FISHERY RESEARCH

WHIRLING DISEASE RESEARCH REPORT: THE DISTRIBUTION OF *Myxobolus cerebralis* INFECTIVITY ON HAYSPUR HATCHERY, AND IN LOVING CREEK, SILVER CREEK, AND THE BIG WOOD RIVER JUNE, 2006

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ABSTRACT

Sentinel groups of juvenile rainbow trout were exposed to Loving Creek, Silver Creek, and the Big Wood River District Canal in south-central Idaho during two consecutive sets of trials between June 5 and June 26, 2006. The purpose of the trials was to investigate possible *Myxobolus cerebralis* infectivity at these locations. These were done subsequent to similar trials conducted in 2003 and 2004, during which the parasite was detected in the Big Wood River and the District Canal, but not in Loving Creek or Silver Creek. Heavy snows during the winter of 2005-2006 lead to greater summer runoff water levels in the Big Wood River drainage compared to the drought conditions experienced in the previous sample years. The District Canal carries irrigation water from the Big Wood River to farm fields at the head of Loving Creek above Hayspur Hatchery, and it was speculated that there would be a greater chance of excess water spilling into the creek during a high runoff year. A high level of *Myxobolus cerebralis* infectivity (100% prevalence with high individual intensities) was detected in 2006 from sentinel fishes exposed in the District Canal, while a single infected individual was found in one group exposed at the railroad trestle site in Loving Creek (1.2% prevalence with low intensity). Infectivity was not detected at the diversion dam or hatchery trash rack locations in Loving Creek, or at the Kilpatrick Bridge site in Silver Creek. These results support the conclusions that *Myxobolus cerebralis* has not become established at detectable levels in Loving Creek or Silver Creek, but is intermittently carried into the upper reaches of Loving Creek with Big Wood River irrigation water. This would also explain why detections of the parasite at Hayspur Hatchery since 1989 have been sporadic and at consistently low levels.

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INTRODUCTION

Idaho Department of Fish and Game's (IDFG) Hayspur Hatchery is located in Blaine County, on Loving Creek, a major tributary to Silver Creek in the Little Wood River drainage (Figure 1). *Myxobolus cerebralis* (MC), a myxosporean parasite that is the causative agent of salmonid whirling disease, was first confirmed from adult rainbow trout *Oncorhynchus mykiss* reared on the hatchery in 1988 and has since been intermittently detected in hatchery production fish and in wild/feral fish in Loving Creek (Eagle Fish Health Laboratory database, unpublished). Surface water from Loving Creek was historically used by the hatchery to rear up to 350,000 catchable-size rainbow trout. That portion of the hatchery production was discontinued after 1995 due to the presence of MC infectivity in the Loving Creek water source.

The presence of MC in the Big Wood River was first confirmed by the Eagle Fish Health Laboratory in 1995 and has been frequently detected since. The District Canal carries irrigation water from the Big Wood River to fields in the upper portions of the Silver Creek drainage, including those fields that contain the springs that give rise to Loving Creek. Reports that excess irrigation water has periodically spilled directly into the springs (Douglas Megargle, Magic Valley Regional Fish Biologist, personal communication) have led to speculation that MC triactinomyxons (TAMS) carried by these surges of water from the Big Wood River are a likely source of the historic infections in Loving Creek and Hayspur Hatchery. Exposure trials conducted in 2003 and 2004 to test this supposition found high levels of infectivity in the Big Wood River and District Canal, but failed to detect the presence of the parasite at any location in the Loving Creek/Silver Creek system (Burton 2006). An extended drought during these trials may have lead to minimal spillage of irrigation water into the Loving Creek springs. However, the occurrence or duration of irrigation water spills during the exposure trials could not be confirmed because permission was not granted to enter the private land where the Loving Creek springs arise. In contrast, the heavy snow pack in the Big Wood River Drainage during spring 2006 indicated that there would be an abundance of water available during the irrigation season, almost assuring that excess from the Diversion Canal would spill into upper Loving Creek. More exposure trials were conducted in June 2006 to learn if there would be detectable MC infectivity in Loving Creek or Silver Creek during a high-water year. The results from those trials are contained in this report.

STUDY SITE

Hayspur Hatchery is located in Blaine County, Idaho, approximately 17.6 kilometers southeast of the town of Bellevue (Figure 1). Three live-box locations were chosen near the hatchery: the first at the diversion dam that diverts water from the original Loving Creek channel to the hatchery, a second suspended from the trash rack walkway above the hatchery intake, and the third at the old railroad trestle where the hatchery diversion returns to the original Loving Creek channel. A fourth location was in Silver Creek under the Kilpatrick Bridge, approximately 2.9 km southeast of the hatchery. A final site was in the District Canal 11.6 km southeast of Bellevue, on the east side of the Gannett Highway. This canal lateral provides water to the agricultural fields where the spring sources of Loving Creek are located, approximately 1.5 km downstream from the exposure site. With the exception of the diversion dam site, all were previously used in the 2003/2004 trials (Burton 2006). Map coordinates for all sites are included in Table 1.

OBJECTIVES

The objectives of this study were to investigate the possibility that the infective triactinomyxon (TAM) stage of MC may be transferred via an irrigation canal into the Loving Creek Springs and may then infect susceptible juvenile rainbow trout at locations on or near Hayspur Hatchery, and to confirm previous findings regarding the distribution of the parasite in the upper Silver Creek drainage. Secondary purposes of the study were to increase understanding of any effects of the parasite on trout populations in the Silver Creek drainage and of the possibility that the parasite may spread within the drainage.

METHODS

The general methods used in this trial have been previously documented (Burton and Johnson 2003; Burton 2004; Munson et al. 2005). In this study, two exposure trials using live-boxes with 50 rainbow trout fry per box were completed from June 5 to June 26, 2006. The first trial was for 10 days, the second for 11 days. Dissolved oxygen, pH, conductivity and water velocity parameters were collected at deployment and retrieval of the sentinel groups (Table 1), and water temperatures were recorded every 30 minutes using Stowaway XTI temperature loggers inside the boxes (Table 2).

Following exposure, the fish were held in isolation at the IDFG's Eagle Fish Health Laboratory in specific pathogen-free well water with constant temperature of 13° C for 100 days to allow for spore development. The fish were then euthanized using an overdose of MS-222 anesthetic, decapitated, and heads were halved along a sagittal line. One set of half-heads was pooled (5-fish) and tested using the pepsin-trypsin digest method (Bartholomew 2001). If spores were detected in any pool, the corresponding half-heads were digested individually using a quantitative methodology (Markiw and Wolf 1974) to determine actual prevalence in the population as well as the intensity of individual infections. If clinical signs were observed before the fish were sacrificed, the initial pooling step was skipped.

RESULTS

All of the fish in the first exposure group at the Diversion Canal site were dead at retrieval. Spikes in the temperature graph from the Stowaway logger suggest that the water in the canal was probably shut off on the seventh and eighth days of the exposure. Fish from the second exposure period in the canal were 100% infected (Table 3) with a mean estimated number of spores per head at nearly 36,000 (Table 4). These results were consistent with those reported from the 2003-2004 trials.

One lightly infected fish was detected from the first exposure group at the railroad trestle site in Loving Creek, (1 of 49 individuals for 2% prevalence; 13,333 estimated spores/head), but none were detected in the second exposure group of 35 individuals (Table 3). This is a notable difference from the 2003-2004 trials, where no infectivity was detected in 7 separate groups of fish (197 individuals) exposed at this site. No MC spores were detected from any group of fish exposed at Kilpatrick Bridge (Silver Creek), at the Loving Creek diversion dam, or at the hatchery trash rack.

The optimum temperature range for MC infectivity has been reported to be between 9° and 17° C (Vincent 1998, Burton et. al. 2000). Mean water temperatures at all sites fell within this optimal range (Table 2), although the mean in Silver Creek at Kilpatrick (16.1° C) approached the upper limit. The coolest recorded temperatures were in the District Canal, with a mean of 12.4° C for the second exposure. (The mean temperature for the District Canal was actually 11.9° C during the first exposure when the fish were lost).

DISCUSSION

The most significant information gathered from this trial is that MC infectivity was again present in the Loving Creek system after having been undetected for a number of years. High levels of infectivity have been detected in every group of fish exposed in the District Canal, indicating a high probability of infectivity persisting in any irrigation water that reaches the Loving Creek Springs. It may be assumed that any irrigation water entering the springs would be of smaller volume relative to the spring flow, leading to dilution of the number of TAMs carried down Loving Creek toward the trial sites. This is supported by the detection of only one infected fish out of 247 individuals tested from 3 separate sites. With only one infected fish detected in a year when it is believed that significant irrigation water spills occurred, then it is consistent that none were detected in years when such spills were likely minimal or did not occur at all. The Hayspur Hatchery manager (Brad Dredge, personal communication) observed daily fluctuations in water levels and turbidity in Loving Creek during the trial period consistent with variable irrigation water spills. However, since access to the private property where the mixing of the irrigation and spring water is thought to have occurred was still not available, there is no incontrovertible proof that this happened. This newest detection of MC in upper Loving Creek does prove that the abandonment of Loving Creek water for use in production at Hayspur Hatchery and the significant reconstruction efforts and costs on the facility were justified.

The apparent lack of established MC infectivity in Loving Creek in spite of numerous historic detections of infected trout in the creek and hatchery continues to be unexplained. Silver Creek and its tributaries are low gradient spring creeks with significant silt and nutrient loads from both natural and agricultural sources. The creeks also contain susceptible natural-spawning populations of both rainbow and brown trout *Salmo trutta*. All indications suggest that the system should be ideal habitat for the parasite. Little is known about the population status of *Tubifex tubifex,* the alternate host for MC, in Loving Creek or Silver Creek. Dr. Daniel L. Gustafson (Montana State University, personal communication) has taken samples of benthic invertebrates from Silver Creek and observed a varied population of worms with *T. tubifex* being relatively rare. Beauchamp et al. 2001 reports that different populations or strains of *T. tubifex* have different levels of susceptibility to the parasite, varying from high to totally resistant. This varying susceptibility of the worms leads to greater or lesser production of TAMs and thus has a direct correlation with the levels of infectivity to which fish in a system are exposed (Stevens et al. 2001). With all other factors seeming to favor the presence of MC in the Silver Creek drainage, it is logical to assume that there must be something involving the worm host that limits that portion of the parasite's life cycle. Further investigation into the *T. tubifex* population of this ecosystem is beyond both the expertise and resources of the author, but a definitive answer to the question of why MC has not flourished might be extremely valuable in managing the parasite in other aquatic systems.

The lack of detectable MC infectivity at the Kilpatrick Bridge exposure site continues to support the conclusions that the parasite is not established in that portion of Silver Creek, and thus has no impact on trout populations in that portion of the drainage.

The transfer of surface water between drainages for irrigation or other purposes is a common practice in the United States and has probably occurred ever since European settlers arrived. Traditionally, almost no consideration has been given to the likely transfer of aquatic organisms with that water, or the impacts that the introduction of an exotic species (or a different genetic population of an existing species) may have on the receiving ecosystem. In the case of Silver Creek, an apparent fortuitous ecological condition is the only likely reason that clinical whirling disease has not had an impact on an acknowledged "blue ribbon" rainbow trout fishery. The appearance of MC in production fish at Hayspur Hatchery did prompt IDFG to significantly modify the production goals of the hatchery and spend millions of dollars on new wells, pumps, and ponds on the facility. The intent of this trial was to provide useful information to those persons or agencies involved in surface water and aquatic species management. While it is unlikely that this knowledge will lead to a change in irrigation water management in the Big Wood River/Silver Creek systems, it may be useful in the planning of any new water diversion systems.

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Table 1. Water quality parameters collected at deployment and retrieval of 2006 Silver Creek Exposure Trials. All UTM coordinates use map datum WGS 84.

Exposure Site	Loving Creek @ diversion dam			Hayspur Hatchery @ trash rack			Loving Creek @ railroad trestle			Silver Creek @ Kilpatrick Bridge			District Canal		
UTM (11T)	730789mE, 4802570mN			731124mE, 4802626mN			731317mE, 4801899mN			732269mE, 4799749mN			727726mE, 4806277mN		
Date	06/05	06/15	06/26	06/05	06/15	06/26	06/05	06/15	06/26	06/05	06/15	06/26	06/05	06/15	06/26
Time (24h)	11:50	13:06	11:05	12:10	14:09	11:31	11:05	12:19	10:30	10:20	10:38	10:10	12:36	15:10	12:05
Temp (C)	14.64	14.96	14.37	15.14	16.13	15.10	13.47	13.63	13.58	13.40	11.71	14.70	15.61	10.91	15.53
Conductivity (mS/cm^3)	0.356	0.348	0.352	0.352	0.342	0.344	0.374	0.370	0.369	0.380	0.381	0.380	0.174	0.179	0.186
pH	8.20	8.45	8.49	8.34	8.65	8.68	8.21	8.35	8.37	8.13	8.29	8.34	8.68	8.48	8.59
Velocity (m/s)	0.60	0.20	0.70	0.10	0.05	0.03	0.60	0.91	1.40	0.30	0.70	0.55	0.50	1.20	0.90
Turbidity (FTU)	6.79	5.71	5.71	5.21	6.14	3.09	6.98	5.23	6.48	2.86	2.96	3.70	28.36	26.91	10.85

Table 2. Water temperature data for Silver Creek Exposure Trial sites, recorded in degrees Celsius at 30-minute intervals using Stowaway XTI temperature loggers (Onset Computer Corp.).

Location	Group / Dates	1 6/05/06 to 6/15/06	2 6/15/06 to 6/26/06	Combined 6/05/06 to 6/26/06
Loving Creek @ diversion dam	Mean	13.9	14.3	14.1
	Minimum	9.3	9.5	9.3
	Maximum	19.9	20.4	20.4
Hayspur Hatchery @ trash rack	Mean	14.4	14.9	14.7
	Minimum	9.5	9.6	9.5
	Maximum	21.2	21.7	21.7
Loving Creek @ railroad trestle	Mean	14.3	14.7	14.5
	Minimum	9.2	9.3	9.2
	Maximum	21.0	21.3	21.3
Silver Creek @Kilpatrick Bridge	Mean	16.1	16.1	16.1
	Minimum	10.3	11.0	10.3
	Maximum	22.7	22.7	22.7
District Canal	Mean	11.9	12.4	12.6
	Minimum	7.6	7.7	7.6
	Maximum	20.0	19.5	20.0

Table 3. Test results using pepsin-trypsin digest to detect *Myxobolus cerebralis* from rainbow trout exposed to various sites in Silver Creek and the Big Wood River Diversion Canal, Blaine County, Idaho.

Exposure Dates	6/05 to 6/15/06	6/15 to 6/26/06	Cumulative fish tested
Loving Creek @ diversion dam	0/41[a]	0/34	0/75
Hayspur Hatchery @ trash rack	0/46	0/42	0/88
Loving Creek @ railroad trestle	1/49	0/35	1/84
Silver Creek @ Kilpatrick Bridge	0/36	0/50	0/86
District Canal	NA	47[b]/47	47/47

[a]Number of positive individuals / number individuals tested.
[b]Whirling behavior observed prior to sampling.

Table 4. Intensity of infection data for individual rainbow trout in the *Myxobolus cerebralis*-positive exposure groups from the Loving Creek (Railroad Trestle) and Diversion Canal sites.

Location	Exposure Dates	Prevalence (% Positive)	Intensity of Infection	
			Range of Spores/Head	Mean (std. error)
Loving Creek @ railroad trestle	6/5 to 6/15/06	2	13,333[c]	NA
Diversion Canal	6/15 to 6/26/06	100	1,667 – 263,333	35,922 (6,473)

[c] Observation from 1 fish.

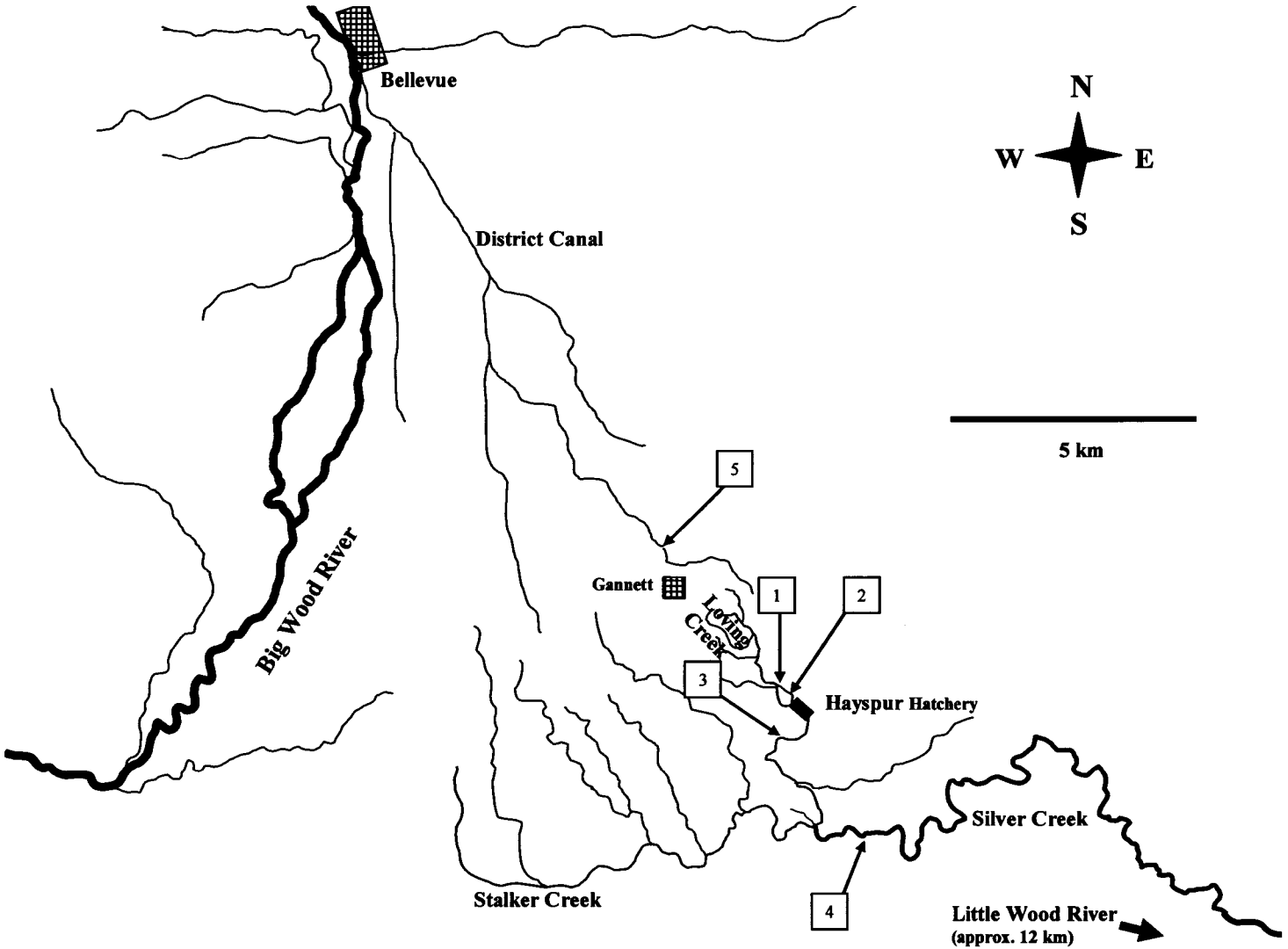

Figure 1. The Big Wood and upper Little Wood River drainages of Idaho, showing the relative locations of Hayspur Hatchery, the main tributaries of Silver Creek, and the District Canal. Live-box exposure sites were: 1) Loving Creek diversion dam; 2) Hatchery trash rack; 3) Railroad trestle; 4) Kilpatrick Bridge; 5) District Canal

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