Relating *Myxobolus cerebralis* infection in native Yellowstone cutthroat trout and *Tubifex tubifex* with environmental gradients at multiple spatial scales

Principle Investigators:

Dr. Billie L. Kerans
Associate Professor
Montana State University
Department of Ecology
311B Lewis Hall
Bozeman, MT 59717
bkerans@montana.edu
phone (406) 994-3725
fax (406) 994-3190

Dr. Todd M. Koel
Supervisory Fisheries Biologist
National Park Service
Fisheries and Aquatic Sciences Section
P.O. Box 168
Yellowstone National Park, WY 82190
todd_koel@nps.gov
phone (307) 344-2281
fax (307) 344-2211

Collaborators: Dr. Charlotte Rasmussen, Researcher, Western Fisheries Research Center, USGS-BRD, 6550 NE 65th St., Seattle, WA 98115
Crystal Hudson, Researcher, U. S. Fish and Wildlife Service, Bozeman Fish Health Center, Bozeman, MT.

Date: 1/9/2003

The spread of animal or plant pathogens to new hosts or geographic areas has caused incalculable ecological costs that include extinctions or range restrictions of native species with subsequent changes in community composition. For example, the invasion of avian malaria to the Hawaiian Islands reduced populations and restricted ranges of several native bird species. *Myxobolus cerebralis* (Myxozoa: Myxosporea), the metazoan parasite that causes "whirling disease" (WD) in several species of trout and salmon, has the potential to similarly affect native fauna in the Intermountain West of the USA. Endemic to Eurasia, the parasite invaded the US in the 1950's and has now spread to 23 states, where it has caused dramatic and highly publicized reductions in populations of nonindigenous, wild rainbow trout (*Oncorhynchus mykiss*). Cutthroat trout (CT) are indigenous to the Intermountain West, coexist in watersheds with RBT, and are highly susceptible to the parasite. This raises the possibility of further damage to many CT populations which are already under stress from the introduction of nonnative salmonids and other anthropogenic activities. Although Sipher and Bergersen suggested that infected Snake River CT (identical to the Yellowstone CT used in this study) may grow and survive better than infected RBT when subjected to similar conditions, little is known about effects of *M. cerebralis* on CT populations. Information is needed on these populations in order to conceive appropriate management strategies. The Yellowstone CT (YCT) subpopulations occurring in the Yellowstone Lake basin provide an excellent opportunity to study the parasite and its affects on YCT with varying life history patterns.

Much progress has been made examining how life history characteristics of RBT coincide with their infection risk and the physicochemical and biological attributes that correlate with infection risk. For example, Krueger found significant positive correlations between RBT infection risk and abundant fine sediments, slow water velocities and stable water temperatures in the Madison River, MT. In addition, *M. cerebralis* has a complex, two-host life cycle that alternates between salmonids and *Tubifex tubifex* (the oligochaete alternative host): salmonids produce myxospores that are infective to *T. tubifex* and *T. tubifex* produces triactinomyxons that are infective to salmonids. Krueger found that areas of high RBT infection risk had high abundance of *T. tubifex* releasing triactinomyxons. Moreover, geographic isolates of *T. tubifex* are genetically distinct and have widely different levels of triactinomyxon production. Such studies need to be extended to native CT populations so that appropriate management strategies can be undertaken.

Goals, Milestones and Outcomes

The goal of this study is to determine possible management strategies for native CT in the Intermountain West. We will examine spatial and temporal variation in WD risk to YCT populations, the biological (including infection in tubificids and wild YCT) and physicochemical factors which correlate with infection risk, and the life history characteristics of YCT that possibly allow some subpopulations or individuals to have low risk of infection. The objectives of the study were as follows:

1) Conduct a detailed examination of YCT infection risk in three spawning streams (Yellowstone River, Pelican Creek, Clear Creek) where past studies have shown varying disease risk using sentinel YCT, tubificid assemblages, and prevalence of infection in tubificids,

2) Examine infection in spawning adult YCT to determine potential spore loading of streams and in wild reared young to determine infection prevalence and severity in wild fish,

3) Examine the potential physical and chemical features of these three streams and determine

which factors best explain infection risk, prevalence and severity in YCT and tubificids, and

4) Determine the YCT life history patterns most likely to avoid infection by *M. cerebralis*.

5) Continue to monitor large-scale spatial and temporal variation in risk of infection to YCT in the other YCT spawning tributaries representing a wide-range of temperature and flow conditions, and correlate infections with large, tributary-basin scale explanatory physical variables.

Progress to Date

Objective 1: Detailed examination of YCT infection risk

Part A. Salmonid in situ enclosure studies: We monitored disease risk using in situ enclosures of sentinel YCT at three reaches in each of the Yellowstone River, Pelican Creek and Clear Creek during the summer of 2002 Two in situ enclosures were placed in each reach during three, 10 day time periods: early July, early August and early September. In each enclosure, 60 age-zero YCT (3+ weeks post hatch) were exposed for a 10-day period. During the period of exposure, both water temperatures (continuous temperature loggers) and stream discharge will be monitored. After the exposures, all fish were removed and transported to the Wild Trout Laboratory at Bozeman and held for an additional 90 or 150 days before being sacrificed for histological examination and confirmation of *Myxobolus cerebralis* by PCR. The fish from the first exposure period have been sacrificed and prepared for histological examination and PCR. The remaining fish will be sacrificed during the next two months.

Part B. Tubificid assemblages and prevalence of disease in worms: We examined the assemblages of tubificids and other invertebrates in each of the nine reaches. We took six core samples iin the soft sediments of each of the nine reaches close to the time that the in situ enclosures were deployed in the streams. Invertebrates were preserved in 10% formalin and brought back to the laboratory where the invertebrates were removed from the cores and worms were slide mounted. To date we are in the process of identifying invertebrates and worms.

Prevalence of infection in *T. tubifex* was examined by collecting live worms from the reaches in each intensive stream. Worms were brought back to the laboratory and placed in 12-well plates and water will be examined microscopically for TAMs. Worms releasing TAMs were sent to Charlotte Rasmussen, USGS Western Fisheries Research Center, for PCR analyses to confirm that the TAMs are *M. cerebralis* and to examine the genetics of the geographic variants of *T. tubifex* found in Yellowstone National Park. 185 live oligochaetes were collected from streams in YNP with 112 worms collected from the three study reaches. Of these, 46 out of 185 and 4 out of 112 were found to be releasing actinospores; however, PCR tests showed that none of the actinospores were *M. cerebralis*. Although the PCR tests for most of the remaining worms have not been completed, we recently found two worms (one from the Yellowstone River and one from Pelican Creek) that tested positive for *M. cerebralis* using PCR (the first detection of *M. cerebralis* is worms in the Park).

Objective 2: Determination of *M. cerebralis* spore loading and infection in wild young in spawning streams

In addition to examination of sentinel cage YCT, we sacrificed several adult YCT as they ascended the Yellowstone River, Pelican Creek, and Clear Creek. Heads of these adult trout were sent to the Bozeman Fish Health Laboratory for pepsin-trypsin digest and PCR

confirmation for the presence or absence of *M. cerebralis*. Spore counts of these adult heads will also be conducted. In early June, Yellowstone National Park fisheries biologists attempted collection of adult YCT in lower Pelican Creek near the site of the historic spawning migration trap. At one time this trap was used to collect thousands of upstream migrating YCT. Few adult YCT were found in Pelican Creek during what was once a seasonal period of intense use by these fishes.

We also collected wild-reared, age-zero YCT at multiple locations of these intensive streams. Wild YCT fry collected for infection examination will be transported to the NPS Lake Aquatic Sciences Laboratory and held in living stream tanks for at least 90 days to allow disease development, prior to being sacrificed and tested for *M. cerebralis* infection by the Bozeman Fish Health Center (methods as in Objective 1). Wild fry were collected from the Yellowstone River and Clear Creek, although no wild fry were found in Pelican Creek. Post-collection mortality was high for the wild fry collected in the Yellowstone River, but not in those collected in Clear Creek. The early collections of these fish have been sacrificed and are being prepared for histology and PCR. Although we need to examine Pelican Creek even more closely during 2003, evidence from fry and adult collections suggests that we have already experienced a significant loss of the Pelican Creek spawning population. Since *M. cerebralis* has been detected in adult YCT lakewide, the potential exists for this parasite to cause similar declines in other tributaries.

Objective 3: Explanatory physicochemical attributes

Each reach will be mapped and physical features will be measured during baseflow conditions in the summer of 2003, thus we have no results to date.

Objective 4: Determination of YCT life history patterns most likely to avoid infection

We will use historical and on-going NPS long-term counts of spawning YCT in streams to determine peak and duration of spawning times in intensive streams. Determination of seasonal variation in *M. cerebralis* infection risk will allow prediction of potential shifts in timing of YCT peak spawning migration.

Objective 5: Large-scale variation in WD risk and explanatory physical variables

In situ enclosures of sentinel YCT were also established at 12 tributaries to Yellowstone Lake and the Yellowstone River. These streams have been monitored for *M. cerebralis* infection in both YCT and *T. tubifex* in previous years. Onsite remote thermographs were maintained at each site for daily temperature monitoring.

Explanatory large-scale physical variables were derived from NPS geo-spatial datasets located at the Yellowstone Center for Resources. As *M. cerebralis* infection disperses into additional tributaries to Yellowstone Lake, we will be able to relate infection spread to basin characteristics and stream temperature regime. Nearly all (50 total) lake cutthroat spawning tributaries now are monitored for hourly temperatures throughout the year. The 12 streams studied in 2001 were also fitted with water level loggers. In these streams we will be able to also relate flow regime to any future *M. cerebralis* infections. Quantification of environmental characteristics preferred by *M. cerebralis* in the Yellowstone Lake basin will assist fisheries managers in predicting probable areas with high risk of infection. Tributary basins with landscape-level characteristics similar to Pelican Creek include Beaverdam Creek, Trail Creek, and Chipmunk Creek in the remote South

and Southeast arms of Yellowstone Lake. This research will assist efforts to preserve these YCT, as Yellowstone Lake and its tributaries represent the last stronghold for what is the largest genetically pure inland cutthroat trout population in the world.