling Disease Initiative

National Partnership for the Management of Wild & Native Cold Water Fisheries

Montana University System, MSU-Bozeman

Contract Dates: May 1, 2005 – December 31, 2006 or December 31, 2007

Project #	Title	Principal Investigator	Other Partners	Award / Match
Project #1.	<i>Resolving uncertainties in Myxobolus cerebralis introduction and establishment risks</i>	Jerri L. Bartholomew, Assistant Professor, Senior Researcher Oregon State University, Dept of Microbiology 220 Nash Hall Corvallis, OR 97331-3804 Phone: 541-737-1856; Fax: 541-737-0496 Jerri.bartholomew@oregonstate.edu	Antonio Amandi, Acting Project Leader, Oregon Dept of Fish and Wildlife, Fish Health Services Oregon State University Dept of Microbiology 220 Nash Hall, Oregon State University amandia@onid.orst.edu	\$61,313 \$66,975
<p>Project Summary: <i>Tubifex tubifex</i> are obligate invertebrate hosts in the life cycle of <i>Myxobolus cerebralis</i>, the myxozoan parasite that causes whirling disease in salmonid fishes. The exotic parasite is established to varying degrees across Oregon's Columbia River System (Pacific Northwest, USA) and <i>T. tubifex</i> are likely to play a role in its pattern of occurrence. To better understand these patterns, the research team collected <i>T. tubifex</i> from 3 Oregonian river basins (Grande Ronde, Deschutes and Willamette), determined their genotype (mitochondrial lineage and RAPD pattern) and exposed 10 different populations to <i>M. cerebralis</i> in the laboratory. Three lineages were identified: I, III and VI. Lineage III was found in all river basins but dominated central and eastern sites. The RAPD assay further divided the lineages into geographic sub-populations. No RAPD genotype was common to all basins. There was a significant difference in prevalence of infection and level of parasite production among the populations exposed to <i>M. cerebralis</i> that was attributable to genotype composition, and even within lineage III, the only worms to release actinospores. The distribution of susceptible <i>T. tubifex</i> explains the occurrence of <i>M. cerebralis</i> in Oregon, and these studies further support the influence of oligochaete genotypes on the manifestation of whirling disease in salmonid populations.</p>				

Project #	Title	Principal Investigator	Other Partners	Award / Match
Project #2.	<i>Effect of benthic invertebrate populations, riparian zone and associated water quality on infection rates of Tubifex tubifex with Myxobolus cerebralis</i>	Deborah Cartwright Iwanowicz, PhD SCEP Student University of Georgia ; USGS, National Fish Health Research Laboratory 11700 Leetown Road Kearneysville, WV 25430 Phone: 304-724-4439; Fax: 304-724-4435 deborah_cartwright@usgs.gov	(1) Vicki Blazer, USGS, National Fish Health Laboratory vicki_blazer@usgs.gov (2) W. Bane Schill, USGS, National Fish Health Laboratory bane_schill@usgs.gov	\$83,070 \$74,188
<p>Project Summary: Few studies have addressed environmental effects (other than temperature) on <i>Tubifex tubifex</i> populations, their ability to become infected with <i>M. cerebralis</i> and produce infective triactinomyxon (TAM) spores, particularly in Eastern states. In previous studies <i>T. tubifex</i> were collected from streams with four land-cover types – meadow or agricultural, deciduous, coniferous and deciduous/meadowland. Previous field and laboratory experiments suggested that substrate affected infectivity, TAM production and oligochaete assemblages. However, data also suggested that factors other than substrate also affected infectivity and viable TAM production. Hence, we proposed to further evaluate the oligochaete populations and water quality at these sites. Water chemistry was analyzed for differences between sites with different land-cover. Although many differences were observed, only barium was significantly higher in coniferous sites than all other sites. In addition, laboratory exposures were completed evaluating the ability of <i>T. tubifex</i> from the individual sites to be infected in a reference substrate (sand), and exposures in site specific substrate and water to determine differences in infectivity in similar lineages from different environments. <i>Tubifex tubifex</i> were not only used from sites with different land-covers, but also from both the East coast (New York) and West coast (California and Colorado). It was found that worms on the West coast have a higher infectivity level than worms on the East coast, and that the mixture of substrate and water also influenced the production of TAMs from <i>T. tubifex</i>.</p>				

Project #	Title	Principal Investigator	Other Partners	Award / Match
Project #3.	<i>Movements of resident and non-resident anglers in Montana: implications of transferring whirling disease among drainages</i>	Christopher S. Guy Montana Cooperative Fishery Research Unit Montana State University Department of Ecology Bozeman, MT 59717 Phone: 406-994-3491 cguy@montana.edu	(1) Dr. Alexander V. Zale Montana Cooperative Fishery Research Unit Montana State University Department of Ecology Bozeman, MT 59717 Phone: 406-994-2380 zale@montana.edu (2) Travis B. Horton Montana Dept of Fish Wildlife and Parks 4600 Giant Springs Road Great Falls, MT 59405 Phone: 406-454-5853 thorton@state.mt.us	\$56,648 \$16,862

Project Summary:

Movement of anglers among rivers in southwestern Montana presents a potential pathway for the spread of whirling disease and other aquatic nuisance species (ANS) on soil laden angling equipment. The objectives of this study were to 1) determine the effectiveness of a density extraction to isolate myxospores from soil and the effectiveness of polymerase chain reaction (PCR) at detecting *M. cerebralis* myxospores in soil; 2) quantify movement of resident and non-resident anglers in southwestern Montana and soil quantity carried on angling equipment; and 3) determine myxospore adherence to wading equipment materials. Myxospores were extracted from soil using a soil particle density separation technique. A single blind study was used for PCR experiments with varying quantities of myxospores and soil. Angler movement was assessed with a survey at fishing access sites on six southwestern Montana rivers. Soil samples were taken during the survey from boots and waders with a pressure sprayer to assess quantity of soil carried on angling equipment. Myxospore adherence to wading materials (lightweight, neoprene, rubber, and felt) was tested by exposing myxospores to material and rinsing with a pressure sprayer. Mean percent myxospore recovery with density extraction declined as soil quantity increased. Polymerase chain reaction experiments detected myxospores in treatments with ≤ 0.25 g of soil and ≥ 100 myxospores/0.25 g soil. Resident and non-resident anglers did not differ significantly in the number of fishing access sites used or drainages fished in during the previous 30 days. Non-residents fished in more states in the previous 30 days than residents and traveled greater distances to fish in the previous 7 and 30 days than residents. Mean quantity of sediment carried on one boot-wader leg was 8.39 g (± 1.5 , 95% CI). Lightweight waders and felt soled boots were the most prominent types of wading equipment materials used by anglers and myxospores adhered to felt more than rubber and the glass control. Integration of angler movement patterns and mean sediment quantities transported with angler numbers suggests that anglers in southwestern Montana are potentially moving tons of sediment among fishing access sites every year, thereby making transport of ANS likely.

Project #	Title	Principal Investigator	Other Partners	Award / Match
Project #4.	<i>Forensic applications of otolith microchemistry for tracking sources of illegally stocked whirling disease positive trout</i>	Brett M. Johnson, Associate Professor Department of Fishery and Wildlife Biology, Colorado State University 1474 Campus Delivery Fort Collins, CO 80523-1474 Phone: 970-491-5002 Fax: 970-491-5091 brett@cnr.colostate.edu	(1) Daniel Gibson-Reinemer, Graduate Research Assistant, Dept of Fishery and Wildlife Biology Colorado State University 970.491.2749; dangr34@hotmail.com (2) Patrick J. Martinez, Aquatic Researcher Aquatic Research Section Colorado Division of Wildlife 970-255-6141; Pat.martinez@state.co.us (3) Dana Winkelman, Leader Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University Phone: 970.491.1414; dlw@cnr.colostate.edu (4) Gregory Whitley, Postdoctoral Research Assistant Dept of Fishery and Wildlife Biology Colorado State University 970.491.2749; whitgw@cnr.colostate.edu	\$64,696 \$25,483
<p>Project Summary: The investigators used naturally-occurring chemical markers to trace the environmental history of hatchery trout. Analysis of water and otolith chemistry at hatcheries revealed a high degree of temporal stability, coupled with high variation among hatcheries relative to variation within hatcheries. Proportional relationships between water and otolith chemistry for Sr:Ca, Ba:Ca, and ⁸⁷Sr/⁸⁶Sr allowed them to use these three quantities as environmental markers in otoliths to classify trout to their hatchery of origin. Multivariate models used to discriminate among hatcheries performed best when all three markers were used, achieving an average accuracy of up to 96% for a group of five hatcheries. Using only Sr:Ca and Ba:Ca, the research team was able to identify the hatchery of origin with average accuracy rates which varied from 59% using a group of 11 hatcheries to 90% when groups of only two hatcheries were considered. In a rigorous test of the forensic capabilities of otolith chemistry, multivariate models classified a blind sample of at-large fish stocked from hatcheries with 79% accuracy. These results indicate the most effective use of otolith chemistry in a forensic context will require collaboration with investigators using traditional methods of inquiry to reduce the number of hatcheries classified with otolith markers. The investigators advocate an eclectic approach to source identification using elemental and isotopic markers as a powerful new source of information that can be used to strengthen cases based on multiple lines of evidence.</p>				

Project #	Title	Principal Investigator	Other Partners	Award / Match
Project #5.	<i>Whirling disease risk at multiple spatial scales</i>	Billie Kerans, Associate Professor Department of Ecology Montana State University 311B Lewis Hall Bozeman, MT 59717 Phone: 406-994-3725 Fax: 406-994-3190 bkerans@montana.edu	E. Richard Vincent, Whirling Disease Research Coordinator Montana Fish, Wildlife and Parks Bozeman, MT 59715 406.994.3551 rvincent@montana.edu	\$74,345.25 \$21,000
<p>Project Summary: <i>Myxobolus cerebralis</i>, the causative agent of whirling disease, has been a major contributor to the loss of young trout in numerous streams within the Intermountain West (Colorado, Idaho, Montana, Utah, Wyoming). Currently there are no effective management procedures for mitigating the effects of this disease because it is not fully known why the parasite has severe effects in some trout populations while remaining fairly benign in others. Characteristics of the parasite, hosts, environment, and their interactions, may partially explain varying responses of wild rainbow trout populations to whirling disease. The goal of this study was to examine possible contributors to, and indicators of, stream degradation and their relationship to whirling disease risk. Specifically, the objectives were to quantify relationships between whirling disease risk and 1) land use 2) biological stream integrity and 3) physicochemical parameters within four major drainages in western Montana. The hypothesis was that whirling disease risk is influenced by anthropogenic land use practices that create favorable habitat for the oligochaete worm host, <i>Tubifex tubifex</i>, which is reflected in the biological integrity and physicochemical features of the stream. Whirling disease risk was quantified by the severity and prevalence of infection in caged sentinel trout. A geographic information system (GIS) was used to model land use (e.g., agriculture, mines) within drainages. Bioassessment metrics specific to Montana (e.g., total taxa richness, sensitive taxa richness) and those directly related to whirling disease (e.g., density of <i>T. tubifex</i>) were used to assess biological stream integrity. Physicochemical characteristics included those associated with favorable <i>T. tubifex</i> habitat (e.g., substrate), and those that have been associated with an increased incidence of whirling disease (e.g., temperature). Importance of predictor variables was assessed using Spearman's rank correlation and regression tree analyses. Final regression trees identified the proportion of riparian forest, road density, oligochaete density, <i>Limnodrilus hoffmeisteri</i> density, Plecoptera taxa richness, and the proportion of Plecoptera as the most important predictors of whirling disease risk among drainages. A greater understanding of the linkage between land use, biological stream integrity, physicochemical features and whirling disease risk is needed before effective management techniques can be implemented in Montana watersheds and elsewhere.</p>				

<p>Project #6.</p>	<p><i>The viability of Myxobolus cerebralis myxospores after passage through the alimentary canal of avian piscivores in the Greater Yellowstone Ecosystem</i></p>	<p>Todd Koel, Supervisory Fisheries Biologist, Fisheries and Aquatic Sciences Section, Center for Resources PO Box 168, Yellowstone National Park, WY 82190 and Affiliate Professor, Department of Ecology, Montana State University Phone: 307-344-2281 todd_koel@nps.gov</p>	<p>Billie Kerans, Associate Professor Department of Ecology Montana State University 311B Lewis Hall Bozeman, MT 59717 Phone: 406-994-3725 Fax: 406-994-3190 bkerans@montana.edu</p>	<p>\$59,415 \$34,800</p>
<p>Project Summary: Whirling disease, caused by the exotic parasite <i>Myxobolus cerebralis</i> is responsible for severe declines in wild trout populations in the Intermountain West, including a decline in native Yellowstone cutthroat trout <i>Oncorhynchus clarki bouvieri</i> from Yellowstone National Park. Unclear is the vector for dissemination of whirling disease. Obvious vectors include the movement of myxospores by humans (anglers and their gear) or by fish-eating wildlife, especially those capable of traveling long distances in a short period of time such as avian piscivores. The overall goal of this study was to determine the potential of highly mobile avian piscivores, including American white pelicans, great blue herons, great egrets, and double-crested cormorants as dispersal vectors for <i>M. cerebralis</i>. The specific objectives were to determine if 1) <i>M. cerebralis</i> can be detected, and 2) <i>M. cerebralis</i> remains viable, following consumption and passage through the gastrointestinal tract of these avian piscivore species. Rainbow trout <i>Oncorhynchus mykiss</i> (6 weeks post hatch) were infected by <i>M. cerebralis</i> by exposure to TAMs. Biologists at the USDA APHIS National Wildlife Research Center Mississippi Field Station captured 6 each of American white pelicans, double-crested cormorants, great blue herons, and great egrets and transported them to an aviary at Ft. Collins, Colorado for disease challenges. Three birds of each species were simultaneously fed 10 infected trout, whereas three were given certified disease-free placebos. Fecal material was collected prior to experimental feeding and subsequently each day for 10 days after feeding. A one-gram fecal sub-sample from each bird were sent to Pisces Molecular, Colorado for genetic analysis to detect <i>M. cerebralis</i> DNA. Another 1-gram sub-sample was used to test for infectivity in <i>T. tubifex</i> in the ecology laboratory at Montana State University. The investigators found <i>M. cerebralis</i> DNA in the feces of all birds (12 total) fed infected fish; however only the feces from the great blue herons fed infected fish induced triactinomyxon (TAM) production by <i>T. tubifex</i> held in laboratory cultures. Thus, the study confirms the ability of herons to vector <i>M. cerebralis</i> among aquatic habitats in the Greater Yellowstone Ecosystem and elsewhere. The results were more equivocal for the other three bird species as <i>M. cerebralis</i> DNA was found in their feces, but no TAMs were produced by tubificids exposed to infected feces. These results may be caused by an unknown aspect of the experimental protocol or perhaps real differences in effects (digestion/deactivation) of the gastrointestinal tracts of these bird species on myxospores. Only through replication of this work will confirmation of the ability (or lack thereof) of pelicans, cormorants, and egrets to vector <i>M. cerebralis</i> be obtained.</p>				

Project #7.	<i>Characterization of whirling disease resistance patterns in rainbow trout from Harrison Lake, Montana: classification of resistant and susceptible individuals and elucidation of the effects of recent natural selection</i>	Eric Wagner, Director of Research Fisheries Experiment Station Utah Division of Wildlife Resources 1465 West 200 North Logan, UT 84321 Phone: 435-752-1066; Fax: 435-797-6977 ericwagner@utah.gov	Chris Wilson, Certified Fish Pathologist, Utah Division of Wildlife Resources chriswilson@utah.gov	\$83,816.24 \$13,725
<p>Project Summary: The goals of this project were to:</p> <ol style="list-style-type: none"> 1) Understand temporal changes in whirling disease resistance patterns that may have occurred within the population following parasite establishment in the mid-1990's; 2) Identify a panel of genetic markers that may be associated with whirling disease resistance in rainbow trout; and 3) Determine if genetic markers identified in goal #2 hold utility for better understanding processes that have occurred within the Harrison Lake population. <p>To address the first goal, the investigators performed a quasi-temporal analysis of resistance patterns within the Harrison Lake population by examining resistance phenotypes of progeny from different age classes within the population. Their findings suggest that natural selection for parasite resistance has made the population more resistant over time. For the second goal, they performed a Quantitative Trait Locus (QTL) mapping study to identify resistance-linked genetic markers. This investigation examined over 700 genetic markers, and identified a subset of 62 loci on a small subset of the rainbow trout genome that are linked to disease resistance. In the third investigation, they examined markers identified under objective #2 in a temporal cross section of 170 fish from Harrison Lake. This analysis allowed them to determine if any loci bore the signal of the natural selection process and provided additional evidence for tight linkage to resistance associated genes. Though additional confirmations may be required, the researchers identified a subset of 6 loci that may be more closely associated with resistance.</p>				

<p>Project #8.</p>	<p><i>Assessing the density and distribution of Tubifex tubifex lineages in Windy Gap Reservoir, Colorado</i></p>	<p>Dana Winkelman, Leader Colorado Cooperative Fish and Wildlife Research Unit Room 201, Wagar Building Colorado State University Fort Collins, CO 80523 Phone: 970-491-1414 Fax: 970-491-1413 dlw@cnr.colostate.edu</p>	<p>R. Barry Nehring, Aquatic Researcher Colorado Division of Wildlife 2300 S. Townsend Avenue Montrose, CO 81401 970.252.6008 barry.nehring@state.co.us</p>	<p>\$31,758 \$83,726</p>
<p>Project Summary: Windy Gap Reservoir (WGR), a 38 hectare impoundment on the Colorado River in Middle Park, Colorado, has been a major point source of production of triactinomyxon (TAM) actinospores of <i>Myxobolus cerebralis</i> (<i>Mc</i>) since the 1990s. Two separate studies in 1998 identified the area of the lake receiving the inflow of water from the Colorado River as the zone where <i>Mc</i> infection was highest among <i>Tubifex tubifex</i> worms and also the area where TAM production was highest. Beginning in 2001, TAM production in WGR declined dramatically and remained low through December 2006. The objectives of this 2005 study were two-fold: to develop a spatial distribution of the various lineages of <i>T. tubifex</i> in WGR during the open water period, and, determine if TAM production in WGR is inversely proportional to the relative abundance of non-susceptible (lineage V) <i>T. tubifex</i> and/or positively correlated with the relative abundance of the <i>M. cerebralis</i>-susceptible lineages. Results suggest that the density of aquatic oligochaetes and <i>T. tubifex</i> increased compared to 1998, and that lineage I and VI <i>T. tubifex</i> dominate the oligochaete population. This was not the case in 1998. The 2005 data also demonstrate that TAM production declined dramatically compared to the levels observed in 1998. The decline in TAM production is congruent with the dominance of the lineage I and VI <i>T. tubifex</i> in the oligochaete population. Field and laboratory studies indicate that these two lineages as well as lineage V worms are largely refractory to infection by <i>M. cerebralis</i>. The results of the study taken together with the findings of others support the hypothesis that the non-susceptible <i>T. tubifex</i> lineage worms (I, V and VI) are acting as biofilters that deactivate myxospores of <i>M. cerebralis</i> and thereby reduce ambient levels of TAM production. Colonization of aquatic habitats by lineage I, V and VI <i>T. tubifex</i> where <i>M. cerebralis</i> is enzootic could reduce ambient levels on infection and ameliorate negative impacts on wild trout populations by this parasite.</p>				
<p>Project #9</p>	<p><i>Investigating competition among lineages of T. tubifex and the potential for biological control of whirling disease in natural streams</i></p>	<p>Dana Winkelman, Leader Colorado Cooperative Fish and Wildlife Research Unit Room 201, Wagar Building Colorado State University Fort Collins, CO 80523 Phone: 970-491-1414 Fax: 970-491-1413; dlw@cnr.colostate.edu</p>	<p>Kevin Thompson, Aquatic Research Biologist Colorado Division of Wildlife 2300 S. Townsend Avenue Montrose, CO 81401 970.252.6037 kevin.thompson@state.co.us</p>	<p>\$56,080 \$35,747</p>
<p>Project Summary: The investigators did not report on the results of their project.</p>				

