Threemile Creek
Beneficial Use Assessment
Idaho County, Idaho

August 2008

Idaho Department of Environmental Quality
Lewiston Regional Office

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Executive Summary

Threemile Creek is located in Idaho County, Idaho. The water quality in Threemile Creek is influenced by both point and nonpoint sources of pollution. In the Idaho Water Quality Standards (IDAPA 58.01.02), Threemile Creek is protected for cold water aquatic life, salmonid spawning, and secondary contact recreation designated beneficial uses. DEQ staff conducted a beneficial use assessment study for Threemile Creek from 2005 through 2006. The purpose of the study was to evaluate the salmonid spawning aquatic life beneficial use designation for Threemile Creek, and to characterize the creek's temperature profile above and below the City of Grangeville wastewater treatment plant outfall to determine whether Idaho's point source natural background temperature provision (IDAPA 58.01.02.401.01.e) is applicable.

Data gathered during the study indicate that the salmonid spawning designated beneficial use is appropriate, as salmonid spawning and cold water aquatic life are currently existing uses in Threemile Creek. Temperature data collected indicate that Threemile Creek water temperatures measured immediately below the City of Grangeville's wastewater treatment plant were increased by more than 0.3 degree Celsius above water temperatures measured immediately above the City of Grangeville's wastewater treatment plant during periods when water temperatures upstream exceeded the applicable aquatic life numeric temperature criteria.

Introduction

Department of Environmental Quality (DEQ) staff conducted a beneficial use assessment study for Threemile Creek from 2005 through 2006, to evaluate the salmonid spawning aquatic life beneficial use designation for Threemile Creek and to characterize the creek's temperature profile above and below the City of Grangeville wastewater treatment plant outfall to determine whether Idaho's point source natural background temperature provision (IDAPA 58.01.02.401.01.e) is applicable.

Background

Threemile Creek originates 4 miles south of Grangeville, Idaho, in forested headwaters (Figure 1), and flows north for approximately 16 miles to its confluence with the South Fork Clearwater River at river mile 7.6. The watershed is approximately 24,966 acres in size, with 99% of the land privately owned, approximately 0.5% owned and managed by the Bureau of Land Management, and the lower 5 miles flowing within the Nez Perce Tribal Reservation. The Grangeville wastewater treatment plant is the dominant point source, and dry land farming, forestry, livestock grazing, and urban development are the dominant nonpoint sources in the watershed. For the 8 miles before it enters the South Fork Clearwater River, Threemile Creek flows through a relatively undeveloped, steep-sloped canyon. There are two potential fish migration barriers within the canyon: a waterfall approximately halfway down the creek, and a land slide near the mouth that seasonally causes the creek to flow subsurface.

Figure 1. Forested Headwaters of Threemile Creek

The designated beneficial uses for Threemile Creek are cold water aquatic life, salmonid spawning, and secondary contact recreation, as established in the Idaho Water Quality

Standards (WQS) (IDAPA 58.01.02.120.07). The standards require that waters designated for salmonid spawning be protected if they "provide or could provide a habitat for active, self-propagating populations of salmonid fishes" (WQS 100.01.b). In 2004, a temperature Total Maximum Daily Load (TMDL) analysis was completed for Threemile Creek by the Nez Perce Tribe, the U.S. Environmental Protection Agency (EPA), and the Idaho Department of Environmental Quality (DEQ 2003). The South Fork Clearwater River TMDL allocated a temperature wasteload allocation for the City of Grangeville's wastewater treatment plant based on Threemile Creek's salmonid spawning season and aquatic life temperature criteria.

Compliance with the TMDL will require a substantial cost increase to the City of Grangeville for upgrading the facility. Treatment cost concerns have led the city to investigate the creek's water quality and fisheries in hopes that, with further analysis, the salmonid spawning beneficial use designation might be revised.

The city's evaluation of the creek documented the following:

- There are existing salmonids, but no young of the year (YOY), in reaches above or below the City of Grangeville.
- Existing salmonids may be escaped hatchery stock from private ponds located adjacent to the creek.
- There are two potential fish migration barriers: a waterfall located approximately mid-point longitudinally in the creek, and a land slide near the mouth in the canyon, which appears to be a barrier only during low flows when it causes the creek to flow subsurface, not during spawning.

The EPA issued a national pollution discharge elimination system (NPDES) permit to the City of Grangeville's wastewater treatment plant on June 20, 2005, without a limitation on effluent temperature. A temperature limitation was excluded from the permit in response to a request by DEQ that the temperature limits be excluded while DEQ determines whether salmonid spawning is an existing use in Threemile Creek and whether the city's wastewater discharge is in compliance with Idaho's point source temperature provision for natural background conditions.

Project Goals and Objectives

The goal of this project is to determine the appropriateness of the TMDL-developed temperature wasteload allocation for the Grangeville wastewater treatment facility.

The project's two objectives are:

- Evaluate the salmonid spawning aquatic life beneficial use designation for Threemile Creek (IDAPA 58.01.02.120.07).

- Characterize the creek's temperature profile above the wastewater treatment plant outfall (Figure 2) and below it, to determine whether Idaho's point source natural background temperature provision (IDAPA 58.01.02.401.01.e) is applicable.

Figure 2. Temperature Meter Site Above Grangeville Wastewater Treatment Plant Outfall

Salmonid Fisheries Analyses

Fish data was collected by DEQ staff during the beneficial use assessment, to determine existing salmonid populations at key locations and to determine the genetic origin of these fish. Electrofishing (Figure 3) was employed as the preferred sampling method, given the inherent variability associated with snorkeling survey efforts, the need to maximize accuracy in species and age class determinations with minimal cost, and the need to collect fin clips for genetic analysis.

Figure 3. Electrofishing in Threemile Creek

The length of the stream segment that was electrofished varied depending on the conditions at each site. The summertime presence of juvenile salmonids in first- through fourth-order streams is considered sufficient evidence that salmonid spawning has occurred and is an existing use in the near vicinity (Grafe et al. 2002).

Sampling efforts were conducted in July and September 2005 during the salmonid rearing time periods for this area. Non-lethal fin clip tissue samples were collected from salmonids and preserved for mitochondrial DNA analysis. Additional sampling efforts took place within Spencer's Pond (Figure 4), to assess whether the salmonids found in Threemile Creek are not native and, in fact, are escapees or descended from stocked salmonids from Spencer's Pond.

Figure 4. Spencer's Pond With Hoop Net

Population Survey

Electrofishing was conducted at transects approximately one-half mile apart, from the mouth of Threemile Creek to an area near the headwaters. Sixty-five salmonids greater than 100 millimeters in length and 39 juvenile salmonids less than 100 millimeters in length were observed and measured. Dace were observed at every sampling site, excluding the archery range and headwaters sites (Appendix A). Figure 5 identifies the sites on the creek where salmonids were captured.

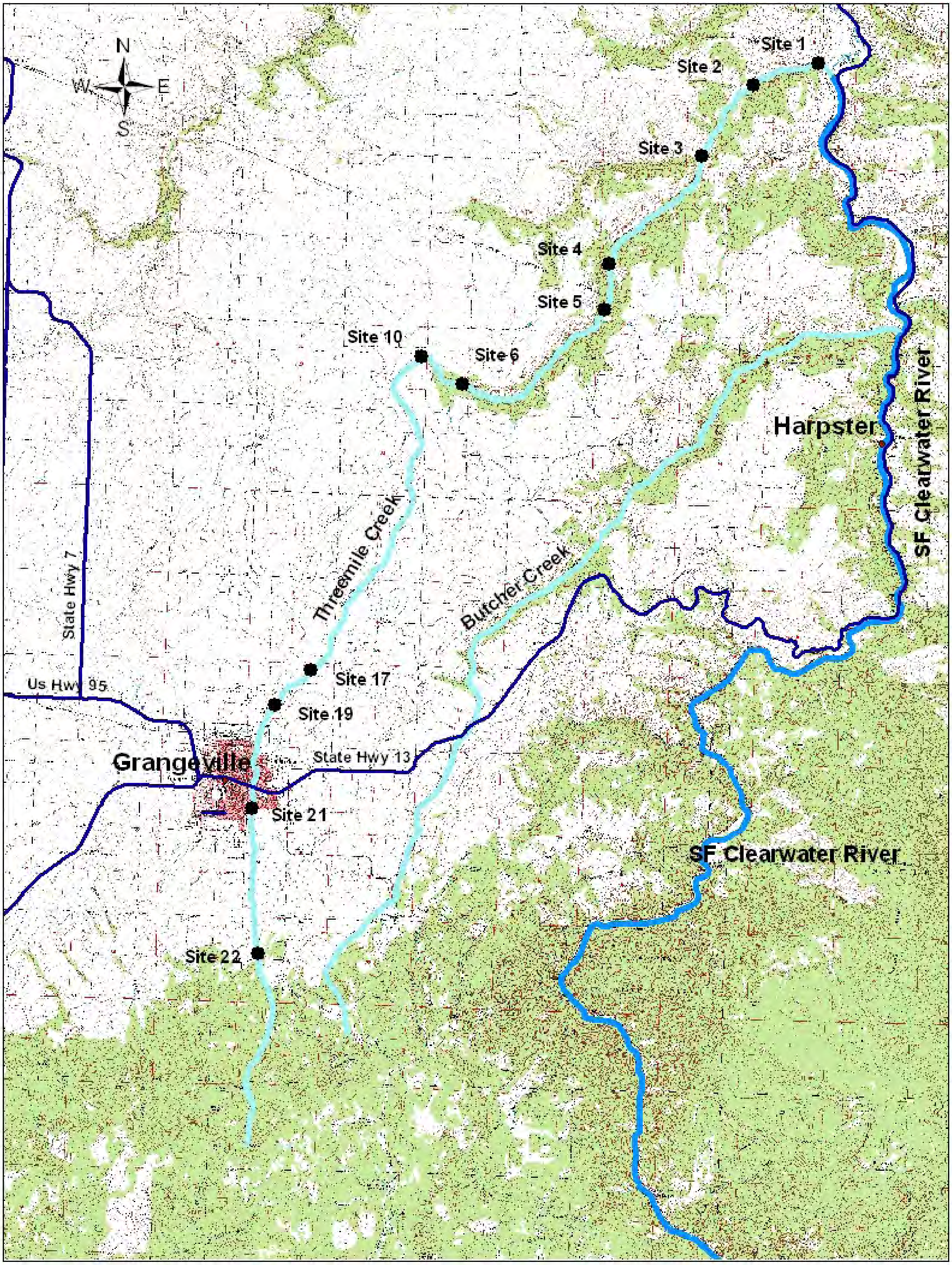

Figure 5. Electrofishing Survey Sites Where Salmonids Were Collected in Addition to Dace

DNA Genetic Analysis

DEQ field crew members collected more than 100 representative fish tissue samples for analysis by the Idaho Department of Fish and Game Eagle Fish Genetics Lab. The DNA analysis of fin clip samples (Figures 6, 7, 8, and 9) indicates rainbow trout in Threemile Creek do not all originate from private stocked ponds. Fish samples collected above the potential fish barriers include central Idaho native origin populations, and fish samples collected below the potential barriers include both hatchery origin and central Idaho native origin populations (Appendix B).

Twelve fish tissue samples were collected from Spencer’s Pond. Genetic screening identified three different haplotypes in samples collected from Spencer’s Pond common to hatchery strains. None of these haplotypes were found in fish tissue samples from Threemile Creek.

Figure 6. Fin Clipping

Figure 7. Preparing Gel

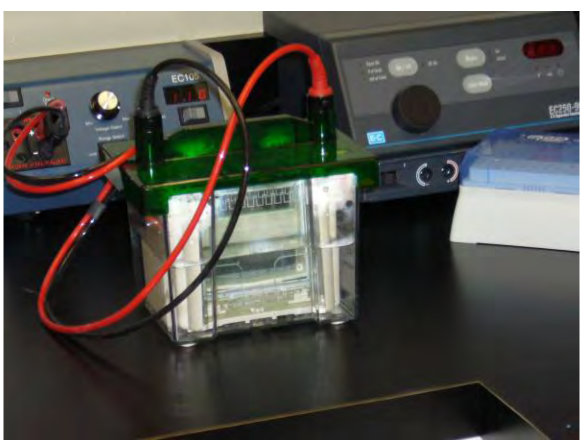

Figure 8. Electrophoresis Process

Figure 9. DNA Process

Stream Temperature Conditions

Idaho's point source background temperature provision allows receiving waters to be increased by 0.3 degree Celsius above natural background conditions when the stream temperature exceeds criteria. In-stream temperature data has been collected and analyzed to determine whether Grangeville's wastewater treatment plant discharge increases stream temperature by more than 0.3 degree Celsius above upstream temperature when the stream temperature above the wastewater treatment plant exceeds aquatic life criteria.

Temperature data was collected using DEQ-deployed Onset™ temperature loggers following DEQ's *Protocol for Placement and Retrieval of Temperature Data Loggers in Idaho Streams* (DEQ 2000). Each temperature logger was calibrated and programmed to record hourly water temperatures from April 2005 through November 2006. Temperature loggers were replaced if they were found to be lost or missing.

Paired Watersheds

John's Creek is a Camas Prairie stream located directly south of Threemile Creek, with attributes and land uses comparable to those of Threemile Creek. Excluding the wastewater treatment plant discharge, John's Creek can be considered a paired watershed with Threemile Creek. Temperature loggers were placed in John's Creek to provide data to help illustrate the common water temperature trends found in Camas Prairie streams (Appendix C).

Analysis of Temperature Data

Temperature data collected for this study were evaluated by the DEQ IDASA temperature analysis program for the critical time periods identified in the TMDL when wastewater effluent may cause stream temperature exceedances. The TMDL identifies these time periods as April 1 through May 31 for salmonid spawning and July 15 through September 15 for cold water aquatic life.

At the headwaters and the forest break site on Threemile Creek, temperatures in 2006 were in compliance with the salmonid spawning criteria and the cold water aquatic life criteria. Throughout the remaining watershed, numerous temperatures exceeded the salmonid spawning and cold water aquatic life temperature criteria (Appendix D).

Above and Below Grangeville Wastewater Treatment Plant

Temperature loggers were placed above and below the wastewater treatment plant from October 2005 through June 2006. The temperature logger below the wastewater treatment plant was placed 110 meters downstream of the outfall to insure a well-mixed location. Upstream and downstream temperature data from 2006 were compared to determine if temperatures increased more than 0.3 degree Celsius, using the critical time periods identified in the South Fork Clearwater River TMDL (as noted above). Between April 1 and May 31 in 2006, on 56 of the 61 days, the provision for an increase of 0.3 degree Celsius above background temperature was exceeded (Appendix E).

Idaho Water Quality Standards Ambient Air Temperature Exemption

The Idaho Water Quality Standards include an exemption for application of the State's aquatic life temperature standard when ambient air temperature exceeds the 90th percentile of the maximum weekly maximum temperature (IDAPA 58.01.02.080.03) compiled using a 7-day rolling average over a 30-year period. The exemption is intended to exclude application of the State's aquatic life stream temperature standard during periods of very extreme temperature conditions.

In DEQ's regional application of the Idaho water quality standards temperature exemption, Threemile Creek is within the Moscow Zone. In the Moscow Zone, DEQ applies 96.04 degrees Fahrenheit as the 90th percentile of the maximum weekly maximum temperature, compiled using a 7-day rolling average over a 30-year period. Grangeville's 2005 and 2006 ambient air temperatures were obtained from the University of Utah Mountain Meteorological Group. The data were analyzed to determine the number of daily maximum temperatures exceeding 96.04 degrees Fahrenheit. In 2005, August 6 was the only day on which this temperature was exceeded in Grangeville, at 96.44 °F. On four days in 2006 in Grangeville, the target temperature was exceeded: July 22 (96.98 °F), July 23 (97.16 °F), July 24 (97.34 °F) and July 27 (97.34 °F). In Moscow in 2005, two days had temperatures exceeding 96.04 °F: July 21 (96.1 °F) and August 21 (97.0 °F). In Moscow in 2006, this temperature was exceeded on five days: July 21 (99 °F), July 22 (99 °F), July 23 (100 °F), July 24 (97 °F) and August 21 (98.1°F).

Water temperature measurements that met or exceeded the temperature exemption value, and were recorded on the above dates, have been flagged in the data set as not applicable due to the air temperature exemption and have not been considered in the analysis of the data set.

Quality Assurance/Quality Control

All field equipment and meters were calibrated and maintained prior to use, in accordance with the manufacturer's instructions.

All sample sites were digitally located using a global positioning system (GPS) and mapped using geographic information system (GIS) technology. Sample sites were marked in the field for easy recognition by field crew members during repeat visits.

A pair of temperature loggers was placed, one at the site below the lower waterfall and one at the headwaters site, to track any potential temperature reading discrepancies. Data from these paired loggers were compared for temperature recording discrepancies. None were found.

Quality assurance/quality control (QA/QC) methods and procedures for genetic analysis were provided by the Idaho Department of Fish and Game Eagle Fish Genetics Lab.

Conclusions

Data gathered during the study indicate that the salmonid spawning designated beneficial use is appropriate, as salmonid spawning and cold water aquatic life are currently existing uses in Threemile Creek.

Temperature data were collected during periods when water temperatures upstream exceeded the applicable aquatic life numeric temperature, so the natural background provision might apply. These data indicate that Threemile Creek water temperatures measured immediately below the City of Grangeville's wastewater treatment plant were more than three-tenths (0.3) degree Celsius greater than water temperatures measured immediately above the City of Grangeville's wastewater treatment plant, more than allowed by the natural background provision.

Air temperatures for several days during 2005 and 2006 exceeded the value required for exemption under the Idaho water quality standards ambient air temperature provision. Stream temperatures on these days were not included in the data set analyzed.

References Cited

DEQ. 2000. Protocol for Placement and Retrieval of Temperature Data Loggers in Idaho Streams. Idaho Department of Environmental Quality. Boise, Idaho. 41p.

DEQ. 2003. South Fork Clearwater River Subbasin Assessment and Total Maximum Daily Loads. Idaho Department of Environmental Quality. Boise, Idaho. 680p.

DEQ. 2005. Threemile Creek Beneficial Use Assessment Project Plan. Idaho Department of Environmental Quality. Lewiston, Idaho. May.

Grafe, C.S., C.A. Mebane, M.J. McIntyre, D.A. Essig, D.H. Brandt, and D.T. Mosier. 2002. The Idaho Department of Environmental Quality water body assessment guidance, second edition-final. Idaho Department of Environmental Quality. Boise, ID. 114 p.

IDAPA 58.01.02. Idaho water quality standards and wastewater treatment requirements.

Appendix A. Threemile Creek Electrofishing Survey

Threemile Creek Electrofishing Survey

Prepared by:
Idaho Department of Environmental Quality
Lewiston Regional Office
August 2005

Electrofishing Survey

An intensive electrofishing survey was conducted from July 25, 2005 through July 27, 2005, on Threemile Creek by Idaho Department of Environmental Quality-Lewiston Regional Office personnel in accordance with the *Threemile Creek Beneficial Use Assessment Project Plan* (DEQ 2005). Threemile Creek, which originates 4 miles south of Grangeville, Idaho, in forested headwaters, flows north approximately 16 stream miles to its confluence with the South Fork Clearwater River. Currently designated beneficial uses are cold water aquatic life, salmonid spawning, and secondary contact recreation.

The purpose of this survey was to document and verify whether salmonid spawning is occurring above a potential fish barrier on Threemile Creek, and if so, when and to what extent this is occurring. Project objectives are contained in the project plan, which provides for conclusions based on the presence of young of the year (YOY) or multiple age classes of salmonids. Salmonids are considered to be juvenile if they are less than 100 mm in length.

Electrofishing conducted for this first sampling effort occurred at transects approximately one-half mile apart, from the mouth of Threemile Creek to an area near the headwaters.

Dace were observed at every sampling site, excluding the archery range and headwaters sites. More than 50 dace were collected and released throughout each reach.

Salmonids were collected at 11 of the 22 electrofishing sites. Non-lethal fin clips (fingernail size) were obtained from 65 salmonids greater than 100mm in length and six juvenile salmonids (< 100mm) and placed into 2-milliliter tubes filled with 100% non-denatured alcohol. An additional 33 juveniles were measured and observed at site 22 but no fin clips were taken from them. Data on all captured salmonids are shown in Tables A-1 and A-2. Genetic samples were sent to the Idaho Department of Fish and Game Eagle Fish Genetics Lab upon completion of the electrofishing study in September. Figure A-1 identifies sites where salmonids were collected.

Sampling Locations

All sampling locations were identified by a permanent landmark (road and/or landowner name) and the geographic coordinates were recorded using a *Garmin eTrex Legend* GPS instrument. All sites are described in the results section below. Sites 1 through 9 are located downstream from the potential fish barrier at the waterfall; sites 10 through 24 are located above the waterfall.

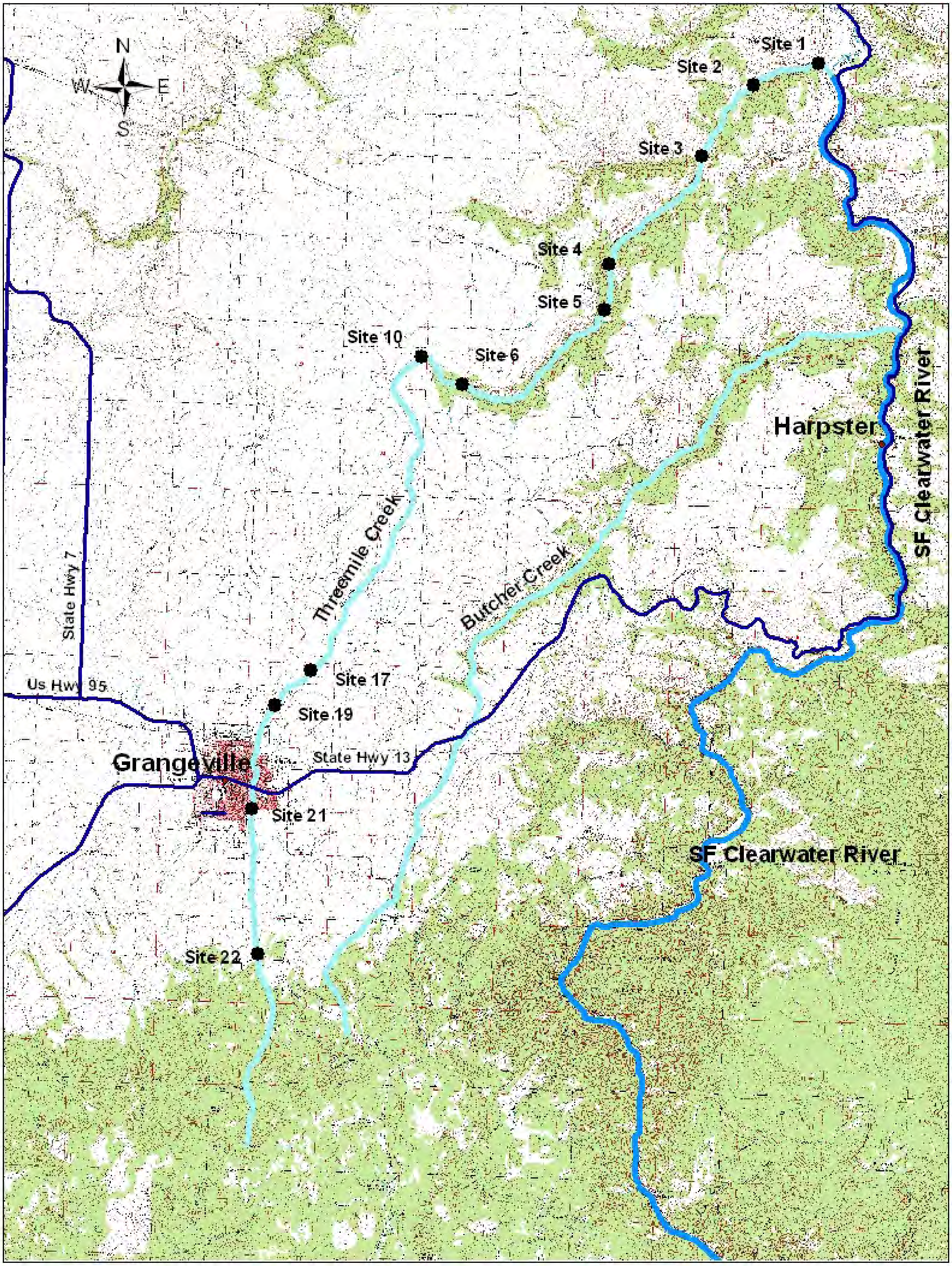

Figure A-1. Electrofishing Locations Where Salmonids Were Collected in Addition to Dace

Results

Site 1: Six salmonids were collected throughout the reach, which extended approximately 160 meters. Sizes ranged from 65mm to 150mm. Substrate consisted of loose cobble with an absence of fines. Grasses and thistles were the dominant riparian species; with alder, willow, hawthorn, and some conifers on the steep left bank.

Site 2: Seven salmonids were collected. Reach length was 150 meters. Substrate consisted of loose cobble with an absence of fines. Grasses and thistles were the dominant riparian species, with alder, willow, hawthorn, and some conifers on the steep left bank.

Site 3: Nine salmonids were collected and fin clipped. Reach length 340 meters. Sizes ranged from 180 mm to 250 mm. This site was above the mouth of Milt Springs, approximately 2.5 miles above the mouth of Threemile Creek. From this point, downstream the creek was dry for 1.2 miles. Substrate consisted of loose cobble with an absence of fines. Grasses and thistles were the dominant riparian species; with alder, willow, hawthorn, and some conifers on the steep left bank. The right bank also contained yellow star-thistle and spotted knapweed.

Site 4: Six salmonids were collected and fin-clipped. Sizes ranged from 170mm to 240mm. The reach length was approximately 200 meters. Substrate consisted of large cobble, characterized by an absence of fines. Some stretches of exposed bedrock were noted. Riparian vegetation included alder, willow, fruit trees, hawthorn, various thistles and grasses, with pines and firs on the steep right bank.

Site 5: Five salmonids were collected and fin clipped. Sizes ranged from 190 mm to 240 mm. This reach began 800 meters upstream of site 1. Reach length was 250 meters. Substrate consisted of loose large cobble, with limited stretches of exposed bedrock. The stream channel was braided in certain stretches and bordered by riparian vegetation including: alder, willow, fruit trees, hawthorn, various thistles and grasses, with pines and firs on the steep right bank.

Site 6: Six salmonids collected and fin-clipped. Sizes ranged from 180 mm to 345 mm. Substrate consisted primarily of bedrock. Reach length extended approximately 250 meters. Riparian vegetation on both banks consisted of alder, willow, hawthorns, dogwoods, pines, firs, thistles and grasses.

Site 7: No salmonids were collected. Substrate consisted of silt. Reach length was 200 meters.

Site 8: No salmonids were collected. Substrate consisted of silt. Reach length was 150 meters.

Site 9: No salmonids were collected. Substrate consisted of silt. Reach length was 150 meters.

Site 10: Two salmonids (220mm and 250mm) were collected and fin clips were obtained from the dorsal fin. The reach extended approximately 200 meters upstream. The substrate was composed of large and small cobbles, with mixed gravel sizes in the lower reach, changing to a predominantly silt/clay bottom at the top of the sampling site. Riparian vegetation consisted of hawthorns, willows, alders, and various grasses and pines further upland.

Site 11: No salmonids were collected at this site. The electrofishing length extended approximately 200 meters. Substrate consisted predominately of silt/clay.

Site 12: No salmonids were collected at this site. The reach length was approximately 150 meters, adjacent to agricultural fields. Substrate consisted of smaller pebbles, predominately silt/clay.

Site 13: Sampling occurred for 250 meters and no salmonids were observed or collected. The substrate consisted primarily of silt/clay.

Site 14: No salmonids were collected at this site. The reach extended approximately 220. The substrate consisted primarily of silt, making it difficult to wade due to sinking. Large willows were present throughout the reach.

Site 15: No salmonids were collected at this site, with the reach totaling approximately 180 meters. Substrate consisted primarily of silt, with small cobble and gravels in riffle habitats. Large willows were present throughout the reach.

Site 16: No salmonids were collected at this site, but were observed in a large pool too deep to wade safely. DEQ staff spoke with the renter of the house located adjacent to the site, who reported that there are trout ranging from 20 to 24 inches in the pool located directly below the bridge. The reach extended approximately 110 meters. Substrate was composed of small cobble, gravel, and silt in the pooled habitat.

Site 17: One salmonid (250mm) was collected at this site, and a sample from the dorsal fin was taken. The reach extended approximately 180 meters. Substrate differed in size throughout the reach, from small cobble in the riffle and run habitats to silt/clay in most pooled habitats. Large willows lined both banks throughout the reach. The channel was straight, adjacent to an agricultural field on the right bank, and an animal feeding operation on the left bank, approximately 60 meters from the creek.

Site 18: No salmonids were collected at this sampling location. The reach totaled 250 meters, and large willows lined both banks throughout the sampling area.

Site 19: One salmonid (260mm) was collected at this site, and a sample from the dorsal fin was obtained. The reach extended approximately 200 meters. The substrate was predominantly silt/clay. The riffled habitat throughout the reach was almost too shallow in certain areas to electrofish. Large willows dominated the riparian area, with few alders and hawthorns present. Access to Threemile Creek was through a hay field that parallels the right bank.

Site 20: No salmonids were collected at this sampling location. Adjacent to the right bank is a grass area of 10-20 feet. The reach extended approximately 250 meters, and the substrate consisted predominantly of silt/clay.

Site 21: Two salmonids (170mm and 250mm) were collected at this site, and fin clips were obtained from their dorsal fins. Two salmonids were measured but no fin samples were taken. The reach had a mix of cobble, gravel, and undercut banks, extending 200 meters upstream to the landowner's property line. Riparian vegetation included willow, alder, hawthorn, red osier dogwood, and various grasses.

Site 22: Five separate reaches, totaling approximately 1,300 meters and ending just below Mountain View Road, were electrofished. Twenty-three salmonids were collected and measured that ranged from 130mm to 270mm. One juvenile salmonid (35mm) was vouchered and wholly placed into a prepared vial. In addition, 33 juveniles (less than 50mm) were observed and measured from throughout the reach. These 33 juveniles did not have fin clips taken from them. Riparian vegetation included mostly large willows. These sites contained riffle, run, and pool habitats with substrate consisting of cobble, gravels, pebbles, and finer sediment. We were informed that each year a pond located adjacent to Threemile Creek is stocked with 1,000 Rainbow Trout.

Site 23: No salmonids were collected at this site. Approximately 40 tailed frog larvae were observed, along with an adult Columbia Spotted Frog. Total reach length was 200 meters encompassed within the city's archery range. There was a mix of cobble, gravel, and other fish cover (undercut banks, overhead cover) with plunge and lateral scour pools. Riparian vegetation included alder, willow, and hawthorn, and the upland tree species consisted of Grand fir, Douglas fir, and Spruce.

Site 24: No salmonids were collected. The reach length extended 250 meters. The substrate consisted of smaller cobbles and coarse pebbles, dominated by riffle habitat. Riparian vegetation consisted of alder and hawthorn, with Ponderosa Pine and Grand Fir along the channel and further upland.

Tributary to Threemile Creek:

Three sites on Whiskey Bill Creek, a tributary to Threemile Creek, were found to be dry and therefore were not electrofished.

Table A-1. Site number, length, vial number, and location of captured salmonids.

Location	*Length (mm)	Vial Number	Reach Length (m)	Geographic Coordinates
Site 1	150	B32	160	Unable to obtain due to geomorphology of canyon
Site 1	**65**	B31	160	
Site 1	**80**	B30	160	
Site 1	**80**	B29	160	
Site 1	**75**	B28	160	
Site 1	**85**	B27	160	
Site 2	185	B39	150	Unable to obtain
Site 2	185	B38	150	
Site 2	210	B37	150	
Site 2	150	B36	150	
Site 2	165	B35	150	
Site 2	180	B34	150	
Site 2	160	B33	150	
Site 3	170	B20	340	46 02 06.0 116 00 41.2
Site 3	250	B19	340	
Site 3	190	B18	340	
Site 3	180	B17	340	
Site 3	220	B16	340	
Site 3	220	B15	340	
Site 3	180	B14	340	
Site 3	185	B13	340	
Site 3	185	B12	340	
Site 4	230	B11	250	46 00 29.9 116 02 03.6
Site 4	220	B10	250	
Site 4	220	B9	250	
Site 4	240	B8	250	
Site 4	230	B7	250	
Site 4	170	B6	250	
Site 5	220	B5	250	46 00 57.7 116 02 00.8
Site 5	210	B4	250	
Site 5	190	B3	250	
Site 5	240	B2	250	
Site 5	200	B1	250	
Site 6	210	B26	250	45 59 42.0 116 04 07.0
Site 6	210	B25	250	
Site 6	180	B24	250	
Site 6	210	B23	250	
Site 6	240	B22	250	
Site 6	345	B21	250	

Location	*Length (mm)	Vial Number	Reach Length (m)	Geographic Coordinates
Site 10	220	A32	200	45 59 58.2 116 04 43.1
Site 10	250	A31	200	
Site 17	250	A30	180	45 56 43.5 116 06 13.3
Site 19	260	A1	200	45 56 21.2 116 06 44.7
Site 21	170	A3	200	45 55 18.0 116 07 02.6
Site 21	250	A2	200	
Site 22	N/A	A29	350	45 53 48.7 116 06 53.6
Site 22	N/A	A28	350	
Site 22	N/A	A27	350	
Site 22	135	A26	350	
Site 22	120	A25	350	
Site 22	270	A24	350	
Site 22	135	A23	350	
Site 22	140	A22	350	
Site 22	210	A21	350	
Site 22	140	A20	350	
Site 22	135	A19	350	
Site 22	145	A18	350	
Site 22	35	A17	350	
Site 22	250	A16	350	
Site 22	160	A15	350	
Site 22	150	A14	350	
Site 22	140	A13	150	45 54 10.5 116 06 57.3
Site 22	145	A12	150	
Site 22	145	A11	150	
Site 22	160	A10	300	45 54 18.0 116 06 59.0
Site 22	145	A9	300	
Site 22	160	A8	300	
Site 22	170	A7	300	
Site 22	135	A6	250	45 54 30.1 116 07 00.8
Site 22	130	A5	250	
Site 22	140	A4	250	45 54 51.6 116 06 58.4

Table A-2. Thirty-three juveniles measured, but not fin clipped, analyzed or included in the genetic study.

Location	*Length (mm)
Site 22	**45**
Site 22	**45**
Site 22	**45**
Site 22	**35**
Site 22	**40**
Site 22	**35**
Site 22	**30**
Site 22	**40**
Site 22	**35**
Site 22	**45**
Site 22	**45**
Site 22	**35**
Site 22	**45**
Site 22	**40**
Site 22	**45**
Site 22	**30**
Site 22	**45**
Site 22	**40**
Site 22	**45**
Site 22	**30**
Site 22	**30**
Site 22	**25**
Site 22	**25**
Site 22	**25**
Site 22	**20**
Site 22	**35**
Site 22	**35**
Site 22	**35**
Site 22	**35**
Site 22	**30**
Site 22	**30**
Site 22	**30**
Site 22	**35**

Appendix B. DNA Genetic Analysis

IDAHO DEPARTMENT OF FISH AND GAME
EAGLE FISH GENETICS LAB
1800 Trout Road
Eagle, Idaho 83616

James E. Risch / Governor
Steven M. Huffaker / Director

M E M O R A N D U M

To:	Cindy Barrett, Water Quality Analyst (Idaho Department of Environmental Quality)
Cc:	Ed Schriever, Paul Kline
From:	Matthew Campbell, Eagle Fish Genetics Lab
Subject:	Genetic results, Threemile Creek, ID

Cindy,

We have completed the genetic analyses on rainbow trout samples from Threemile Creek and Spencer's Pond near Grangeville, Idaho. The primary objective of this work was to test the hypothesis that *Oncorhynchus mykiss* found in Threemile Creek are not native and in fact are escapees or decedents of escapees from Spencer's Pond (Figure 1). Spencer's Pond (located near the headwaters of Threemile Creek) is privately owned and has been stocked with rainbow trout from the Sweetwater Hatchery (Lewiston). Previous studies in our lab have suggested that a mitochondrial DNA (mtDNA) Restriction Fragment Length Polymorphism (RFLP) assay may be useful in distinguishing native redband trout in Idaho from several hatchery rainbow trout strains that have been stocked in Idaho and other Western states (Campbell and Cegelski 2005). We used this mtDNA/RFLP assay to screen samples from Spencer's Pond (N = 11) as well as samples collected from two areas in Threemile Creek: "Below Barrier" and "Above Barrier". Samples from "Below Barrier" (N = 37) were collected in a three kilometer section between a rock slide (considered a likely migration barrier) and the mouth of Threemile Creek (Figure 1). Samples from "Above Barrier" (N = 58) were collected in a twelve kilometer section from a waterfall (a documented upstream migration barrier) to approximately .5 km above Spencer's Pond (Figure 1).

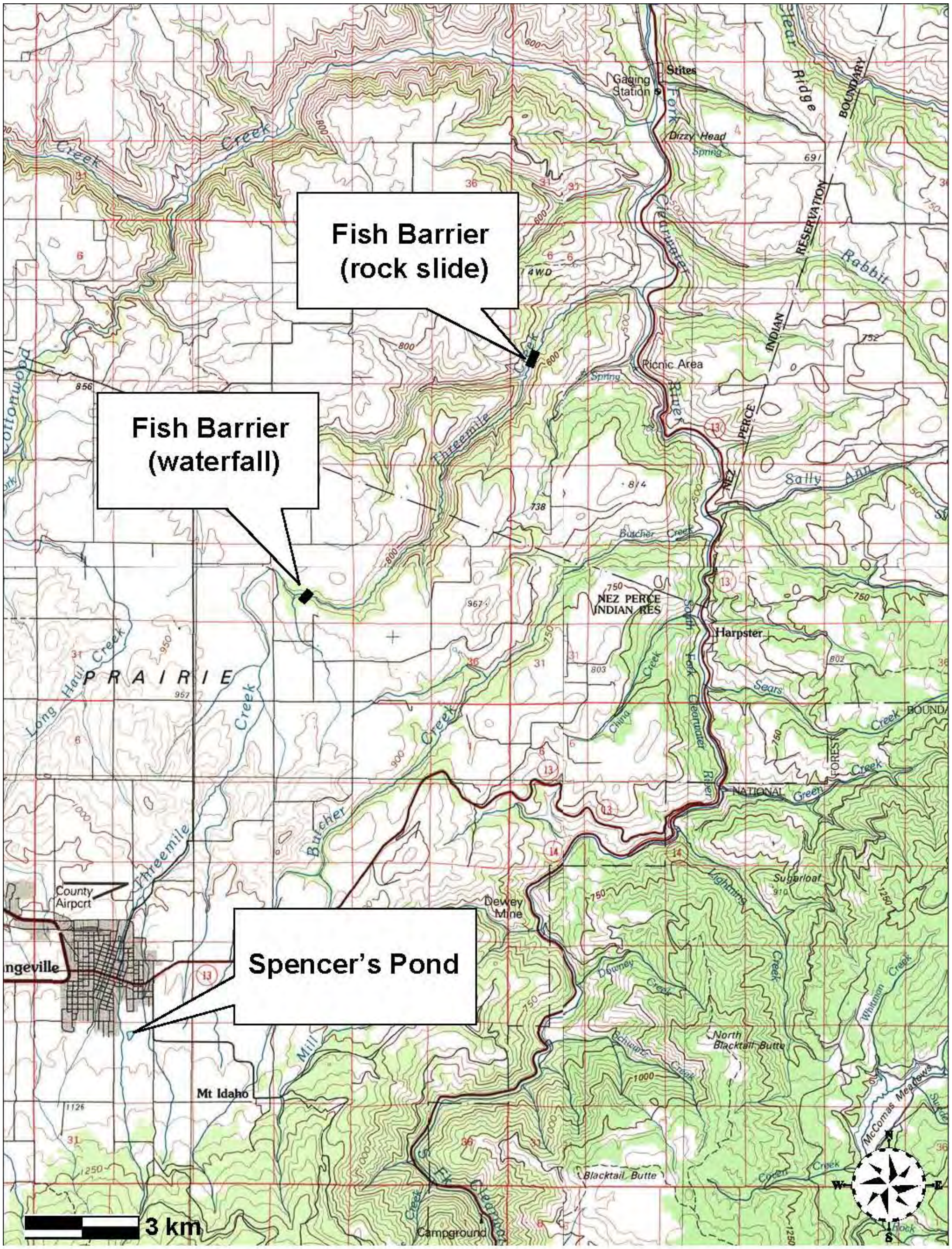

Figure B-1. Study Sample Locations. "Below Barrier" Samples Were Collected in a 3 km Section Between the Rock Slide Fish Barrier and the Mouth of Threemile Creek. "Above Barrier" Samples Were Collected in a 12 km Section From the Waterfall Fish Barrier to Approximately .5 km Above Spencer's Pond

Laboratory procedures followed standard protocols. Total genomic DNA was extracted from a 1 x 1 mm piece of fin clip following methods described by Paragamian et al. (1999). DNA was re-suspended in 100 µl TE. Extracted DNA was then amplified using the Polymerase Chain Reaction (PCR) with primers specific for the combined NADH Dehydrogenase 1 and 2 gene regions of the mitochondrial genome (~3500 b.p.). Amplification products were digested with 4 restriction enzymes (*Hae-III, Hha-I, Hinf-I,* and *Mse-I)* and electrophoresed on 3% agarose gels or 6% polyacrylamide gels to reveal polymorphic fragment patterns (Figure 2).

Screening identified three different haplotypes in samples from Spencer's Pond (Figure 3, Appendix A). Seven samples exhibited composite haplotype "C", a haplotype common to all of the hatchery strains we have previously screened (Figure 4). Three samples exhibited composite haplotype "E". This haplotype is also common in many hatchery strains we have screened previously (Figure 4). A final sample exhibited a new haplotype not previously identified ("M"). None of these haplotypes were found in any samples from Threemile Creek. All samples collected from "Above Barrier" exhibited composite haplotype "A", a haplotype found fixed or nearly fixed in 10 isolated, putatively pure, populations of *O. mykiss* previously examined (Figure 4-Campbell and Cegelski 2005). Haplotype "A" was also the dominant haplotype exhibited in samples from "Below Barrier" (28/37 = 75.7%). However, four other haplotypes were also observed ("F", "L", "N", and "O"). Of these, only haplotype "F" has been observed previously (hatchery RBT from Hayspur and Fish Lake). Haplotype "O" was so different than the other haplotypes we have observed in *O. mykiss* that it is conceivable that this sample is actually a cutthroat trout. I'll try to include this sample in a future project where we are examining cutthroat X rainbow hybridization.

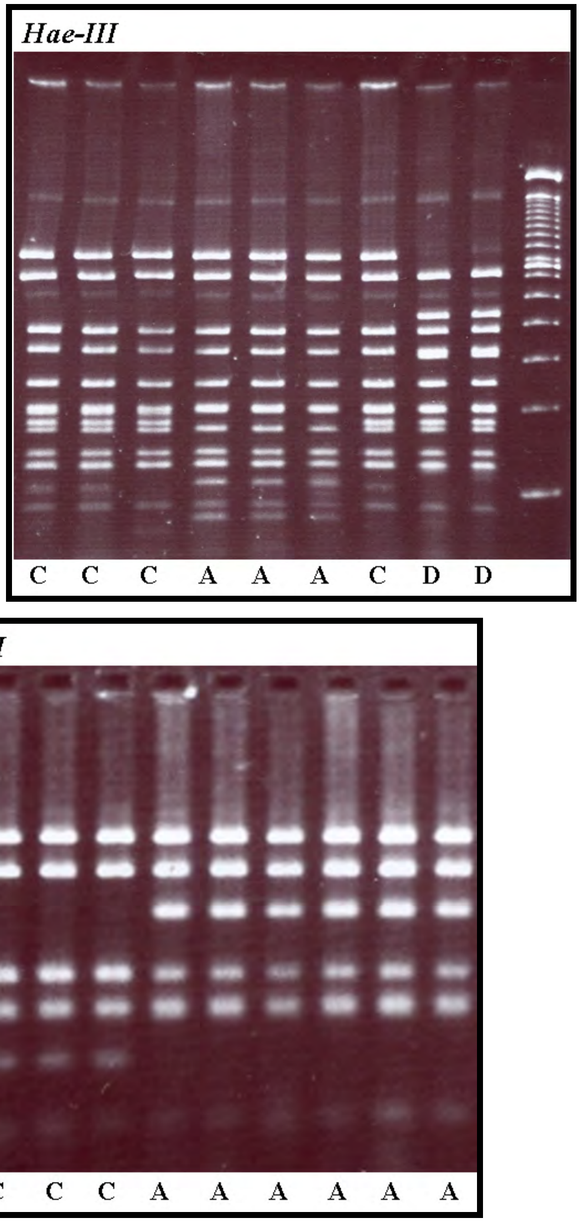

Figure B-2. Examples of Gels Exhibiting Polymorphic Banding (fragment) Patterns. Samples Shown for Both Enzymes (Hae-III and Hinf-I) Enclude TM-70 Through TM-78 (Spencer's Pond and "Below Barrier"). The Final Lane of the Hae-III Gel and the First Lane of the Hinf-I Gel Contain a DNA Ladder

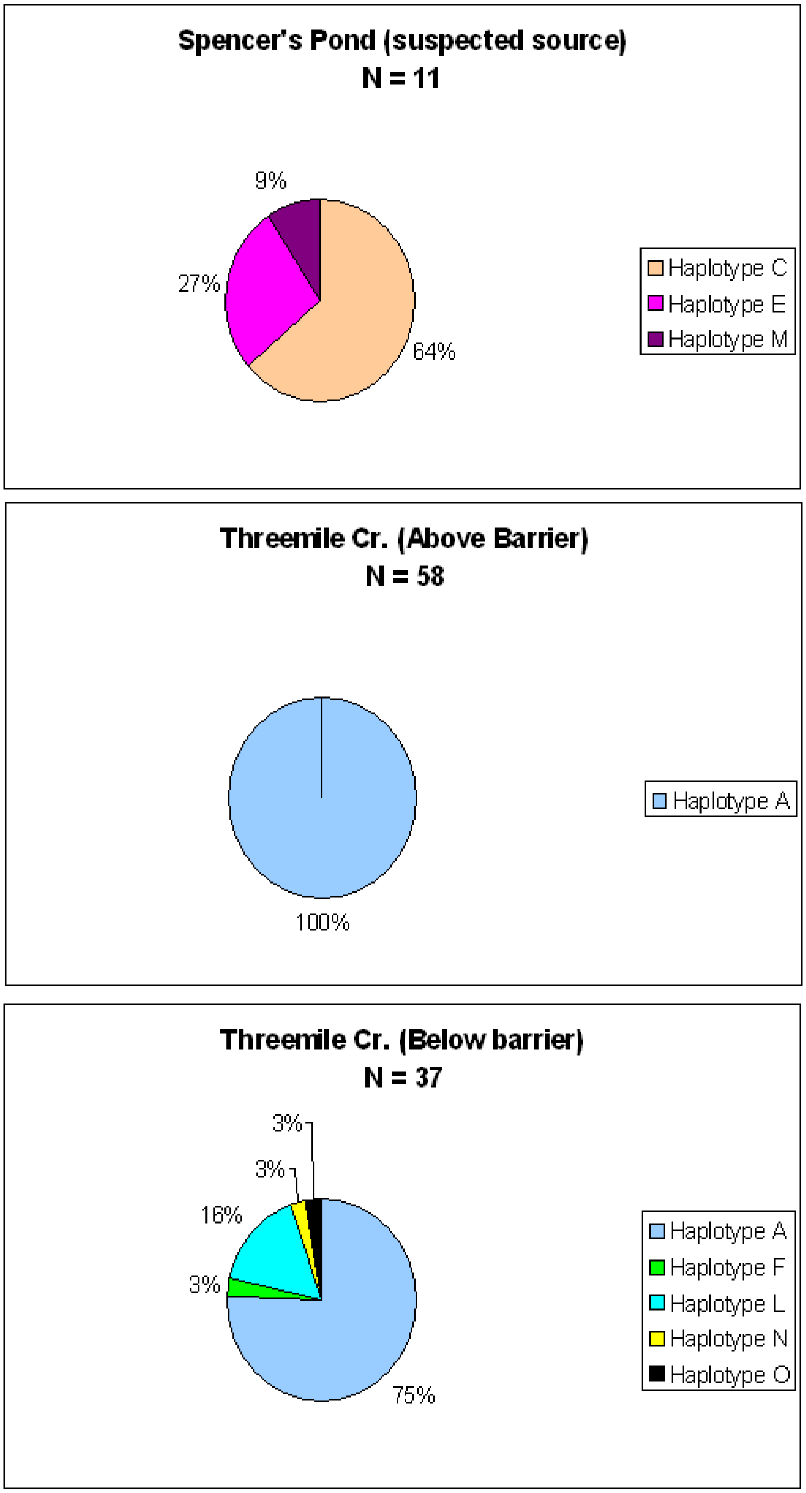

Figure B-3. Frequency of mtDNA Haplotypes in Samples From Spencer's Pond and Threemile Creek (Above Barrier and Below Barrier)

These results are inconsistent with the hypothesis that the origin of rainbow trout in Threemile Creek is from escapees from Spencer's Pond. Instead, samples collected "Above Barrier" exhibited an mtDNA haplotype common to native, interior *O. mykiss* populations that we have sampled in central Idaho (Figure 4.-Campbell and Cegelski 2005). This haplotype was also the dominant haplotype observed in samples collected "Below Barrier". However, the presence of additional multiple *O. mykiss* haplotypes (at least three), one of which (Haplotype F) has been observed previously in other non-native hatchery populations we have screened, suggests that the population below the lowest barrier, is likely of mixed ancestry, containing both native as well as non-native hatchery haplotypes. This may not be particularly surprising given that the S.F. Clearwater River and tributaries to the S.F. Clearwater River have been stocked previously with hatchery rainbow trout (http://fishandgame.idaho.gov/apps/stocking/).

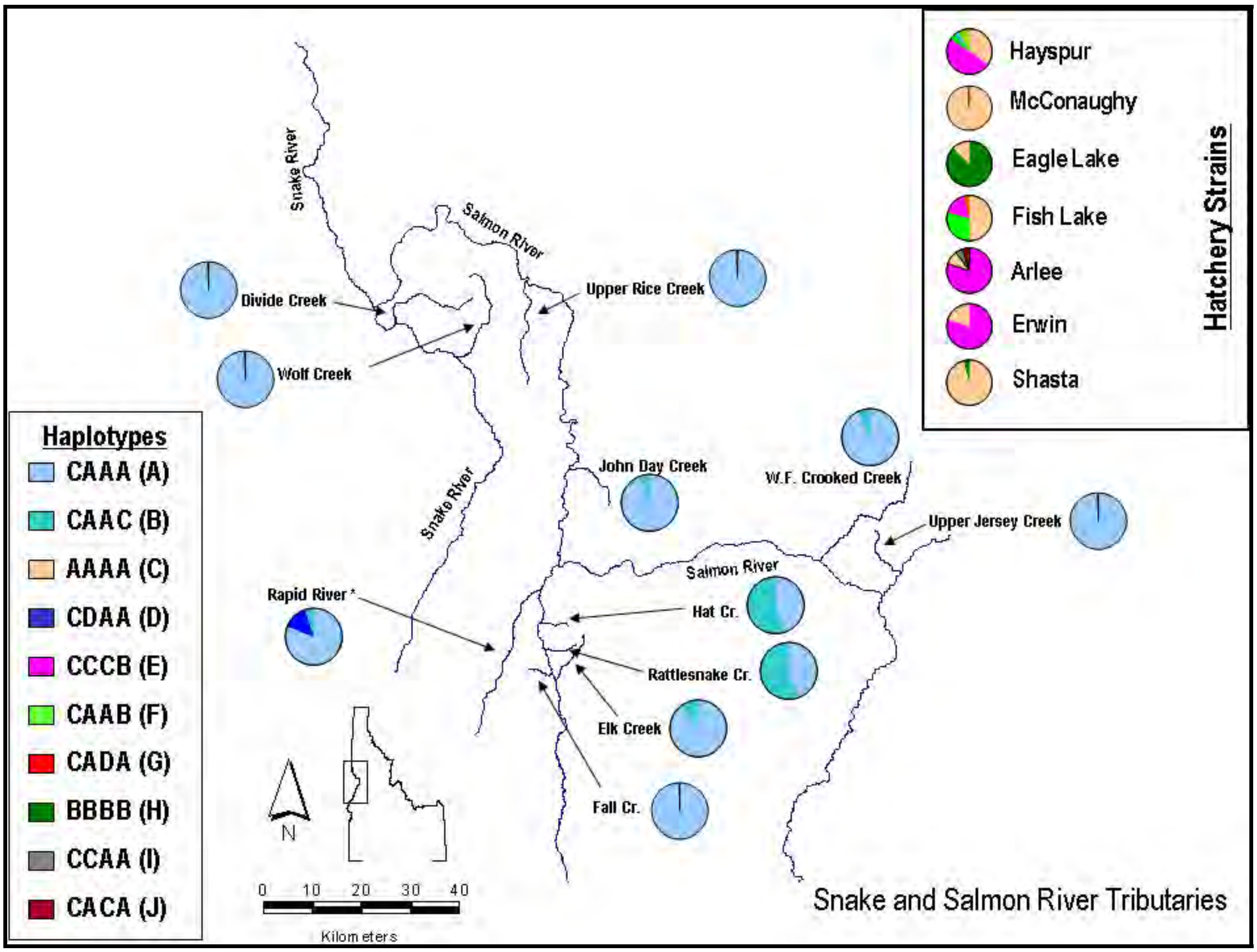

Figure B-4. Distribution of mtDNA Haplotypes in Tributaries to the Snake and Salmon Rivers, ID and Reference Hatchery Rainbow Trout Strains (Campbell and Cegelski 2005)

As I mentioned to you previously, our lab is participating with two other labs (Washington State University and the Columbia River Inter-tribal Fish Commission) in the development and screening of diagnostic nuclear DNA, single nucleotide polymorphisms (SNPs) assays to assess intraspecific hybridization. The development of these markers is important because they would allow us to assess introgression (the actual incorporation of genes from one taxa into another) rather than simply report admixture. We will keep you informed of our progress on this new marker development in case you have additional projects in the future that focus on intraspecific hybridization issues. Thanks again for coming down. If you have any questions or comments please give me a call.

Sincerely,

Matt

Campbell, M.R. and C.C. Cegelski. 2005. Native Species Investigations, Project 2. Idaho Department of Fish and Game. Annual performance report, Grant # F-73-R-25, Report Number 05-19

Paragamian, V. L., M. S. Powell, and J. C. Faler. 1999. Mitochondrial DNA analysis of burbot stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. Transactions of the American Fisheries Society 128:868-874.

Appendix A. Sample Name, Sample Location, Access Code, Polymorphisms observed at each enzyme (Hae-III, Hha-I, Hinf-I, and Mse-I), Haplotype, and Composite Haplotype.

Sample Name	Sample Location	Access Code	Hae-III	Hha-I	Hinf-I	Mse-I	Haplotype	Composite Haplotype
TM-01	Above Barrier	1293-1339-01	C	A	A	A	CAAA	A
TM-02	Above Barrier	1293-1339-02	C	A	A	A	CAAA	A
TM-03	Above Barrier	1293-1339-03	MISS	MISS	MISS	MISS	MISS	MISS
TM-04	Above Barrier	1293-1339-04	MISS	MISS	MISS	MISS	MISS	MISS
TM-05	Above Barrier	1293-1339-05	C	A	A	A	CAAA	A
TM-06	Above Barrier	1293-1339-06	C	A	A	A	CAAA	A
TM-07	Above Barrier	1293-1339-07	C	A	A	A	CAAA	A
TM-08	Above Barrier	1293-1339-08	MISS	MISS	MISS	MISS	MISS	MISS
TM-09	Above Barrier	1293-1339-09	C	A	A	A	CAAA	A
TM-10	Above Barrier	1293-1339-10	C	A	A	A	CAAA	A
TM-11	Above Barrier	1293-1339-11	C	A	A	A	CAAA	A
TM-12	Above Barrier	1293-1339-12	MISS	MISS	MISS	MISS	MISS	MISS
TM-13	Above Barrier	1293-1339-13	C	A	A	A	CAAA	A
TM-14	Above Barrier	1293-1339-14	C	A	A	A	CAAA	A
TM-15	Above Barrier	1293-1339-15	C	A	A	A	CAAA	A
TM-16	Above Barrier	1293-1339-16	C	A (light)	A (light)	MISS	MISS	MISS
TM-17	Above Barrier	1293-1339-17	C	A	A	A	CAAA	A
TM-18	Above Barrier	1293-1339-18	C	A	A	A	CAAA	A
TM-19	Above Barrier	1293-1339-19	C	A	A	A	CAAA	A
TM-20	Above Barrier	1293-1339-20	C	A	A	A	CAAA	A
TM-21	Above Barrier	1293-1339-21	C	A	A	A	CAAA	A
TM-22	Above Barrier	1293-1339-22	C	A	A	A	CAAA	A
TM-23	Above Barrier	1293-1339-23	C	A	A	A	CAAA	A
Control-01	Control-01	Control-01	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
TM-24	Above Barrier	1293-1339-24	C	A	A	A	CAAA	A
TM-25	Above Barrier	1293-1339-25	C	A	A	A	CAAA	A
TM-26	Above Barrier	1293-1339-26	C	A	A	A	CAAA	A
TM-27	Above Barrier	1293-1339-27	C	A	A	A	CAAA	A
TM-28	Above Barrier	1293-1339-28	C	A	A	A	CAAA	A
TM-29	Above Barrier	1293-1339-29	C	A	A	A	CAAA	A
TM-30	Above Barrier	1293-1339-30	C	A	A	A	CAAA	A
TM-31	Above Barrier	1293-1339-31	C	A	A	A	CAAA	A
TM-32	Above Barrier	1293-1339-32	C	A	A	A	CAAA	A
TM-33	Above Barrier	1293-1339-33	C	A	A	A	CAAA	A
TM-34	Above Barrier	1293-1339-34	C	A	A	A	CAAA	A
TM-35	Above Barrier	1293-1339-35	C	A	A	A	CAAA	A
TM-36	Above Barrier	1293-1339-36	C	A	A	A	CAAA	A
TM-37	Above Barrier	1293-1339-37	C	A	A	A	CAAA	A
TM-38	Above Barrier	1293-1339-38	C	A	A	A	CAAA	A
TM-39	Above Barrier	1293-1339-39	C	A	A	A	CAAA	A
TM-40	Above Barrier	1293-1339-40	C	A	A	A	CAAA	A
TM-41	Above Barrier	1293-1339-41	C	A	A	A	CAAA	A
TM-42	Above Barrier	1293-1339-42	C	A	A	A	CAAA	A

Sample Name	Sample Location	Access Code	Hae-III	Hha-I	Hinf-I	Mse-I	Haplotype	Composite Haplotype
TM-43	Above Barrier	1293-1339-43	C	A	A	A	CAAA	A
TM-44	Above Barrier	1293-1339-44	C	A	A	A	CAAA	A
TM-45	Above Barrier	1293-1339-45	C	A	A	A	CAAA	A
TM-46	Above Barrier	1293-1339-46	C	A	A	A	CAAA	A
Control-02	Control-02	Control-02	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
TM-47	Above Barrier	1293-1339-47	C	A	A	A	CAAA	A
TM-48	Above Barrier	1293-1339-48	C	A	A	A	CAAA	A
TM-49	Above Barrier	1293-1339-49	C	A	A	A	CAAA	A
TM-50	Above Barrier	1293-1339-50	C	A	A	A	CAAA	A
TM-51	Above Barrier	1293-1339-51	C	A	A	A	CAAA	A
TM-52	Above Barrier	1293-1339-52	C	A	A	A	CAAA	A
TM-53	Above Barrier	1293-1339-53	C	A	A	A	CAAA	A
TM-54	Above Barrier	1293-1339-54	C	A	A	A	CAAA	A
TM-55	Above Barrier	1293-1339-55	C	A	A	A	CAAA	A
TM-56	Above Barrier	1293-1339-56	C	A	A	A	CAAA	A
TM-57	Above Barrier	1293-1339-57	C	A	A	A	CAAA	A
TM-58	Above Barrier	1293-1339-58	C	A	A	A	CAAA	A
TM-59	Above Barrier	1293-1339-59	C	A	A	A	CAAA	A
TM-60	Above Barrier	1293-1339-60	C	A	A	A	CAAA	A
TM-61	Above Barrier	1293-1339-61	C	A	A	A	CAAA	A
TM-62	Above Barrier	1293-1339-62	C	A	A	A	CAAA	A
TM-63	Above Barrier	1293-1339-63	C	A	A	A	CAAA	A
TM-64	Spencer's Pond, above	1293-1339-64	A	A	A	A	AAAA	C
TM-65	Spencer's Pond, above	1293-1339-65	A	A	A	A	AAAA	C
TM-66	Spencer's Pond, above	1293-1339-66	E*	A	A	A	**EAAA**	M
TM-67	Spencer's Pond, above	1293-1339-67	A	A	A	A	AAAA	C
TM-68	Spencer's Pond, above	1293-1339-68	MISS	MISS	MISS	MISS	MISS	MISS
TM-69	Spencer's Pond, above	1293-1339-69	A	A	A	A	AAAA	C
Control-03	Control-03	Control-03	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
TM-70	Spencer's Pond, above	1293-1339-70	C	C	C	B	CCCB	E
TM-71	Spencer's Pond, above	1293-1339-71	C	C	C	B	CCCB	E
TM-72	Spencer's Pond, above	1293-1339-72	C	C	C	B	CCCB	E
TM-73	Spencer's Pond, above	1293-1339-73	A	A	A	A (partial)	AAAA	C
TM-74	Spencer's Pond, above	1293-1339-74	A	A	A	A (partial)	AAAA	C
TM-75	Spencer's Pond, above	1293-1339-75	A	A	A	A (partial)	AAAA	C
TM-76	Below barrier	1293-1339-76	C	A	A	A	CAAA	A
TM-77	Below barrier	1293-1339-77	D	A	A	A	DAAA	L
TM-78	Below barrier	1293-1339-78	D	A	A	A	DAAA	L
TM-79	Below barrier	1293-1339-79	D	A	A	A	DAAA	L
TM-80	Below barrier	1293-1339-80	C	A	A	A	CAAA	A
TM-81	Below barrier	1293-1339-81	C	A	A	A	CAAA	A
TM-82	Below barrier	1293-1339-82	C	A	A	A	CAAA	A
TM-83	Below barrier	1293-1339-83	C	A	A	A	CAAA	A
TM-84	Below barrier	1293-1339-84	D	A	A	A	DAAA	L
TM-85	Below barrier	1293-1339-85	D	A	A	A	DAAA	L

Sample Name	Sample Location	Access Code	Hae-III	Hha-I	Hinf-I	Mse-I	Haplotype	Composite Haplotype
TM-86	Below barrier	1293-1339-86	D	A	A	A	DAAA	L
TM-87	Below barrier	1293-1339-87	C	A	A	A	CAAA	A
TM-88	Below barrier	1293-1339-88	C	A	A	A	CAAA	A
TM-89	Below barrier	1293-1339-89	C	A	A	A	CAAA	A
TM-90	Below barrier	1293-1339-90	C	A	A	A	CAAA	A
TM-91	Below barrier	1293-1339-91	C	A	A	A	CAAA	A
TM-92	Below barrier	1293-1339-92	C	A	A	A (light)	CAAA	A
Control-04	Control-04	Control-04	Neg.	Neg.	Neg.	Neg.	Neg.	
TM-93	Below barrier	1293-1339-93	C	A	A	A	CAAA	A
TM-94	Below barrier	1293-1339-94	C	A	A	A	CAAA	A
TM-95	Below barrier	1293-1339-95	C	A	A	A	CAAA	A
TM-96	Below barrier	1293-1339-96	C	A	A	B	CAAB	F*
TM-97	Below barrier	1293-1339-97	C	A	A	A	CAAA	A
TM-98	Below barrier	1293-1339-98	C	A	A	A	CAAA	A
TM-99	Below barrier	1293-1339-99	C	A	A	A	CAAA	A
TM-101	Below barrier	1293-1339-101	C	A	A	A	CAAA	A
TM-102	Below barrier	1293-1339-102	F	B	E	D	FBED	O
TM-103	Below barrier	1293-1339-103	C	E	A	A	CEAA	N
TM-104	Below barrier	1293-1339-104	C	A	A	A	CAAA	A
TM-105	Below barrier	1293-1339-105	C	A	A	A	CAAA	A
TM-106	Below barrier	1293-1339-106	C	A	A	A	CAAA	A
TM-107	Below barrier	1293-1339-107	C	A	A	A	CAAA	A
Control-05	Control-05	Control-05	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
TM-109	Below barrier	1293-1339-109	C*	A	A	A	CAAA	A
TM-110	Below barrier	1293-1339-110	C	A	A	A	CAAA	A
TM-111	Below barrier	1293-1339-111	C	A	A	A	CAAA	A
TM-112	Below barrier	1293-1339-112	C	A	A	A	CAAA	A
TM-113	Below barrier	1293-1339-113	C	A	A	A	CAAA	A
TM-114	Below barrier	1293-1339-114	C	A	A	A	CAAA	A
FC-43, reference	Fish Creek steelhead	544-572-43	C	A	A	A	CAAA	A
FC-44, reference	Fish Creek steelhead	544-572-44	C	A	A	A	CAAA	A
FC-45, reference	Fish Creek steelhead	544-572-45	C	A	A	A	CAAA	A

Appendix C. Common Temperature Trends: Threemile and John's Creeks

Common Water Temperature Trends: Threemile Creek and John’s Creek

John’s Creek is a Camas Prairie stream located directly south of Threemile Creek, with attributes and land uses comparable to those of Threemile Creek. Excluding the wastewater treatment plant discharge, John’s Creek can be considered a paired watershed with Threemile Creek. Temperature loggers were placed in John’s Creek to provide data to help illustrate the common water temperature trends found in Camas Prairie streams. Figure C-1 is a graph showing temperatures in John’s Creek and in Threemile Creek above the wastewater treatment plant (also called sewage treatment plant and labeled stp on the graph).

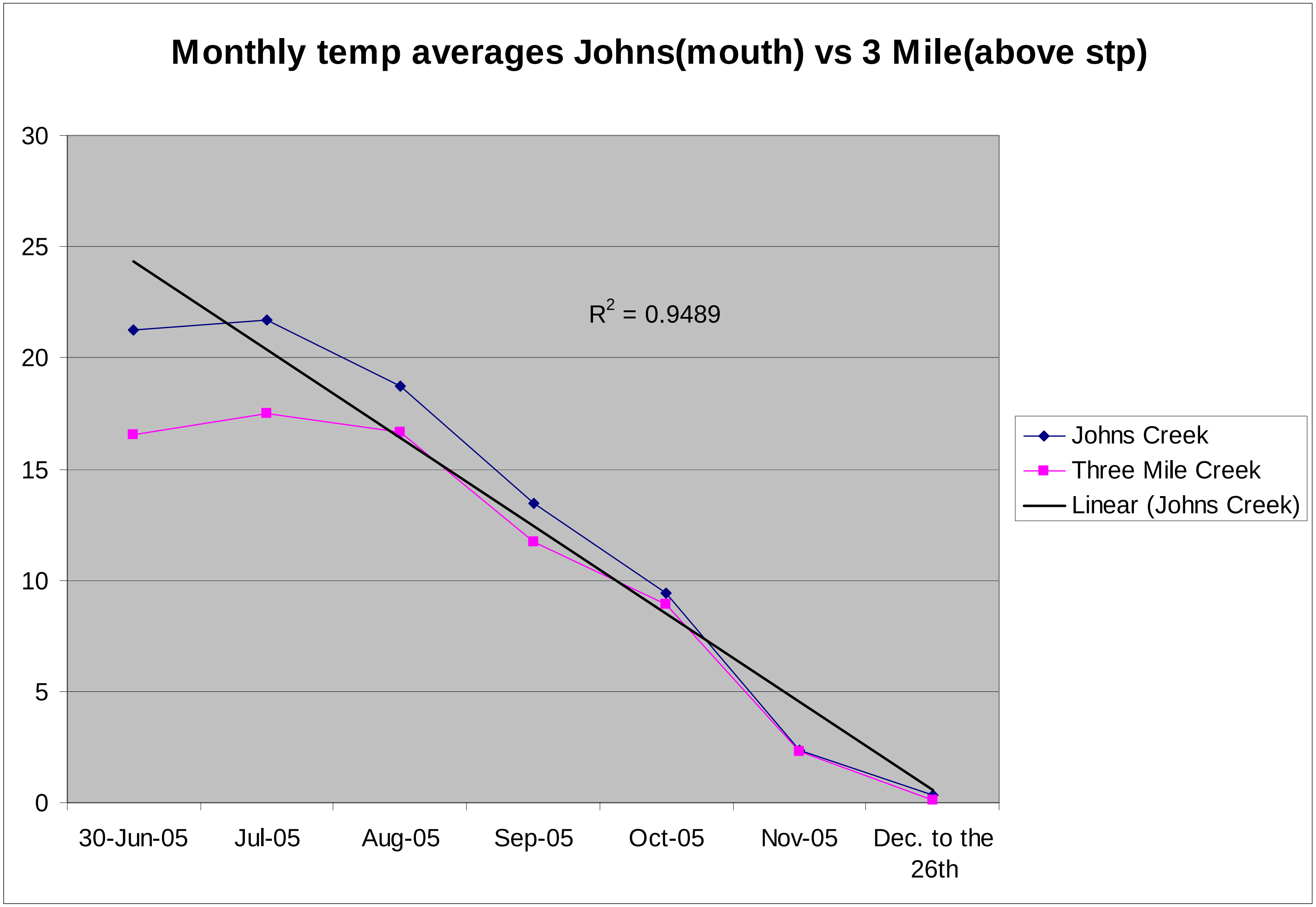

Figure C-1. Monthly Average Temperatures, Mouth of John's Creek and Threemile Creek Above the Wastewater Treatment Plant (degrees Celsius)

Appendix D. Summary of Temperature Exceedances

Table D-1. Threemile Creek Temperature Project Exceedance Summary.

Data entered into DEQ IDASA temperature program with Spring Dates adjusted to TMDL Dates:
Salmonid Spawning April 1 to May 31; Cold Water Aquatic Life July 15 to September 31

Creek:	Location/Year		Exceedance # days	% Exceedance
Threemile	Headwater/Spring 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	0	0
		9 degrees C Average	0	0
		Cold Water Aquatic Life		
		22 degrees C instantaneous	0	0
		19 degrees C Average	0	0
	Forest break 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	0	0
		9 degrees C Average	0	0
		Cold Water Aquatic Life		
		22 degrees C instantaneous	0	0
		19 degrees C Average	0	0
	Above STP 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	29	48
		9 degrees C Average	25	40
		Cold Water Aquatic Life		
		22 degrees C instantaneous	7	10
		19 degrees C Average	8	12
	Below STP 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	27	44
		9 degrees C Average	31	51
		Cold Water Aquatic Life		
		22 degrees C instantaneous	4	6
		19 degrees C Average	15	24
	Above fish barrier 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	37	60
		9 degrees C Average	47	77
		Cold Water Aquatic Life		
		22 degrees C instantaneous	11	17
		19 degrees C Average	12	19
	Below fish barrier 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	37	60
		9 degrees C Average	47	77
		Cold Water Aquatic Life		
		22 degrees C instantaneous	11	17
		19 degrees C Average	12	19

Creek:	Location/Year		Exceedance # days	% Exceedance
	Above Milt Springs 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	no data	
		9 degrees C Average	no data	
		Cold Water Aquatic Life		
		22 degrees C instantaneous	5	8
		19 degrees C Average	8	13
	Below Slide 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	0	0
		9 degrees C Average	32	52
		Cold Water Aquatic Life		
		22 degrees C instantaneous	0	0
		19 degrees C Average	0	0
	Mouth 2006	**Spring Salmonid Spawning**		
		13 degrees C instantaneous	36	59
		9 degrees C Average	48	78
		Cold Water Aquatic Life		
		22 degrees C instantaneous	44	70
		19 degrees C Average	46	73
	Forest Break 2005	**Spring Salmonid Spawning**		
	John's Creek	13 degrees C instantaneous	no data	
		9 degrees C Average	no data	
		Cold Water Aquatic Life		
		22 degrees C instantaneous	48	76
		19 degrees C Average	29	46

Appendix E. Comparison of Stream Temperatures Above and Below the Grangeville Wastewater Treatment Plant

Comparison of Stream Temperatures Above and Below the Grangeville Wastewater Treatment Plant

To compare Threemile Creek water temperatures recorded above the Grangeville wastewater treatment plant with those recorded below the plant, temperature loggers were placed above and below the wastewater treatment plant from October 2005 through June 2006. The temperature logger below the wastewater treatment plant was placed 110 meters downstream of the outfall to insure a well-mixed location.

Monthly averages from each location are shown in Figure E-1, along with some data from John's Creek for comparison. Daily temperatures from above and below the wastewater treatment plant are shown for the spring period, April 1 through May 31, in Figure E-2, and for the summer period, July 15 through September 15, in Figure E-3. Table E-1 lists monthly average water temperatures for Threemile Creek at the headwaters, above the wastewater treatment plant, and below the plant, along with John's Creek at the mouth.

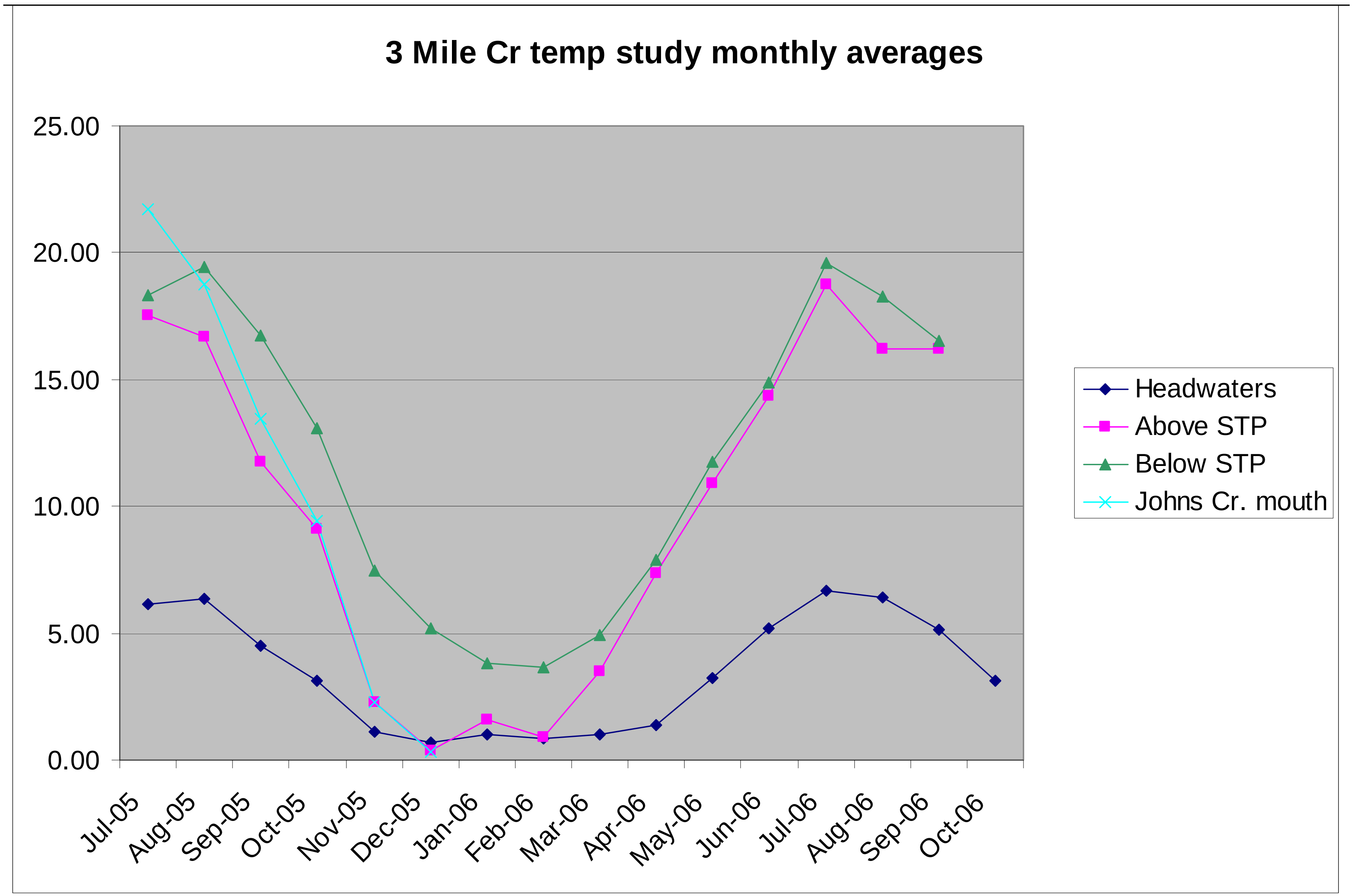

Figure E-1. Threemile Creek Monthly Temperature Averages, Above and Below Wastewater Treatment Plant (degrees Celsius)

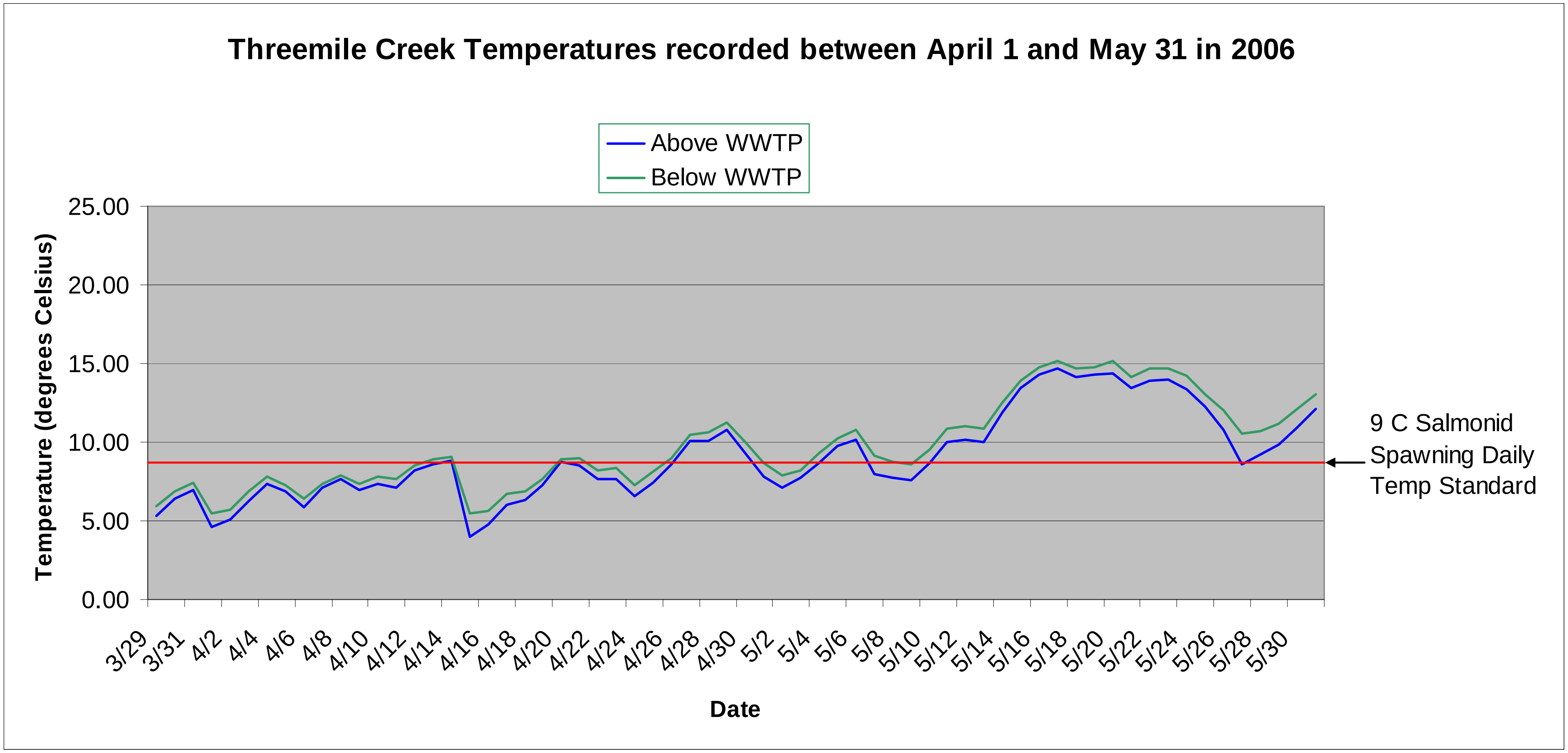

Figure E-2. Threemile Creek Temperatures Above and Below Wastewater Treatment Plant, April 1 through May 31, 2006

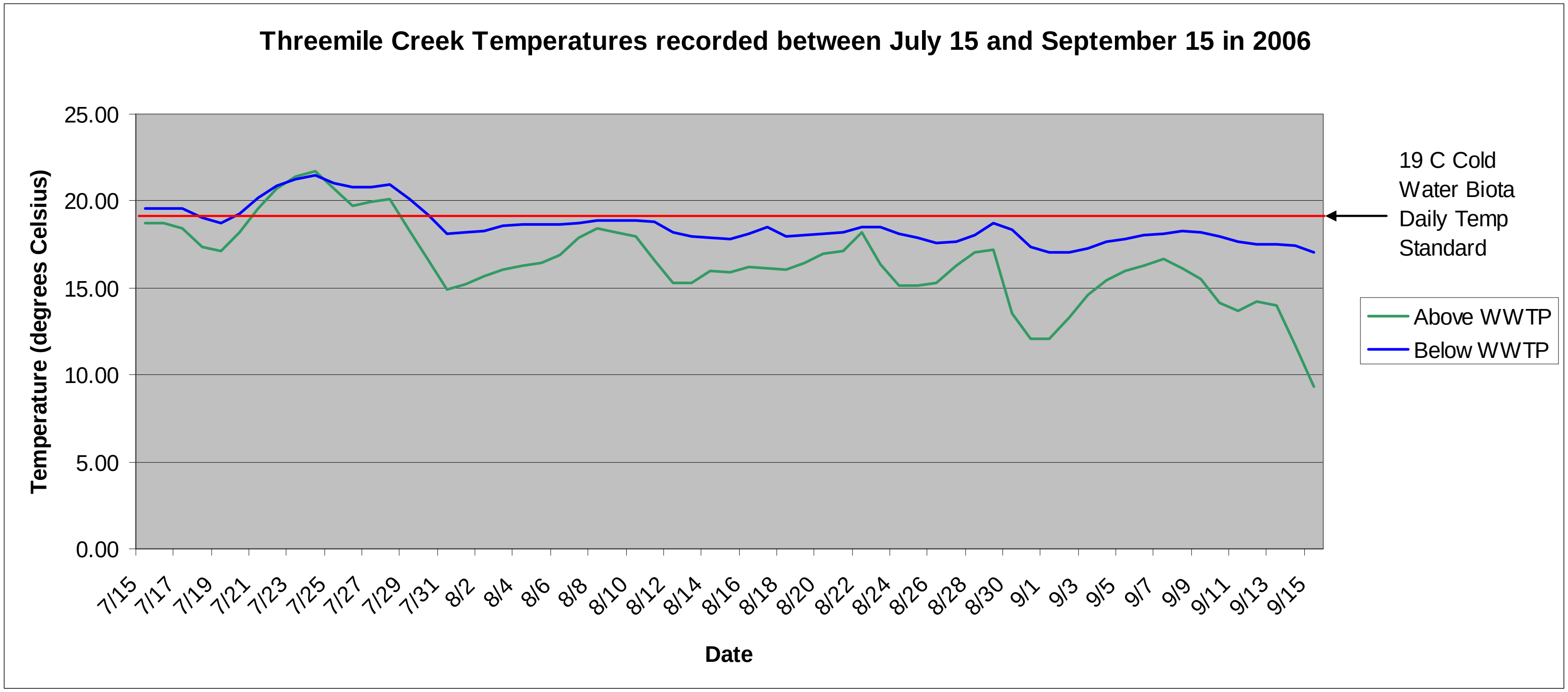

Figure E-3. Threemile Creek Temperatures Above and Below Wastewater Treatment Plant, July 15 through September 15, 2006

Table E-1. Monthly averages compared and plotted against each other for the mouth of John's Creek, above and below the Wastewater Treatment Plant and the headwaters of Threemile Creek.

	Head-waters	Above STP	Below STP	John's Cr Mouth
Jun-05	5.05			
Jul-05	6.13	17.52	18.32	21.71
Aug-05	6.38	16.67	19.42	18.75
Sep-05	4.52	11.74	16.73	13.46
Oct-05	3.13	9.09	13.08	9.42
Nov-05	1.12	2.29	7.45	2.27
Dec-05	0.69	0.37	5.18	0.32
Jan-06	1.02	1.60	3.82	
Feb-06	0.87	0.92	3.66	
Mar-06	1.01	3.52	4.91	
Apr-06	1.40	7.39	7.91	
May-06	3.25	10.93	11.78	
Jun-06	5.19	14.36	14.90	
Jul-06	6.69	18.77	19.62	
Aug-06	6.44	16.23	18.28	
Sep-06	5.12	16.23	16.55	
Oct-06	3.12			

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