INVESTIGATING WHIRLING DISEASE in
the UPPER BIG LOST RIVER BASIN
of MACKAY, IDAHO

by

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DEDICATION

To the Corn Palace of Mitchell, South Dakota.
ABSTRACT

Whirling Disease is a parasitic disorder causing skeletal deformities in susceptible salmonids. Trout Unlimited, a non-profit conservation and sportsfishing organization, is interested in the disease because it has been implicated in the population declines of inland trout species that occurred in the early 1980s in the upper Big Lost River Basin of Mackay, Idaho. Presence/absence and severity surveys were conducted in the basin previously, but not in the past decade. Rainbow trout sentinel fry exposures and wild fish monitoring was conducted in connected tributaries to determine the current distribution and prevalence of the disease, to identify streams where trout populations are unaffected and reproducing successfully and to guide future management. Distribution of the disease is similar to prior assessments, but severity of infection seems to have declined. Waters testing positive for Whirling Disease have temperature regimes that are significantly warmer than negative waters 36.5% of days surveyed. Infected waters also have significantly larger brook trout fry, suggesting early emergence of trout fry may coincide with high concentrations of the parasite that causes Whirling Disease.
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INTRODUCTION

Whirling Disease

Whirling Disease (WD) is a salmonid disorder caused by the myxozoan parasite, *Myxobolus cerebralis*. It was first discovered in Germany in 1903 and described by Hofer (1903). The disease was first discovered in the United States in 1956 in Pennsylvania (Hoffman 1962). WD was confirmed in Idaho in 1987 (Hauck et al. 1988). Its distribution throughout North America has been summarized by Bergensen and Anderson (1997) and Bartholomew and Reno (2002).

Despite its century-old discovery, little was confirmed about the ecology of *Myxobolus cerebralis* until Wolf et al. (1986) determined the parasite has a two-host life cycle and exists as two distinct life forms between the hosts (Figure 1). The first host is the obligate oligochaete, *Tubifex tubifex*. The alternate host is a salmonid fish. Whereas *T. tubifex* is the only known oligochaete that can host the parasite, many salmonids can carry *M. cerebralis*, albeit with varying levels of susceptibility (MacConnell and Vincent 2002, Vincent 2002).

Rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) are the salmonids relevant to this study in the upper Big Lost River basin. Rainbow trout are considered the salmonid species most susceptible to *M. cerebralis* infections. Brook trout are also highly susceptible to the parasite (Hedrick et al. 1998).

*Myxobolus cerebralis* is released into waters in the form of multi-cellular elliptical myxospores when infected fish die. The parasite can also be distributed via the feces of piscivorous birds or animals that have eaten infected fish and when fishing gear used in infected waters is subsequently used in clean waters without being properly disinfected (Stromberg 2006). The myxospore form of the parasite is very hardy and various experiments have shown it to be capable of surviving drought, freezing temperatures and other severe conditions (Kerans and Zale 2002, Gilbert and Granath 2003).
Tubifex worms ingest myxospores released into the water column. Tubifex can be found in various habitats, but are best known for being well adapted to surviving in silty, eutrophic, organic and polluted waters (Hedrick et al. 1998). Inside the guts of *T. tubifex*, the parasite transforms into its alternate form: a three-legged triactinomyxon, also known as a TAM.

Mature TAMs are released back into the water by the worm based on a temperature cue. While various studies have investigated the temperature range when TAM release occurs, the most widely accepted estimate is water temperatures ranging from 10 to 15°C (El-Matbouli et al. 1999). TAM release drops dramatically above and below this range. *M. cerebralis* in its TAM form is not as hardy as a myxospore and can only survive in the water column from a few hours to two weeks (Markiw 1992).

If susceptible salmonids are in the water column when TAMs are present, the parasite can infect the fish by attaching to its epithelial cells. From there, TAMs quickly enter the skin of the fish and progress through the nervous system to the skeletal cartilage (Hedrick et al. 1998, MacConnell and Vincent 2002). The parasite will attack cartilage, forming lesions, deforming the skeleton and causing inflammation. This can put pressure on the central nervous system and cause the fish to swim in an erratic, *whirling* manner, thus giving the disease its name. Skeletal deformations altering swimming behavior can make foraging difficult and can increase the likelihood of fish predation. Other clinical signs of the disease include a dark discoloration of the fish tail known as Black Tail. *M. cerebralis* can also leave infected fish more vulnerable to other environmental stressors and severe infections can cause direct fish mortality (Hedrick et al. 1998, MacConnell and Vincent 2002, Gilbert and Granath 2003).

Young fish are more susceptible to *M. cerebralis* than older fish because their skeleton is still largely cartilaginous. Ossified skeletal structures are more resistant to *M. cerebralis* spores, so resistance to infection increases with increasing size and age (Hoffman and Byrne 1974, O’Grodnick 1979, Markiw 1991). Ryce and Zale (2001) showed rainbow trout fry gained some age-based resistance to infection when their first exposure to *M. cerebralis* was delayed to 9
weeks, compared to 7 weeks post hatch. TAMs can still infect grown salmonids because fish skeletons never completely ossify. However, grown fish are unlikely to suffer clinical signs of the disease. When they die, they are still capable of releasing myxospores into the water column, continuing the *M. cerebralis* life cycle (MacConnell and Vincent 2002).

**History of the Upper Big Lost River Basin**

Whirling Disease has been implicated in the severe declines of inland trout populations that occurred in the early 1980s and continued through the mid-90s in the upper Big Lost River basin of Mackay, Idaho (Gregory 2005).

Located in central Idaho, the Big Lost River is the largest of the region’s “sinks” drainages. The Big Lost River earns its name because downstream geology causes its waters to sink completely underground approximately 90 river miles below its headwaters. The upper region of the Big Lost River encompasses approximately 508 square miles and officially includes waters upstream of Chilly, where the river makes a right turn, changing flow direction from northeast to southwest. For this analysis, the study area also includes river hydrology downstream to the Mackay Dam (Figure 2).

The upper Big Lost River basin is comprised of several major forks. The North Fork flows from the west. Summit creek flows northeast from the central region into the North Fork. Wildhorse Creek flows north from the central region of the basin. Starhope Creek also flows north to drain much of Copper Basin and converges with the northwest-flowing East Fork. The Big Lost River proper begins at the convergence of the North and East forks approximately 34 river miles upstream from the town of Mackay, Idaho.

Paiute sculpin (*Cottus beldingi*), shorthead sculpin (*Cottus confuses*) and mountain whitefish (*Prosopium williamsoni*) are native to the upper Big Lost River (Gamett 2003). The upper Big Lost River (UBL) also hosts several salmonid species, including wild brook trout
(Salvelinus fontinalis) and rainbow trout (Oncorhynchus mykiss). However, because the UBL is disconnected from other rivers, trout are not native (Gamett 2003). Trout species were first stocked in the river in the early 1800s and the region grew notorious for being an excellent fishery into the next century.

Trout populations declined in the early 1980s. While anecdotal, stories of the population declines during the early 1980s were confirmed by notable sources, including many local fly-fishing outfitters who stopped bringing clients to the UBL because the fishing became poor (Gregory 2005). Regular population monitoring had not previously been conducted in the UBL and Corsi (1989) provided the first estimates of trout numbers in 1986.

Dramatic declines in trout populations during the 70s and 80s were taking a toll in the world-renowned freshwater fisheries of neighboring states, like Colorado and Wyoming, where fly-fishing presents a significant contribution to the state’s ecotourism economy. Whirling Disease was being investigated as the culprit (Nehrig and Walker 1996, Vincent 1996). In 1987, officials first tested for Whirling Disease in the major rivers of Idaho and discovered WD-positive waters in the Big Lost River (Hauck et al. 1988, Elle 1998). Gamett (2004) provided estimates a decade later, showing that 1996 populations for brook and rainbow trout had continued to fall to less than 10% of Corsi’s 1986 estimates. A series of Wild Fish Health Surveys, conducted by the US Fish & Wildlife Service, began in the UBL in 1996 but were mostly collected in 1999. These helped to better illustrate the distribution of Whirling Disease in tributaries throughout the UBL (USFWS National Wild Fish Health Survey).

The analysis of the changing dynamics of Whirling Disease in the upper Big Lost River basin is important to Trout Unlimited. TU is a national non-profit conservation and sports-fishing organization with a mission “to conserve, protect and restore North America’s coldwater fisheries and their watersheds.” The organization became interested in the Big Lost River basin in 2004 during the proposal period to petition the native Big Lost Mountain Whitefish, Prosopium williamsoni, for protection under the Endangered Species Act. Additionally, the local chapter of
TU identified the UBL as a priority site for protection in light of the unexplained declines in trout populations there (K. Goodman, Trout Unlimited, *pers. comm.*).

While investigating threats to fish populations in the basin, TU discovered a concern regarding sufficient fish spawning habitat and a few seasonal obstructions to fish migrations along the river, caused by irrigation diversions. Additionally, the Big Lost Mountain Whitefish is susceptible to Whirling Disease. In 2005, TU commissioned a “desk assessment” of threats to fish populations in the UBL. Through a process of elimination, Gregory (2005) narrowed down likely scenarios for the drop of trout populations, ruling out fish related mortality, fish habitat degradation, water quantity, grazing, water quality and fish stocking. The desk assessment pointed to Whirling Disease as the final probable cause for trout population declines in the UBL. In response, in 2006 Trout Unlimited commissioned this investigation to focus on the current state of Whirling Disease in the basin and learn about its potential role in the trout population declines of the early 1980s.

**Goals of the Study**

*Determine the current status of Whirling Disease in the upper Big Lost River basin.*

Data regarding the presence, distribution and severity of WD infections in the upper Big Lost River basin are sparse, with the last (and only) sentinel exposures collected a decade ago (Elle 1997) and the most recent wild fish surveys occurring in 1999 (USFWS National Wild Fish Health Survey). As such, it is important to collect information on prevalence and severity of Whirling Disease from tributaries within the basin to determine the current state of the disease and how it has changed over time.

After several years of drought, 2006 proved to be a high water year (J. Gregory, Gregory Aquatics, *pers. comm.*). Previous studies of the distribution of WD in this basin all occurred
during drought conditions. Collection of WD data during this high water year will be a pertinent component of the long-term monitoring and comparison of the disease in the basin over time.

**Identify major WD-positive and negative tributaries in the upper Big Lost River basin.**

Although trout population estimates conducted in 2003 have suggested recent increases in brook trout populations, rainbow trout abundance is still low relative to historic numbers. Stocking resistant fish and restoring spawning habitat may be necessary to assist in recovery of the fishery (Gregory 2005). Identification of “clean” waters and WD infected streams is critical to this approach. Because grown fish can become infected with *M. cerebralis* without showing clinical signs and release spores when they die, it is desirable that fish be stocked into WD-free waters to decrease the likelihood they spread the parasite. Identifying infected tributaries will also help Trout Unlimited, state and federal agencies prioritize management approaches that attempt to disrupt the Whirling Disease life cycle.

**Investigate connections between fry emergence timing and water temperature regimes.**

Release by tubifex worms of the triactinomyxon, or TAM, form of *M. cerebralis* occurs during a temperature cue of 10-15°C (El-Matbouli et al. 1999). Young-of-year fish fry emerging from the redd before water temperatures enter this range may be able to grow to or past size- or age-based resistance to the disease before TAMs are released, thus decreasing infection rates in those tributaries. Collecting fry emergence data and comparing the temperature regimes of streams that test WD-positive against those testing WD-negative may reveal insight regarding how the timing of fry emergence determines the distribution of the disease in the basin.
METHODS

Sentinel Fish Exposures

Lab-reared, disease-free, Hayspur-strain rainbow trout fry (mean size of 0.9g) were obtained from the Idaho Department of Fish and Game hatchery in Mackay, Idaho. Fifty to fifty-five fry were placed in sentinel live cages at each of five sites.

Cages were homemade of 3/16” Nytex mesh aluminum and cylindrical-shaped (250 sq in, 18” length, 12” diameter). These cages have been used by IDFG for a decade and have proven more sensitive to Whirling Disease infection in deep water than those recommended by the Whirling Disease Foundation and used by Elle (1997) (K. Johnson, IDFG, pers. comm.).

Five sentinel exposure sites were selected in the Big Lost River Drainage (Figure 2). Two sites were selected to replicate sentinel exposures in 1996 (Elle 1997) on the East Fork of the Big Lost River. These sites include Copper Basin, located in the headwaters, and Castle Rock, located between Starhope and Wildhorse Creeks.

New sites for the sentinel exposures were selected at Chilly Slough, Warm Creek and Trout Haven above their respective confluences with the Big Lost River. Chilly Slough is located near the intersection of Highway 93 and Trail Creek Road. Warm Creek is in the Barton Flats region approximately 7 river miles above the Mackay Dam and 1 mile below the outflows of two fish hatcheries (one private, one operated by IDFG). The Trout Haven site is located approximately one river mile below the Mackay Dam along a private well spring.

Sentinel live cages were submerged and fixed in place at areas of relatively slow velocity so as not to excessively tax the animals. Onset StowAway XTI model temperature loggers were placed in white hard plastic water-proof housings and secured to the interior of the sentinel exposure cages with wire (Onset Computer Corporation, Bourne, Massachusetts). Loggers recorded temperature in Celsius at 30 minute intervals. Temperature loggers at sentinel fish study sites recorded throughout the 10 day exposure trials.
Additional landscape and water chemistry parameters were measured at time of initial fish exposure and at retrieval. Location and altitude was recorded using a Garmin eTrex Legend model hand-held GPS unit, according to the 1984 World Geodetic System (Garmin International, Inc., Olathe, KS). Flowing-water stream width was recorded with measuring tape. Habitat type was qualified generally according to topography (i.e. “flat valley,” “v-shaped”) and stream behavior (i.e. “meandering,” “high flows”) near the field sites. Dissolved oxygen (% saturation), conductivity (µS/ccm) and pH were measured using a Yellow Springs Instrument model 556 MPS (Yellow Springs Instruments, Yellow Springs, Ohio). Water flow velocity (ft/sec) at the sentinel cage was measured using a Marsh-McBirney Flo-mate model 2000 (Hach/Marsh-McBirney, Inc., Frederick, Maryland). Turbidity (FTU) was measured with a Hanna Instruments model 93703 meter (Hanna Instruments, Woonsocket, Rhode Island).

Exposure of sentinel fish began at each site on 12 June 2006 and lasted until 22 June 2006, which was near the time of season when Elle (1997) found the greatest clinical signs of WD among sentinels.

Sentinel trout fry were collected after 10-day exposure. Fry were placed in 10L restaurant milk bags, with their groupings maintained according to site. Transport bags were marked with site ID using indelible labels. The bags held 2L of water from the site and 5L of pure medical-grade oxygen, which was delivered from regulators to the bag using new disposable pipettor tips at each site. During transportation, the bags were stored in a large cooler and ice was added to maintain the temperature at 13°C (±3°C) to match the water temperature at the final destination.

Sentinel fry were immediately transported to the Idaho Department of Fish & Game (IDFG) Eagle Fish Health Lab (EFHL) wet labs in Eagle, Idaho. Each group was placed in its own 37L tank. Trout were reared in parasite-free, flowing well water, maintained at 13°C. Trout were fed three times a week and checked for mortalities daily.
Ten randomly-selected sentinel trout from each exposure location were sacrificed at 936 Celsius Temperature Units (CTU). This occurred on 1 September 2006, at 72 days post-exposure (72 d * 13°C = 936 CTU). Heads from each fish were bisected along the mid-sagittal line. Infection severity was measured in half-heads using the MacConnell/Baldwin (M/B) scale according to IDFG EFHL wet lab protocols (K. Johnson, IDFG, pers. comm.). The M/B scale ranks infection severity from 0 (no infection) to 5 (extreme infection) (Vincent 2002). (See Appendix I for further description of M/B histology lab scores.)

The remaining sentinel trout were lethally anesthetized at 1313 CTU on 1 October 2006 at 101 days post-exposure. Heads from each fish were split along the mid-sagittal line. *Myxobolus cerebralis* spores were quantified in the cranial tissues of half-heads using the quantitative Pepsin-Trypsin Digest (qPTD) method according to IDFG EFHL wet lab protocols (K. Johnson, IDFG, pers. comm.). The qPTD test is normalized to estimate the number of *M. cerebralis* spores per fish head.

**Wild Fish Monitoring**

Wild trout fry and juvenile trout were surveyed in various tributaries of the upper Big Lost River to understand the prevalence and distribution of Whirling Disease.

Site selection for wild fish monitoring was based on previously conducted Wild Fish Health Survey sites (USFWS National Wild Fish Health Survey). Additional sites were selected based on hydrological connectivity, local knowledge of potentially vulnerable trout populations and suggestions from environmental organizations and state and federal agencies interested in obtaining additional information within the basin. Wild fish data was collected along tributaries just above their confluence with the larger river fork. To explore the effects of elevation on trout reproduction and WD distribution, some larger tributaries hosted both upper- and lower-reach field sites (Figure 2).
Final site locations for wild fish monitoring were chosen after stretches of each tributary were walked to identify locations of suitable salmonid habitat and available spawning locations. Accessibility and hydrological connectivity were also taken into account in the site selection process, ensuring there were no barriers to potential fish movements along the tributaries. Site locations were recorded in 1984 World Geodetic System (WGS 84) using a Garmin eTrex Legend model hand-held GPS unit (Garmin International, Inc., Olathe, KS). Landowner permission was obtained for sampling on private property.

StowAway XTI model temperature loggers were placed in white hard plastic water-proof housings and installed in fixed, shaded locations at each study site (Onset Computer Corporation, Bourne, Massachusetts). The temperature loggers were affixed to weighted anchors with wire and fully submerged throughout the duration of the study. Loggers recorded temperature in Celsius at 30 minute intervals. Temperature loggers were deployed at wild fish monitoring sites between 27 and 29 June 2006, with one site beginning on 6 July 2006, because road flooding had previously prevented access to the site. Loggers were removed between 15 and 20 August, 2006, in approximately the same order they were first installed.

Collection of wild trout fry (age 0) was conducted concurrently with collection of wild juvenile trout (ages 1-2). Tributaries were electro-fished beginning 25 meters downstream and moving 25 meters upstream of each temperature logger (50m total) using a Smith-Root 15c, gas-powered backpack electro-fisher (40Hz, 4ms, 300-400 volts) (Smith-Root, Inc., Vancouver, Washington). Wild trout were collected with dip nets.

Wild trout fry were collected to determine average population growth rates and species emergence dates for wild young-of-year brook and rainbow trout fry at each site. Fry surveys were conducted twice at each site. Sites were first surveyed between 11 and 20 July 2006 and again between 7 and 20 August 2006 in approximately the same order.

To collect trout fry, pools and eddies of relatively low to no flow were electro-fished. Brook and Rainbow trout fry were captured and placed in a shaded 5-gallon bucket partially filled
with river water, without anesthetics. When 20-30 fry were captured or the end of the 50m stretch was reached, species and total fry length was recorded for each fish. After being measured, fry were immediately returned to a low flow portion of the river. Absence of fry was recorded for appropriate sites.

Wild juvenile trout, ages 1-2, were collected to determine the distribution of WD among salmonids. At this age, infected wild fish would host *M. cerebralis* spores, but are unlikely to have migrated from the stream in which they were born. Fish Age 1 and older were immediately placed in shaded 5-gallon buckets partially filled with river water. Any fish that was obviously larger than 200mm in total length was immediately returned to the river down stream of the surveyor.

Captured juvenile fish were measured for total length, species type was recorded and fish were sacrificed by asphyxiation. A maximum of 20 fish each of rainbow and brook trout were sacrificed at each site, based on requirements for wet lab techniques. Sacrificed fish were separated by site, species and date (if collection occurred on more than one day), stored in sealed zip-loc freezer bags, labeled with indelible marker and placed in a cooler of ice. Fish samples were frozen immediately upon return to the field station.

Frozen wild juvenile fish samples were sent to the IDFG Eagle Fish Health Laboratory in Eagle, Idaho. After thawing, fish heads were bisected along the mid-sagittal line. Half-head samples of the same species collected on the same site and date were pooled into groups. *Myxobolus cerebralis* spores were quantified in the cranial tissues of half-heads using the quantitative Pepsin-Trypsin Digest (qPTD) method according to IDFG EFHL wet lab protocols to test for prevalence of WD infection among the wild juvenile trout (K. Johnson, IDFG, *pers. comm.*). If the pool of fish returned WD-negative, all fish in the pool were marked as negative. If the pool tested WD-positive and time permitted, the second half-heads from each fish in the pool were tested by qPTD individually to estimate severity of infection in each fish.
Data Analysis

Raw data were available for M/B histological examinations of 1996 sentinels at Copper Basin and Castle Rock. These data were compared to 2006 results at the same exposure sites using t-Tests assuming unequal variances. Exposure intervals in 2006 did not overlap with those conducted in 1996. June 2006 data were compared both to the data from the 1996 exposures occurring immediately before and after the 2006 exposure interval (June and July only) and to the entire set of 1996 data (June through August).

Some wild fish monitoring sites were omitted from further analysis for various reasons. Pinto and Rock Creeks and the Upper Reach of the North Fork did not host any wild fish, most likely because they proved hydrologically disconnected. Wild fry and juvenile trout were collected from Broad Canyon Creek, but the site was omitted because lab samples were not returned.

Daily mean water temperatures were calculated from temperature logger outputs. Field sites were separated into two groups based on whether wild juvenile fish caught at the site tested positive and negative for Whirling Disease. Daily mean water temperatures for sites were analyzed between the two groups using T-tests to determine if temperature regimes of WD-positive sites significantly differed from those of WD-negative sites. A separate T-test was performed for each day of the summer.

Regression analysis was run to determine if wild brook trout fry length measurements collected at field sites within a single period (i.e. 11-20 July or 7-20 August) changed significantly with Julian date. No significant correlation was found during the mid-July ($R^2 = 0.003, P = 0.33$) or mid-August ($R^2 = 0.003, P = 0.33$) periods. Within each period, the total fry length data was averaged by field site. Sites were then sorted based on whether they tested positive or negative for Whirling Disease by the sentinel exposure or wild juvenile fish monitoring results. Two T-tests were performed to compare average fry lengths of WD-positive
sites to WD-negative sites for each period. Too few wild rainbow trout fry (n = 2 in July, n = 37 in Aug) were collected to perform the same analysis on that species.

For statistical analyses, significance was considered to be P < 0.5. Statistical analysis was conducted with JMP (SAS Institute, Cary, NC).

Wild juvenile trout lab results were compared to previous analyses of identical species at the same field sites. Current and historic lab results for sentinel and wild trout were plotted along maps using ArcGIS 9.2 for spatial analysis of the distribution of Whirling Disease (ESRI, Redlands, CA).
RESULTS

Sentinel Exposures

Ten randomly-selected sentinel rainbow trout from each of five exposure sites were examined for prevalence and severity of *Myxobolus cerebralis* infection after 10-d exposure using the modified MacConnell/Baldwin (M/B) 0-5 histological scale at 936 CTUs (Table 1). The remaining 40-45 sentinel rainbow trout from each of the five exposure sites were tested for prevalence and severity using quantitative Pepsin-Trypsin Digest (qPTD) at 1313 CTUs (Table 2). Two exposures, Copper Basin and Castle Rock, were replicated from 1996 exposures and the earlier M/B and qPTD data are repeated for comparison (Elle 1997).

According to histological data, sentinel trout infections by *M. cerebralis* for June 2006 exposures at Copper Basin were significantly lower than the combined exposures from June and July 1996 (P < 0.001) and the combined 1996 exposures from the entire summer, June through August (P < 0.001). Infections for June 2006 exposures at Castle Rock were significantly lower than the combined 1996 exposures in June and July (P < 0.05). However, infections from the 2006 Caste Rock exposures were not significantly less severe than the combined 1996 exposure data from the entire summer, June through August (P = 0.197).

Mean test scores for the five 2006 exposure sites show similar trends between the M/B histological and qPTD studies (Figure 3). With the exception of the Warm Creek site, sentinels in 2006 had lower mean infections of *M. cerebralis* than sentinels a decade prior, according to M/B histological test results.

All 2006 exposure sites yielded lower mean infection severity by qPTD than in 1996, with the exception of the August 1996 Castle Rock exposure (Figure 3). In 2006, only Warm Creek had an infection above 30,000 spores, the level above which clinical signs (i.e. whirling behavior) are seen in the fish. A single fish from this Warm Creek group was seen to exhibit
whirling behavior beginning 22 August 2006 at 82 d post exposure (K. Johnson, IDFGH, pers. comm.).

Infection was not detected in any sentinel fish (0% prevalence) from the Chilly Slough site (n = 52) by either M/B histology or qPTD. Sites that tested positive for *M. cerebralis* infection showed high levels of prevalence among sentinel trout, with 70-90% prevalence among fish examined by histological tests, and 90-100% prevalence among fish examined by qPTD (Figure 4).

**Temperature Regimes and Wild Fry Length Monitoring**

Throughout the summer, the average daily mean water temperature of the WD-positive sites was warmer than the average daily mean water temperature of the WD-negative group. On average, WD-positive sites had daily mean water temperatures 1.3°C warmer than those of WD-negative sites. T-tests performed between the two groups each day showed that the difference in temperature was significant (P < 0.05) 36.5% of the days of the summer.

Wild fry length data suggests brook trout fry most likely emerged during the last three weeks of June 2006 (Figure 5). Temperature regimes of individual streams show that with the exception of the Upper Reaches of Wildhorse Creek and the North Fork, brook trout fry at all sites would have spent at least five of the first nine weeks post-emergence in waters with average daily temperatures within the range that correlates to releases of the highest concentrations of TAMs (10-15°C) (El-Matbouli et al. 1999). (See Appendix II for temperature regimes of individual streams.)

Regression analysis showed there was no correlation between wild brook trout fry length and Julian date within either of the two periods of data collection in mid-July (R² = 0.003, P = 0.33) or mid-August (R² = 0.003, P = 0.33). Therefore, data were combined into "mid-July" and
“mid-August” collection groups. Within each of these collection groups, fry length data at each site were averaged and sites were split between those testing positive or negative for Whirling Disease based on sentinel exposures or wild juvenile fish monitoring test results (Figure 6). In mid-July, mean brook trout fry length was significantly larger (P < 0.01) in WD-positive waters (\( \bar{x} = 52.07 \) mm, \( n = 4 \)) than WD-negative ones (\( \bar{x} = 36.45 \) mm, \( n = 6 \)). In mid-August, mean brook trout fry length was again significantly larger (P < 0.05) in WD-positive waters (\( \bar{x} = 67.76 \) mm, \( n = 7 \)) than WD-negative ones (\( \bar{x} = 53.71 \) mm, \( n = 6 \)).

Too few wild rainbow trout fry were collected at field sites in mid-July or mid-August to identify trends in the data (Figure 7).

**Wild Juvenile Trout Monitoring**

Wild juvenile brook trout tested positive for *M. cerebralis* infection by qPTD at 6 of 14 field sites where the species was collected (Bartlett, Cabin, Corral and Deep creeks, Copper Basin and North Fork Low Elevation). Two of these sites, Bartlett and Deep creeks, did not previously test positive for WD. The lower reach of the North Fork had not previously been tested. No sites that tested positive for WD in the late 1990s tested negative for WD in 2006. (See Appendix III for full wild juvenile trout lab results and their comparisons to previous surveys.)

Wild juvenile rainbow trout tested positive for *M. cerebralis* infection by qPTD at 1 of 6 field sites where the species was collected (Cabin Creek). Rainbow trout at Cabin Creek previously tested positive for Whirling Disease.

Wild juvenile rainbow and brook trout testing positive for Whirling Disease can be found along the North Fork and East Forks. Summit and Wildhorse Creeks and the tributaries flowing into the Starhope Fork did not test positive for Whirling Disease. This general distribution reflects the findings of previous studies on wild trout along those forks, although far fewer wild rainbow trout could be collected than brook trout.
Lab tests on wild juvenile trout were conducted by testing pools of fish half-heads from each site. If tests for the pool were WD-negative, each fish in the test was considered negative. If the pool tested WD-positive, the remaining half-heads could be tested individually to determine exactly how many fish became infected. Many of the positive-testing pools were not followed-up with individual fish tests. Additionally, the number of fish in each pool was not kept consistent. This made it difficult to determine exactly how many wild trout tested positive at each site.

Figure 8 shows the spatial distribution of the lab results from the wild juvenile brook trout monitoring. Figure 9 represents the same data for wild juvenile rainbow trout. Solid pie charts represent data from 2006 and hollow pie charts represent previous years’ data. The size of each pie chart is proportional to the total number of fish collected and tested at that site, with the number repeated in text in the center of the chart. Wedges in red represent the number of known WD-positive fish collected at that site. Wedges in blue represent the number of known WD-negative fish. Purple wedges represent the number of fish of “unknown” status – those fish that were included in positive-testing pools, but were not further tested for individual status. If a pool tested positive and no follow-up was done on individual fish, a single fish from the pool was considered positive and the rest were classified as unknown. If a pool tested negative, all fish within the pool were considered negative.
DISCUSSION

Lab results from the sentinel trout data suggest that while Whirling Disease is still present in the Upper Big Lost River basin, severity has decreased in the past decade.

It is noteworthy that the 1996 histological studies (Elle 1997) graded *M. cerebralis* infection against the original MacConnell/Baldwin scale, which described infection severity from 0 (no infection) to 4 (extreme infection) (Baldwin et al. 2000). The 2006 samples were graded on a modified version of the M/B scale, which extended the scale to grade severity from 0 to 5. In the modified scale, the score of 5 was added to represent infections even more extreme that previously witnessed (Vincent 2002; K. Johnson, IDFG, *pers. comm.*). (See Appendix I for a comparison and full description of the modified scale.)

Of the 34 sentinel rainbow trout analyzed by the original 0-4 M/B scale in the Elle (1997) study, 23 (67.6%) were graded with the maximum score of 4. No additional information on the severity of those samples was available beyond their M/B scale scores. It is possible that some of these 23 fish may have had *M. cerebralis* infections so severe it would have warranted a score of 5 of the modified scale. If that is the case, some of the 1996 data may actually represent more severe infections on the modified 0-5 scale, a possibility that further supports the conclusions that severity of infection has dropped.

The Warm Creek site, which showed the highest sentinel infections in 2006, is downstream of a public and a private fish hatchery (Figure 4). The public hatchery is operated by IDFG and is the site where the sentinel rainbow trout fry were reared. This site has tested negative for WD in annual inspections since 1987, although the settling pond just below the hatchery has tested positive at minimal levels. The settling pond is disconnected from the fish runways by elevation. The private hatchery has consistently tested strongly positive for Whirling Disease (K. Johnson, IDFG, *pers. comm.*).
It is surprising that sentinels at Chilly Slough show no sign of *M. cerebralis* infection by either test, especially in light of such high prevalence of infection among the other four sentinel exposure sites in relative close proximity (Figure 4). By qualitative analysis, Chilly Slough hosted the habitat most anticipated to produce Whirling Disease infections. The site has a low gradient and is full of silty anoxic sediments and leaf litter, which was expected to be ideal habitat for tubifex worms and the release of TAMs (Arndt et al. 2002).

Chilly Slough should be a focus for additional research to learn what characteristics of the site allow it to remain WD-negative. Due to its proximity to other near-fully infected waters, it is more likely that Whirling Disease infection is being limited by the ecology of the site (i.e. tubifex lineage, TAM concentrations), rather than by vectors for the disease (i.e. distribution by fish-eating birds). The site should be investigated as a potential site for stocking healthy fish and precautions should be taken to ensure anglers fishing in these waters are using clean gear that will not transfer *M. cerebralis* spores into the waters.

Temperature regimes at nearly all sampled streams show fry emerging at a time when they will spend most of their early weeks inhabiting water temperature ranges (10-15°C) conducive to high concentrations of TAMs being released by tubifex worms. (See Appendix II for temperature regimes of all field sites.) While most streams have daily mean temperatures just entering the lower bounds of this range, WD-positive sites have waters that are on average 1.3°C warmer, suggesting they may be exposed as fry to higher or more regular concentrations of TAMs.

Warmer daily mean temperatures within streams testing positive for Whirling Disease suggest that fry in these streams would have emerged earlier than those in WD-negative streams. In further support of this theory, mean brook trout fry lengths were significantly higher in mid-July and mid-August in waters that tested positive than those that did not.
While Elle (1997) concluded that *M. cerebralis* infection would occur throughout the summer, regardless of when fry emerge, there is conflicting evidence that TAM releases spike seasonally, based on water temperature changes in the spring and fall, regardless of summertime temperature regimes (Lukins et al. 2003). If this is the case and fry in positive waters emerged earlier due to warmer water temperatures, the fry may have endured the spiked releases of TAMs into the waters during late May and early-to-mid June.

In the initial stages of this study, it was suspected that some streams’ water temperature regimes might be warm enough to allow fry to emerge early and attain size-based resistance to *M. cerebralis* before the temperatures induced the release of TAMs into the water. However, this data suggests the opposite may be happening. Fry emerging earlier may enter waters in which they would be bombarded by the fresh release of TAMs. Fry with a delayed emergence might avoid the initial surge of TAM concentrations in the stream.

Temperature loggers were not placed in field sites until the final week of June, which was after these differences in temperature regimes would have played out. To confirm these conclusions, temperature regime data should be collected in streams as early as 1 April, to record water temperatures through April, May and June. Additionally in-stream TAM concentrations should be measured and compared across WD-positive and WD-negative waters throughout the spring and summer months.

The distribution of Whirling Disease among wild trout appears to have remained the same during the past decade, despite data suggesting additional sites have become WD-positive in 2006. For instance, brook trout from Bartlett Creek tested negative for WD in previous surveys, but positive in 2006. This is misleading. In 1996 only 4 brook trout were tested at the site. In 2006, brook trout were gathered on 2 separate occasions: 13 brook trout were collected on 17 July, 3 were collected on 18 August. Of the set of 13, and individual-fish follow-up was conducted and a single fish tested positive for WD with the lowest possible recordable infection. Of the 3 brook trout tested later, the pool tested positive, but no individual-fish follow-up was
conducted. It is therefore likely that too few fish were tested in 1996, potentially yielding a false-negative for that site.

Additionally, Bartlett Creek flows into the North Fork, which was previously untested. In 2006, brook trout from the North Fork Lower Elevation site, located approximately 3500 ft upstream of the Bartlett Creek confluence, tested positive for Whirling Disease. Although time, funding or other resources may limit the extent to which individual fish can be tested, if possible in the future, positive-testing pooled fish samples should be followed-up to the individual-fish level to avoid ambiguity.

The Summit, Wildhorse and Starhope forks of the Big Lost, and tributaries flowing into them, appear to remain free of Whirling Disease in 2006. Neither wild brook trout nor the few rainbow trout collected from these waters tested positive by qPTD. As with Chilly Slough, these tributaries should be targeted for additional research to learn what characteristics. These rivers should be considered for restoration projects designed to increase or improve trout spawning habitat and for stocking fish.

By introducing known WD-negative fish in these “clean” waters rather than infected streams, fewer fish will come in contact with TAMs and subsequently release them when they die. While it is the current practice to ensure hatchery fish are WD-negative before they are stocked in streams, recent problems in western Maryland – where 80,000 hatchery trout (rainbow and brown) had to be killed after showing clinical signs for Whirling Disease – show the implications of making mistakes (Pelton 2007).

Few rainbow trout were collected during this study. Rainbow trout are highly susceptible to Whirling Disease, but it is unclear if low collection data is due to dwindling populations, or is a by-product of an as-of-yet unknown bias in sampling methods. The field work conducted in 2006 should not be considered an estimator or indicator of trout population trends. Field work concluded in August as rainbow trout fry were emerging from the redds. Additionally, many wild juvenile rainbow trout collected for previous years’ Wild Fish Health Surveys were collected
from August through October. Extensive, systematic trout population surveys should be conducted throughout the upper Big Lost to determine if trout populations have recovered, have stabilized or continue to drop in the basin.

There is a good deal of interest in stocking WD-infected waters with the Hofer strain of rainbow trout. The Hofer strain is resistant to Whirling Disease because it originates from the same German ecosystem as the disease itself (El-Matbouli et al. 2002). However, some popular publications are reporting that the Hofer strain is earning a reputation for being too docile to be a suitable replacement for the prized rainbow trout sports fish that once populated these streams (Berwyn 2007). If the end goal of solving the Whirling Disease problem is to return inland fisheries to something that will rejuvenate the sports fishing economy in these regions, some hesitation is warranted with this approach.
Figure 1. Schematic of the two-host Whirling Disease life cycle. The parasite Myxobolus cerebralis can be seen in its two distinct life forms: as the triactinomyxon, or TAM, that is released by tubifex worms, and as the hardy multicellular myxospore released from the cartilage of dead infected salmonids. (Image used with permission. Courtesy of Dave Kumlien, Executive Director, Whirling Disease Foundation.)
<table>
<thead>
<tr>
<th>Key</th>
<th>Site Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (ft)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Castle Rock</td>
<td>43.90051299</td>
<td>-114.00148359</td>
<td>7202</td>
<td>Sentinel</td>
</tr>
<tr>
<td>B</td>
<td>Chilly Slough</td>
<td>43.80729525</td>
<td>-113.83538302</td>
<td>7837</td>
<td>Sentinel</td>
</tr>
<tr>
<td>C</td>
<td>Copper Basin</td>
<td>43.94419375</td>
<td>-113.65707975</td>
<td>5973</td>
<td>Sentinel</td>
</tr>
<tr>
<td>D</td>
<td>Trout Haven</td>
<td>43.97825152</td>
<td>-113.77789519</td>
<td>6158</td>
<td>Sentinel</td>
</tr>
<tr>
<td>E</td>
<td>Warm Creek</td>
<td>43.91487647</td>
<td>-114.18093276</td>
<td>7168</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>1</td>
<td>Bartlett Creek</td>
<td>43.74406678</td>
<td>-113.94126448</td>
<td>7932</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>2</td>
<td>Bear Canyon Creek</td>
<td>43.76990810</td>
<td>-113.94499417</td>
<td>7822</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>3</td>
<td>Broad Canyon Creek</td>
<td>43.82711191</td>
<td>-113.84919925</td>
<td>7761</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>4</td>
<td>Cabin Creek</td>
<td>43.86830921</td>
<td>-113.83200050</td>
<td>7947</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>5</td>
<td>Corral Creek</td>
<td>43.89274127</td>
<td>-114.12503838</td>
<td>6996</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>6</td>
<td>Deep Creek</td>
<td>43.7963611</td>
<td>-113.9030851</td>
<td>8016</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>7</td>
<td>Lake Creek</td>
<td>43.75084044</td>
<td>-113.88774276</td>
<td>8213</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>8</td>
<td>Muldoon Creek High Elevation</td>
<td>43.70160313</td>
<td>-113.92047325</td>
<td>7888</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>9</td>
<td>Muldoon Creek Low Elevation</td>
<td>43.92416515</td>
<td>-113.32266035</td>
<td>7748</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>10</td>
<td>North Fork High Elevation</td>
<td>43.92448523</td>
<td>-114.18253927</td>
<td>7211</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>11</td>
<td>North Fork Low Elevation</td>
<td>44.00438212</td>
<td>-114.03165643</td>
<td>6733</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>12</td>
<td>Pinto Creek</td>
<td>43.99940112</td>
<td>-113.92975981</td>
<td>6958</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>13</td>
<td>Rock Creek</td>
<td>43.74386343</td>
<td>-113.94052369</td>
<td>7885</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>14</td>
<td>Starhope Creek</td>
<td>43.86278017</td>
<td>-114.20884426</td>
<td>7421</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>15</td>
<td>Summit Creek</td>
<td>43.81815408</td>
<td>-114.09724709</td>
<td>7362</td>
<td>Wild Fish Monitoring</td>
</tr>
<tr>
<td>16</td>
<td>Wildhorse Creek High Elevation</td>
<td>43.8995270</td>
<td>-114.09730316</td>
<td>7049</td>
<td>Wild Fish Monitoring</td>
</tr>
</tbody>
</table>

Figure 2. Locator map for field sites and major tributaries of the upper Big Lost River basin of Mackay, Idaho.
Table 1. Results of MacConnell-Baldwin (M/B) histological analysis performed on sentinel rainbow trout after 10d exposures at sites in the upper Big Lost River basin in 2006.
Analysis performed at 936 CTUs. The M/B scale ranks severity of *M. cerebralis* infection on a scale of 0 (no infection) to 5 (most extreme infection). Data in *italics* represent 1996 sentinel exposures with the same species at the same site by Elle (1997). Previous study was conducted on original 0-4 scale. (See Appendix for comparison of 0-4 and 0-5 M/B grading scales.)

<table>
<thead>
<tr>
<th>Location</th>
<th>IDFG Accession #</th>
<th># of Fish Sampled</th>
<th>MacConnell-Baldwin Histo Score</th>
<th>Date of First Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper Basin</td>
<td>06-313</td>
<td>10</td>
<td>2 2 2 3 1 -</td>
<td>6/12/06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>- - - 1 9 n/a 3.9</td>
<td>6/3/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>- - - 5 n/a 4</td>
<td>7/8/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>- - - 4 n/a 4</td>
<td>8/5/96</td>
</tr>
<tr>
<td>Castle Rock</td>
<td>06-314</td>
<td>10</td>
<td>1 3 2 2 2 -</td>
<td>6/12/06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1 - - 2 2 n/a 2.8</td>
<td>6/3/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>- - 2 3 n/a 3.6</td>
<td>7/8/96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1 2 1 - n/a 1.4</td>
<td>8/5/96</td>
</tr>
<tr>
<td>Chilly Slough</td>
<td>06-315</td>
<td>10</td>
<td>10 - - - -</td>
<td>6/12/06</td>
</tr>
<tr>
<td>Trout Haven</td>
<td>06-317</td>
<td>10</td>
<td>3 5 2 - -</td>
<td>6/12/06</td>
</tr>
<tr>
<td>Warm Creek</td>
<td>06-316</td>
<td>10</td>
<td>1 - 1 2 5 1</td>
<td>6/12/06</td>
</tr>
</tbody>
</table>
Table 2. Results of quantitative Pepsin-Trypsin Digest (qPTD) analysis performed on sentinel rainbow trout after 10d exposures at sites in the upper Big Lost River basin in 2006. Analysis performed at 1313 CTUs. Multiplied by 1000, qPTD results reveal the number of *M. cerebralis* spores found per fish head. Data in *italics* represent 1996 sentinel exposures with the same species at the same site by Elle (1997).

<table>
<thead>
<tr>
<th>Location</th>
<th>IDFG Accession #</th>
<th># of Fish Sampled</th>
<th>Positive</th>
<th>Prevalence</th>
<th>Digest Results (x1000)</th>
<th>Date of First Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper Basin</td>
<td>06-374</td>
<td>45</td>
<td>42</td>
<td>93.33%</td>
<td>7.02</td>
<td>0-26.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td>100%</td>
<td>93.3</td>
<td>0-400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>18</td>
<td>90%</td>
<td>176.9</td>
<td>0-740</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td>100%</td>
<td>192.2</td>
<td>23-623</td>
</tr>
<tr>
<td>Castle Rock</td>
<td>06-375</td>
<td>43</td>
<td>41</td>
<td>95.35%</td>
<td>9.13</td>
<td>0-93.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td>100%</td>
<td>149.1</td>
<td>7-353</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>17</td>
<td>85%</td>
<td>78.8</td>
<td>0-247</td>
</tr>
<tr>
<td></td>
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<td>20</td>
<td>14</td>
<td>70%</td>
<td>14.6</td>
<td>0-43</td>
</tr>
<tr>
<td>Chilly Slough</td>
<td>06-376</td>
<td>42</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0-0</td>
</tr>
<tr>
<td>Trout Haven</td>
<td>06-378</td>
<td>40</td>
<td>36</td>
<td>90.00%</td>
<td>4.91</td>
<td>0-16.7</td>
</tr>
<tr>
<td>Warm Creek</td>
<td>06-377</td>
<td>40</td>
<td>40</td>
<td>100.00%</td>
<td>46.41</td>
<td>6.7-196</td>
</tr>
</tbody>
</table>
Figure 3. Comparison of M/B histological (left) and qPTD (right) analyses of infection severity among sentinel rainbow trout after 10d exposures at sites in the upper Big Lost River basin in 2006. Filled symbols represent 2006 data. Hollow symbols represent data from 1996 exposures performed by Elle (1997). Trends across current and historic exposure sites are similar between both tests. Sentinels at Chilly Slough (n=52) experienced no infection.
Figure 4. Spatial distribution and prevalence of infection at sentinel exposure sites in the upper Big Lost River basin in 2006 after 10d exposures of rainbow trout. Multiplying mean qPTD scores by 1000 quantifies *M. cerebralis* spores per fish head. Sentinels at Chilly Slough suffered no infection, while all other sites experienced 90-100% infection prevalence.
Figure 5. Total length of wild brook trout fry and date of capture, sorted by field site. Distribution of fry lengths suggest brook trout emerged during the last three weeks of June. Same-sized fish will have overlapping data points.
Figure 6. Average wild brook trout fry length vs. date measured, sorted by 2006 Whirling Disease status of streams. This is a summary of the data presented in Figure 5. Wild brook trout fry lengths were averaged for each day at each field site. Sites were then sorted based on whether they tested positive or negative for Whirling Disease in 2006. Within the mid-July and mid-August sampling periods, no correlation was found between sampling date and average fry length ($R^2=0.003$, $P=0.33$ for both periods). T-tests comparing positive to negative sites in mid-July showed fry in WD-positive waters were significantly larger ($P < 0.01$). In mid-August, fry in WD-positive waters were again significantly larger ($P < 0.05$) than fry from negative waters.
Figure 7. Total length of wild rainbow trout fry and date of capture, sorted by field site. Larger rainbow fry (> 60mm) were possibly small Age 1 fish from the year before. Field work ended as wild rainbow trout fry were emerging from the redds in late August, which precluded further analysis of the data.
Figure 8. Proportion of wild juvenile brook trout testing positive and negative for Whirling Disease in 2006. Solid circles represent 2006 data. Hollow circles represent previous studies (USFWS NWFHS). Circle size represents number of fish tested, with number repeated in center. WD-status shown as a proportion of the circle in red (positive), blue (negative) and purple (unknown).
Figure 9. Proportion of wild juvenile rainbow trout testing positive and negative for Whirling Disease in 2006. Solid circles represent 2006 data. Hollow circles represent previous studies (USFWS NWFHS). Circle size represents number of fish tested, with number repeated in center. WD-status shown as a proportion of the circle in red (positive), blue (negative) and purple (unknown).
WORKS CITED


Hofer, B. 1903. Ueber die Drehkrankheit der Regenbogenforelle. Allgemeine Fischerei Zeitung 28:7-8. (Translated to English from the original German by Karolina Krauss, Montana State University for the Whirling Disease Initiative.)


**APPENDIX I: Comparison of MacConnell/Baldwin Histological Scales**

Histological grading scales used for quantification of *M. cerebralis* infection severity, taken from Baldwin et al. (2002) and Vincent (2002).

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormalities noted.</td>
</tr>
<tr>
<td>1</td>
<td>Discrete, rare (usually single), small foci of cartilage degeneration with intralesional <em>M. cerebralis</em> myxospores/generative stages present. Any associated leukocyte infiltrates are small.</td>
</tr>
<tr>
<td>2</td>
<td>Single, locally extensive focus or several smaller foci (usually 2) of cartilage degeneration/necrosis with intralesional <em>M. cerebralis</em> myxospores/generative stages. Lytic foci typically surrounded and/or infiltrated by few to moderate numbers of leukocytes.</td>
</tr>
<tr>
<td>3</td>
<td>Multiple foci of cartilage degeneration/necrosis (usually 3 or 4) with intralesional <em>M. cerebralis</em> myxospores/generative stages. Moderate numbers of leukocytes typically associated with lytic cartilage.</td>
</tr>
<tr>
<td>4</td>
<td>Multifocal (usually ≥4 sites) to coalescing, often locally extensive areas of cartilage degeneration/necrosis with intralesional <em>M. cerebralis</em> myxospores/generative stages. Moderate to large numbers of leukocytes typically border and/or infiltrate degenerate cartilage.</td>
</tr>
<tr>
<td>5</td>
<td>Multifocal (6 or more) to coalescing areas of cartilage necrosis, with locally extensive destruction in at least one focus (preferably more) are present and have intralesional <em>M. cerebralis</em> myxospores/generative stages. Moderate to large numbers of leukocytes typically border and/or infiltrate necrotic cartilage.</td>
</tr>
</tbody>
</table>
APPENDIX II: Field Site Temperature Regimes

Field sites testing positive for Whirling Disease:

- Bartlett Creek
- Cabin Creek
- Corral Creek
- Deep Creek
- North Fork Low Elevation
**Field sites testing negative for Whirling Disease:**

- Bear Canyon Creek
- Lake Creek
- Muldoon Creek High Elevation
- Muldoon Creek Low Elevation
- Starhope Creek
- Summit Creek
- Wildhorse Creek High Elevation
- Wildhorse Creek Low Elevation

---

**Bear Canyon Creek (Tested WD Negative)**

![Graph showing temperature trends for Bear Canyon Creek](image)

**Lake Creek (Tested WD Negative)**

![Graph showing temperature trends for Lake Creek](image)
Sites omitted from analysis:

- Broad Canyon Creek (lab results not returned)
- North Fork High Elevation (no fish found)
- Pinto Creek (no fish found – dry bed mid-summer)
- Rock Creek (no fish found – dry bed mid-summer)
### APPENDIX III: Wild Juvenile Fish Lab Results

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Pos/Neg</th>
<th>Total Fish Sampled</th>
<th>Known WD-Pos</th>
<th>Known WD-Neg</th>
<th>Unknown Status</th>
<th>2006 IDFG Accession #</th>
<th>Comments</th>
<th>Previous Status</th>
<th>Prev Study</th>
<th>Prev IDFG Accession #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartlett Creek</td>
<td>Brook Trout</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>06-263</td>
<td></td>
<td></td>
<td></td>
<td>99-087</td>
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