

**FISH & RY RESEARCH**



**IRON CREEK PRE-CONNECTION WHIRLING DISEASE TRIAL**

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**Keith A. Johnson  
Fishery Pathologist Supervisor**

**Douglas R. Burton Fish Pathologist**

**Carla I. Hogge  
Fish Health Technologist**

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## Table of Contents

ABSTRACT .....	1
INTRODUCTION .....	2
STUDY SITE .....	2
OBJECTIVES .....	2
METHODS .....	2
<i>Tubifex</i> Worm Collections .....	3
RESULTS .....	3
DISCUSSION .....	4
ACKNOWLEDGEMENTS .....	5
LITERATURE CITED .....	6

## List of Tables

Table 1. Site locations of live-boxes and water quality parameters collected from Iron Creek and the Salmon River, 30 April through 9 May, 2005 .....	7
Table 2. Quantitative pepsin/trypsin digest enumeration of <i>Myxobolus cerebralis</i> spores in fish heads sampled from exposure trials performed in Iron Creek and the Salmon River, 30 April through 9 May, 2005.....	8

## List of Figures

Figure 1. Scale map of Iron Creek and the Salmon River showing the relative locations of the live-box exposure sites for 30 April through 9 May, 2005.....	9
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## ABSTRACT

Two sentinel groups of rainbow trout *Oncorhynchus mykiss* exposed in Iron creek yielded dramatically different results. No exposure to *Myxobolus cerebralis* was demonstrated at the upstream site while a prevalence of 100% with high spore counts was obtained four stream miles downstream. A third group exposed in the Salmon River immediately upstream of the confluence of Iron Creek had a high prevalence but lower spore counts compared to the positive Iron Creek site. Parameters of the aquatic environment measured in this sentinel trial failed to explain the differences in prevalence and intensity of infection. The exposures were made prior to the establishment of reconnection of migration of salmonids with the main stem Salmon River and serve as a pre-connection test which could be repeated at a later time to examine the time-course of natural expansion of the parasite when migration opportunity is provided in 2006.

Author:

Keith A. Johnson

Fishery Pathologist Supervisor

## INTRODUCTION

Iron Creek is a tributary to the Salmon River that enters from the west and drains an area of 15,540 hectares. Four irrigation diversions located on the lower section seasonally dewater the creek and prevent connection to the Salmon River. Migration of bull trout, steelhead and Chinook salmon into the drainage (for spawning and rearing) is hindered from utilizing this habitat. IDFG Salmon Region fisheries staff has evaluated the species composition and population structure of existing salmonids in Iron Creek prior to reconnection. The wild fish health database of the Eagle Fish Health Laboratory (EFHL) contains an observation of a rainbow trout positive for *M. cerebralis* but the documentation of the collection by NMFS designated the location as being downstream of the disconnected stream section. Consequently, this observation may not be representative of an exposure upstream of the disconnected section and the exposure may have occurred in the Salmon River.

This reconnection provides an opportunity to examine whether *M. cerebralis* would become established or expand its distribution in Iron Creek possibly negatively impacting the existing resident salmonids.

## STUDY SITE

Three sites were selected for this exposure series (Figure 1). Two sites were in Iron Creek upstream of the irrigation withdrawal barrier and the third was in the Salmon River at the Iron Creek Road bridge located about 0.3 km upstream of the confluence (UTM coordinates in Table 1).

## OBJECTIVES

The objective of this sentinel trial was to examine the pre-connection status of this parasite within this system. Having this information would also allow a future evaluation of the rate of invasion and expansion of the parasite as the result of this connection. The rate of natural invasion is currently unknown.

## METHODS

Hayspur strain triploid rainbow trout *Oncorhynchus mykiss* (rbt) were obtained as eyed-eggs and reared at the Eagle Fish Health Laboratory wet lab to a mean size of 0.55 g. Sentinel groups of fifty trout were randomly selected from the main group and transported to and from the exposure site in plastic bags with water and oxygen. At the exposure site, each group of fish was placed in a cylindrical aluminum live-box that measured 47 cm in length x 30.0 cm in diameter. Each live-box was equipped with a STOWAWAY XTI temperature logger to monitor water temperatures at 30 minute intervals. Fish were exposed at each sentinel location for a ten-day period beginning on April 30, 2005. This coincided with a time when whirling disease was demonstrated in the Salmon River both upstream and downstream of the Iron Creek

confluence in 2001 as reported by Cavender, et al 2003 using the same protocols employed in this test.

Characteristics that were measured at each exposure site included GPS-UTM coordinates, elevation, stream width, water depth of live box placement, water velocity adjacent to the live box, dissolved oxygen in mg/l, conductivity, pH, and turbidity. Data was collected at deployment and retrieval. Average values are presented in Table 1.

Groups were returned to the wet laboratory at the Eagle Fish Health Laboratory on May 9, 2005 and held in separate 37-L tanks supplied with 13°C flowing well water previously determined to be parasite free. Groups were fed three times weekly at 2%bw/d and checked daily for mortalities. Sentinel trout were lethally anesthetized and cranial tissues sampled 100 days post-exposure (1300 cumulative Celsius temperature units). This period has become the standard post-exposure holding period and allows comparing relative challenge dose between sentinel trials performed at different locations or season.

Heads were split along the mid-sagittal line and half heads were bagged separately. One half was examined by quantitative spore enumeration (QPTD, EFHL Protocol Manual) while the other retained frozen. Prevalence and mean intensity of infection was determined for each group of sentinels. A t-test was used to examine for statistically significant differences ( $\alpha = 0.05$ ) between exposure groups made at different locations. ANOVA was used to test for correlation of mean values for parameters of the aquatic environment with infection prevalence and intensity of sentinels exposed at the three sites.

### **Tubifex Worm Collections**

An attempt was made in July, 2006 to collect *Tubifex* worms from substrate samples obtained near the exposure sites. This was done to investigate whether the pattern in infection in the sentinel trial could be explained by susceptibility lineage of *Tubifex* (Beauchamp et al. 2001) in Iron Creek. Technicians were unable to recover any Oligochaetes resembling *Tubifex* at four sites near the exposure locations in Iron Creek.

## **RESULTS**

Survival during the 100-day post-exposure holding period was very good and no signs of whirling disease were noted. No spores were found from sentinels exposed at the uppermost site on Iron creek while four miles downstream the infection prevalence was 100% and spore counts were rated extremely high (Table 2). A similar prevalence of infection was obtained in the Salmon River sentinels yet spore counts were about half of that in the positive Iron Creek sentinels. This difference in intensity was significant at  $\alpha = 0.05$ .

Two environmental parameters yielded a modest level of correlation with exposure prevalence and intensity. These were: water velocity adjacent to the live box (R-square = 0.758), and water depth adjacent to the live box (R-square = 0.944). However, analysis of variance (ANOVA) did not find that the differences were significant at  $\alpha = 0.05$ . The other aquatic parameters were not correlated with prevalence or intensity of infection.

## DISCUSSION

The dramatic difference in infection prevalence and intensity between the two exposure sites on Iron Creek was not expected since the sites were separated by only four stream miles. The upper site might be considered ideal for exposure since it was selected because of evidence of high degree of beaver activity which has been suggested as contributing to ideal habitat for the *Tubifex* primary host of *M. cerebralis* (Baldwin et al. 1998). These locations present an ideal opportunity to examine the effect of the aquatic habitat on both the prevalence and intensity of the host(s)-parasite relationship. This test also provides a further opportunity to examine the time-course by which the range of the parasite is affected by re-establishing permanent migration opportunity for salmonids between Iron Creek and the main stem Salmon River. This provides a rare opportunity with a demonstrated but limited distribution within a relatively small geographical area increasing the likelihood of obtaining meaningful results compared to much larger, more complicated drainages such as the Pahsimeroi River (Colvin, 2005).

## **ACKNOWLEDGEMENTS**

Valuable on-site assistance with the exposure phase of this work was provided by Greg Lowell, Salmon Region Fisheries Technician. Valued fish culture aid was freely given by the staff of Eagle Hatchery (IDFG) during the post-exposure holding period. The fish health technologists of EFHL Carla Hogge, Sharon Landin, and Roberta Scott provided consistent spore enumeration in this study. Statistical analysis was made by Martin Koenig, IDFG Nampa Fisheries Research. The sacrifices of B. Duncan and associates were appreciated during this study.

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Table 1. Site locations of live-boxes and water quality parameters collected from Iron Creek and the Salmon River, 30 April through 9 May, 2005.

Site	UTM Coordinates (NAD27)			Altitude (m) above sea level	Stream Width (m)	Stream Depth <sup>a</sup> (m)	Velocity <sup>a</sup> (m/sec)	Water Temperature		Water Chemistry Parameters			
	Zone	mE	mN					Mean °C (std. dev)	Min/Max	DO mg/l	Cond	pH	Turbidity
Iron Creek- Upper	11	730014	4977684	1,640	5	0.31	0.06	5.03 (1.52)	1.39/8.43	9.03	0.082	8.06	0.88
							0.09			8.6			
Iron Creek- Lower	11	736620	4976315	1,417	2.9	0.55	0.39	7.72 (2.00)	2.78/11.36	10.48	0.106	7.92	0.46
							0.43			8.58			
Salmon R bridge	12	265284	4974251	1,349	68.6	0.46	0.01	11.60 (1.09)	8.69/13.81	9.25	0.159	8.42	73
							0.06			7.15			
							0.10						

<sup>a</sup>Measured next to the live-box.

<sup>b</sup>Mean of values recorded at 30-minute intervals by a Stowaway XTI temperature logger over 10 days.

Table 2. Quantitative pepsin/trypsin digest enumeration of *Myxobolus cerebralis* spores in fish heads sampled from exposure trials performed in Iron Creek and the Salmon River, 30 April through 9 May, 2005.

	No. Fish	Prevalence	Calculated Spores/Fish.	
	<u>Exposure Site</u>		<u>Tested</u>	<u>(%Positive)</u>
	<u>Mean (std. dev.)</u>		<u>Range</u>	
Iron Creek — Upper	47	0	NA	NA
Iron Creek — Lower	35	100	238,667 (169,658)	16,667 — 790,000
<u>Salmon River Bridge</u>	<u>47</u>	<u>100</u>	<u>104,078 (75,336)</u>	<u>1,667 —</u>

353,333

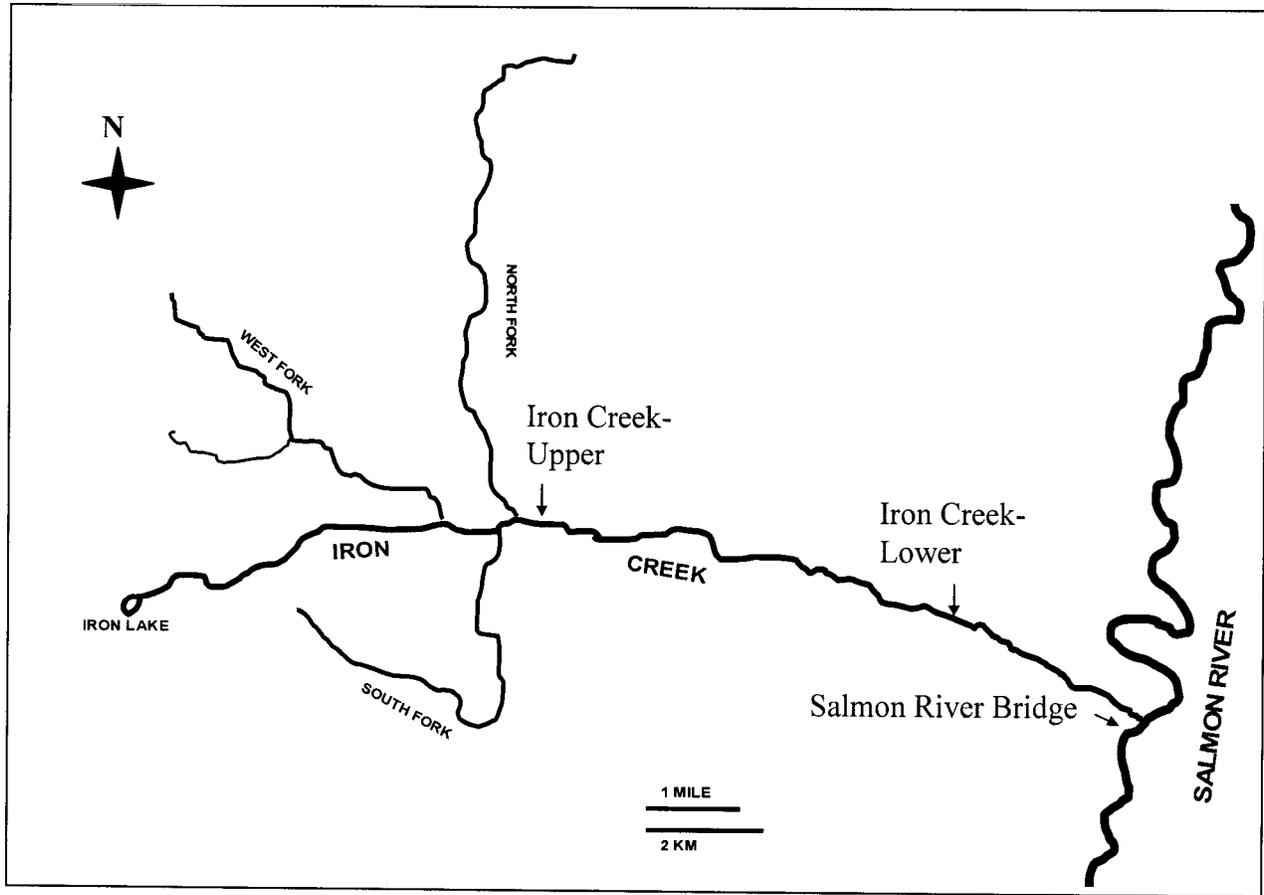


Figure 1. Scale map of Iron Creek and the Salmon River showing the relative locations of the live-box exposure sites for 30 April through 9 May, 2005.

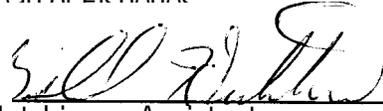
Prepared by:

Keith A. Johnson  
Fishery Pathologist Supervisor

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

Ed chriever, Chief  
Bureau of Fisheries



Bill Hutchinson Assistant  
Bureau Chief