

FISHERY RESEARCH



**LITTLE LOST RIVER WHIRLING DISEASE EXPOSURES**



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## ABSTRACT

Sentinel rainbow trout were used to examine locations in Wet Creek within the Little Lost River basin to investigate whether whirling disease (*Myxobolus cerebralis*) was the likely cause of a decline in bull trout numbers. Two groups of sentinels were held in separate locations in Wet Creek for the ten day period of May 16 to 26, 2003. A third group was exposed in the Little Lost River for the same period. These trout were held an additional 100 days at the wet laboratory of the Eagle Fish Health Laboratory and quantitatively assayed individually for *M. cerebralis* spores. No infection was detected from the uppermost exposure location in Wet Creek while 100% prevalence and high intensity of infection was demonstrated in sentinels exposed at the lower Wet Creek and Little Lost River locations. Water quality parameters measured in this trial demonstrated that the upper exposure site was cooler than those at lower elevations.

Since bull trout are known to have low susceptibility to whirling disease and occupy habitat in the upper reaches of Wet Creek, it is unlikely that the parasite would be the cause of a bull trout population decline. The intensity of the challenge at the lower two locations was sufficient to implicate a role in limiting the abundance of susceptible salmonid species.

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## INTRODUCTION

Salmonids from the Little Lost River (LLR) drainage were sampled by Idaho Department of Fish and Game (IDFG) starting in 1995 because stocking records from Lost River Trout Company indicated potentially positive rainbow trout had been sold to private pond owners within the drainage. Additionally, trout from Hayspur Hatchery, IDFG had also been planted into the drainage during the period of time when that facility was positive for the parasite. Additionally, there is an existing irrigation water connection with the known positive Pahsimeroi River drainage. All of these mechanisms may have contributed to the establishment of *Myxobolus cerebralis* in the LLR. The Fish Health Database of the Eagle Fish Health Laboratory (EFHL) contains ten cases of wild salmonids collected from tributaries of this basin. Most sampling effort occurred in the years 1995, 2001, and 2002. Species examined included rainbow trout, brook trout, and bull trout but only rainbow trout were demonstrated positive. Rainbow trout determined to be positive came from the Little Lost River main stem, Wet Creek, and Sawmill Creek. Rainbow trout and brook trout from Summit Creek were negative. The USFWS Wild Fish Health Survey also reports results from examined wild salmonids from LLR in 1999 and 2000. They found *M. cerebralis* positive rainbow and brook trout collected from "Wet Canal" which may be an irrigation canal extracted from lower Wet Creek (Kessel, personal communication, 2003).

Population estimates performed recently have demonstrated a decline in both rainbow trout and bull trout in Wet Creek. Both Jim Fredericks (IDFG, R6) and Bart Gamett (USFS, Mackay) questioned whether the presence of *M. cerebralis* could be contributing to the decline in these species in Wet Creek. Laboratory challenge studies that compare the susceptibility of bull trout with rainbow trout indicate that bull trout demonstrate susceptibility but that the intensity of infection (histological lesion score and spore count) are greatly reduced in bull trout (Hedrick et al, 1999) compared to rainbow trout.

The EFHL offered to examine this question using sentinel rainbow trout fry exposed to sites in Wet Creek and the main stem Little Lost River in a manner similar to exposures conducted in the Salmon, Pahsimeroi and Lemhi rivers in cooperation with research by the University of Idaho. These exposures have been very effective in demonstrating the presence of the parasite, prevalence of infection and the relative exposure intensity.

## METHODS

### Study Site

A single main stem site on the Little Lost River was an engineered reach for irrigation water withdrawal located 15.5 miles north of Howe, ID and two sites were chosen for Wet Creek (Figure 1, Table 1). The site designated "Wet Creek, upper" was located immediately upstream of the Sawtooth National Forest boundary, had low impact from grazing, numerous beaver ponds, and known as bull trout rearing habitat.

## **Experimental Animals and Exposure Methods**

Kamloops strain rainbow trout were obtained from Hayspur Hatchery as eyed eggs, reared at the EFHL wet lab to a size of 0.96 g mean weight, and counted into three groups of about 35 individuals.

Exposures were initiated on May 16, 2003. Jessica Hollist, USFS Mackay assisted in site selection and collection of water parameters. StowAway XTI (Onset Computer Corporation, Buzzard Bay, MA) temperature recorders were attached to the livebox at each site. Site characteristics recorded included valley shape, GPS coordinates, elevation, substrate type, stream width, and livebox water depth. Water flow adjacent to the livebox was measured with a Flowmate model 2000 (Marsh-McBirley, Frederick, MD). Dissolved oxygen, percent oxygen saturation, conductivity, and pH were measured with an YSI model 556MPS (Yellow Springs Instruments, Yellow springs, OH). The Wet Creek upper location was set in a beaver pond since some observations by other researchers (Baldwin et al 1998) suggest this microhabitat type amplifies infectivity by providing ideal habitat for *Tubifex*. The lower Wet Creek site was heavily grazed and silted. The main stem LLR site was in an irrigation canal.

Fish were retrieved from the sites after 13 days exposure on May 29 and transported to the wet lab at the Eagle Fish Health Laboratory. Mortality (24 of 35 fish) occurred during the exposure in the Little Lost River main stem group and was attributed to a significant increase in river flow during the exposure leading to exhaustion.

## **Post-exposure Handling**

Each group was maintained in separate tanks supplied with 13 °C well water, fed three times a week at about 2% body weight per day, and checked daily for mortalities. Post-exposure holding period extended for 100 days (1300 CTU). Only two fish were lost during this holding period.

## **Collection of Samples, Examination, and Analysis**

Groups were sacrificed on August 29, 2003. Fish were euthanized in an overdose of MS222, fork length measured, weighed, and heads severed and split along the mid-sagittal axis into right and left halves. One half of each fish was processed by the quantitative spore count pepsin/trypsin digest method (AFS/FHS Bluebook) while the second half was retained frozen. Prevalence of infection and mean quantitative spore counts were calculated for each group.

## **RESULTS**

### **Aquatic Parameters**

The chemical and environmental exposure site characteristics were representative of the variety present within most Little Lost River tributaries: extensive grazing in the valley floor with relatively pristine conditions in the headwaters on USFS land. The beaver pond location of the

upper Wet Creek location contained extensive settled silt and organic debris. The lower Wet Creek site reflected extensive grazing in the immediate area upstream of the site with extensive erosion of stream banks and resulting siltation. The Little Lost River main stem site had widely fluctuating flows characteristic of an irrigation canal during spring run-off (Table 1). Another characteristic of the three sites in this study was that the two lower sites were in relatively flat terrain while the stream at the upper Wet Creek site had steep gradient.

Water temperature profiles (Table 1, Figure 2) for the Lower Wet Creek and Little Lost River main stem sites averaged 11.5 and 13.1 °C respectively while the upper Wet Creek site was considerably colder (mean of 5.6 °C).

### **Prevalence and Intensity of *Myxobolus cerebralis* Infection**

The prevalence of infection was 100% in rainbow trout exposed at the lower Wet Creek site and main stem Little Lost River site but the assay failed to detect spores of the parasite at the uppermost Wet Creek exposure site.

The quantitative spore counts obtained from each exposure where the parasite indicated a high level of exposure relative to numerous other exposures performed under similar conditions in the South Fork Boise River, Pahsimeroi River, Salmon River, and Lemhi River. Mean spore counts at the lower Wet Creek site were 200,000 spores per head and 364,000 spores per head at the main stem Little Lost River site. The mean spore count of sentinels exposed demonstrated at the main stem Little Lost River and held under similar conditions is the highest recorded by our laboratory. An individual head from this group also set the record number for the highest number of spores counted by our laboratory for 2003 and earned Jim Fredericks the Eagle lab “Boone and Crockett” spore count trophy.

At our laboratory and laboratories of other state resource agencies where quantitative spore counts are performed a mean count of 70,000 confirms to illustrate a very high level of exposure (Pete Walker, Colorado Division of Wildlife).

## **DISCUSSION**

Exposure of sentinel rainbow trout at a highly susceptible size constitutes a very sensitive method of demonstrating the presence and intensity of *Myxobolus cerebralis* infections. The timing of this series (late May) was selected to coincide with peak infectivity previously demonstrated in three other river systems in Idaho (Cavender et al 2003 Burton and Johnson 2003; Munson and Johnson 2003). This peak in infectivity is the same for other measures of infection potential such as enumeration of the infectious triactinomyxon parasite stage on the water column. Fish exposures demonstrate not only the relative intensity of the infectious stage but also its ability to successfully attach, migrate, and parasitize the host.

There is the need to address the question of whether the level of infection demonstrated in this test would be sufficient to negatively impact the bull trout population of upper Wet Creek. The lack of *M. cerebralis* from rainbow trout exposed at the uppermost site in Wet Creek would indicate that there is a refuge in that area which is free of the parasite. It should also be noted that there was almost no mortality during the 100 day post-exposure holding period. Only a few

sentinel trout from the lower Wet Creek and main stem Little Lost River groups that showed black tail and whirling behavior, the signs of whirling disease.

This test demonstrates that there is the potential for the parasite to limit natural production of a susceptible species, such as rainbow trout but an effect on bull trout is very unlikely since bull trout are very refractory to infection. The Eagle Fish Health Laboratory has examined over 260 naturally-produced bull trout statewide without demonstrating a single individual positive for *M. cerebralis*. Montana Fish, Wildlife and Parks have demonstrated infected, naturally-produced bull trout from the Flathead River drainage of Montana (J. Peterson, MFWP, 2003). Bull trout have been infected under laboratory challenge conditions (Hedrick, et al 1999) but with few spores and little histopathology.

The results of this trial also indicate that the Wet Creek-Little Lost River system could be a fruitful area for further work on the epidemiology of *M. cerebralis*. The two exposure sites on Wet Creek were separated by only seven stream miles and yet the infection rate in this stretch went from zero to 100% progressing downstream. This situation begs further work to define what parameters of the aquatic environment contribute to such a change in the level of infectivity in such a short linear distance with only limited tributary input. The pattern of increasing prevalence of *M. cerebralis* in sentinel groups with decreasing elevation was reported by Sandell, et al (2002) in the Lostine River, OR and Schisler and Bergerson (2002) in CO.

## **ACKNOWLEDGEMENTS**

Jessica Hollist of the USFS Mackay District office provided valuable assistance in selection of the exposure sites and collection of water parameters during the field component of this trial. Staff of the IDFG Eagle Hatchery assisted rearing the sentinel rainbow trout in the wet laboratory. Fish Health Technologists Carla Hogge, Sharon Landin, and Roberta Scott of the Eagle Fish Health Laboratory performed the quantitative spore assays. Lani Clifford prepared this report to be consistent with the Department Fisheries Research format. The constructive reviews of the manuscript by Steve Elle, Jim Fredericks and Steve Yundt of IDFG are appreciated.

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Table 1. Little Lost River Whirling Disease Sentinel Exposure May, 2003

<u>Site</u>	<u>LAT (N)</u>	<u>LONG (W)</u>	<u>Altitude ft</u>	<u>Width</u>	<u>Velocity</u>	<u>Water Temp. Parameters</u>			<u>Water Chemistry Parameters</u>			
						<u>Mean °C</u>	<u>Std. Dev.</u>	<u>CV</u>	<u>Dissolved Oxygen</u>		<u>Cond</u>	<u>pH</u>
									<u>mg/l</u>	<u>% Saturation</u>	<u>uS/ccm</u>	
LLR Main	43. 52.126N	113. 05.080W	5009	18 ft	0.30 m/s	13.14	3.36	25.57	12.67	113.1	0.317	7.70
				ND	0.38 m/s				9.74	101.3	0.202	7.90
Wet Cr Lower	44. 07.707N	113. 23.230W	6518	14.8 ft	0.22 m/s	11.51	4.14	36.00	11.89	105.2	0.275	7.93
				15 ft	0.51 m/s				10.36	100.1	0.264	7.65
Wet Cr Upper	44. 01.887N	113. 27.926W	7346	13.1	0.05 m/s	5.57	2.45	43.99	10.10	89.5	0.264	8.30
				14.1	0.42 m/s				11.17	96.6	0.380	7.96

Table 2. Little Lost River Sentinel Exposures (May 16 to 29, 2003) of rainbow trout subsequently sampled by PTD 100 days post-exposure

<u>Group</u>	<u>Lab Assignment #</u>	<u>#+/#</u>	<u>Percent +</u>	<u>Fish #</u>	<u>Spores (X 1000)</u>	<u>Mean (X 1000)</u>
Wet Crk. upper	03-303	0/34	0	--	--	0
Wet Crk. lower	03-304	32/32	100	29	37	200.6
				19	57	
				25	57	
				28	60	
				30	73	
				7	77	
				5	83	
				1	87	
				12	93	
				13	100	
				17	103	
				2	106	
				10	120	
				22	153	
				14	153	
				27	160	
				8	160	
				32	187	
				15	187	
				3	210	
				9	210	
				26	227	
				4	227	
				24	290	
				11	293	
				18	317	
				31	317	
				20	320	
				23	337	
				21	343	
				16	583	
				6	693	
Little Lost R mainstem	03-305	11/11	100	10	190	363.6
				9	200	
				7	220	
				4	240	
				2	327	
				3	333	
				1	35	
				8	440	
				5	483	
				11	577	
				6	640	

Figure 1. Little Lost River drainage map demonstrating sentinel trout exposure sites used in May, 2003

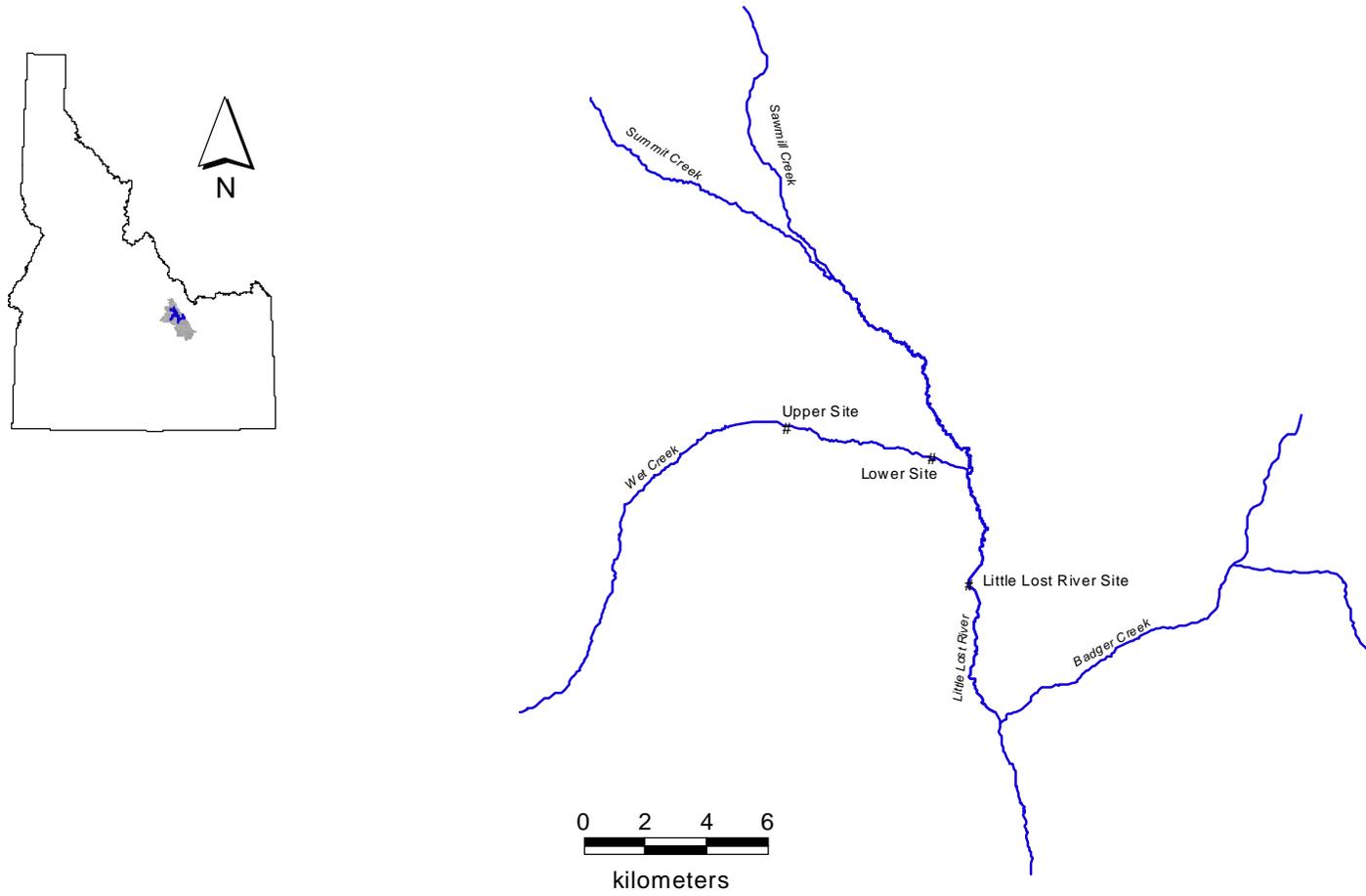
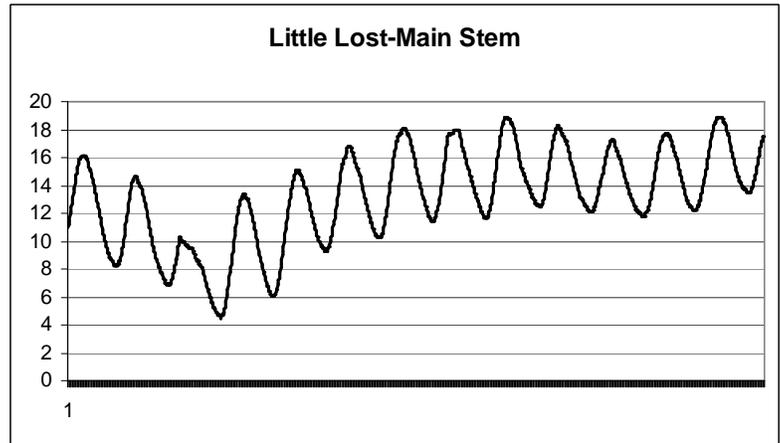
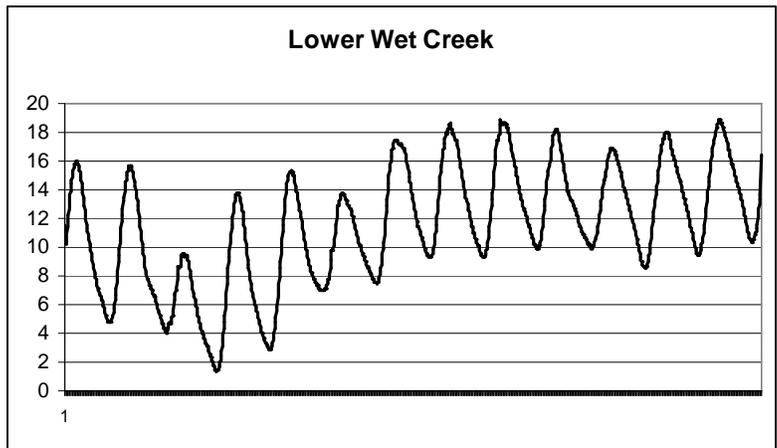


Figure 2: Water Temperature Profiles for each Livebox Location within the Little Lost River during the period of May 16 to 29, 2003

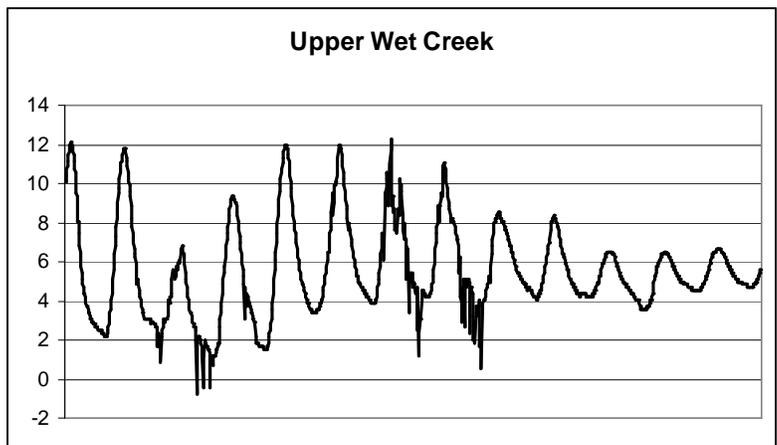
Little Lost-Main Stem	
Mean	13.142516
Standard Error	0.0945825
Median	13.34
Mode	12.26
Standard Deviation	3.3626709
Sample Variance	11.307556
Kurtosis	-0.4539275
Skewness	-0.4341201
Range	14.39
Minimum	4.5
Maximum	18.89
Sum	16612.14
Count	1264



Lower Wet Creek	
Mean	11.505655
Standard Error	0.1169824
Median	11.61
Mode	10.53
Standard Deviation	4.1392601
Sample Variance	17.133474
Kurtosis	-0.6156237
Skewness	-0.2818075
Range	17.54
Minimum	1.32
Maximum	18.86
Sum	14405.08
Count	1252



Upper Wet Creek	
Mean	5.571568
Standard Error	0.0692867
Median	5.13
Mode	4.66
Standard Deviation	2.449655
Sample Variance	6.0008095
Kurtosis	0.1606722
Skewness	0.6599069
Range	13.01
Minimum	-0.74
Maximum	12.27
Sum	6964.46
Count	1250



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