WHIRLING DISEASE REPORT 1997-98

Montana Fish, Wild and Parks

Project 3860

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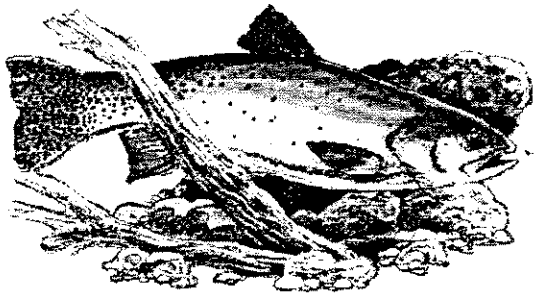

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January 12, 2000

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Acknowledgments

The author wishes to thank all those who assisted with the sentinel cage project with special thanks to Jeff Bagdanov, Jody Hupka and Jennie Miles who maintained and operated the Pony Whirling Disease lab and made sure all the live cage placements and removal were done to insure accurate and useful data. A special thanks to Dr Beth MacConnell and Dr Tom Baldwin for the quality histo-pathology on are the infected test fish. Thanks to Patrick Graham, Montana Fish, Wildlife and Parks Director and Larry Peterman Fisheries Division Administration for their foresight making this project a department priority.

Whirling Disease Studies - Introduction

Whirling disease research in Montana during 1997-98 has been focused in three areas: 1) relative susceptibility of various salmonid species to infections by *Myxobolus cerebralis; 2)* survey of Montana's cool water fisheries for the presence and/or the intensity of infection by *Myxobolus cerebralis*; and 3) specialized more intense field whirling disease studies on specific drainages.

Relative Susceptibility of various salmonids to Myxobolus cerebralis infections.

During 1997 and 1998, a total of nine strains of rainbow trout, three strains of cutthroat trout, bull trout, brown trout, eastern brook trout, kokanee salmon, chinook salmon, and mountain whitefish have been exposed to various levels of triactinomyon to relation each speices or strain's susceptibility to infections by the parasite. These lab infection experiments have either been done at the state lab in Pony, MT or at the Wild Trout Lab in Bozeman. With the exception of mountain whitefish study which is being conducted by Dr Beth MacConnell at the Bozeman lab, all of this work has been completed at the Pony Lab.

Experimental exposures of each fish species tested at the Pony Lab was done using the following test procedures: 1) all test fish were approximately 60 day old fry ; 2) known concentrations of fish infective spore (TAM or triactinomyxon) were used against each lot of test fish; 3) concentration levels of tams were 100 and 1000 per fish; 50 fish were in each exposure lot; 4) the exposure period was two hours in two gallons of water; 5) at the end of f the two hour period, the fish were rinsed with fresh water and placed in a 30 gallon aquarium and kept for 90 days in 50 F water; and 6) at the end of the 90 day period, the fish were sacrificed and sent to the histology lab at Pullman, WA. The results of this research will be included in a future report.

Studies on the susceptibility of mountain whitefish to *Myxobolus cerebralis* is being conducted in three areas: 1) the relative susceptibility to known concentrations of triactinomyxons; 2) relation of age and size to susceptibility; and 3) their contribution to spore production. These studies will continue into 2000-2001.

Statewide Survey for Presence of *Myxobolus cerebralis* is Montana's Cool Water Fisheries

Background

Since the discovery of *Myxobolus cerebralis* in the Madison River in December, 1994, Montana Fish, Wildlife and Parks has been conducting a very intensive survey of the cold water streams in western and central Montana to determine the distribution of the whirling disease parasite. Early sampling generally involved using samples of fish taken from either routine gill netting or electrofishing operations. In many cases, these fish were either too large and/or old to be ideal test fish. In order to collect the correct size and age of salmonids from as many cold water streams as possible, a statewide electrofishing crew was established in 1996. During 1996 and 1997, this crew sampled over 100 stream sites for the presence of *Myxobolus cerebralis* in the existing salmonid populations (Downs 1997b and Grisak, 1999). While this sampling method is

somewhat useful in determining the presence or absence of the parasite, it is not very accurate at quantifying the disease's intensity. At times even the detection of the parasite may be questionable because of such variables as movement and time of infection. It is possible that a stream could test WD positive, but actually not have a *M. cerebralis* infection because the infected fish may have originated from a WD positive water. A good example of this is Squaw Creek in the Madison River drainage which was determined to be positive from a WD positive trout electrofished from its waters, but two subsequent live cage exposures in 1997 and 1998 showed the same stream to be negative. This suggests that the positive trout sampled by electrofishing in Squaw Creek was probably a positive trout moving in from the Madison River. Another problem with electrofishing samples is that an infected fish could have tested negative histologically, but actually been positive. This is due to the fact that the disease is not very detectable in the cranial area until 70-80 days after initial exposure. Artificial exposures of test trout to *M.cerebralis* TAMS in the Pony Lab have shown that it takes at least 80 days in 50 F water (approximately 1500 degree days F) after the initial TAM exposure before the disease can be histologically identified in the infected fish. Since it is difficult to determine date of initial exposure to the parasite, under wild stream conditions a false negative could be obtained from infected fish taken from random electrofishing samples.

Fish sampled from the wild populations or exposed in sentinel cages are evaluated for both the presence and absence of the parasite, plus the degree or intensity of infection. In order to determine the degree of infection, a histological grading system was developed by Dr. Beth MacConnell of the USFWL Service and Dr Tom Baldwin of the Washington State University Animal Disease Diagnostic Laboratory. Using samples from a select portion of the fish's cranium, the degree of WD infection is given a score based on the degree of cartilage damage and tissue inflamation. Rankings were assigned a numerical rating from "0" which had no detectable infection to "4" where there was extensive cartilaginous necrosis and severe inflamation. There is likely a direct relationship between the average infection intensity of young salmonids and the overall impact on the population. At this point in time, it is generally felt that when mean infection intensities exceed 2.50 significant adverse impacts on wild salmonid populations are likely to occur (Table 1).

Table 1. Probable Relationship of Average WD Infection Rate and Wild Salmonid Population Impacts.

Average Infection Grade	Salmonid Population Impacts
Grade 0.00 - 1.00	**No significant impacts**
Grade 1.00 - 2.00	**Minor population impacts**
Grade 2.00 - 2.50	**Possible problems in stream of low recruitment**
Grade 2.50 - 3.00	**Fish population declines may occur**
Grade 3.00 - 4.00	**Fish population declines will occur**

Grab Samples of Wild Salmonids Methodology

There are times when the only method available to sample a body of water to determine whether the parasite is present is through "grab samples" which are obtained through either netting or electrofishing operations. In order to obtain the maximum use from this type of data certain factors which control disease intensity should be kept in mind. The following factors regulate the presence and intensity of the infection.

1) Young-of-the-year salmonids are the best age to collect.

2) Peak infection periods for most species of fish are during their first 10 weeks of life after hatching.

3) Mean daily water temperatures control infection intensity with peak infections occurring between 52-58 F (11-14 C).

4) The best target fish species are spring spawners (rainbow trout).

5) Fish should be collected approximately 1500-2300 degree days F (800-1300 degree days C) after peak infection which is generally occurring during the hatching and early fry period. In most highly productive streams peak infection in the fish is usually late August to early September. Mortality of highly infected fish starts occurring after 2300 degree days F, so only lighter infected fish may be available later in the fall period.

6) Correct preparation of sampled fish is necessary to insure accurate histological ratings.

Live Cage Methodology

Beginning in the spring of 1998, the statewide whirling disease survey began to use sentinel cages to determine the presence or absence and the degree of whirling disease infection to determine likely sites where whirling disease may have population impacts. In this initial live cage survey, streams from regional management areas 1, 2, 3 and 4 were selected to be part of the study. The actual live cage used in the experiment consisted of a 18 X 24 inch cylindrical screened container which could be placed into a stream site allowing stream water to flow through the cage for a given period.. Each live cage exposure contained (60) uninfected 35 - 60 mm rainbow or westslope cutthroat trout supplied by one of the State Fish Hatcheries. The exposure period for each live cage was standardized at 10 days. At the end of the 10 day exposure period, the test trout were removed and taken to Pony, MT, where they were held in 30 gallon aquaria for an additional 80 days at a constant 50 F temperature to insure the WD infection would reach its maximum intensity. Each aquaria has its own independent water supply which is pumped from a well on the lab site. Water temperatures of this well generally hold between 48 F and 52 F. At the end of the 90 day period all the surviving fish were killed and heads sent to the Washington State University Animal Disease Diagnostic Laboratory at Pullman, WA. At this lab, the head were histologically examined and an infection intensity of 0 to 4 was assigned each fish. The results of this histological rating was presented as percent of fish infected and the mean infection

intensity. Every live cage site also had an accompanying thermograph to establish mean daily water temperatures during the exposure periods.

Results

The following streams or stream sites for Regions 1 through 4 had live cage exposures: Region 1 - 3 sites on two streams; Region 2 - 8 sites on 7 streams; Region 3 - 26 sites on 19 streams; and Region 4 - 10 sites on 7 streams. Results from some of the test sites were compromised due to high water and/or vandalism. There also were additional live cage exposures on streams with a specific more intensive WD study, such as Rock Creek and the Big Blackfoot River in Region 2; the Madison River, Willow Creek and the Yellowstone River in Region 3 and the Missouri River and Little Prickley Pear Creek in Region 4. Reports are available on these specific studies. Histological results from the 1998 statewide sentinel cage exposures are shown in Table 2.

Table 2. Statewide Whirling Disease Live Cage Data for 1998. Water temperatures are the average of the high four days within the ten day exposure period.

Date	Lot No. & Location	Av Water Temp	Grade of Infection	% Whirl
Region 1				
7/20 - 30	**SR-1** **Upper Swan River**	**65.1**	**Grade 0 - 43** **Grade 1 - 3** **Grade 2 - 2** **Grade 3 - 0** **Grade 4 - 0** **Av Grade = 0.15** **% Infection = 10.4**	**0%**
7/20 - 30	**GCR-T1** **Goat Creek - Hyway 83 bridge**	**52.9**	**Grade 0 - 49** **Grade 1 - 0** **Grade 2 - 0** **Grade 3 - 0** **Grade 4 - 0** **Av Grade = 0.00** **% Infection = 0.00**	**0%**
7/20 - 30	**GCR-T2** **Woodard Cr - old Squeeze Loop Rd**	**49.8**	**Grade 0 - 50** **Grade 1 - 0** **Grade 2 - 0** **Grade 3 - 0** **Grade 4 - 0** **Av Grade =0.00** **% infection = 0.00**	**0%**

Region 2				
9/28 - 10/8	**WAR-7** **Warren Cr**	**50.5**	**Grade 0 - 50** **Grade 1 - 0** **Grade 2 - 0** **Grade 3 - 0** **Grade 4 - 0** **Av Grade = 0.00** **% Infection = 0.00**	**0%**
9/28 - 10/8	**BLK - 1 -98** **Big Blackfoot R** **near mouth**		**Grade 0 - 50** **Grade 1-4 - 0** **Av Grade = 0.00** **% Infection = 0.00**	**0%**
10/6 - 16	**CFK-1** **Clark Fork River** **Deer Lodge Site**		**Grade 0 - 43** **Grade 1 - 2** **Grade 2 - 3** **Grade 3 - 0** **Grade 4 - 2** **Av Grade = 0.32** **% Infection = 14.0**	**0%**
10/6 - 16	**CFK - 2**		**No Data**	
10/6 - 16	**LBLk-1** **Little Blackfoot River** **County Rd 249 crossing**	**48.0**	**Grade 0 - 50** **Grade 1 - 0** **Grade 2 - 0** **Grade 3 - 0** **Grade 4 - 0** **Av Grade = 0.00** **% Infection = 0.00**	**0%**
10/6 - 16	**Harv - 1** **Harvey Creek**	**45.3**	**Grade 0 - 50** **Grade 1 - 0** **Grade 2 - 0** **Grade 3 - 0** **Grade 4 - 0** **Av Grade = 0.00** **% infection = 0.00**	**0%**

10/7 - 17	Nflint - 1 No. Fork Flint Cr At Fish and Game cabins	39.2	Grade 0 - 1 Grade 1 - 0 Grade 2 - 2 Grade 3 - 3 Grade 4 - 32 Av Grade = 3.71 % Infection = 97.4	7.9%
10/7 - 17	Warm-1 below Washoe Hatchery effluent	44.2	Grade 0 - 46 Grade 1 - 2 Grade 2 - 1 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.14 % Infection = 8.0	0%
Region 3				
9/24 - Oct 4	EGR- 1 East Gallatin River near mouth	52.7	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
9/24 - 10/4	EG-2 East Gallatin River at Nelson Road	54.2	Grade 0 - 48 Grade 1 - 2 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.04 % Infection = 4.0	0%
9/24 - 10/4	WG-3 Gallatin River @ Logan	51.1	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0.00	0%

9/24 - 10/4	16mile-1 Sixteen Mile Creek near mouth	53.4	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
9/28 - 10/8	Umiss - 1 Upper Missouri River @ Toston No Data	55.8	Grade 0 - Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade =	
7/ 8 - 18	Deep - 1 Deep Creek upper station @ Clopton Bridge	59.6	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
7/8 - 7/18	Deep - 2 Deep Creek lower station @ Fish Trap	64.7	Grade 0 - 49 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 2.0	0%
9/28 - 10/8	Deep - 3 Deep Creek lower @ Fish Trap	53.6	Grade 0 - 41 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
9/28 - 10/8	Deep - 4 Deep Creek upper @ Clopton bridge No Data	51.7	Grade 0 - Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade =	0%

7/14 - 7/24	CCR - 1 Confederate Creek near mouth	66.3	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
9/28 - 10/8	CCR-2 Confederate Creek near mouth	49.9	Grade 0 - 49 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
7/14 - 10/8	MCR - 1 Magpie Creek @ a culvert near the mouth	55.7	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
7/14 - 10/8	BCR-1 Beaver Creek @ road crossing	60.4	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection =0	0%
June 15 - 24	Jeff - 1 Hell's Canyon	60.0	Grade 0 - 49 Grade 1 - 0 Grade 2 - 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.044 % Infection = 2.0	0%
July 13 - 23	Jeff - 2-98 Hell's Canyon Cr	60.0	Grade 0 - 34 Grade 1 - 8 Grade 2 - 2 Grade 3 - 4 Grade 4 - 2 Av Grade = 0.64 % Infection = 32.0	0%

July 13 - 23	Jeff - 3-98 Willow Springs	55.2	Grade 0 - 32 Grade 1 - 11 Grade 2 - 3 Grade 3 - 4 Grade 4 - 0 Av Grade = 0.58 % Infection = 36.0	0%
Sept 29 - Oct 9	Jeff - 4 - 98 Sappington Bridge	54.4	Grade 0 - 49 Grade 1 - 0 Grade 2 - 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.04 % Infection = 2.0	0%
Sept 30 - Oct 8	Jeff - 5 -98 Above mouth of Hell's Canyon Cr	54.4	Grade 0 - 49 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.02 % Infection = 2.0	0%
Sept 29 - Oct 9	BH - 1 - 98 Big Hole River near Maiden Rock	50.1	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%
Sept 29 - oct 9	BH - 2- 98 Big Hole River near Melrose	49.9	Grade 0 - 47 Grade 1 - 3 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.06 % Infection = 6.0	0%
Oct 5 - 15	Ruby - 1 - 98 Ruby River near Sheridan	50.9	Grade 0 - 35 Grade 1 - 7 Grade 2 - 4 Grade 3 - 1 Grade 4 - 2 Av Grade = 0.53 % Infection = 27.1	0%

Oct 5 - 15	Beav - 1 -98 Beaverhead River near Dillon Barrett's Diversion	53.4	Grade 0 - 47 Grade 1 - 2 Grade 2 - 0 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.10 % Infection = 6.0	0%
Oct 5 - 15	Poin - 1- 98 Poindexter Slough @ Dillon Frontage Rd	51.3	Grade 0 - 47 Grade 1 - 3 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.06 % Infection = 6.0	0%
Oct 7 - 17	HPR - 1 - 98 Horse Prairie Creek Peck Ranch Crossing	45.9	Grade 0 - 49 Grade 1 - 0 Grade 2 - 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.02 % Infection = 2.0	0%
Oct 7 - 17	Rrock - 2- 98 At Dell	48.3	Grade 0 - 9 Grade 1 - 5 Grade 2 - 4 Grade 3 - 11 Grade 4 - 21 Av Grade = 2.60 % infection = 82.0	20%
Oct 14 - 24	MadR - 4 near mouth	47.7	Grade 0 - 45 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0	0%
Region 4 Oct 9 - 19	Belt - 1 -98 Belt Creek @ Isaak Walton Crossing Fish lost	46.3	Grade 0 - 0 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00	0

Oct 9 - 19	Bspr - 1- 98 Big Spring Creek @ Carroll Trail FAS	49.4	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0
Oct 9 - 19	Jud - 1 - 98 Judith River @ Wertheimer Ranch	46.9	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0	0
Oct 2 - 12	Smith - 1 - 98 Smith River @ Eden bridge	51.9	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0
Oct 2 - 12	Smith - 2 - 98 Smith River @ Camp Baker	44.5	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0
Oct 2 - 12	Hound - 1 -98 Hound Creek MacKamie Bridge	53.0	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0	0
Sept 30 - Oct 9	Sun - 1- 98 Sun River @ Fort Shaw FAS	53.9	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % Infection = 0	0%

Sept 30 - Oct 9	**Sun - 2- 98 Sun River @ Hyway 287 bridge**	**52.8**	**Grade 0 - 49 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0**	**0%**
Sept 30 - oct 9	**Elk - 1 -98 Elk Creek @ Augusta**	**51.6**	**Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0**	
Data lost	**Gaint Springs 5T**		**Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.00 % infection = 0**	**0%**

General Discussion

In order to accurately measure the maximum rate of infection at any particular sample site, one must take into account the various physical factors which determine maximum infection rates. Three known primary factors are: time of year, water temperature and water flow rate. Through the use of time-sequence live cage studies on Willow Creek, Madison River, Missouri River and Little Prickley Pear Creek, a relationship between mean daily water temperature and the intensity of infection has been calculated for the period from early March through mid July when water temperatures are rising. During this period, the peak intensity of infection generally occurs between 52 F and 56 F with infection intensities lower during the cooler early period and in the warmer mid-summer period. When daily mean water temperatures fall below 45 F or exceed 63 F infection intensities are near zero (Vincent 1998). We are not certain at this time, if this water temperature - infection intensity relationship is the same during the fall period, when water temperatures are on the decline. The usable average mean daily water temperature for each 10 day exposure is computed using the highest four mean daily water temperatures for the ten day period. Water flow rates also have some bearing on infection rates through dilution. It appears the only measurable decline in infection intensity occurring with peak flows measured during snow melt runoff. This dilution effect was noted in the time-sequence series in the Madison River and Willow Creek.

Region 1 -
Three live cage were placed in the upper Swan River drainage, one in the Swan River and two tributaries. Only in the upper Swan River exposure did exposed rainbow trout have a positive histological reading which average a 0.15. This is a very low infection, but water temperatures above the optimum infection range, so this site bears watching in the future. The remaining two sites were negative at temperature near optimum, so the chances of a severe infection here at this time are minimal.

Region 2 -
Eight live cage exposures were used in the statewide survey in region 2. Only one site showed a severe infection, the North Fork of Flint Creek, although positive results were found in the Clark Fork River near Deer Lodge and in Warm Springs Creek below the Washoe State Fish Hatchery. The Flint Creek live cage was a late fall site and had a very intense infection. This level of infection should have some negative impacts on existing salmonid numbers. Additional intensive whirling disease research (live cages and aquatic oligochaete studies) is being carried out in the Rock Creek and Big Blackfoot drainages.

Region 3 -
A total of 26 statewide live cage sites were tested in Region three. These live cages were located in the Gallatin, Madison, Jefferson and Missouri River drainages.

Gallatin River drainage - Three late fall sites (mid-October) were exposed, two in the East Gallatin River and one in the main Gallatin River. Only the Nelson road site on the upper East Gallatin River had WD positive trout with infection intensity very low..

Madison Rive drainage - only one cage site was included in the statewide survey and this station was located near the mouth and was negative.

Missouri River drainage above Canyon Ferry Reservoir - Ten sites were tested in the statewide survey with a WD positive being found in only in Deep Creek near the fish trap. These mid-July sites had mean daily water temperatures which were too high to expect a high intensity of infection. A repeat live cage was placed at the same location in late September resulted in a negative WD reading. Further work at this site is advised.

Jefferson Rive drainage - Twelve sites were tested in this drainage in the statewide survey with every site except the Maiden Rock site on the Big Hole River testing positive. All sites had a moderate to light infection intensity, except the Red Rock River at Dell which had an infection intensity which could damage WD sensitive salmonids. Since many of these sites were done during the fall period, it would be premature to conclude that the WD infection rates are benign. There is some suggestion that fall peak infection water temperatures may be lower than found during the spring Additional intensive whirling disease research (live cages and aquatic oligochaete studies) is being carried out in the Madison River, Yellowstone River and Willow Creek drainage.

Region 4 -
A total of ten sites were tested in Region 4. These sites were located on eight tributaries of the Mid-Missouri River. Cage placement occurred during the early to mid-October period, except the site at Giant Springs near Great Falls. The Giant Springs site was placed in the Missouri River in early spring. At all sites, the sentinel fish tested negative to *Myxobolus cerebralis*.

Special Whirling Disease Studies

Big Blackfoot Study

Background

Whirling disease research on the Big Blackfoot River system during 1998 fell into the category of a survey of the mainstem Big Blackfoot and some of the primary tributaries. Table 3 shows the results for the July 1998 live cage tests for whirling disease in the Big Blackfoot River Drainage.

Table 3. Whirling Disease test results for 12 sites in Big Blackfoot River Drainage for the July 1 - 11,1998, period. Water temperatures are the average of the high four days within the ten day exposure period.

Site	No of fish	Percent Infected	Mean Grade Infection	Mean daily temp using high 4 days
BBR-1 Lower Big Blackfoot	36	14%	0.22	64.7 F
BBR-2 Big Blackfoot below Elk Cr	41	15%	0.21	No Data
BBR-3 Big Blackfoot above Clearwater River	20	52%	1.10	63.0
BBR-4 Big Blackfoot Below N. Fork	41	155	0.25	No Data
BBR-5 Big Blackfoot below Nevada Creek	27	0	0	No Data
BBR-6 Big Blackfoot at Nevada/Ogden Road	39	0	0	57.2 F
Tributaries				
NFK-1 N. Fork above Kleinschmidt	43	0	0	57.3*
NFK-2 N. Fork below Kleinschmidt	50	12%	0.14	52.2*
LFS-3 Lower Kleinschmidt	48	90%	2.83	54.4 F
CCR-4 Lower Chamberlin Cr	50	8%	0.16	57.3 F
COT-5 Cottonwood Cr	50	94%	3.66	58.2 F
WAR-6 Warren Creek	47	19%	0.21	63.2 F

Discussion

Using the chart in Table 1 as a guide to when whirling disease infections may cause population problems, most of the WD infection intensities measured during this period are below the 2.4-2.7 line. Only Lower Kleinschmidt and Cottonwood Creeks are high enough to cause population problems through loss of recruitment. Some of the sites were measured when water temperatures were too high to measure accurately the maximum infection rate, such as most of the sites in the mainstem Big Blackfoot and Warren Creek. We also do not know at what stage of infection each of these sites are, so they could escalate to higher intensities or stay low. The site in the mainstem above the Clearwater River is also troubling in that the 1.10 mean grade infection is very high considering the high mean daily water temperature of 63.0 F. This infection at the optimum water temperatures could well be in the low three's. This site bears watching. The Bonner site on the lower Big Blackfoot is too warm during the exposure period to produce any meaningful data, as was Warren Creek, thus we re-exposed fish there in late September - early October.

Madison River Whirling Disease Studies - 1998

Background

Whirling disease was first detected in the Madison River in December, 1994. The discovery cumulated a four-year search for factors(s) which could explain a large population decline of wild rainbow trout in the upper Madison River. This decline in wild rainbow trout numbers in the Pine Butte Study section began in 1991 through the loss of yearling rainbow trout, the youngest age class estimated. Subsequent population estimates made in 1992-94 showed even a greater decline in yearling rainbow trout numbers. Initially these declines were only measured in the upper two study sections, Pine Butte and Snoball, but by the fall of 1993, yearling wild rainbow trout numbers the Varney Study Section (approximately 48km downstream) also showed large declines. Only the lowest downstream study site, Norris, has yet to show wild yearling rainbow trout declines. As a result of these large declines in recruitment, the adult wild rainbow trout numbers have experienced a steady decline in numbers to the point where the number of two-year-old and older rainbow had decreased to nearly 90% of the long term average. By 1998, some increase in recruitment of yearling rainbow trout has been measured, so the 1998 wild rainbow trout numbers are about 30% of their long term average. Wild brown trout in the upper Madison River have shown no significant change during this 1991-1998 period.

Objectives

The Madison River whirling disease study objectives are: 1) determine the spatial distribution of whirling disease in the Madison River between Quake Lake and Ennis Lake; 2) determine the change in WD infection intensity with regards to time and water temperature; 3) determine the relationship between WD infection rates and primary spawning sites for wild rainbow trout; and 4) determine the relationship between spatial and water temperature spawning requirements for wild rainbow and brown trout and the intensity of whirling disease infections.

Methods

In order to determine these infection rates and intensities, the use of live cages was determined to best method to determine both infection rate and intensity. These live cages containing 60 (35 - 50 cm) were placed in each stream site and left for 10 days upon which the fish were removed and taken to the whirling disease research lab at Pony, MT, and kept in 10 C well water aquaria for an additional 90 days. At that time fish were sacrificed and sent to the Washington Animal Disease Diagnostic Laboratory at Washington State University for histological analysis. Histological analysis included both the presence or absence of the parasite and a histological grading of the disease severity. Histological samples were taken from the cranial area of each fish and with the aid of a microscope presence or absence and severity of infection was determined. The infection severity was scored using a 0 - 4 scale which ranks the infection based off of the degree of cartilage damage and inflamation. Grade 0 was no detectable infection and grade 4 being extremely severe. Each stream station also contained a recording thermometer to determine water temperature during the 10 day stream exposure.

Results

During 1998, a time-sequence site was placed approximately 100m upstream from the Kirby Bridge on the upper Madison River. A total of 11 ten day time periods were exposed from May 5 through October 18, 1998 (Table 4).

Table 4. Whirling Disease infection rates compared to time and water temperature (F) measurements from sentinel cages located at Kirby Bridge, 1998. Water temperatures are the average of the high four days within the ten day exposure period.

Date	Lot No.	Av Water Temp F	Grade of Infection	Flows (cfs)
May 5 - 15	Kirby -1	45.9	Fish Lost	1525
May 15- 25	Kirby -2	48.5	Grade 0 - 5 Grade 1 - 3 Grade 2 - 3 Grade 3 - 5 Grade 4 - 6 Av Grade = 2.18 % Infection =77.3	1410
May 25 - June 4	Kirby -3	50.0	Grade 0 - 3 Grade 1 - 1 Grade 2 - 2 Grade 3 - 2 Grade 4 - 39 Av Grade = 3.55 % Infection = 93.4	2117

June 4 - 14	Kirby -4	51.2	Grade 0 - 7 Grade 1 - 2 Grade 2 - 2 Grade 3 - 6 Grade 4 - 33 Av Grade = 3.12 % Infection = 86.0	2580
June 14 -24	Kirby -5	51.9	Grade 0 - 6 Grade 1 - 3 Grade 2 - 4 Grade 3 - 6 Grade 4 - 31 Av Grade = 3.06 % Infection = 88.0	2821
June 24 - July 5	Kirby - 6	55.4	Grade 0 - 8 Grade 1 - 13 Grade 2 - 8 Grade 3 - 13 Grade 4 - 8 Av Grade = 2.00 % Infection = 84.0	3354
July 5 - 15	Kirby - 7	57.5	Grade 0 - 8 Grade 1 - 1 Grade 2 - 11 Grade 3 - 6 Grade 4 - 24 Av Grade = 2.74 % Infection =86.0	2504
July 15 - 25	Kirby - 8	60.6	Grade 0 - 20 Grade 1 - 13 Grade 2 - 11 Grade 3 - 5 Grade 4 - 1 Av Grade = 1.08 % Infection =60.0	1627
Sept 16 - 26	Kirby - 9	62.3	Grade 0 - 22 Grade 1 - 3 Grade 2 - 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.19 % Infection =15.4	1181
Sept 26 - Oct 8	Kirby - 10	55.2	Grade 0 - 34 Grade 1 - 12 Grade 2 - 3 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.42 % Infection = 32.0	

Oct 8 - 18	Kirby - 11	50.8	Grade 0 - 22 Grade 1 - 5 Grade 2 - 9 Grade 3 - 10 Grade 4 - 4 Av Grade = 1.38 % Infection = 56.0	1887

During the 1998 sampling period, the time sequence station located at the Kirby Gauge showed a peak infection of 3.55 occurring in late May and early June. The 1998 peak infection was similar to that found at this site in 1997, but the 1998 peak infection period was in late May versus mid June in 1997 (Figure 1). A total of twelve sentinel cage were placed in a spatial

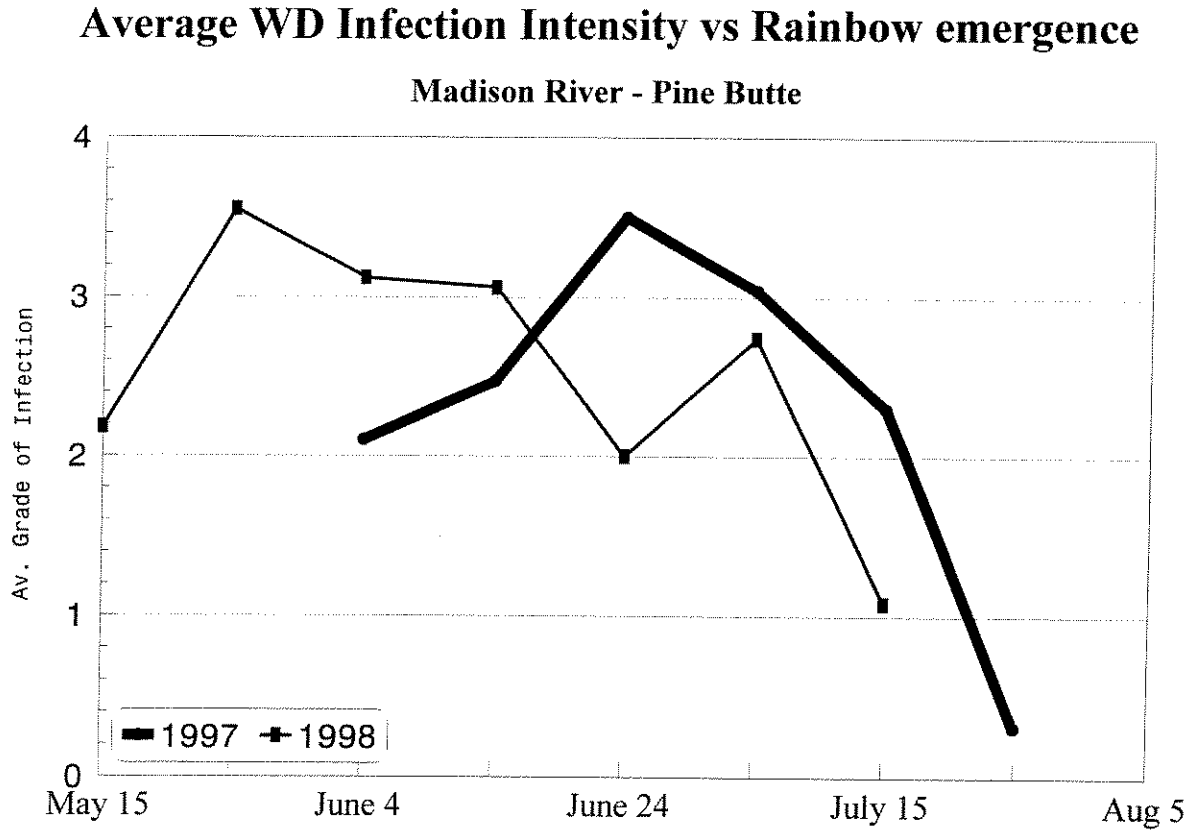

Figure 1. Comparison of WD Infection Rates between 1997 and 1998.

series from Hebgen Dam downstream to the mouth of Elk Creek (Table 5). The cages were placed to determine if spatial differences in WD infection intensity occurred within the mainstem channel of the Madison River. Data from these spatial cages show a significant difference from site to site with no upstream or downstream trend. This series was run three times (May 28th - June 4th, July 8 - 18th and Sept 28th - Oct 8th). The infection rates were the

Table 5. Whirling Disease infection rates compared to spatial sites on the Madison River for three time periods in 1998. Water temperatures are the average of the high four days within the ten day exposure period.

Site	May 28 - June 4	July 8 - 18	Sept 28 - Oct 8
Norris Bridge	Grade 0 - 48 Av Grade = 0 % infection = 0 Av Water Temp = 53.7F	No Data	No Data
Varney Bridge	Grade 0 - 3 Grade 1 - 5 Grade 2 - 8 Grade 3 - 8 Grade 4 - 17 Av Grade = 2.76 % Infection = 92.7 Av Water Temp =53.5	Grade 0 - 12 Grade 1 - 12 Grade 2 - 10 Grade 3 - 7 Grade 4 - 9 Av Grade = 76.0 % Infection = 1.78 Av Water Temp = 60.4	Grade 0 - 39 Grade 1 2 Grade 2 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.95 % Infection = 7.1 Av Water Temp = 54.3
McAtee Bridge	Grade 0 - 15 Grade 1 - 9 Grade 2 - 11 Grade 3 - 7 Grade 4 - 8 Av Grade = 1.68 % Infection = 70% Av Water Temp =50.8	Grade 0 - 24 Grade 1 - 10 Grade 2 - 9 Grade 3 - 5 Grade 4 - 1 Av Grade =0.96 % Infection =49.0 Av Water Temp =	Grade 0 - 28 Grade 1 - 0 Grade 2 - 0 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.10 % Infection =3.5 Av Water Temp =53.9
Lower Palisades	Grade 0 - 5 Grade 1 - 2 Grade 2 - 5 Grade 3 - 10 Grade 4 - 19 Av Grade = 2.88 % Infection =12.2 Av Water Temp =49.9	Grade 0 - 19 Grade 1 - 7 Grade 2 - 9 Grade 3 - 5 Grade 4 - 4 Av Grade = 1.27 % Infection = 56.8 Av Water Temp = 56.0	Grade 0 - 18 Grade 1 - 1 Grade 2 - 0 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.20 % Infection =10.0 Av Water Temp =54.5
Upper Palisades	Grade 0 - 2 Grade 1 - 3 Grade 2 - 1 Grade 3 - 6 Grade 4 - 15 Av Grade = 3.07 % Infection =92.6 Av Water Temp =49.9	Grade 0 - 18 Grade 1 - 12 Grade 2 - 7 Grade 3 - 5 Grade 4 - 0 Av Grade = 0.98 % Infection =57.1 Av Water Temp = 56.0	Grade 0 - 26 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.04 % Infection =3.7 Av Water Temp =54.5

Lyon's Bridge	Grade 0 - 10 Grade 1 - 7 Grade 2 - 10 Grade 3 - 6 Grade 4 - 3 Av Grade = 1.58 % Infection =72.2 Av Water Temp =	Grade 0 - 10 Grade 1 - 8 Grade 2 - 6 Grade 3 - 9 Grade 4 - 6 Av Grade = 1.82 % Infection =74.4 Av Water Temp =58.5	Grade 0 - 30 Grade 1 - 7 Grade 2 - 4 Grade 3 - 2 Grade 4 - 2 Av Grade = 0.64 % Infection =33.3 Av Water Temp =53.7
Kirby's Bridge	Grade 0 - 3 Grade 1 - 1 Grade 2 - 2 Grade 3 - 2 Grade 4 - 39 Av Grade = 3.55 % Infection =93.6 Av Water Temp =50.0	Grade 0 - 8 Grade 1 - 1 Grade 2 - 11 Grade 3 - 6 Grade 4 - 24 Av Grade = 2.74 % Infection =84.0 Av Water Temp =57.5	Grade 0 - 34 Grade 1 - 12 Grade 2 - 3 Grade 3 - 1 Grade 4 - 0 Av Grade = 0.42 % Infection =32.0 Av Water Temp =55.2
Lower Pine Butte	Grade 0 - 20 Grade 1 - 4 Grade 2 - 5 Grade 3 - 5 Grade 4 - 4 Av Grade = 1.18 % Infection =47.4 Av Water Temp =46.2	Grade 0 - 25 Grade 1 - 11 Grade 2 - 0 Grade 3 - 1 Grade 4 - 2 Av Grade = 0.56 % Infection =35.9 Av Water Temp =57.5	Grade 0 - 38 Grade 1 - 1 Grade 2 - 3 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.17 % Infection = 9.5 Av Water Temp =55.2
Upper Pine Butte	Grade 0 - 20 Grade 1 - 4 Grade 2 - 5 Grade 3 - 5 Grade 4 - 4 Av Grade = 1.18 % Infection =47.4 Av Water Temp =46.2	Grade 0 - 29 Grade 1 - 12 Grade 2 - 1 Grade 3 - 2 Grade 4 - 1 Av Grade = 0.53 % Infection =35.6 Av Water Temp =57.5	Grade 0 - 47 Grade 1 - 1 Grade 2 - 2 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.10 % Infection = 6.0 Av Water Temp =55.2
Raynolds Pass Bridge	Grade 0 - 22 Grade 1 - 10 Grade 2 - 7 Grade 3 - 8 Grade 4 - 3 Av Grade = 1.20 % Infection =56.0 Av Water Temp =48.7	Grade 0 - 29 Grade 1 - 10 Grade 2 - 9 Grade 3 - 2 Grade 4 - 0 Av Grade = 0.68 % Infection =42.0 Av Water Temp =57.6	Grade 0 - 34 Grade 1 - 4 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.11 % Infection =10.5 Av Water Temp =56.4
North Slide	Grade 0 - 8 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.11 % Infection =11.1 Av Water Temp =	Grade 0 - 18 Grade 1 - 18 Grade 2 - 9 Grade 3 - 5 Grade 4 - 0 Av Grade = 0.68 % Infection =64.0 Av Water Temp =	Grade 0 - 21 Grade 1 - 2 Grade 2 - 1 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.17 % Infection =12.5 Av Water Temp =56.4

South Slide	Grade 0 - 4 Grade 1 - 1 Grade 2 - 0 Grade 3 - 2 Grade 4 - 33 Av Grade = 3.48 % Infection =90.0 Av Water Temp =	Grade 0 - 49 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.02 % Infection =2.0 Av Water Temp =	Grade 0 - 49 Grade 1 - 1 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0.02 % Infection =2.0 Av Water Temp =56.4
Hebgen/Quake	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0 % Infection =0 Av Water Temp =46.5	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0 % Infection =0 Av Water Temp =55.6	Grade 0 - 47 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4 - 0 Av Grade = 0 % Infection =0 Av Water Temp =56.9

highest during the earliest time period with the highest infection rates measured in the Earthquake Slide area and near the West Fork of the Madison River area. Two sites, the station between Hebgen Dam and the Earthquake Slide Area and the Norris station, had no *Myxobolus cerebralis* infected test rainbow trout. A total of four sentinel cage sites in the West Fork of the Madison River of the Madison River area were chosen to run a mini time series (Table 6).

Table 6. Whirling Disease infection rates compared to time and water temperature (F) measurements from sentinel cages located at the Upper and Lower Pine Butte Stations and for the Upper and Lower Palisades Stations, 1998. Water temperatures are the average of the high four days within the ten day exposure period.

Date	Upper Pine Butte	Lower Pine Butte	Upper Palisades
May 26 - June 4	Grade 0 - 21 Grade 1 - 1 Grade 2 - 1 Grade 3 - 3 Grade 4 - 1 Av grade =0.37 % Infection=22.2 Av Temp=46.2	Grade 0 - 20 Grade 1 - 4 Grade 2 - 5 Grade 3 - 5 Grade 4 - 4 Av grade =1.18 % Infection=47.4 Av Temp=46.2	Grade 0 - 2 Grade 1 - 3 Grade 2 - 1 Grade 3 - 6 Grade 4 - 15 Av grade =3.07 % Infection=92.6 Av Temp=49.9
June 4 - 14	Grade 0 - 25 Grade 1 - 6 Grade 2 - 6 Grade 3 - 5 Grade 4 - 8 Av grade 1.30 % Infection=50.0 Av Temp=50.9	Grade 0 - 18 Grade 1 - 10 Grade 2 - 7 Grade 3 - 6 Grade 4 - 6 Av grade 1.40 % Infection= 61.7 Av Temp=50.9	Grade 0 - 2 Grade 1 - 2 Grade 2 - 4 Grade 3 - 4 Grade 4 - 19 Av grade 3.16 % Infection=93.5 Av Temp=51.2

June 14 - 24	**Grade 0 - 26** **Grade 1 - 6** **Grade 2 - 7** **Grade 3 - 2** **Grade 4 - 5** **Av grade 1.00** **% infection = 43.5** **Av Temp = 51.1**	**Grade 0 - 33** **Grade 1 - 2** **Grade 2 - 5** **Grade 3 - 3** **Grade 4 - 2** **Av grade 0.64** **% infection = 26.7** **Av Temp = 51.1**	**Grade 0 - 10** **Grade 1 - 6** **Grade 2 - 7** **Grade 3 - 7** **Grade 4 - 14** **Av grade 2.20** **% infection =77.3** **Av Temp = 50.9**
July 8 - 18	**Grade 0 - 21** **Grade 1 - 1** **Grade 2 - 1** **Grade 3 - 3** **Grade 4 - 1** **Av grade 0.59** **% infection = 22.2** **Av Temp =58.8**	**Grade 0 -29** **Grade 1 -12** **Grade 2 - 1** **Grade 3 - 2** **Grade 4 - 1** **Av grade 0.53** **% infection = 22.2** **Av Temp = 58.8**	**Grade 0 - 18** **Grade 1 - 12** **Grade 2 - 7** **Grade 3 - 5** **Grade 4- 0** **Av grade 0.98** **% infection = 22.2** **Av Temp = 56.0**
Sept 28 - Oct 8	**Grade 0 - 47** **Grade 1 - 1** **Grade 2 - 2** **Grade 3 - 0** **Grade 4- 0** **Av grade 0.10** **% infection = 6.0** **Av Temp = 55.2**	**Grade 0 - 38** **Grade 1 - 1** **Grade 2 - 3** **Grade 3 - 0** **Grade 4- 0** **Av grade 0.17** **% infection = 9.5** **Av Temp =55.2**	**Grade 0 - 26** **Grade 1 - 1** **Grade 2 - 0** **Grade 3 - 0** **Grade 4- 0** **Av grade 0.04** **% infection = 3.7** **Av Temp = 54.5**

The upper two sites had significantly lower infection intensities with peak in infection intensity occurring in the June 4 - 14th period in all four sites. Sentinel cages were placed in two sites on the West Fork of the Madison River, one near the mouth and one below the mouth of Soap Creek (Table 7). Infection rates are very low in both sites with the lower site showing a slightly higher rate of WD infection.

Table 7. Whirling Disease infection rates compared to time and water temperature (F) measurements from sentinel cages located in the West Fork of the Madison River at two sites, one near the mouth and one immediately above the mouth of Soap Creek, 1998. Water temperatures are the average of the high four days within the ten day exposure period.

Date	Upper Station - immediately about mouth of Soap Creek.	Lower Station - near mouth at USFS Bridge
June 14 - 24	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4- 0 Av grade =0.00 % infection = 0 Av Temp = 45.9 F	Grade 0 - 50 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4- 0 Av grade = 0.00 % infection =0 Av Temp = 51.3 F
July 9 - 17	Grade 0 - 45 Grade 1 - 2 Grade 2 - 1 Grade 3 - 0 Grade 4- 2 Av grade = 0.24 % infection =10.0 Av Temp = 57.4 F	Grade 0 - 23 Grade 1 - 14 Grade 2 - 7 Grade 3 - 3 Grade 4- 3 Av grade =0.98 % infection =54.0 Av Temp = 57.0 F
Sept 17 - 25	Grade 0 - 46 Grade 1 - 2 Grade 2 - 2 Grade 3 - 0 Grade 4- 0 Av grade =0.12 % infection =8.0 Av Temp = 51.4 F	Grade 0 - 47 Grade 1 - 0 Grade 2 - 0 Grade 3 - 0 Grade 4- 0 Av grade = 0.00 % infection = 0 Av Temp = 52.8 F

Missouri River Studies

The Missouri River/Little Prickley Pear Creek studies began in 1997 following the discovery of whirling disease positive fish in both the Missouri River near Craig and in Little Prickley Pear Creek. The 1997 study employed sentinel cages in the following sites:1)a time series site in Little Prickley Pear Creek (near mouth of Wolf Creek- Wirth Diversion); 2) a time series site in the Missouri River at Craig and three spatial sites in the mainstem Missouri River (below Holter Dam, Mid Cannon and Pelican Point). Peak whirling disease infections were considered low in all sites where positive fish were found in sentinel cage exposures with no positive rainbow trout found in the three mainstem Missouri River cages (exception Craig site which was positive for the parasite). In 1998, the sentinel cage studies was expanded to include a spatial series on Little Prickley Pear Creek and sentinel cages in two tributaries to Little Prickley Pear Creek (Lyons and Wolf Cr) and two additional tributaries to the Missouri River (Dearborn River and Sheep Creek).

Little Prickley Pear Creek

Table 8 compares the whirling disease infection rates by 1997 and 1998 for time series of sentinel cages on the Wirth Diversion site. Infection rates have show a significant increase in intensity from 1997 to 1998. During 1997, there was a short period during late May and early June where infection rates were above the 2.7 level where young-of-the-year rainbow trout could suffer some losses from the disease (Figure 2). In 1998, nfection rates had increased to very high levels to where WD infection intensities exceeded 3.00 from at least late April through early July. These levels should cause extensive mortality to the 1998 year class of wild rainbow trout. During 1998
a spatial series of sentinel cages were placed in Little Prickley Pear Creek beginning at the mouth and extending upstream approximately 22 miles to the mouth of Canyon Creek (Table 9).

Table 8. Comparison of whirling disease infection rates between 1997 and 1998 from the time series station(Wirth Diversion) on Little Prickley Pear Creek. Water temperatures are an average of the high four day mean in F.

Time Period 1998 is 2 days later	**1997** No. Fish	Infection Intensity	**1998** No. Fish	Infection Intensity
4/26 - 5/5	37	Av Grade = 0.14 Av Temp = 47.8 F % infection = 10.8	50	Av Grade = 3.30 Av Temp = 54.2 F % infection = 94.0
5/5 - 5/15	39	Av Grade = 2.31 Av Temp = 51.8 % infection = 82.0	Lost	Av Grade = Av Temp = % infection =
5/15 - 5/25	59	Av Grade = 2.56 Av Temp = 53.2 % infection = 83.1	10	Av Grade = 4.00 Av Temp = 55.8 % infection = 100.0
5/25 - 6/3	47	Av Grade = 2.94 Av Temp = 54.2 % infection = 83.0	50	Av Grade = 3.66 Av Temp = 54.3 % infection = 96.0
6/3 - 6/13	33	Av Grade = 2.85 Av Temp = 56.7 % infection = 81.8	50	Av Grade = 3.58 Av Temp = 56.0 % infection = 96.0
6/13 - 6/23	lost	Av Grade = Av Temp = 57.2 % infection =	lost	Av Grade = Av Temp = 52.6 % infection =

6/23 - 7/3	50	Av Grade = 1.50 Av Temp = 58.0 % infection = 52.0	31	Av Grade = 3.84 Av Temp = 57.6 % infection = 96.7
7/3- 7/13	50	Av Grade = 0.98 Av Temp = 60.1 % infection = 46.0	50	Av Grade = 3.60 Av Temp = 60.1 % infection = 94.0
7/13 - 7/23-1997	50	Av Grade = 0.58 Av Temp = 61.3 % infection = 32.0	No data	
7/23 - 8/1-1997 7/28-8/6 -1998	25	Av Grade = 0.00 Av Temp = 63.2 % infection = 0.00	50	Av Grade = 1.12 Av Temp = 62.7 % infection = 66.0
8/1 - 8/12 -1997	50	Av Grade = 0.02 Av Temp = 62.7 % infection = 2.0	No data	
8/12 - 8/22	9	Av Grade = 0.11 Av Temp = 61.4 % infection = 11.1	50	Av Grade = 0.52 Av Temp = 61.1 % infection = 34.0
8/22 - 8/31	34	Av Grade = 0.15 Av Temp = 61.7 % infection = 11.8	49	Av Grade = 0.45 Av Temp = 59.0 % infection = 32.7
8/31 - 9/10	49	Av Grade = 0.05 Av Temp = 60.0 % infection = 2.0	No data	
9/10 - 9/20-1997 9/14 - 9/24-1998	50	Av Grade = 0.64 Av Temp = 56.2 % infection = 2.0	45	Av Grade = 2.49 Av Temp = 58.8 % infection = 26.7
9/20 - 10/1-1997	45	Av Grade = 0.82 Av Temp = 54.7 % infection = 42.0	Lost data	
10/1-10/11-1997 10/4-10/14-1998	50	Av Grade = 0.78 Av Temp = 51.5 % infection = 42.0	50	Av Grade = 0.88 Av Temp = 49.6 % infection = 36.0
10/11 - 10/20	34	Av Grade = 0.62 Av Temp = 47.9 % infection =44.0	No data	

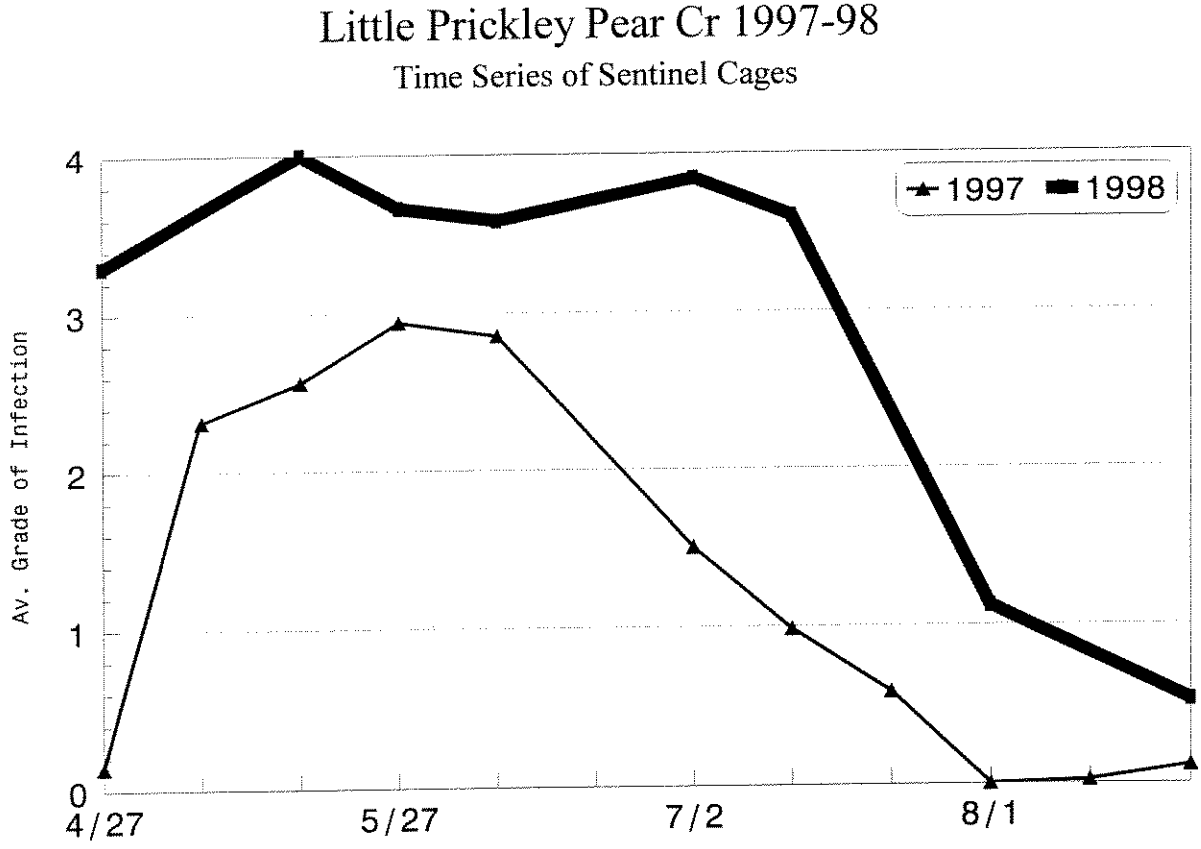

Figure 2. Comparison of whirling disease infection rates over time at the Little Prickley Pear time series station(Wirth Diversion) for 1997 and 1998.

Table 9. Spatial sentinel whirling disease cages located in Little Prickley Pear Creek for two time periods in 1998. Water temperatures are an average of the high four day mean in F.

Site	Stream mile	May 27 - June 6	Sept 24 - Oct 4
1S Wolf Cr Alt	4.6	Av grade = 3.96 Av Temp = 55.3 F Percent whirl/BT = 73.3	No Cage
Wolf Cr Time/Seq Station Lots 7 and 21	4.6	Av grade = 3.66 Av Temp = 55.3 F Percent Whirl/BT = 84.0	PPCR-21 - lost
2S Exit 219	11.5	Av grade = 3.84 Av Temp = 55.8 F Percent Whirl/BT = 84.0	Av grade = 1.74 Av Temp = 52.4 F % Whirling/BT = 0.0
3S Upper Sieben Cage 1	18.0	Av grade = 3.76 Av Temp = 55.8 F Percent Whirl/BT = 26.0	Av grade = 0.90 Av Temp = 51.8 F %Whirling/BT = 0.0
Upper Sieben Cage 2 4S	18.0	Av grade = 3.45 Av Temp = 55.8 F Percent whirl/BT = 35.7	
Upper Chevallier 5S	21.7	Av grade = 3.45 Av Temp = 55.3 F Percent Whirl/BT = 38.7	Av grade = 1.52 Av Temp = 51.1 F % Whirl/BT = 0.0
Lower Chevallier 6S	21.7	Av Grade = 3.42 Av Temp = 55.3 F Percent Whirl/BT = 15.6	Av grade = 3.00 Av Temp = 51.1F % Whirl/BT = 0.0
Putsch Ranch 7S	23.3	Av grade = 2.51 Av Temp = 55.3 F Percent Whirl/BT = 2.2	Av grade = 1.46 Av Temp = 51.4 F % Whirl/BT = 4.0
Above Canyon Cr 8S	26.4	Av grade = 0.00 Av Temp = 49.0 F Percent Whirl/BT = 0.0	Av grade = 0.00 Av Temp = 49.7 F % Whirl/BT = 0.0

Missouri River and Tributaries

Four sites on the mainstem Missouri River were chosen for sentinel cages to measure presence and intensity of whirling disease infections in the river between Holter Dam and Great Falls. One of these sites (Craig) was chosen for a time series of cages starting in late April and continuing every ten days through early August (Table 10). During 1997, whirling disease infection rates were very low peaking in mid May at 0.66. Infection intensities at these low levels are unlikely to cause any significant problems to young-of-the-year rainbow trout in this reach of river. In 1998, infection rates increased to a peak infection of 1.83 in late May - early June which was about three times that found in 1997. Even this threefold increase should not have been enough to cause population damage to the 1998 rainbow year class. Table 11. shows the sentinel cage results from three addition sites on the mainstem Missouri River in 1997 and four in 1998. In 1997, no mainstem site, except the Craig site, show in positive test fish, but a repeat in 1998 showed that the Pelican Point station had a very low incidence of the parasite (2.0% infection of 50 test rainbow trout). Tributary sentinel cage tests in 1998 showed only two tributaries infected with the parasite (Wolf and Sheep Creeks) and the remaining two (Lyons Cr and the Dearborn River) were negative. Infection intensities in Wolf and Sheep Creek were very low and it is doubtful that any damage would have occurred to young-of-the-year rainbow trout either stream during 1998.

Table 10. Comparison of whirling disease infection rates between 1997 and 1998 from the time series station at Craig on the Missouri River. Water temperatures are an average of the high four day mean in F.

Date	1997		1998	
	No. Fish	Infection Intensity	No. Fish	Infection Intensity
4/26 - 5/5	17	Av Grade = 0.00 Av Temp =45.0 % infection =0	46	Av Grade =0.59 Av Temp = 48.4 % infection =41.0
5/5 -5/15	64	Av Grade =0.66 Av Temp =48.4 % infection =23.4	Lost	Av Grade = Av Temp = 51.0 % infection =
5/15 - 5/25	55	Av Grade =0.20 Av Temp =50.2 % infection =12.7	29	Av Grade =1.69 Av Temp = 53.3 % infection =72.0
5/25 - 6/3	31	Av Grade =0.29 Av Temp =53.8 % infection =16.1	48	Av Grade =1.83 Av Temp = 54.4 % infection =71.0
6/3 - 6/13	38	Av Grade =0.03 Av Temp =57.5 % infection =2.6	50	Av Grade = 0.60 Av Temp = 55.7 % infection =

6/13 - 6/23	**50**	**Av Grade =0.00 Av Temp =60.2 % infection =0**	**50**	**Av Grade =0.58 Av Temp =55.6 % infection =32.0**
6/23 - 7/2	**50**	**Av Grade =0.00 Av Temp =60.9 % infection =0**	**41**	**Av Grade =0.54 Av Temp =59.9 % infection =39.0**
7/2 - 7/12	**50**	**Av Grade =0.08 Av Temp =62.8 % infection =2.0**	**50**	**Av Grade =0.58 Av Temp =63.5 % infection =42.0**
7/12 - 7/22	**50**	**Av Grade =0.32 Av Temp =65.3 % infection =16.0**	**No data**	
7/22 - 8/1	**45**	**Av Grade =0.09 Av Temp =66.9 % infection =6.7**	**No data**	
8/1 - 8/11	**26**	**Av Grade =0.00 Av Temp =67.2 % infection =0**	**No data**	

Table 11. Comparison or whirling disease infection rates between 1997 and 1998 for the spatial sentinel cage sites on the mainstem Missouri River and selected tributaries. Water temperatures are an average of the high four day mean in F.

Sample Site	**1997 No. Fish**	**Infection Intensity**	**1998 No. Fish**	**Infection Intensity**
Missouri River		**1997**		**1998 June 6 - 16**
Holter Dam	26	Av Grade = 0.00 Av Temp = % infection =0	50	Av Grade = 0.00 Av Temp =55.4 % infection =
Mid Cannon	8	Av Grade = 0.00 Av Temp = % infection =0	50	Av Grade = 0.00 Av Temp =56.4 % infection =

Pelican Point		No data	50	Av Grade = 0.02 Av Temp =56.9 % infection =2.0
Gaint Springs	no data		50	Av Grade = 0.00 Av Temp = % infection =
Tributaries	no data			
Sheep Creek	no data		46	Av Grade = 0.11 Av Temp = 57.4 % infection = 9.0
Wolf Cr	no data		46	Av Grade = 0.11 Av Temp = 56.6 % infection = 9.0
Dearborn River	no data		50	Av Grade = 0.00 Av Temp = 53.4 % infection = 0
Lyons Cr	no data		48	Av Grade = 0.00 Av Temp =47.7 % infection =0

Rock Creek Studies

Background & Disscussion

Whirling disease research on the Rock Creek and tributaries during 1998 comprised of a spatial series within the mainstem Rock Creek, a mini time series on the Hogback section and a survey of five important tributaries. The mini time series on the Hogback section showed an increasing average infection rate with increasing water temperatures with the peak measured during the July 12 - 22 period (Table 12). During the peak infection time period, infection rates were high enough to cause some level of salmonid population damage, especially spring spawning species, such as the Westslope cutthroat trout and rainbow trout. Two downstream sites in the mainstem Rock Creek showed only minor levels of infection, probably below levels that would cause salmonid population declines. Of the seven tributaries studied, only the East Fork of Rock Creek showed WD infection levels high enough to cause population damage. Further sentinel cage studies in 1999 is recommended.

Table 12 . Whirling Disease sentinel cage results for Rock Creek and tributaries. Mini time series for the Hogback section of Rock Creek tested for the June 18 - July 32, 1998 period. Spatial series for two time periods (June 18-26 & July 2-12). Water temperatures are an average of the high four day mean in F.

Site	No. Fish	Percent Infected	Mean Grade of Infection	Mean Daily Water Temp F
Mini Time Series				
Hogback (RCR-1) June 18-26	**28**	**71**	**1.71**	**50.1**
Hogback (RCR-2) July 2 -12	**50**	**88**	**2.36**	**53.0**
Hogback (RCR-3) July 12-22	**50**	**88**	**2.80**	**61.9**

Spatial Stations	**June 18 - 26 No. Fish**	**Av Grade**	**July 2 - 12 No. Fish**	**Av Grade**
Hogback Cr near mouth	50	Av Grade = -0.06 Av Temp =53.4F % infection =6.0	**0**	
Stoney Cr	50	Av Grade - 0.00 Av Temp = 44.7F	50	Av Grade =0.00 Av Temp =49.8F
Fish & Game	49	Ave Grade = 0.94 Av Temp = 50.5F % infection =49.0	50	Av Grade = 1.30 Av Temp = 59.0F % infection =62.0
Valley of the Moon	47	Av Grade = 1.15 Av Temp = 51.0**F**	50	Av Grade = 1.48 Av Temp = 56.4F % infection= 66.0
East Fork Rock Cr	51	Av Grade= 3.57 Av Temp = 50.0F % infection = 92.0	50	Av Grade = 3.84 Av Temp = 61.4F % infection=98.0
West Fork Rock Cr-	27	Av Grade = 0.00 Av Temp =48.9F	50	Av Grade = 0.00 Av Temp= 55.8F
Willow Cr	50	Av Grade = 0.14 Av Temp= 48.2F	Lost	
Ross Cr	43	Av Grade = 0.00 Av Temp = 48.1F	50	Av Grade = 0.06 Av Temp = 63.1F % infecion = 4.0

Yellowstone River Studies

Background

Prior to 1998, whirling disease had not been found in the Yellowstone River drainage. All testing for the presence of *Myxobolus cerebralis* was done through the use of electrofishing samples of either yearling or young-of-the-year wild brown, rainbow or cutthroat trout. These samples were taken from various sites in the mainstem Yellowstone River from sites located on the upper river near Yankee Jim Canyon to sites downstream near the town of Springdale, MT. No sample taken prior to 1998 in the mainstem Yellowstone River was determined to be positive to the presence of *M. cerebralis*. During April, 1997, a routine sample of young-of-the-year and yearling rainbow and brown trout sampled from the lower reaches of Armstrong Spring Creek showed a mild infection of *Myxobolus cerebralis* (16.3% of sampled fish). Previous samples taken in Armstrong Spring Creek in 1996 were negative.

Objectives

To determine the presence or absence of *Myxobolus cerebralis* in the mainstem upper Yellowstone River system from the mouth of Yankee Jim Canyon to the Sheep Mountain Fishing Access Site. In addition, sites which were found to be positive, the degree or intensity of infection would be determined. This survey would also include Armstrong Spring Creek where a light whirling disease infection was found in 1997.

Methods

In order to determine these infection rates and intensities, the use of live cages was determined to best method to determine both infection rate and intensity. These live cages containing 60 (35 - 50 mm) were placed in each stream site and left for 10 days upon which the fish were removed and taken to the whirling disease research lab at Pony, MT, and kept in 10 C well water aquaria for an additional 90 days. At that time fish were sacrificed and sent to the Washington Animal Disease Diagnostic Laboratory at Washington State University for histological analysis. Histological analysis included both the presence or absence of the parasite and a histological grading of the disease severity. Histological samples were taken from the cranial area of each fish and with the aid of a microscope presence or absence and severity of infection was determined. The infection severity was scored using a 0 - 4 scale which ranks the infection based off of the degree of cartilage damage and inflamation. Grade 0 was no detectable infection and grade 4 being extremely severe. Each stream station also contained a recording thermometer to determine water temperature during the 10 day stream exposure. A total of seven mainstem live cage sites were used on the mainstem Yellowstone River and two in Armstrong Spring Creek.

Results and Discussion

The seven live cage exposures on the mainstem Yellowstone River were exposed beginning on October 8, 1998 and removed on October 18, 1998. Five of the live cage exposures showed no detectable infections of *M. cerebralis* (Sites Yankee Jim Canyon, Emigrant, mouth of Mill Cr, near the mouth of the Shields River and Sheep Mountain Fishing Site Access). Two sites

the **9th Street Bridge site** in Livingston and the **Hyway 89 Bridge site** north of Livingston did show infections of *M. cerebralis*. The 9th Street site showed an 18% infection with an intensity of 0.44.The Hyway 89 Bridge Site showed a 6% infection rate with an intensity of infection of 0.06. Neither site shows a whirling disease infection rate with a high enough intensity to cause any wild rainbow or cutthroat trout population impact. It is generally thought that infection intensity has to excess 2.50 before wild trout populations would show any measurable impact. Since early electrofishing samples in 1996 and 1997 at these sites did not detect any *M. cerebralis* infections, these infection found in October, 1998, probably are new and are likely to increase in intensity. The degree of increase is uncertain at this time and continued monitoring in future years is advised. The two Armstrong Spring Creek live cage sites showed a much higher level of whirling disease infection with percent of infection reaching 56 and 80% in the lower and upper cages, respectively. The intensity of infection was still below 2.50, but was higher than found in the river sites, 1.46 and 1.98, respectively. Again this level of whirling disease infection should not cause a detectable impact on the wild trout fisheries in this spring creek at this time, but any future increases could cause population losses. Since the 1996 sampling showed no detectable infection; the April, 1997, showed a presence but low rate of infection and the July, 1998, a further increase in infection intensity future increases are likely. Continued sampling in 1999 and 2000 is advised.

References

Downs, C.C. 1997b. Supplemental Whirling Disease Testing Project. Final Report. Montana Cooperative Fishery Research Unit, Department of Biology for Montana Fish, Wildlife and Parks, Bozeman, Montana.

Grisak, Grant G. 1999. Missouri River Rainbow Trout Spawning Study, Missouri River Young of the Year Trout Investigations II and Montana Statewide Whirling Disease Investigations. Progress Report. Montana Cooperative Fishery Research Unit, Department of Biology for Montana Fish, Wildlife and Parks, Bozeman, Montana.

Vincent, E. R. 1999. The Relationship of Time, Temperature and Fish Life Histories to Whirling Disease Infections. Whirling Disease Symposium. Montana Fish, Wildlife and Parks.