FISHERY RESEARCH

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

March 2003 to October 2004

Douglas R. Burton
Fish Pathologist

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ABSTRACT

A series of exposure trials were conducted between March 2003 and October 2004 in the Big Wood River, Loving Creek, and Silver Creek drainages of south-central Idaho for the purpose of pinpointing areas where *Myxobolus cerebralis* infectivity could be detected, and to determine the potential of cross-drainage transfer of the parasite from the Big Wood River drainage into the Silver Creek drainage. Study results could be used to evaluate the risks of *M. cerebralis* infection and clinical salmonid whirling disease to both wild and hatchery trout in Silver Creek and Hayspur Hatchery. Susceptible rainbow trout fry were placed in live-boxes for 10 days at three locations on Hayspur Hatchery (Loving Creek Intake, below the Brood Pond, and at the bottom of Gaver Lagoon), in Loving Creek below the hatchery (Railroad Trestle), in Silver Creek (Kilpatrick Bridge), in the Big Wood River at Bellevue, and in the District Canal channels when irrigation water was present. *Myxobolus cerebralis* infections were detected in fish exposed in the Big Wood River and in the District Canal lateral leading to the fields above the Loving Creek Springs, but no infectivity was detected anywhere in Loving Creek, on Hayspur Hatchery, or in Silver Creek. These results indicate that *M. cerebralis* was not established at detectable levels in Loving Creek and Silver Creek during the trial period, and so could not have had an adverse impact on the wild salmonid populations at that time. Results also indicate that no cross-drainage transfer of *M. cerebralis* occurred during the trial periods, but could not disprove that such has not occurred in the past. Central Idaho experienced drought conditions during the study that may have lead to limited overflow of irrigation water into upper Loving Creek. This study should be repeated when water supplies return to average levels or above.

Author:
Douglas R. Burton
Fishery Pathologist

INTRODUCTION

Hayspur Hatchery, operated by Idaho Department of Fish and Game (IDFG), is located in the Little Wood River drainage of Blaine County Idaho, near the head of Loving Creek, a major tributary to Silver Creek (Figure 1). Historically, the hatchery used both spring and surface water from Loving Creek to annually produce over a million fingerling and catchable-size (>250 cm) rainbow trout *Oncorhynchus mykiss*. *Myxobolus cerebralis*, a myxosporean parasite that is the causative agent of salmonid whirling disease, was first confirmed from adult rainbow trout reared on the hatchery in 1988 and has since been periodically detected in both production fish and in wild/feral fish from Loving Creek both above and below the facility (Eagle Fish Health Laboratory database, unpublished). Over $1.5 million has been spent since 1990 to develop well water and to build enclosed concrete ponds at Hayspur Hatchery. These steps were taken for the purpose of avoiding infection by the parasite in production fish. Surface water is no longer used to rear fish for stocking. The hatchery now produces 10-15 million trout eggs per year from fish reared on pathogen-free well water.

The presence of *M. cerebralis* in the Big Wood River was first confirmed in 1995 and has since been periodically detected. The District Canal carries irrigation water from the Big Wood River to fields in the top of the Silver Creek drainage, including those containing the springs that give rise to Loving Creek. Douglas Megargle, the IDFG Magic Valley Regional Fishery Manager, reports that excess irrigation water from the District Canal periodically spills directly into the springs (personal communication). It has been speculated that *M. cerebralis* triactinomyxons carried by these intermittent surges of irrigation water are a possible source of the historic infections in Loving Creek and Hayspur Hatchery.

Myxobolus spores were detected once (1995) in wild trout from Stalker Creek, another nearby tributary to Silver Creek, but the species was not confirmed. Periodic sampling has never detected *Myxobolus* spores in any wild fish from main stem of Silver Creek. Anglers reported poor fishing in the Nature Conservancy's Silver Creek Preserve, and the question was asked if whirling disease was having a negative impact on the wild trout population. Historic sampling had been sporadic, with no objective other than to monitor for the presence or absence of the parasite. Therefore, a series of exposure trials was initiated to learn if, and to what extent, *M. cerebralis* has become established in Silver Creek and Loving Creek, and to attempt to quantify the presence of the parasite by measuring the level of infectivity at various sites.

OBJECTIVES

The purposes of this study were to learn the extent to which *M. cerebralis* has become established in Loving Creek, Silver Creek, and the earthen ponds of Hayspur Hatchery, and to document the possibility that water containing the infective stage of the parasite transferred from the Big Wood River via irrigation canals may have been the source of the historic infections detected in trout at some of those locations. Efforts were made to quantify the levels of *M. cerebralis* at any positive location by measuring the prevalence of infection within exposed sentinel populations of rainbow trout, and by measuring the intensity of infection within individuals by enumerating spore loads.

STUDY SITE

Hayspur Hatchery is located in Blaine County, Idaho, approximately 17.6 kilometers southeast of the town of Bellevue (Figure 1). Four live-box locations were chosen on or near the hatchery: the first at the hatchery intake screen structure on the Loving Creek diversion, another at the downstream end of Gaver Lagoon, a third below the outlet of the abandoned earthen brood pond, and the last at the Loving Creek railroad trestle just below the confluence of the original Loving Creek channel and the hatchery outlet stream. A fifth live-box was placed in Silver Creek under the Kilpatrick Bridge, approximately 2.9 km southeast (downstream) of the hatchery. The final three sites were in the Big Wood River system. One site in the river was within Bellevue city limits, approximately 15 meters below the Broadford Street Bridge and 0.5 km above the District Canal diversion. The primary site in the District Canal was 11.6 km southeast of Bellevue, just on the east side of the Gannett Highway. This canal lateral provides water to the agricultural fields where the Loving Creek springs rise, approximately 1.5 km downstream from the exposure site. The springs themselves are on private property to which access was not available. The final alternate canal site, used only once when the primary site had no water, was located 2.7 km south of Bellevue, on the east side of Highway 75. Map coordinates for all sites are presented in Appendix 1.

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, seven 10-day exposure trials using live-boxes with 50 rainbow trout fry per box were completed from March 2003 through October 2004. Mean fish weight at exposure ranged from 0.27 to 0.91 grams. Dissolved oxygen, pH, conductivity and water flow parameters were collected at deployment and retrieval of the sentinel groups (Appendix 1), and water temperatures were recorded every 30 minutes using Stowaway XTI temperature loggers placed inside the live-boxes (Table 1).

Following exposure, the fish were reared in isolation on pathogen-free well water at the Eagle Fish Health Laboratory (Idaho Department of Fish and Game) at constant water temperature of 13^{o} C for approximately 100 days to allow for spore development. The fish were then sacrificed using an overdose of MS-222 anesthetic, decapitated, and heads were halved. 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 intensity of the individual infection. If clinical signs were observed before the fish were sacrificed, the initial pooling step was skipped.

RESULTS

No infected fish were detected in any groups exposed in Loving Creek, on Hayspur Hatchery, or in Silver Creek (Table 2). Every group exposed in the Big Wood River or in the irrigation canal immediately above the Loving Creek Springs (Canal I) did demonstrate infectivity. Prevalence was very high in both exposures at the Canal I site (100% in June 2003 and 97.5% in June 2004) and in two of three exposures in the Big Wood River (96.4% in May

2004 and 100% in October 2004). The prevalence in March 2004 Big Wood River exposure was very low (2.1% or 1 fish out of 41 tested). The mean water temperature during this exposure was 5.71° C. Vincent (1998) and Burton et al. (2000) both report that *M. cerebralis* infectivity is significantly reduced at temperatures below 9° C. No infectivity was detected in fish exposed at the alternate Canal II site (September 2003).

Individual levels of infection, (i.e. numbers of spores per fish head), were variable within each positive exposure group (Table 3). The May and October 2004 exposures in the Big Wood River were very similar in both mean number of spores per fish head and in range of spores per head observed, while the single observation from the March exposure was much lower than the means for May and October. The two data sets from the irrigation canal were a full year apart. Prevalence was similar, but the mean number of spores per fish was almost 10-fold greater in 2003 than in 2004.

DISCUSSION

Based on these trial results, *M. cerebralis* was not established in Silver Creek above the Kilpatrick Bridge at a level great enough to produce detectable infectivity during 2003 or 2004. Without a detectable presence of the causative organism, whirling disease could not be implicated as a cause of a decline of the wild trout population.

These results also indicate that the parasite was not present at detectable levels in Loving Creek or in the earthen ponds on Hayspur Hatchery during 2003 and 2004, in spite of previous detections at those locations. It may then be deduced that the historic detections of infected fish were either the result of a temporarily established population of *M. cerebralis* organisms that failed to persist, or because the creek experiences intermittent pulses of infectivity from an outside source that have resulted in infected fish but have not lead to an established population of the parasite.

This study did demonstrate that *M. cerebralis* is present in the Big Wood River and in irrigation water drawn from the river, and that infectivity is very high during the time that irrigation water may reach the Silver Creek Springs. But because of drought conditions during the study period, irrigation water may not have been spilled into the springs during the relatively short-duration exposure trials. The evidence suggests that pulses of irrigation water spilling into the Loving Creek Springs during high-water years could have contained the infective stage of *M. cerebralis* and been the source of historic infections in Loving Creek and at Hayspur Hatchery. However, this study failed to either prove or disprove that theory. Even though the presence of the parasite was not demonstrated in upper Loving Creek during these trials, the risk that it could reappear persists. Therefore, it can not be concluded that trout production at the hatchery can be resumed on surface water without risk of *M. cerebralis* infection. A limited repeat of these trials during a year with average or above water supplies is planned.

Another unanswered question is why *M. cerebralis* could not be detected in the Loving Creek/Hayspur Hatchery area during these trials in spite of historic detections. 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 populations of both rainbow and brown trout. All indications suggest that the system should be ideal habitat for the parasite. Beauchamp et al. 2001 reports that different populations or strains of *Tubifex tubifex*, the alternate host for *M. cerebralis*, have different levels of susceptibility to the parasite, ranging

from highly susceptible to totally resistant. This varying susceptibility of the worms leads to greater or lesser production of triactinomyxons and thus has a direct correlation with the levels of infectivity to which fish in a system are exposed (Stevens et al. 2001). There is no published information currently available regarding the makeup and abundance of *T. tubifex* in the Silver Creek system, and further investigations concentrating on the nature and biology of this invertebrate population are warranted.

ACKNOWLEDGEMENTS

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Table 1. 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.).

	Exposure Dates:	3/31/03 to 4/10/03	6/9/03 to 6/19/03	9/16/03 to 9/26/03	3/8/04 to 3/19/04	4/26/04 to 5/6/04	6/1/04 to 6/11/04	10/4/04 to 10/14/04
Hatchery Intake	Mean (error)	9.7(0.14)	19.6(0.16)	11.3(0.12)	9.7(0.14)			
	Minimum	5.6	13.1	7.0	5.8			
	Maximum	18.9	27.4	15.9	15.9			
Brood Pond	Mean (error)	11.1(0.05)	12.2(0.05)	11.2(0.03)	11.2(0.04)			
	Minimum	9.8	10.6	10.2	10.1			
	Maximum	14.3	14.4	12.9	13.5			
Gaver Lagoon	Mean (error)	9.1(0.12)	15.9(0.09)	11.6(0.06)	8.1(0.06)			
	Minimum	5.0	11.5	8.6	5.4			
	Maximum	17.0	21.5	14.1	11.6			
Loving Creek	Mean (error)	9.9(.09)	14.9(0.09)	11.4(0.06)	9.8(0.08)	12.9(0.19)	14.9(0.14)	10.8(0.09)
@Railroad	Minimum	6.8	11.0	9.0	7.0	6.8	9.6	7.4
Trestle	Maximum	16.1	19.2	14.4	13.1	20.6	21.2	14.6
Silver Creek	Mean (error)	8.4(0.14)	17.4(0.10)	11.5(0.09)	7.8(0.11)	14.0(0.16)	16.5(0.13)	11.2(0.08)
@Kilpatrick	Minimum	3.1	12.5	7.4	3.9	6.9	10.4	7.9
Bridge	Maximum	17.1	23.0	15.6	13.0	20.8	22.0	15.2
Big Wood River	Mean (error)				5.7(0.08)	10.1(0.09)		10.2(0.08)
	Minimum				2.4	5.2		7.2
	Maximum				9.3	13.6		14.1
Canal Site I	Mean (error)		12.5(0.11)				13.3(0.14)	
	Minimum		9.0				7.2	
	Maximum		17.0				24.2	
Canal Site II	Mean (error)			10.7(0.09)				
	Minimum			7.0				
	Maximum			14.4				

Table 2. 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, Idaho.

Exposure Dates:	3/31 to 4/10/03	6/9 to 6/19/03	9/16 to 9/26/03	3/8 to 3/19/04	4/26 to 5/6/04	6/1 to 6/11/04	10/4 to 10/14/04	Cumulative fish tested
Hatchery Intake	0/20[a]	0/19	0/46	0/48				0/133
Brood Pond	0/20	0/20	0/35	0/26				0/101
Gaver Lagoon	0/20	0/20	0/37	0/45				0/122
Loving Creek	0/20	0/20	0/40	0/26	0/23	0/24	0/44	0/197
Silver Creek	0/20	0/20	0/40	0/37	0/22	0/28	0/50	0/217
Canal I		41/41[b]				39/40[b]		80/81
Canal II			0/41					0/41
Big Wood River				1/48	27/28[b]		36/36[b]	64/112

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

Table 3. Intensity of infection data for individual rainbow trout from *Myxobolus cerebralis*-positive exposure groups in the Big Wood River and Diversion Canal.

Location	Exposure Date	PREVALENCE (Percent Positive)	INTENSITY OF INFECTION Range of Spores/Head	Mean (std. error)
Canal I	June 2003	100	30,000 - 699,993	316,664 (21,740)
Canal I	June 2004	98	0 - 190,000	37,875 (6,206)
Big Wood River	March 2004	2	13,333[a]	
Big Wood River	May 2004	96	0 - 296,667	57,083 (12,756)
Big Wood River	October 2004	100	1,667 - 286,664	59,675 (11,670)

[a] Observation from 1 fish.

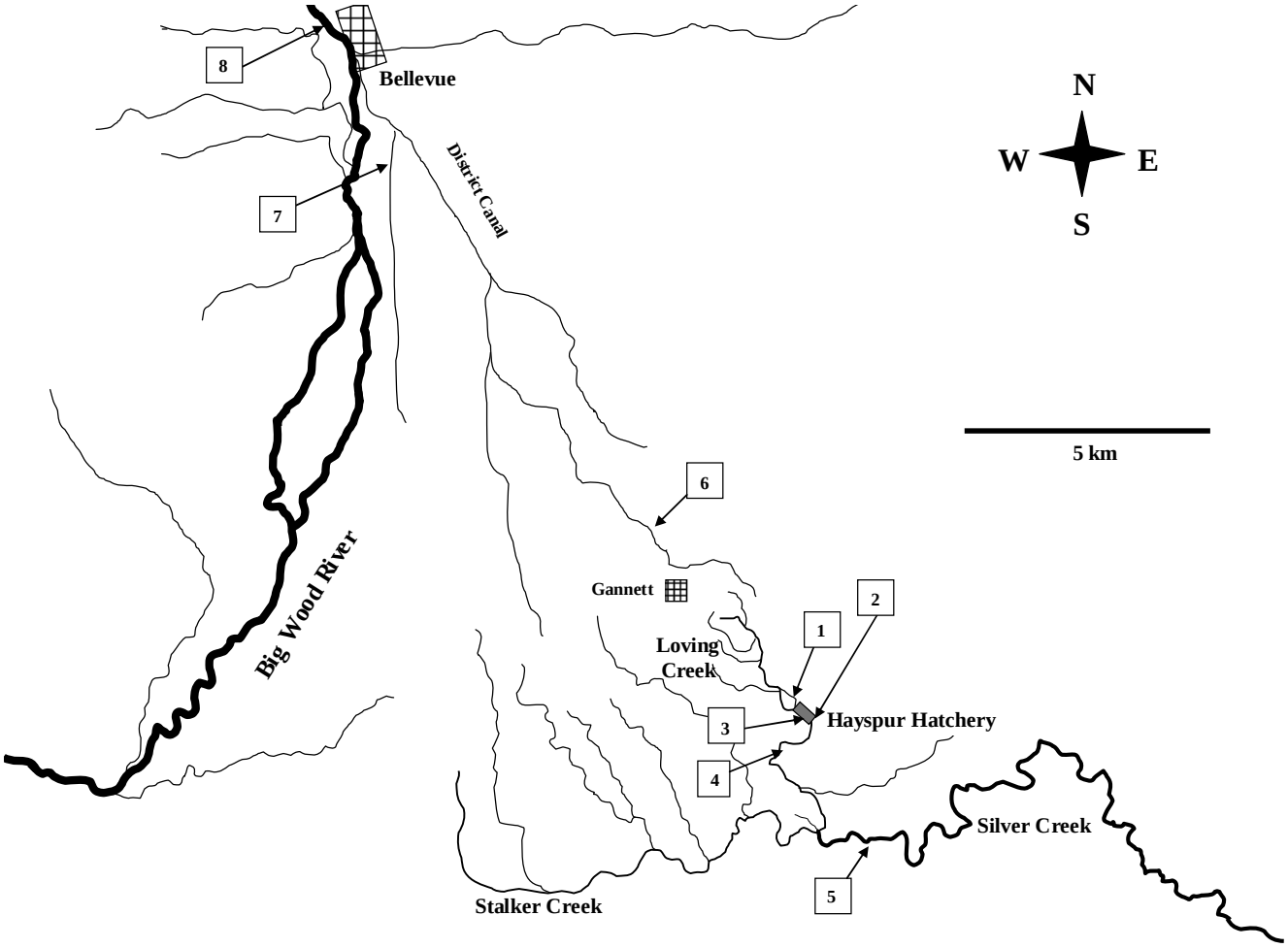

Figure 1. The Big Wood and upper Little Wood River drainages, showing the relative locations of Hayspur Hatchery, the main tributaries of Silver Creek, and the District Canal. Live-box exposure sites were: 1) Hatchery intake; 2) Brood pond; 3) Gaver Lagoon; 4) Railroad trestle; 5) Kilpatrick Bridge; 6) Canal I; 7) Canal II; 8) Big Wood River at Bellevue.

APPENDICES

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

Hayspur Hatchery Intake (Trash Rack) UTM 11T, 731124mE, 4802626mN

Exposure Trial	I		II		III		IV		
Date	3/31/03	4/10/03	6/9/03	6/19/03	9/16/03	9/26/03	3/8/04	3/19/04	
Time (24h)	13:20	10:00	12:20	9:07	9:51	11:00	11:15	11:44	Means
DO (%)	119	78.5	145.8	88.8	91	119	97.6	108.7	106.05
DO (mg/l)	12.0	9.07	14.65	9.19	10.26	13.13	11.65	12.04	11.50
Conductivity (mS/cm^3)	0.397	0.419	0.317	0.368	0.422	0.416	0.426	0.437	0.400
pH	8.07	7.37		7.47	7.53	7.76	7.4	7.6	7.60
Flow Velocity (m/s)	0.01	0.05	0.03	0.01	0.06		0.02	0.06	0.03
Turbidity (FTU)				2.81	2.93	4.36	6.84	5.51	4.49

Hayspur Brood Pond Outlet UTM 11T, 731335mE, 4802464mN

Exposure Trial	I		II		III		IV		
Date	3/31/03	4/10/03	6/9/03	6/19/03	9/16/03	9/26/03	3/8/04	3/19/04	
Time (24h)	13:00	10:30	12:35	9:30	10:28	11:16	11:40	12:02	Means
DO (%)	132.3	118.6	116.2	101.2	83.5	79.9	99.4	104.3	104.43
DO (mg/l)	14.08	12.74	12.22	10.88	9.06	8.67	10.73	11.10	11.19
Conductivity (mS/cm^3)	0.411	0.413	0.417	0.412	0.416	0.416	0.415	0.413	0.414
pH	7.71	7.52		7.84	7.26	7.89	7.64	7.41	7.61
Flow Velocity (m/s)	0.05	0.08	0.07	0.01	0.02		0.01	0.03	0.04
Turbidity (FTU)				1.09	2.51	4.10	4.95	5.18	3.57

Appendix A. Continued.

Hayspur Gaver Lagoon Outlet UTM 11T, 731312mE, 4802361mN

Exposure Trial	I		II		III		IV		
Date	3/31/03	4/10/03	6/9/03	6/19/03	9/16/03	9/26/03	3/8/04	3/19/04	
Time (24h)	13:45	10:50	12:50	9:45	10:51	11:30	12:05	12:20	Means
DO (%)	122.4	79.8	114.1	51.4	82.8	88.5	70.9	93.2	87.89
DO (mg/l)	13.83	9.37	12.11	5.21	9.18	9.66	8.71	11.12	9.90
Conductivity (mS/cm^3)	0.420	0.429	0.346	0.376	0.417	0.419	0.452	0.484	0.418
pH	7.63	7.27		7.47	7.52	7.70	7.79	7.44	7.55
Flow Velocity (m/s)	0.10	0.03	0.04	0.12	0.02		0.01	0.09	0.06
Turbidity (FTU)				2.67	2.57	6.34	5.70	5.88	4.63

Big Wood River at Bellevue UTM 11T 721063mE, 4816377mN

Exposure Trial	IV		V		VII		
Date	3/8/04	3/19/04	4/26/04	5/6/04	10/4/04	10/14/04	
Time (24h)	14:23	14:02	12:54	10:58	13:07	12:18	Means
DO (%)	90.0	103.0	86.5	69.0		89.7	87.64
DO (mg/l)	10.75	12.0	9.6	8.03			10.10
Conductivity (mS/cm^3)	0.300	0.269	0.257	0.191		0.274	0.258
pH	8.9	7.8		7.59		8.21	8.13
Flow Velocity (m/s)	0.07	0.31	0.09	0.02	0.24	0.08	0.14
Turbidity (FTU)	6.34	7.44	5.82	12.14	1.31	0.30	5.56

Appendix A. Continued.

Loving Creek Railroad Trestle UTM 11T, 731317mE, 4801899mN

Exposure Trial	I		II		III		IV	
Date	3/31/03	4/10/03	6/9/03	6/19/03	9/16/03	9/26/03	3/8/04	3/19/04
Time (24h)	13:30	11:15	13:12	10:00	11:05	11:45	12:30	12:37
DO (%)	133.5	112	114.7	86.9	77.4	116.9	96.3	106.8
DO (mg/l)	14.3	12.32	11.89	8.95	8.45	12.52	10.61	11.9
Conductivity (mS/cm^3)	0.413	0.417	0.373	0.389	0.413	0.413	0.421	0.49
pH	7.7	7.68		8.04	7.62	7.12	7.8	7.54
Flow Velocity (m/s)	0.24	0.02	0.01	0.03	0.02		0.19	0.11
Turbidity (FTU)				3.54	1.85	7.12	5.9	7.71

Loving Creek Railroad Trestle (continued)

Exposure Trial	V		VI		VII		
Date	4/26/04	5/6/04	6/1/04	6/11/04	10/4/04	10/14/04	
Time (24h)	11:45	10:30	12:09	12:24	12:09	12:24	Means
DO (%)	86.5	83.8	80.2	65.8	80.2	65.8	93.34
DO (mg/l)	9.6	9.2	8.24	6.87	8.24	6.87	10.00
Conductivity (mS/cm^3)	0.257	0.418	0.397	0.387	0.397	0.387	0.398
pH		6.82	7.62	7.91	7.62	7.91	7.62
Flow Velocity (m/s)	0.09	0.14	0.07	0.17	0.07	0.17	0.10
Turbidity (FTU)	5.82	7.47					5.63

Appendix A. Continued.

Silver Creek, Kilpatrick Bridge UTM 11T, 732269mE, 4799749mN

Exposure Trial	I		II		III		IV	
Date	3/31/03	4/10/03	6/9/03	6/19/03	9/16/03	9/26/03	3/8/04	3/19/04
Time (24h)	13:00	11:45	13:25	10:20	11:35	12:00	12:55	12:58
DO (%)	155	134.6	107.7	110.3	109.1	123.7	104.7	105.2
DO (mg/l)	17.6	14.91	10.52	10.74	11.94	13.31	11.9	12.07
Conductivity (mS/cm^3)	0.380	0.378	0.350	0.362	0.369	0.373	0.404	0.446
pH	8.01	8.1		7.99	7.76	7.76	7.8	7.27
Flow Velocity (m/s)	0.03	0.03	0.02	0.06	0.1		0.05	0.18
Turbidity (FTU)				2.64	0.41	2.06	6.88	8.72

Silver Creek, Kilpatrick Bridge (continued)

Exposure Trial	V		VI		VII		
Date	4/26/04	5/6/04	6/1/04	6/11/04	10/4/04	10/14/04	
Time (24h)	11:18	9:40	12:37	12.05	11:20	10:25	Means
DO (%)	110.9	78.4	108.5	72.5		80.40	107.77
DO (mg/l)	12.18	8.34	11.1	7.55			11.85
Conductivity (mS/cm^3)	0.392	0.372	0.356	0.353		0.379	0.378
pH		6.98	7.16	7.26		7.92	7.64
Flow Velocity (m/s)	0.11	0.07	0.12	0.08	0.07	0.08	0.08
Turbidity (FTU)	5.72	5.62			0.26	0.69	3.67

Appendix A. Continued.

District Canal Site I UTM 11T, 731312mE, 4802361mN

Exposure Trial	II		VI		Site I Means
Date	6/9/03	6/9/03	6/1/04	6/11/04	
Time (24h)	13:46	10:55	13:00	13:08	
DO (%)	119.1	90.3	78.0	68.1	88.88
DO (mg/l)	12.61	9.28	7.67	7.03	9.15
Conductivity (mS/cm^3)	0.175	0.183	0.235	0.211	0.201
pH		8.2	7.33	7.22	7.58
Flow Velocity (m/s)	0.06	0.06	0.05	0.16	0.08
Turbidity (FTU)		16.01			

District Canal Site II UTM 11T, 721063mE, 4816377mN

Exposure Trial	III		Site II Means
Date	9/16/03	9/26/03	
Time (24h)	12:50	12:45	
DO (%)	105.0	104.9	104.95
DO (mg/l)	11.57	11.25	11.41
Conductivity (mS/cm^3)	0.301	0.308	0.305
pH	8.37	7.82	8.10
Flow Velocity (m/s)	0.38		
Turbidity (FTU)	3.39	1.70	2.55

Prepared by:

Douglas R. Burton
Fish Pathologist

Approved by:

IDAHO DEPARTMENT OF FISH AND GAME

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Virgil K. Moore, Chief
Bureau of Fisheries

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Bill Hutchinson, Assistant Bureau Chief
Bureau of Fisheries