

FISHERY RESEARCH



LAPWAI CREEK, SWEETWATER CREEK, AND POTLATCH RIVER

***MYXOBOLUS CEREBRALIS* INFECTIVITY TRIALS**

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ABSTRACT

A sentinel exposure series of six live-boxes was conducted in Nez Perce County, Idaho to determine the distribution of *Myxobolus cerebralis* in the Lapwai Creek drainage, a tributary of the Clearwater River. Two locations in Sweetwater Creek, three sites in Lapwai Creek, and a site on the Potlatch River were chosen. Rainbow trout fry were exposed for ten days at each site and transferred to the disease-free water at the Eagle Fish Health Laboratory for an additional 100 days. Pepsin/trypsin digest analysis indicated that only the two sites in Sweetwater Creek yielded positive trout. The site downstream of the Sweetwater Creek confluence on Lapwai Creek was positive by use of the nested polymerase chain reaction (PCR) technique for *M. cerebralis*. The highest prevalence was obtained at the upper site on Sweetwater Creek, upstream of the effluent discharge of Sweetwater Aquaculture demonstrating that the infection has become established in Sweetwater Creek.

The limited distribution combined with the low prevalence and intensity of infection obtained in this sentinel trial indicated that the aquatic environment in this vicinity is not conducive for amplification of the parasite to levels which might cause detrimental effects on salmonids. The current condition should be monitored in the future to determine if this situation changes. Management implications for disposition of trout from Sweetwater Aquaculture are presented. Suggestions for management of the permitting process employed by the Idaho Department of Fish and Game for private trout ponds are also discussed.

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INTRODUCTION

Two observations and confirmations of the presence of *Myxobolus cerebralis* were made by the United States Fish and Wildlife Service's Idaho Fish Health Center in 2005 (www.fws.gov/wildfishsurvey/). These indicated that rainbow trout *Oncorhynchus mykiss* reared by Sweetwater Aquaculture of Lapwai, ID were positive for the parasite. Two feral rainbow trout of 20 total collected by electro-fishing on Sweetwater Creek were also demonstrated positive. These positive feral trout could not be determined to have originated from Sweetwater Aquaculture. Sweetwater Aquaculture was informed in February, 2006 that rainbow trout produced at the facility were no longer permitted to be sold to private ponds in Idaho.

The IFHC observations represent only the second time the parasite had been confirmed within the Clearwater River drainage. The other positive location is the American River, a tributary of the South Fork Clearwater River near Elk City, ID. The American River was first determined positive in rainbow trout in 1995 by the Eagle Fish Health Laboratory (EFHL) and again in 1998 by the IFHC.

Exposure of small rainbow trout in sentinel live-boxes and subsequent holding in disease-free water has been documented to be a sensitive method to demonstrate the presence, prevalence, and intensity of *M. cerebralis* infection. These observations have been made not only in Idaho but also Colorado, Montana, Oregon, Wyoming, Utah, and on the East Coast. The Lapwai Creek sentinel series provided the opportunity to add a live-box site in the Potlatch River since *M. cerebralis*-infected trout have potentially been planted into private ponds from an Oregon private hatchery during a period in the late 1980s. The Potlatch River sentinels were used to examine whether the parasite had become established as the result of this importation.

STUDY SITE

Lapwai Creek enters the Clearwater River from a southerly direction 19 rkm upstream of the city of Lewiston, ID and drains an area of 5,751 hectares. Sweetwater Creek is the largest tributary of Lapwai Creek with its confluence 2 rkm upstream of Lapwai, ID. The Potlatch River joins the Clearwater River from the north 5 rkm upstream of Lapwai Creek. Three sites were used for exposure of sentinel groups in Lapwai Creek, two in Sweetwater Creek, and a single site was used in the Potlatch River (Figure 1). Site selections were made with the assistance of Clint Chandler, biologist with Nez Perce Tribal Fisheries Department. Map coordinates for each site are presented in Table 1.

OBJECTIVES

Following an observation of the presence of *M. cerebralis* in hatchery origin rainbow trout reared at Sweetwater Aquaculture of Lapwai, ID, we wanted to determine the presence, prevalence, and intensity of *M. cerebralis* in Lapwai Creek and Sweetwater Creek. Examination of the nearby Potlatch River was included since *M. cerebralis*-infected fish may have been introduced in the 1980s by a private Oregon hatchery.

METHODS

Troutlodge (Sumner, WA) strain rainbow trout *Oncorhynchus mykiss* (rbt) were obtained as eyed-eggs and reared at the EFHL wet lab to a mean size of 0.6 g. Groups of fifty trout were randomly selected from the main group and transported to and from the exposure sites 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. Fish were exposed at each sentinel location for a ten-day period beginning on October 13, 2005.

Site measurements were taken at deployment on 13 October 2005 (Table 1). Dissolved oxygen, conductivity, pH, and water flow parameters were collected (Table 2). A StowAway XTI temperature logger (Onset Computer Corporation, Pocasset, MA) was attached to each live-box and set to measure at 30 minute intervals for the duration of the exposure.

Groups were returned to the wet laboratory at the Eagle Fish Health Laboratory 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 and checked daily for mortalities. Surviving sentinel trout were lethally anesthetized and cranial tissues sampled on December 15, 101 days post-exposure (1,313 cumulative Celsius temperature units). Heads were split along the mid-sagittal line and half heads were analyzed with the quantitative pepsin/trypsin digest (QPTD) method. Confirmation of spores detected by QPTD was made with the nested polymerase chain reaction (PCR) using the method of Andree et al, (1997).

RESULTS

Mortalities due to *Ichthyophthirius multifiliis* were observed in all groups of sentinel trout exposed at sites in Lapwai Creek, but did not occur in those exposed in Sweetwater Creek or the Potlatch River.

Examination of head tissues of sentinels by QPTD indicated that both locations on Sweetwater Creek were positive for *Myxobolus* spores. Spores were confirmed to be *M. cerebralis* with nested PCR. The prevalence of infection of sentinels exposed at the upper site on Sweetwater Creek was 48.1%, while the lower Sweetwater Creek site was 6.5%. The mean spore count per head for these sentinels was 2,450 and 470 for the upper and lower sites, respectively. The single highest spore count was 16,700 spores per head and came from a trout exposed at the most upstream Sweetwater Creek site. This intensity of infection was much lower compared to results of sentinel trials conducted at numerous locations in the Salmon and Snake rivers and their tributaries (Cavender et al. 2003, Hiner and Moffitt 2001, Burton and Johnson 2003, and Munson and Johnson 2003). No spores were observed in any of the sentinel trout exposed at the three sites in Lapwai Creek nor from those of the Potlatch River.

Nested PCR did illustrate that *M. cerebralis* genetic material was present in 2 of 11 sentinels exposed at the Lapwai Creek site immediately downstream of the confluence with Sweetwater Creek. This observation demonstrates that a very slight infection was obtained at that site but was below the limits of detection using QPTD analysis (3,300 spores per head). The duration of post-exposure holding also has been demonstrated to affect the number of spores detected. Spores of *M. cerebralis* have been recovered intact following QPTD at 830

CTU (accumulated Celsius temperature units) in other sentinel trials. The holding period of 1,313 CTU in this study was long enough that spores would have been sufficiently mature to survive enzymatic digestion.

DISCUSSION

This trial illustrates that *M. cerebralis* has become established in Sweetwater Creek upstream of the effluent discharge of Sweetwater Aquaculture. The intensity of infection was low compared to sentinel trials made in locations in the Salmon River, upper Snake River basin, and in locations in the Sinks Basin (Big Lost River, Little Lost River, and Birch Creek) of Idaho.

The sentinels exposed at three locations in Lapwai Creek were determined to be negative for spores by QPTD, but two of eleven from the Lapwai Creek site immediately downstream of Sweetwater Creek confluence were determined positive for *M. cerebralis* DNA using nested PCR. This demonstrates that while positive for the parasite, the intensity of infection was less than the limits of detection by the QPTD method as performed by the EFHL (3,300 spores per head). These findings suggest that the infection may be present in both Sweetwater Creek and to a more limited extent in Lapwai Creek, but that the infection intensity is very low. This level of infection would not contribute to detrimental effects on resident salmonid populations at the time and under the conditions that this sentinel series was done.

The failure to detect *M. cerebralis* in sentinel trout exposed near the mouth of the Potlatch River illustrates that the infection has not become established nor proliferated in this river system as the result of planting potentially infected trout into private ponds in this drainage in the 1980s. Evaluations made shortly after these ponds were stocked showed that most did not have an outlet to the Potlatch River and this might be the reason the infection was not established. It is also possible that *M. cerebralis* has become established, but at an intensity below our limits of detection.

Most of the enhancement activities being conducted by the Nez Perce Tribal Fisheries Department on Lapwai Creek and the Potlatch River are with coho salmon *O. kisutch*. Coho salmon is a species with low susceptibility to *M. cerebralis*. Consequently, it is unlikely that this parasite would have any negative impact on these enhancement activities.

RECOMMENDATIONS

The discrepancy between the QPTD and PCR results for sentinel heads from trout exposed at Lapwai Creek downstream of Sweetwater Creek is likely due to the lower sensitivity of QPTD compared with nested PCR. Unanalyzed half-heads from sentinels exposed at the upper Sweetwater Creek site, the lower Sweetwater Creek site, the Lapwai Creek at Jacques site, and the Lapwai Creek at Spalding site are archived and should also be analyzed individually by nested PCR.

The Idaho Department of Fish and Game (IDFG) should continue the prohibition of sales of any rainbow trout raised at Sweetwater Aquaculture until trout raised at this facility have demonstrated negative *M. cerebralis* inspection results in three consecutive fish health inspections. This criterion is consistent with the former requirements of the USFWS for imported

salmonids under Title 50, with the importation recommendations of the Pacific Northwest Fish Health Protection Committee (PNFHPC), and is similar to the import and transport requirements of the Canadian Department of Fisheries and Oceans' Fish Health Protection Regulations.

It would be advantageous for IDFG, the Idaho State Department of Agriculture, tribal aquaculture programs, and the USFWS to communicate inspection results in order for aquaculture-produced trout, both those intended for stocking into public waters of the State (private ponds and fee-fishing operations included) and those under tribal authority, not to pose a health risk to naturally-produced salmonids.

Additionally, Sweetwater Aquaculture should effectively disinfect its facilities and restart any trout production from disinfected eyed eggs, never bringing any live salmonids onto the station since this method may have been responsible for the infection becoming established. If trout production intended for private stocking is part of the business plan for Sweetwater Aquaculture, they must initiate the three inspection process with trout raised a minimum of 1,800 CTU at their facility for these inspections.

ACKNOWLEDGEMENTS

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Table 1. Locations and physical parameters for exposure trial sites on Lapwai Creek, Sweetwater Creek, and Potlatch River, collected 13 October, 2005. All UTM coordinates use mapping datum WGS 84.

Site	UTM Coordinates		Width (m)	Depth (m)	Habitat Type	
	Zone	mE				mN
Lapwai Cr. @ Jacques	11T	522740	5135505	2	1	Pool; U valley
Lapwai Cr. @ Bone Yard	11T	515853	5136027	7	0.3	Pool; U valley
Lapwai Cr. @ Spalding	11T	514167	5143775	4	0.3	Glide; U valley
Sweetwater Cr. above aqua site	11T	514722	5134263	1.5	0.3	Pool; V valley
Sweetwater Cr. @ Packers Cr.	11T	511810	5129501	1.5	0.2	Pool; V valley
Potlatch River @ NPTF fish trap	11T	517978	5147954	8	0.7	Glide; V valley

Table 2. Water temperature and chemistry parameters for exposure trial sites on Lapwai Creek, Sweetwater Creek, and Potlatch River, collected 13 October, 2005

Site	Water Temperature (C) ^a			Conductivity (mS/ccm)	pH	Turbidity (FTU)
	Mean	Minimum	Maximum			
Lapwai Cr. @ Jacques	13.3	10.7	16.2	0.253	8.4	2.79
Lapwai Cr. @ Bone Yard	12.2	8.4	16.4	0.272	8.2	4.67
Lapwai Cr. @ Spalding				0.280	8.5	1.69
Sweetwater Cr. above aqua site	12.0	9.0	14.6	0.303	8.3	0.02
Sweetwater Cr. @ Packers Cr.	11.0	7.5	14.3	0.263	8.4	
Potlatch River @ NPTF fish trap	13.6	9.9	16.7	0.127	8.2	

^aData recorded every 30 minutes using a StowAway XTI Temperature Logger attached to each live box.

Table 3. Quantitative pepsin/trypsin digest enumeration of *Myxobolus cerebralis* spores in fish heads sampled from exposure trials performed in Lapwai Creek, Sweetwater Creek, and Potlatch River Exposure series (Oct 13 to 23, 2005).

Group	LAS#	#+/total	%+	Individual spores(x000) / head	Total spores/n	Mean (x000)
SWC upper	06-019	13/27*	48.1	1.7; 1.7; 1.7; 1.7; 3.3; 3.3; 10; 16.7	36.7/15	2.45
SWC lower	06-020	3/46	6.5	1.7; 3.3; 16.7	21.7/46	0.47
Lapwai @Jacques	06-021	0/11	0		0/11	0
Lapwai<SWC	06-022	2/11**	0		0/11	0
Lapwai @ Spalding	06-023	0/21	0		0/21	0
Potlatch R	06-018	0/47	0		0/47	0

* 22 fish examined by QPTD + 5 fish examined by PCR

** positives by PCR only

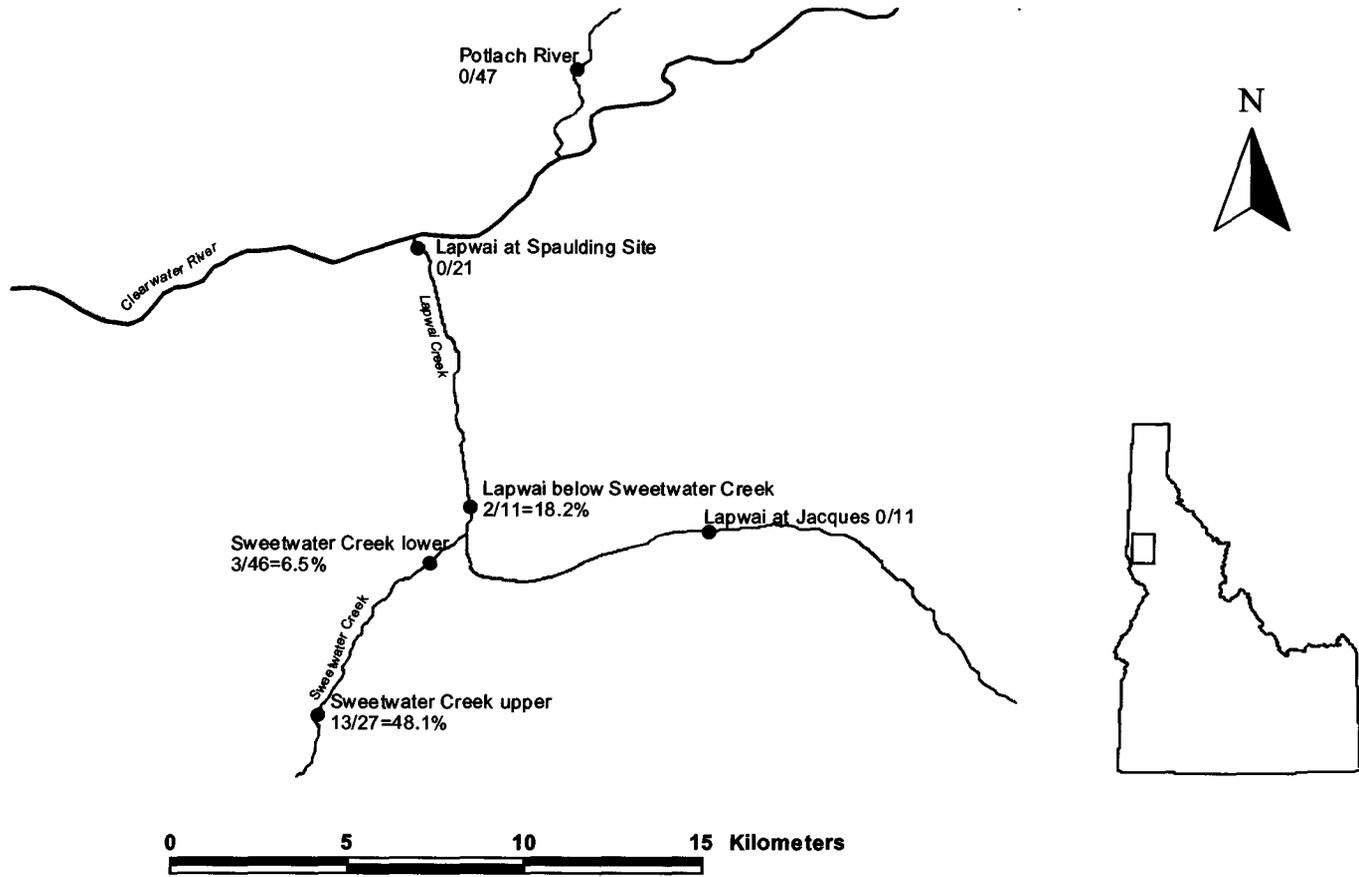


Figure 1. Sites of Sentinel Trout Exposures made October 13 to 23, 2005 at sites on Sweetwater Creek and Lapwai Creek, Nez Perce County, Idaho to detect *Myxobolus cerebralis*.

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