

Distribution of *Myxobolus cerebralis* within a Free-Flowing River System during the Migration Period for Juvenile Anadromous Salmonids in Idaho

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Abstract.—To gain a better understanding of the pathogen-associated risk to resident and anadromous salmonid populations, the distribution, prevalence, and intensity of *Myxobolus cerebralis* infections were assessed during the smolt migration period in the Salmon, Snake, and Clearwater rivers of Idaho. Sentinel exposures of rainbow trout *Oncorhynchus mykiss* fry (0.6 g) occurred at 10 locations beginning at the headwaters of the Salmon River and extending downstream to the Snake and Clearwater rivers from April 18–28 and May 15–25, 2001. Following exposure, fish were examined for the prevalence and severity of infection by means of histological, pepsin-trypsin digest, and nested polymerase chain reaction analyses. Infections did not occur in the portions of the Snake or Clearwater rivers included in this study but were found in the Salmon River at five locations during April and seven locations during May. The results during April demonstrated moderate to high prevalence of infection (65% and 75% at 900 and 1,800 Celsius temperature units [CTUs], respectively) at the most upstream headwater site (river kilometer [rkm] 619, measuring from the mouth of the Salmon River), but intensity decreased 28 km downstream (rkm 591) to 10% and 0% at 900 and 1,800 CTUs, respectively. Fish exposed at two sites further downstream (rkm 454 and 416) showed 100% prevalence in both April and May. The most downriver sites at which infections were detected in April and May were sites 5 (rkm 308) and 7 (rkm 86). Significant correlations were not found between *M. cerebralis* infection and the environmental conditions examined in this study. However, the findings from this study show a temporal (April–May) increase in distribution and prevalence and a trend toward decreasing prevalence downstream of rkm 416. Therefore, resident and anadromous salmonids emerging or migrating through the Salmon River during April and May are probably exposed to the infectious stage of *M. cerebralis*.

The parasite *Myxobolus cerebralis* was first confirmed in Idaho in 1987 by the Idaho Department of Fish and Game (IDFG) and has since been detected in 21 drainages throughout the state (IDFG, unpublished data). Infections within the Salmon River were first confirmed in Chinook salmon *Oncorhynchus tshawytscha* reared in river water at Sawtooth (STFH) and Pahsimeroi (PAH) fish hatcheries (Figure 1). Within this system, the parasite has been identified in juvenile and adult Chinook salmon, steelhead *O. mykiss* (anadromous rainbow trout), and west slope cutthroat trout *O. clarki lewisi* upstream from and including the Mid-

dle Fork of the Salmon River (MFSR), which enters at river kilometer (rkm) 319, measuring from the mouth of the Salmon River. Infections have not been detected in salmonids sampled from the main stem or tributaries downstream of the MFSR. There is concern that the distribution of the parasite may be expanding from the upper Salmon River to downriver tributaries. Such an expansion may impact hatchery and other management programs, reinforcing the need to define the extent of the parasite's dissemination.

Steelhead stocking programs for the Salmon River utilize smolts produced in springwater hatcheries in southern Idaho. Smolts are generally acclimated on river water at STFH for a period of 3 weeks during April prior to release and are then released immediately downriver from the hatchery

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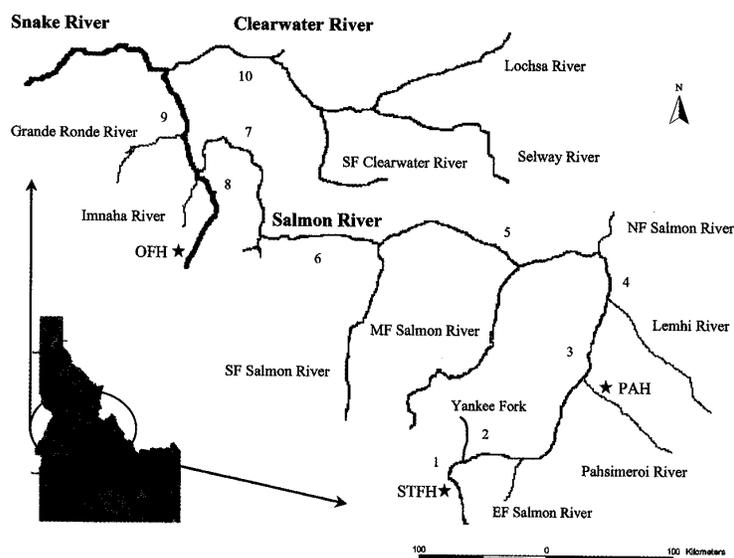


Figure 1.-Live-box locations (sites 1-10) in the Salmon, Snake, and Clearwater rivers of Idaho. Abbreviations are as follows: OFH, Oxbow Fish Hatchery; PAH, Pahsimeroi Fish Hatchery; STFH, Sawtooth Fish Hatchery, SF, South Fork; MF, Middle Fork; NF, North Fork; and EF, East Fork.

facility. Pahsimeroi and Oxbow (OFH) fish hatcheries (Figure 1) also utilize hatchery smolts reared in southern Idaho but do not acclimate fish on river water before releasing them immediately downriver from each facility. Although sampling by IDFG has demonstrated annually that fish are free from *M. cerebralis* spores at the time of release, infections have occurred in a portion of the steelhead adults from these stocks returning to STFH and PAH (IDFG, unpublished data). This suggests that exposure to the parasite occurs during downstream migration in the Salmon River but not the Snake River. These studies also suggest that the upper Salmon River contains triactinomyxon spores during the months corresponding to anadromous smolt migration (April and May). By defining sites where resident and migrating juvenile anadromous fish may contact the parasite, the potential risk of infection can be determined.

Additional observations by IDFG suggest that downstream tributaries such as the Pahsimeroi and Lemhi rivers provide environmental conditions favorable to the parasite (unpublished data). It appears that the prevalence of infection within wild salmonid populations in those rivers is higher than that observed in the upper Salmon River (i.e., above Sawtooth Hatchery), the Yankee Fork of the Salmon River, the East Fork Salmon River, and

MFSR (Figure 1). Investigating *M. cerebralis* dispersion throughout the main-stem Salmon River is further warranted by the fact that all wild and naturally reproducing spring and summer Chinook and sockeye salmon and steelhead in the Salmon River drainage are listed as endangered species (NMFS 1991, 1992, 1997). Since there are limited opportunities to lethally sample these populations for *M. cerebralis*, the use of sentinel fish exposures throughout the system provides the best means of assessing the risk of infection during the primary migration period for salmonid smolts.

Understanding the occurrence of disease with regard to host, pathogen, and environmental interactions is integral to developing strategies to reduce or eliminate infection within fish populations. Therefore, the primary objective of this study was to identify the temporal and spatial distribution in the prevalence and severity of *M. cerebralis* infection within the migration corridor of the Salmon, Snake, and Clearwater rivers in Idaho. In addition, the prevalence and intensity of infection were examined in relation to environmental conditions.

Methods

Study Area

The Salmon River originates in south-central Idaho, flows northwesterly, and empties into the

TABLE 1.-Live-box locations within the Salmon, Snake, and Clearwater rivers of Idaho.

Drainage	Site no.	Name	Location	River km
Salmon River	1	Sawtooth	Adjacent to Sawtooth Fish Hatchery	619
	2	Sunbeam	Upstream from Challis	591
	3	Pahsimeroi	Downstream of Pahsimeroi River	454
	4	Salmon	Downstream of Lemhi River	416
	5	Corn Creek	Downstream of Middle Fork Salmon River	308
	6	Vinegar Creek	Near Wind River pack bridge	179
	7	White Bird	Downstream of White Bird Creek	86
Snake River	8	Pittsburg Landing	Downstream of Imnaha River	824
	9	Heller Bar	Downstream of Grande Ronde River	793
Clearwater River	10	Spalding		12

Snake River (Figure 1). Major Salmon River tributaries include the Yankee Fork, East Fork Salmon River, Pahsimeroi River, Lemhi River, North Fork Salmon River, Middle Fork Salmon River, South Fork Salmon River, and Little Salmon River. On route to the Columbia River basin, the Snake River flows north until it intersects the Clearwater River. The Snake River continues west and empties into the Columbia River.

Fish Exposures

Steelhead.-Forty-one prerelease steelhead smolts (mean weight, 204 g) were collected from STFH in April to determine whether exposure to *M. cerebralis* during acclimation could be demonstrated. These fish were reared in springwater hatcheries in southern Idaho before being transported to STFH, where they were acclimated on Salmon River water for approximately 3 weeks prior to release. Following this acclimation period, smolts were collected and transported to the University of Idaho's Fish Health Laboratory (Moscow), where they were maintained in a 1,100-L circular flow-through tank supplied with 13°C dechlorinated municipal water.

Sentinel fish exposures.-Two lots of triploid Hayspur strain rainbow trout eggs were acquired from Hayspur Fish Hatchery, IDFG (Blaine, Idaho) prior to each exposure. Two thousand fertilized eggs were transferred to both the University of Idaho and Eagle Fish Health Laboratory (EFHL), IDFG (Eagle, Idaho), where incubation and early rearing were completed. At 170-degree-days post-hatch, the trays containing the fry were transferred to shallow flow-through troughs, where the fry were raised for approximately 40 d. Fry weights averaged 0.6 g, and fish were 7.75 weeks of age at the beginning of each exposure. Sentinel fish exposures were performed April 18-28 and May 15-25, 2001. These periods coincided with the prerelease acclimation periods at STFH and have been shown to correspond with a high incidence

of *M. cerebralis* infection in the Pahsimeroi and upper Salmon rivers (IDFG, unpublished data). These dates also overlap peak migration for spring Chinook and steelhead smolts within the mainstem Salmon River drainage (B. Leth, IDFG, personal communication). Ten sites within the mainstem corridor of the Salmon, Snake, and Clearwater rivers were selected for the sentinel fish exposures (Figure 1). The sites were located in quiescent areas downstream from tributaries to provide a means of identifying point source areas of infection (Table 1). Elevations ranged from 245 to 2,119 m above sea level.

Each site was outfitted with one or two aluminum live-boxes constructed from 3.2-mm aluminum plates. The aluminum was shaped into open-ended cylinders 47 cm in length and 30.0 cm in diameter. Each container possessed four openings (one on each end and one on each side) that were covered with plastic mesh to facilitate water flow and prevent fish from escaping. Containers were anchored to the river bottom with lead weights and to the riverbank with steel cable. Project size, live-box availability, and restricted rearing space limited our ability to use multiple boxes across all sites; therefore, sites 1, 4, and 6 were randomly selected to receive duplicate containers. Prior to each trial, fish from the University of Idaho and EFHL were mixed and redistributed between facilities. At the beginning of each trial, the live-boxes at sites 1-5 were each stocked with 100 rainbow trout fry from EFHL and those at sites 6-10 were stocked with 100 fry from the University of Idaho. Controls for both April and May were composed of 100 fry held in flow-through tanks at each rearing facility.

Environmental Variables

Dissolved oxygen (DO) and pH were measured within close proximity to the live-boxes at the beginning and end of each exposure with a YSI 55

DO meter (Yellow Springs Instruments, Yellow Springs, Ohio) and a pH test kit (Hach, Loveland, Ohio). Water samples were acquired during this period and returned to the University of Idaho, where conductivity and turbidity were measured with a YSI conductivity meter and a Hach ratio turbidity meter. Water temperature was measured at 15-min intervals throughout the 10-d exposures with submersed HOBO stowaway temperature meters (HOBO, Bourne, Massachusetts). Discharge information was obtained from the U.S. Geological Survey's water resource Web site (<http://id.waterdata.usgs.gov>) for all sites except site 1; discharge at that location was obtained manually at the beginning and end of each exposure with a Swoffer Model 3000 flowmeter (Swoffer Instruments, Inc., Seattle, Washington).

Laboratory Samples

Steelhead.-A group of 10 fish from the original 41 steelhead smolts maintained at the University of Idaho were sampled at 900 Celsius temperature units (CTUs, defined as the sum of the average daily temperature units [°C] over time). Fish were euthanized with 200 mg/L MS-222 (tricaine methanesulfonate; Argent Laboratories, Redmond, Washington), and a cranial wedge located dorsally to the eye and anterior to the operculum was excised with a sterile scalpel blade. Cranial wedges were split sagittally, and one half was designated for nested polymerase chain reaction (PCR) and the other half for myxospore enumeration through pepsin-trypsin digest (PTD) analysis (Markiw and Wolf 1974). An additional group of 20 smolts was taken at 1,800 CTUs and examined for infection as described above.

Rainbow trout.-Following the sentinel fish exposures, fish from all exposure groups were returned to the facility from which they originated (sites 1-5: EFHL; sites 6-10: the University of Idaho) and maintained in separate flow-through tanks. Water supply was maintained at 13°C. Fish from sites 1-5 were divided and one-half of each group was transferred to the University of Idaho, while fish from sites 6-10 were divided and one-half of each group was transferred to EFHL. This allowed duplicate groups of fish from sites 1-10 for both April and May to be maintained at both the University of Idaho and EFHL for the duration of the study. This provided security in case high fish losses occurred at one of the two facilities.

900-CTU Samples

Subsamples of 10 rainbow trout from each exposure site and their corresponding control group

were taken at 900 CTUs postexposure. These subsamples were composed of five fish from those held at the University of Idaho and five from those at EFHL. Fish were euthanized, decapitated at the posterior end of the operculum with a razor blade, and had their heads split sagittally. Half-heads from each specimen were placed in 10% solutions of neutral buffered formalin. Samples were processed for histology at the Washington Animal Disease and Diagnostic Laboratory at Washington State University (Pullman). Slides were examined under a light microscope to determine the severity of infection according to the MacConnell-Baldwin scale of infection (0-5) for *M. cerebralis*, as modified from Baldwin et al. (2000).

The second half-head from each specimen was prepared for nested PCR analysis by excising tissue containing cartilage and bone from the base of the skull with a 6-mm biopsy punch (Miltex, Japan). Deoxyribonucleic acid (DNA) was extracted using standard reagents, microfiltration tubes, and the mouse-tail protocol outlined in Qiagen's DNeasy tissue kit (Qiagen, Valencia, California). Reagents for all amplifications were obtained from Qiagen's Taq PCR Core kit. Reaction volume was 50 µL and conditions consisted of 1X Qiagen PCR buffer, 0.1 mM MgCl₂, 400 µM deoxynucleotide triphosphates, 2 U *Taq* polymerase per re-action, and 40 pmol of each primer. Amplification was performed with a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts). Primers and gradient conditions for this analysis followed previously established protocols (Andree et al. 1998). Only fish determined to be negative by histology were further examined by PCR analysis.

1,800-CTU Samples

At 1,800 CTUs, subsamples of 20 rainbow trout from each site and control group were taken as described above (10 from EFHL and 10 from the University of Idaho). Five fish from each facility (10 samples total) were prepared for examination, and the remaining fish were frozen (-20°C) as potential reference material. Sampled fish were euthanized and decapitated and their heads were split as described previously. Half-heads from each specimen were prepared for histopathology, and in contrast to the 900-CTU sample, the second half was designated for PTD analysis. Half-heads designated for spore enumeration by PTD analysis were submersed in a 60°C water bath for 10 min and manually defleshed to remove hard cranial elements. The proteolytic enzymes pepsin and trypsin were used to degrade the cartilage and release

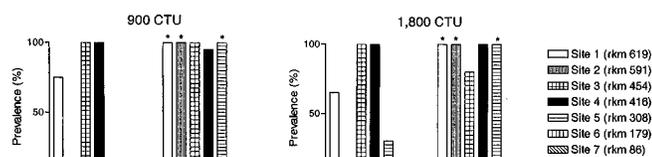


FIGURE 2.-Prevalence of infection among sentinel rainbow trout sampled at 900 and 1,800 Celsius temperature units (CTUs) for Salmon River exposures in April and May, 2001 (see text for details). Asterisks indicate significant ($P < 0.01$) increases from April to May.

myxospores into the solution (Markiw and Wolf 1974). The final product was eluted through a 55% dextrose gradient to remove debris, and myxospores were enumerated with a hemocytometer (Hausser Scientific, Horsham, Pennsylvania).

Statistical Analysis

To ensure that the data within sites could be combined for statistical analysis, differences in prevalence, histology, and PTD score between affiliated replicate boxes and groups of sentinel fish sampled from EFHL and the University of Idaho for April and May were examined with multivariate analysis of variance using the model $y = \text{replicate} + \text{fish} + \text{interaction}$. As no differences were observed within replicate sites ($F = 0.68$, $P = 0.568$) or between sentinel fish from either rearing facility ($F = 0.96$, $P = 0.413$), data were combined so that all site differences could be further analyzed. Pepsin-trypsin digest data did not meet normality assumptions and so were \log_{10} transformed prior to examination. Histology and PTD scores were analyzed across all sites from April to May using Hotelling's T^2 -test and analysis of variance (ANOVA) with the model $y = \text{month} + \text{site} + \text{interaction}$. Prevalence was examined as a categorical variable across sites by means of a comparison of binomial proportions (Ramsey and Schafer 1997).

Relationships between the mean values for environmental variables and those for prevalence, histology, and PTD score were examined by means of multivariate linear regression and general linear regression for both April and May. Throughout this study significance was defined as $P < 0.01$. Statistical analysis was conducted with SAS (SAS Institute 2001).

Results

Analysis of Steelhead Smolts by PTD and PCR

In April 2001, 41 pre-release steelhead smolts were obtained from STFH to determine whether infection could be detected in migrating juveniles

from the upper Salmon River. Following a 3-week exposure to Salmon River water, fish were taken to the University of Idaho and maintained for 20 weeks to allow pathology and spore development to occur. All fish were examined by PTD and/or PCR analysis and were determined to be negative for infection by *M. cerebralis*.

Analysis of Sentinel Fish by Histology, PTD, and PCR

Ten groups of sentinel rainbow trout were examined for infection by *M. cerebralis* following 10-d exposures in April and May within the Salmon, Snake, and Clearwater rivers. Infections were not detected in control fish or sentinel rainbow trout sampled from those portions of the Snake (sites 8 and 9) and Clearwater rivers (site 10) included in this study. The prevalence of infection in sentinel fish sampled at 900 CTUs was determined from combined data from histology scores and PCR analysis, while that in fish sampled at 1,800 CTUs was determined from combined data from histology and PTD scores for both April and May (Figure 2).

900-CTU samples.-Infection in fish sampled at 900 CTUs was found at four locations in April and six locations in May (Figure 2). Prevalence in April increased from 75% at the uppermost headwater location (site 1; rkm 619) to 100% downstream at sites 3 (rkm 454) and 4 (rkm 416) before decreasing to undetectable levels at site 5 (rkm 308) further downstream. Infected fish (10%) at site 2 (rkm 591) in April were only detected by PCR during this sample period. However, prevalence reached 100% at this site in May.

1,800-CTU samples.-Infection in fish sampled at 1,800 CTUs was found at four locations in April and seven locations in May (Figure 2). When all samples were evaluated it was found that infections did not occur at sites 6 (rkm 179) and 7 (rkm 86) in April but reached 15% and 10%, respectively, at those sites in May. The most downstream locations in which the parasite was detected were

TABLE 2.-Prevalence (Prev.), mean histology scores (Histo.), pepsin-trypsin digest scores (PTD), and values of environmental variables at sentinel fish exposure sites during April and May 2001. Fish were exposed at 1,800 Celsius temperature units; see text for other details. There were significant ($P < 0.01$) correlations between prevalence, histology score, and PTD score. Other abbreviations are as follows: Cond., conductivity; Temp., temperature; and DO, dissolved oxygen.

Site	Prev. (%)	Histo.	PTD (X1,000)	Cond. ($\mu\text{S}/\text{cm}^2$)	Temp. ($^{\circ}\text{C}$)	Turbidity (NTU) ^a	pH	DO	Discharge (m^3/s)
April									
1	65	1.05	8.25	92	8.0	1.0	7.8	9.2	8.3
2	0	0	0	74	7.7	6.1	8.0	7.5	18.4
3	100	3.6	85.1	193	10.9	6.9	8.0	11.7	26.2
4	100	3.3	131.3	169	12.1	12.9	8.5	10.9	34.1
5	30	0.5	4.3	142	10.9	21.5	8.0	10.6	93.2
6	0	0	0	60	10.4	20.3	7.2	11.3	109.5
7	0	0	0	50	9.4	13	7.5	11.3	240.8
8	0	0	0	270	9.9	4.7	7.8	12.2	407.8
9	0	0	0	110	11.1	14.6	6.2	11.6	648.5
10	0	0	0	20	9.5	24.7	7.0	12.4	356.8
May									
1	100	3.0	118.9	63	10.9	15.1	7.9	8.9	13.5
2	100	3.9	159.3	46	9.8	9.3	7.3	7.0	54.4
3	80	2.5	118.3	89	12.8	10.9	7.5	8.4	67.7
4	100	3.2	170.1	96	13.8	16.6	7.5	8.8	81.0
5	100	2.2	67.3	52	11.8	16.3	7.5	8.4	268.6
6	15	0.1	0.45	48	11.4	5.5	7.0	10.6	330.5
7	10	0	0.33	38	11.9	11.3	7.2	11.1	762.6
8	0	0	0	248.5	13.0	4.0	8.2	11.1	443.7
9	0	0	0	90.0	13.0	9.0	7.7	11.5	1,468.5
10	0	0	0	15.5	9.8	5.8	7.0	12.0	936.4

a Nephelometric turbidity units.

sites 5 and 7 in April and May, respectively. Overall, infections within the Salmon River were detected in sentinel fish sampled from five locations (sites 1-5) in April and seven locations (sites 1-7) in May (Figure 2).

Infections were detected by histological analysis in sentinel fish sampled from four of the Salmon River sites in April (sites 1 and 3-5) and six during May (sites 1-6) (Table 2). Histology scores for April and May parallel prevalence, moderate to high scores increasing with high prevalence scores. When prevalence decreased below 30% in April and 15% in May, infections were below levels detectable by histological analysis. Infections were not detected by histological examination at sites 2 and 6 in April, but the scores at these locations reached averages of 3.9 and 0.1, respectively, in May.

Myxospore numbers, as determined by PTD analysis, were obtained from sentinel fish sampled at four of the Salmon River sites (1 and 3-5) during April and seven of those sites (1-7) during May (Table 2). Mean spore counts per head ranged from 4,300 to 131,300 in April and from 330 to 170,100 in May. It was found that when spore numbers were below an average of 450 spores/head, infec-

tion was not detected by histology. Myxospore numbers and histology scores were significantly correlated with one another ($P = 0.0001$, $R^2 = 0.73$). Prevalence, histology scores, and myxospore numbers increased significantly ($P < 0.01$) from April to May at sites 1, 2, and 5.

Environmental Variables

Conductivity, temperature, turbidity, pH, and dissolved oxygen values fluctuated between exposure sites in both April and May (Table 2). Discharge increased with decreasing river kilometer. Prevalence, histology scores, and myxospore counts decreased when discharge exceeded 93.2 m^3/s in April and 268.6 m^3/s in May. No significant correlation was observed between environmental variables and level of infection ($P > 0.01$).

Discussion

Sentinel rainbow trout were used to determine the distribution of the infectious stage of *M. cerebralis* within the migration corridor of the Salmon, Snake, and Clearwater rivers of Idaho. Infections were not detected in steelhead smolts (acclimated for 3 weeks in the upper Salmon River), rainbow trout controls, or any sentinel fish from

the Snake or Clearwater rivers. However, infections were detected in sentinel fish exposed at all sites within the Salmon River. These findings confirm that the Salmon River contained triactinomyxons at a level capable of causing infection in rainbow trout fry (0.6 g) exposed for 10 d during the months of April and May 2001. These results support earlier data collected by IDFG (not shown) suggesting that exposure of migrating salmonids to *M. cerebralis* occurs in the Salmon River but not in the portions of the Snake and Clearwater rivers examined in this study.

The absence of infection in prerelease steelhead smolts obtained from STFH supports observations by IDFG indicating that fish from this facility are free of *M. cerebralis* infection at the time of release. This is interesting since a portion of returning adult steelhead are infected with *M. cerebralis*. Infections among sentinel fish exposed at site 1 (Sawtooth) in April indicate that the infectious stage of *M. cerebralis* was present in river water during steelhead acclimation and that these fish did not become infected during this 3-week exposure period. Previous authors have shown steelhead (Hedrick et al. 2001) and rainbow trout to be highly susceptible to *M. cerebralis* (O'Grodnick 1979; Hedrick et al. 1999; Sollid et al. 2002), but the degree of susceptibility decreases with increasing size (O'Grodnick 1979) and age (Hoffman and Byrne 1974; Markiw 1991). The absence of infection among steelhead smolts in the presence of *M. cerebralis* was probably the result of their increased size and age as compared with the sentinel rainbow trout. Fish returning as *M. cerebralis*-positive adults were probably infected as smolts during the extended migration times when high parasite concentrations are found in the Salmon River.

The prevalence of infection in sentinel rainbow trout exposed at Salmon River sites above the Middle Fork (rkm 416-619) ranged from 10% to 100% for both April and May. The consistently high occurrence of infection at locations immediately downstream from the Pahsimeroi (site 3) and Lemhi (site 4) rivers suggests that these tributaries are point sources of infection. Both tributaries have been determined to be positive for *M. cerebralis* by sentinel fish exposures (IDFG, unpublished data) and may be contributing to exposure within the main-stem Salmon River.

This study has demonstrated that infection with *M. cerebralis* can occur below the Middle Fork of the Salmon River in sentinel rainbow trout. Prior to this study, infected salmonids had not been detected in samples collected in the main-stem Salm-

on River or tributaries downstream of the MFSR. Prevalence immediately downriver from the Middle Fork was demonstrated to occur at moderate to high levels, but infections decreased dramatically further downstream. This may have been associated with increasing discharge for both April and May. Triactinomyxon spores have been shown to survive for several days to several weeks under a variety of temperature regimes, but as time passes their viability decreases (Markiw 1992; El-Matbouli et al. 1999; Smith et al. 2002). It is possible that *M. cerebralis* has become established downstream from the Middle Fork but that elevated discharge coupled with high actinospore output from tributaries in May reduces spore travel time, resulting in increased infection in sentinel fish at sites 5, 6, and 7. This discharge pattern may also explain the shift from low infection rates in April to the high prevalence in May observed at Sunbeam (site 2).

Sites 8 and 9 were located within the Snake River. Site 8 was located above the confluence of the Salmon River, but site 9 was located below the confluence of the Imnaha and Grande Ronde rivers, both of which are known to contain fish that have tested positive for the parasite (IDFG, unpublished data; Lorz et al. 1989; Sandell et al. 2002). The absence of *M. cerebralis* infections at site 9 could be related to the elevated discharge in the Snake River, which averaged 648.5 and 1,468.5 m³/s in April and May, respectively.

Environmental variables were monitored during this study in an effort to explain the patterns of *M. cerebralis* infection. However, throughout the study area no significant correlations were found between these variables and the occurrence of infection ($P > 0.01$). Since the inherent interactions among environmental variables make interpreting their direct effects on the occurrence of infection difficult, controlled laboratory studies are needed to address these relationships.

Histology and PTD scores from our study were significantly correlated ($P = 0.0001$, $R^2 = 0.73$), suggesting a strong relationship between spore counts and infection severity. Prevalence, histology, and PTD scores associated with the Salmon River in April were lower than those in May. Taken as a whole, these data suggest that smolts in this system have a greater likelihood of being exposed to the infectious stage of *M. cerebralis* in May than in April. What is also apparent is that resident and anadromous salmonids emerging or migrating at highly susceptible stages of their life cycle (Markiw 1991) are probably exposed to this parasite and may become infected during this period. When at-

tempting to limit the spread of whirling disease, any increase in infection rates in anadromous fish is important since fish may stray to new areas within and outside of this system.

The findings from this project have provided a better understanding of the current status of *M. cerebralis* within the Salmon, Snake, and Clearwater rivers of Idaho. This increased understanding will aid in the development of management actions to limit the spread of whirling disease and the exposure of hatchery-released smolts and wild salmonid populations to the infectious stage of *M. cerebralis*. Strategies that could decrease exposure levels include (1) expanding well water sources at specific hatcheries to delay fish's exposure to river water containing the parasite; (2) limiting the out-planting of fish from potentially positive sources to surrounding drainages; and (3) avoiding nutrient enrichment of streams with potentially infected salmonid carcasses. Increasing our understanding of the many factors associated with the distribution and establishment of this parasite within systems will decrease the risk of further spread.

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References

- Andree, K. B., E. MacConnell, and R. P. Hedrick. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 34:145-154.
- Baldwin, T. J., E. R. Vincent, R. M. Silflow, and D. Stanek. 2000. *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. *Journal of Veterinary Diagnostic Investigations* 12:312-321.
- El-Matbouli, M., T. S. McDowell, D. B. Antonio, K. B. Andree, and R. P. Hedrick. 1999. Effect of water temperature on the development, release, and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. *International Journal of Parasitology* 29:627-641.
- Hedrick, R. P., T. S. McDowell, M. Gay, G. D. Marty, M. P. Georgiadis, and E. MacConnell. 1999. Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. *Diseases of Aquatic Organisms* 37:173-183.
- Hedrick, R. P., T. S. McDowell, K. Mukkatira, M. P. Georgiadis, and E. MacConnell. 2001. Susceptibility of three species of anadromous salmonids to experimentally induced infection with *Myxobolus cerebralis*, the causative agent of whirling disease. *Journal of Aquatic Animal Health* 13:43-50.
- Hoffman, G. L., and C. J. Byrne. 1974. Fish age as related to susceptibility to *Myxosoma cerebralis*, cause of whirling disease. *The Progressive Fish-Culturist* 36:151.
- Lorz, H. V., A. Amandi, C. R. Banner, and J. S. Rohovec. 1989. Detection of *Myxobolus cerebralis* in Oregon. *Journal of Aquatic Animal Health* 1:217-221.
- Markiw, M. E. 1991. Whirling disease: earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis*. *Aquaculture* 92:1-6.
- Markiw, M. E. 1992. Experimentally induced whirling disease, II. determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. *Journal of Aquatic Animal Health* 4:44-47.
- Markiw, E. M., and K. Wolf. 1974. *Myxosoma cerebralis*: Isolation and concentration from fish skeletal elements-sequential enzymatic digestions and purification by differential centrifugation. *Journal of the Fisheries Research Board of Canada* 31:245-251.
- NMFS (National Marine Fisheries Service). 1991. Endangered Status for Snake River Sockeye Salmon, Final Rule. *Federal Register* 56:224(November 20, 1991):58619.
- NMFS (National Marine Fisheries Service). 1992. Threatened Status for Snake River Spring/Summer Chinook Salmon, Threatened Status for Snake River Fall Chinook Salmon, Final Rule. *Federal Register* 57:107(June 3, 1992):23458.
- NMFS (National Marine Fisheries Service). 1997. Listing of Several Evolutionarily Significant Units (ESU) of West Coast Steelhead, Final Rule. *Federal Register* 62:159(August 18, 1997):43937.
- O'Grodnick, J. J. 1979. Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). *Transactions of the American Fisheries Society* 108:187-190.
- Ramsey, F. L., and D. W. Schafer. 1997. *The statistical sleuth: a course in methods of data analysis*. Duxbury Press, Belmont, California.
- Sandell, T. A., H. V. Lorz, S. A. Sollid, and J. L. Bartholomew. 2002. Effects of *Myxobolus cerebralis* infection on juvenile spring Chinook salmon in the Lostine River, Oregon. Pages 135-141 in J. L. Bartholomew and J. C. Wilson, editors. *Whirling disease: reviews and current topics*. American Fisheries Society, Symposium 29, Bethesda, Maryland.
- SAS Institute. 2001. *SAS/STAT user's guide*, version 8.1. SAS Institute, Cary, North Carolina.
- Smith, A. A., E. J. Wagner, and A. Howa. 2002. The

effect of water characteristics on viability of the *Myxobolus cerebralis* actinospore. Pages 227-238 in J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.

Sollid, S. A., H. V. Lorz, D. G. Stevens, and J. L. Bar-

tholomew. 2002. Relative susceptibility of selected Deschutes River, Oregon, salmonid species to experimentally induced infection by *Myxobolus cerebralis*. Pages 117-124 in J. L. Bartholomew and J. C. Wilson, editors. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland.