Burns Paiute Tribe

Natural Resources Department
Fisheries Division

Evaluate the Life History of Native Salmonids in the Malheur Subbasin

FY 2011 Annual Report

Prepared for

Bonneville Power Administration Division of Fish and Wildlife
US Bureau of Reclamation

Background

In 2011 the Burns Paiute Tribe Natural Resources Department Fisheries Program completed separate statements of work funded through five separate federal contracts. In this report we summarize and interpret the work funded through Bonneville Power Administration Project No. 1997-019-00 and through two separate Bureau of Reclamation contracts. Additional federal, current and multi-year projects will be included in future annual reports. These are described below. Bonneville Power Statement of Work for FY2011 included the fourth year of electrofishing survey for redband trout abundance and distribution throughout the basin, continuous temperature monitoring on the Logan Valley Wildlife Mitigation Property, bull trout spawning surveys in the North Fork and Upper Malheur Watershed, and removal of brook trout in Lake Creek via weir operation and electrofishing.

One of the three contracts with the Bureau of Reclamation completed an experimental fall fish salvage program to remove native fish species entrained below Agency Valley Dam and to release captured fish into the North Fork above Beulah Reservoir. This was a cooperative effort between the Bureau of Reclamation, Burns Paiute Tribe, and Vale Oregon Irrigation District. The results of this effort are reported.

A second contract with the Bureau of Reclamation (Native Affairs program) was awarded in 2011 to launch experimental use of environmental DNA (eDNA) to monitor brook trout populations in High Lake. The first year of efforts was aimed at evaluating sample volume with respect to detection sensitivity of field and lab methodology, and at developing the baseline ability to detect brook trout presence. This work occurred in partnership with Cramer Fish Sciences. The results of this effort are reported. In 2012 a pursuant contract was awarded to support three additional years of this project, the goal of which is to refine the field and lab methodologies so that techniques may be used to monitor biomass.

2011 field work not reported in FY2011 Annual Report:

A third contract through the Bureau of Reclamation (Native Affairs program) was utilized to complete the second year of a four year effort to experimentally evaluate the use of mechanical methods to remove nonnative brook trout in High Lake, the seed source for Upper Malheur brook trout populations. In fall of 2011, the Burns Paiute Tribe removed brook trout in the Strawberry Mountain Wilderness via gillnetting, angling, and electrofishing. The results of the second year will be reported in future annual reports, once the multiyear effort nears completion. Future reports will include recommendations for moving forward with treatment efforts after the close of that contract.

Funds were awarded through National Marine Fisheries Service Pacific Coastal Salmon Recovery Fund to continue to evaluate the suitability of habitat for Chinook salmon reintroduction. These funds were used to replicate FY2010 water quality data collection in the Upper Malheur to the North Fork. Grab sampling was utilized to measure turbidity, pH, and dissolved oxygen along with continuous temperature monitoring at select sites. FY2010 and FY2011 efforts will be cumulatively reported in a future annual report to Bonneville Power Administration with recommendations on anadromous outplantings to the Malheur River.

The 2011 field crew consisted of Erica Maltz (Fisheries Program Manager), Drew Harper (Fish Biologist), DJ Brown (Lead Fisheries Technician), Derek Hawley (Fisheries Technician), Zach Adams (Seasonal Fisheries Technician), Gabe First Raised (Seasonal Fisheries Technician), and Keith Kennedy (Seasonal Fisheries Technician).

Erica Maltz, Fisheries Program Manager
31 January 2013

Evaluate the Life History of Native Salmonids in the Malheur Subbasin

FY 2011 Annual Report
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Chapter 1

2011 Bull Trout Spawning Survey Report

Ray Perkins
Oregon Department of Fish and Wildlife
Malheur Fish District
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Bull Trout Spawning Survey Report, 2011

Ray Perkins, ODFW Malheur Fish District
April, 2012

1.1 Introduction

Bull trout *(Salvelinus confluentus*) were known to exist in the North Fork and upper Malheur River watersheds prior to 1992. Due to increased interest in the status of bull trout, ODFW began bull trout spawning surveys in the North Fork Malheur River watershed in 1992 to track trends in spawning bull trout abundance. The North Fork watershed was selected for initial surveys because no brook trout (*Salvelinus fontinalis*) were present in the system. Brook trout are present in the upper Malheur River system, and spawn timing overlaps with that of bull trout.

Survey timing and location have varied slightly over the years. Initial stream reaches were selected using data from stream habitat surveys and population estimates completed in 1991-2 for tributaries of the upper North Fork. From 1992 through 1996, the reach of stream surveyed varied. Many ending points were moved upstream and entire stream reaches were added or dropped as more information on spawning locations was gained. During the early years, survey timing remained similar, with surveys occurring in mid-September and mid-October. In 1997, stream reaches were surveyed in late August for the first time. From 1997 to 2002, crews surveyed stream reaches three times: late August, mid-September, and late September. In 2003, timing changed again. Volunteers surveyed one stream section (upper Little Crane Creek) in late August. The rest of the reaches of streams were surveyed in mid September and mid October. From 2004 through 2007, three stream reaches were surveyed in late August, and all stream reaches were surveyed in mid September and mid October. Conducting surveys from August through mid-October allowed the separation of bull trout and brook trout spawning counts in the upper Malheur River watershed. Comparing data from the North Fork and mainstem Malheur River indicates that bull trout spawn earlier than brook trout. To the best of our knowledge, the core bull trout spawning areas in the North Fork Malheur River basin are being surveyed.

Spawning surveys began in the upper Malheur River watershed in 1998. As with the North Fork watershed, stream reaches were selected from the stream habitat surveys and population estimates conducted in 1993-4. Early surveys were completed in 1994 on Meadow Fork, Snowshoe, Big, and Lake Creeks. Since initiating spawning surveys, streams have been added, dropped, to take advantage of new information and changes in personnel. Stream reaches in lower Summit and Bosonberg creeks were added for a couple of years and then dropped because no bull trout spawning activity was observed. A stream reach on upper Summit Creek was added and surveyed for eight years, but dropped in 2007 due to a lack of personnel and new survey information indicating bull trout were not present. The survey reach on Big Creek has been continually adjusted. From 2004 to 2007, Meadow Fork was surveyed three times, first in late August, then again in mid September and mid October. The reaches on other streams were surveyed in mid-September and again in mid-October. All streams reaches in the upper Malheur River with known bull trout populations are currently being surveyed. Stream reaches may be extended or dropped in the future to incorporate new information. This report summarizes data collected through 2011.

1.2 Methods

We have reduced the number of objectives for this project to the three listed below. All three objectives apply to both watersheds.

Objectives

1. Determine where bull trout spawn.
2. Determine when bull trout spawn.
3. Determine the number of spawning bull trout.

Spawning surveys were completed on streams in the North Fork Malheur and upper Malheur watersheds known or suspected to support bull trout spawning. One or more people surveyed each stream reach in an upstream direction with at least one experienced surveyor per team. If there were multiple surveyors, they usually walk on opposite sides of the stream. From 2003 to 2010, crews counted redd, determined redd size, noted redd visibility, and recorded numbers and estimated total length (inches) of bull trout observed. All redds, except for those first observed on the final survey, were flagged to avoid double counting on subsequent surveys. All flags were pulled on the last survey.

Each crew used a GPS unit to record starting and ending locations of each stream reach as well as the location of each redd. GPS readings were transferred to data sheets manually during surveys. Each GPS unit was set to record coordinates in decimal degrees and use NAD 1983 as the datum. All GPS coordinates were entered into GIS and mapped. Attempts were made to correct for GPS unit or recording errors when points were mapped.

In the upper Malheur Watershed, distinguishing between bull trout and brook trout redds is impossible without identifying the fish creating each redd. Very few fish were identified and associated with redds. Therefore, redds enumerated and mapped in the upper Malheur watershed are an aggregate of both species with estimates for the number of bull and brook trout redds based on spawn timing.

Since 1992, stream reaches have been surveyed multiple times to better identify spawn timing. From 1993 to 1996, we surveyed stream reaches during mid-September and mid-October. From 1997 to 2002, stream reaches were surveyed three times; the last week of August, mid-September, and the last week of September.

Starting in 2003, the number of times streams reaches surveyed was reduced to match reductions in personnel from all participating agencies. In 2003, all stream reaches in both watersheds were surveyed twice, once in mid-September and again in mid-October. In addition, volunteers walked Little Crane Creek on September 1, 2003.

In 2004 and 2005, Little Crane, lower Sheep, upper Swamp, and Meadow Fork stream reaches were surveyed three times; in late August, mid- September and in mid-October. All other stream reaches were surveyed twice, in mid-September and again in mid October.

In 2006, all stream reaches were surveyed three times (similar to 1997-2002 surveys). The Burns Paiute Tribal staff walked a majority of the stream reaches primarily due to further reductions in

Oregon Department of Fish and Wildlife staff time. In 2007, we reverted back to the 2004-2005 survey timing.

In 2008, we modified the timing and survey reaches to accommodate more reductions in personnel and incorporate information on redd locations and redd visibility through time. We conducted an analysis of the available location data from 1999-2007 surveys and visibility data from 2003 to 2007. Location data was used to determine which stream reaches could be dropped with the least impact to the precision of the counts. Visibility data was used to determine the amount of time between surveys without affecting the precision of the counts. In 2008, streams reaches were surveyed during the second week of September and again during the last week of September. Major changes to some stream reaches occurred. These changes included dropping the lower 4.2 miles of Little Crane Creek, the upper 0.5 miles of Sheep Creek, the lower 1.5 miles of Swamp Creek, and the lower 1.0 miles of the North Fork Malheur River. In the upper Malheur River watershed the lower 0.25 miles of Meadow Fork was dropped, and approximately 1.0 mile of Big Creek between Meadow Fork and Snowshoe Creeks was added. The 2009 and 2010 stream reaches and survey periods were similar to the 2008 surveys.

1.3 Results

North Fork Malheur River Watershed

North Fork Malheur River

Upper North Fork Malheur River reach was surveyed twice in 2011. The survey began at the mouth of Horseshoe Creek and ended 1.4 miles upstream. Five redds were observed in total; 4 redds on August 30 and 1 redd on September 28 (Table 1).

Table 1. Bull trout redds observed in the mainstem of the North Fork Malheur River.

YEAR	REDDS	MILES	REDDS/MILE
1992[a]	1	5.9	0.2
1993	1	15.5	0.1
1994	0	7.3	0.0
1995	0	6.0	0.0
1996	6	3.9	1.5
1997	10	2.3	4.4
1998	3	3.8	0.8
1999	9	3.5	2.6
2000	16	3.5	4.3
2001	5	3.0	1.7
2002	8	2.3	3.5
2003	0	3.8	0.0
2004	3	2.5	1.2
2005	3	2.5	1.2
2006	7	2.6	2.7
2007	5	1.5	3.3
2008	7	1.6	4.4
2009	20	1.6	12.5
2010	3	1.6	1.9
2011	5	1.4	3.6

[a]- Does not include 14 questionable redds observed by volunteers included in earlier reports.

Horseshoe Creek

Horseshoe Creek reach was surveyed twice in 2011. The survey began at the confluence with North Fork Malheur River and ended approximately 1.1 miles upstream. A total of 8 redds were observed; all on August 31 (Table 2).

Table 2. Bull trout redds observed in Horseshoe Creek, tributary to North Fork Malheur River.

YEAR	REDDS	MILES	REDDS/MILE
1998	4	0.4	10.0
1999	4	0.8	5.0
2000	7	0.8	6.3
2001	6	0.6	10.3
2002	3	1.2	2.5
2003	1	0.8	1.3
2004	1	0.8	1.3
2005	4	1.2	3.3
2006	15	1.1	11.8
2007	3	1.1	2.7
2008	6	1.2	5.0
2009	2	1.2	1.7
2010	8	1.2	6.7
2011	8	1.1	7.3

Swamp Creek

Swamp Creek reach was surveyed twice in 2011. The lower section began at river mile 1.2 and continued upstream approximately 1.5 miles. The upper section began at the end of the lower section (RM 2.7) and continued upstream an additional 1.2 miles. Ten redds were observed; 4 redds on August 30, and 6 redds on September 27 (Table 3).

Table 3. Bull trout redds observed in Swamp Creek, tributary to North Fork Malheur River.

YEAR	REDDS	MILES	REDDS/MILE
1992	0	1.2	0.0
1993	3	2.2	1.4
1994	9	3.9	2.3
1995	0	3.9	0.0
1996	8	3.8	2.1
1997	21	4.1	5.1
1998	24	4.2	5.7
1999	35	4.1	8.5
2000	40	4.1	9.8
2001	22	4.2	5.3
2002	19	2.0	9.5
2003	13	4.2	3.1
2004	19	4.3	4.5
2005	20	4.2	4.8
2006	32	4.2	7.6
2007	36	4.3	8.4
2008	20	2.6	7.3
2009	18	2.3	7.8
2010	16	2.8	5.7
2011	10	2.7	3.7

Sheep Creek

The lower and upper reaches of Sheep Creek were surveyed twice in 2011. The lower reach began at the confluence with the North Fork and continued upstream approximately 2.0 miles. The upper reach began where the lower section ended and continued upstream an additional 1.9 miles. Ten redds were observed in total; 1 redd on August 30, and 9 redds on September 27 (Table 4).

Table 4. Bull trout redds observed in Sheep Creek, tributary to North Fork Malheur River.

YEAR	REDDS	MILES	REDDS/MILE
1992	0	1.1	0.0
1993	0	2.2	0.0
1994	0	2.2	0.0
1995	2	2.9	0.7
1996	13	3.4	3.8
1997	8	2.9	2.8
1998	17	3.5	4.9
1999	22	3.0	7.3
2000	25	4.0	6.3
2001	15	3.5	4.3
2002	17	3.5	4.9
2003	12	3.9	3.1
2004	14	2.9	4.8
2005	15	3.9	3.8
2006	17	2.9	5.9
2007	34	3.9	8.2
2008	16	3.2	5.0
2009	18	2.9	6.2
2010	11	3.9	2.8
2011	10	3.9	2.6

Elk Creek

Elk Creek was surveyed twice in 2011. The survey began at the culvert under Forest Road 16 and extended approximately 0.6 miles upstream to the confluence of North Fork Elk and South Fork Elk. The survey continued up the South Fork 0.6 miles and up the North Fork 1.4 miles. A total of 10 redds were observed; 8 redds on August 30 and 2 redds on September 27 (Table 5).

Table 5. Bull trout redds observed in Elk Creek and its two tributaries, the North and South forks.

YEAR	REDDS	MILES	REDDS/MILE
1992	1	1.0	1.0
1993	1	2.3	0.4
1994	0	2.0	0.0
1995	1	4.0	0.3
1996	3	4.1	0.7
1997	9	4.1	2.2
1998	6	3.5	1.7
1999	12	3.0	4.0
2000	5	3.0	1.7
2001	3	3.2	0.9
2002	7	2.8	2.5
2003	7	3.2	2.2
2004	5	2.5	2.0
2005	10	3.5	2.9
2006	12	3.7	3.2
2007	9	3.0	3.0
2008	12	2.4	5.0
2009	6	2.5	2.4
2010	8	2.6	3.1
2011	10	2.6	3.8

Little Crane Creek

Little Crane Creek was surveyed twice in 2011. The survey started at Forest Road 16 culvert and continued upstream approximately 1.8 miles to Forest Service Road 1665-0498. A total of 9 redds were observed; 4 redds on August 30 and 5 redds on September 28 (Table 6).

Table 6. Bull trout redds observed in Little Crane Creek, tributary to North Fork Malheur.

YEAR	REDDS	MILES	REDDS/MILE
1992			
1993	3	5.6	0.5
1994	4	7.5	0.5
1995	6	6.0	1.0
1996	8	6.0	1.3
1997	16	4.2	3.8
1998	20	6.0	3.3
1999	33	6.1	5.4
2000	60	6.1	9.8
2001	74	6.2	12.0
2002	45	2.8	16.1
2003	30	6.1	4.9
2004	22	3.2	6.9
2005	15	6.1	2.5
2006	14	2.6	5.4
2007	25	2.0	12.5
2008	14	1.8	7.8
2009	18	1.8	10.0
2010	9	1.9	4.7
2011	9	1.8	5.0

The following streams in the North Fork Malheur River Watershed were not surveyed in 2011; Crane Creek, Cow Creek, Little Cow Creek, Deadhorse Creek, Flat Creek and Spring Creek.

Upper Malheur River Watershed

Snowshoe Creek

Snowshoe Creek was surveyed twice in 2011. This survey began at the confluence with Big Creek and ended approximately 0.9 miles upstream near the wilderness boundary sign. The first 0.4 mile was not surveyed due to a thick stand of alder and brush that made accessing the stream difficult and the use of visual survey methods ineffective. A total of 4 redds were observed. All 4 redds were observed during the second survey on September 28 (Table 7).

Table 7. Redds observed in Snowshoe Creek, tributary to Big Creek, from mid September to mid October.

YEAR	REDDS	MILES	REDDS/MILE
1998	10	1.7	5.9
1999	25	1.7	14.7
2000	3	1.7	1.8
2001	16	1.7	9.4
2002	0	1.4	0.0
2003	6	1.1	5.5
2004	9	0.6	15.0
2005	3	0.8	3.8
2006	8	0.9	8.9
2007	32	0.9	35.6
2008	6	0.7	8.6
2009	2	0.4	5.0
2010	5	0.9	5.6
2011	4	0.4	10.0

Big Creek

Upper Big Creek was surveyed twice and the middle section of Big Creek once in 2011. The middle stream reach begins at the mouth of Meadow Fork and ends 1.8 miles upstream. The upper stream reach begins at the trail crossing and continues upstream 0.3 miles. One redd was observed in this area on September 28 (Table 8).

Table 8. Redds observed in Big Creek, tributary to Upper Malheur River, from mid September to mid October.

YEAR	REDDS	MILES	REDDS/MILE
1998	0	2.3	0.0
1999	8	4.6	1.7
2000	22	4.6	4.8
2001	31	5.2	5.9
2002			
2003			
2004	5	0.8	6.3
2005	2	0.6	3.3
2006	3	0.7	4.3
2007	1	0.5	2.0
2008	4	1.6	2.5
2009	0	0.3	0.0
2010	3	2.1	1.4
2011	1	2.1	0.5

Meadow Fork Big Creek

Meadow Fork was surveyed twice in 2011. The survey reach began at the USFS bridge at river mile 0.2 and continued upstream approximately 3.0 miles to a waterfall. Six redds were observed; 2 redds on August 31 and 4 redds on September 28 (Table 9).

Table 9. Redds observed in Meadow Fork Big Creek, tributary to Big Creek, from mid September to mid October.

YEAR	REDDS	MILES	REDDS/MILE
1998	39	3.3	11.8
1999	25	3.3	7.6
2000	51	3.3	14.8
2001	92	3.2	28.9
2002	16	3.2	5.0
2003	0	3.2	0.0
2004	2	3.2	0.6
2005	7	3.2	2.2
2006	16	3.2	5.0
2007	10	3.2	3.1
2008	17	3.0	5.7
2009	8	3.0	2.7
2010	12	3.0	4.0
2011		3.0	

Lake Creek

Both reaches of Lake Creek were surveyed twice in 2011. The lower stream reach began at the 1648 road culvert and ended approximately 2.0 miles upstream to just downstream of the trailhead. The upper stream reach began at the Lake Creek Trailhead and ended 2.2 miles upstream at a waterfall. A gap of approximately 0.2 miles was not surveyed. Sixteen redds were observed in total; 5 redds on August 31 and 11 redds on September 28 (Table 10).

Table 10. Redds observed in Lake Creek, tributary to Upper Malheur River, from mid September to mid October.

YEAR	REDDS	MILES	REDDS/MILE
1998	34	2.1	16.2
1999	21	4.3	4.9
2000	22	4.3	5.1
2001	44	4.2	10.5
2002			
2003	21	4.2	5.0
2004	55	4.2	13.1
2005	51	4.2	12.1
2006	25	4.3	5.8
2007	74	4.2	17.6
2008	65	4.2	15.5
2009	9	2.0	4.5
2010	52	4.4	11.8
2011	16	4.1	3.9

The following streams in the upper Malheur River Watershed were not surveyed in 2011; Summit Creek and Bosonberg Creek.

1.4 Discussion

Survey data can be compared effectively from 1996 to the present. Survey techniques and timing varied from 1992 to 1995 on the North Fork Malheur. During those years project personnel were struggling with uncertainties related to spawn timing and location. Consequently, there was variation in timing of surveys and areas surveyed. In addition, livestock were abundant in some spawning areas during those years, making identification of redds difficult. Since 1996, survey areas and timing have been standardized. Expertise of surveyors has also increased and some are familiar with all survey reaches. A change in livestock management has reduced stream disturbance and made redds more easily identifiable.

Fifty-three redds were observed in the North Fork Malheur watershed in 2011 compared to 55 redds in 2010, a decrease of 4 percent (Appendix C-1, 2). Redd counts in individual streams ranged from 5 to 10, which is the most uniform count to date. Little Crane, Swamp, and Sheep creeks continue to be prime spawning areas for bull trout in this watershed, containing 55 percent of all redds counted. Based on spawn timing comparisons between the North Fork and upper Malheur Rivers, redds observed prior to mid-September in the upper Malheur River have the highest probability of being bull trout redds. In 2011, 7 redds were counted in the upper Malheur watershed prior to mid-September, with 2 of those observed in Meadow Fork. The redd count continues to indicate that the adult spawning population in the upper Malheur River watershed is very low.

In 2011, sufficient personnel participated in the surveys to complete all stream reaches during the scheduled survey period.

Snowpack for water year 2011 was much higher than normal. It is likely that redd counts during 2012 would increase in the North Fork.

No law enforcement issues were reported by surveyors in 2011. Surveyors observed livestock in the exclosure on Little Crane Creek again during 2011.

1.5 Literature Cited

Hemmingsen, A. R., S.L. Gunckel, J.K.Shappart, B.L. Bellerud, D.V. Buchanan, and P.J. Howell. 1997. Bull Trout Life History, Genetics, Habitat Needs, and Limiting Factors in Central and Northeast Oregon. 1997 Annual Report. Report to Bonneville Power Administration, Contract No.00000228, Project No.199505400, 42 electronic pages (BPA Report DOE/BP-00000228-1).

Appendix

Total redds observed and redds per mile in the upper Malheur River and North Fork Malheur Watersheds from Aug-Oct. 1992-2011, Baker and Grant Counties, Oregon.

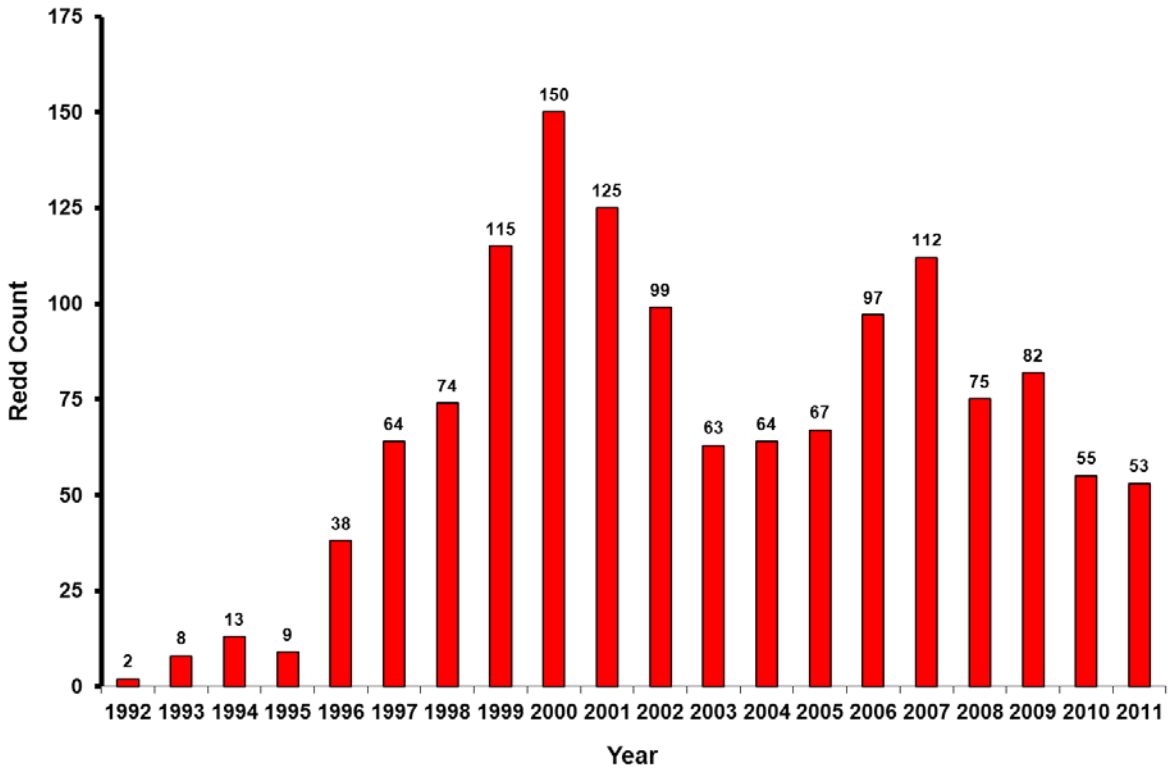

Figure A-1. The number bull trout redds observed in the North Fork Malheur River watershed from 1992-2011.

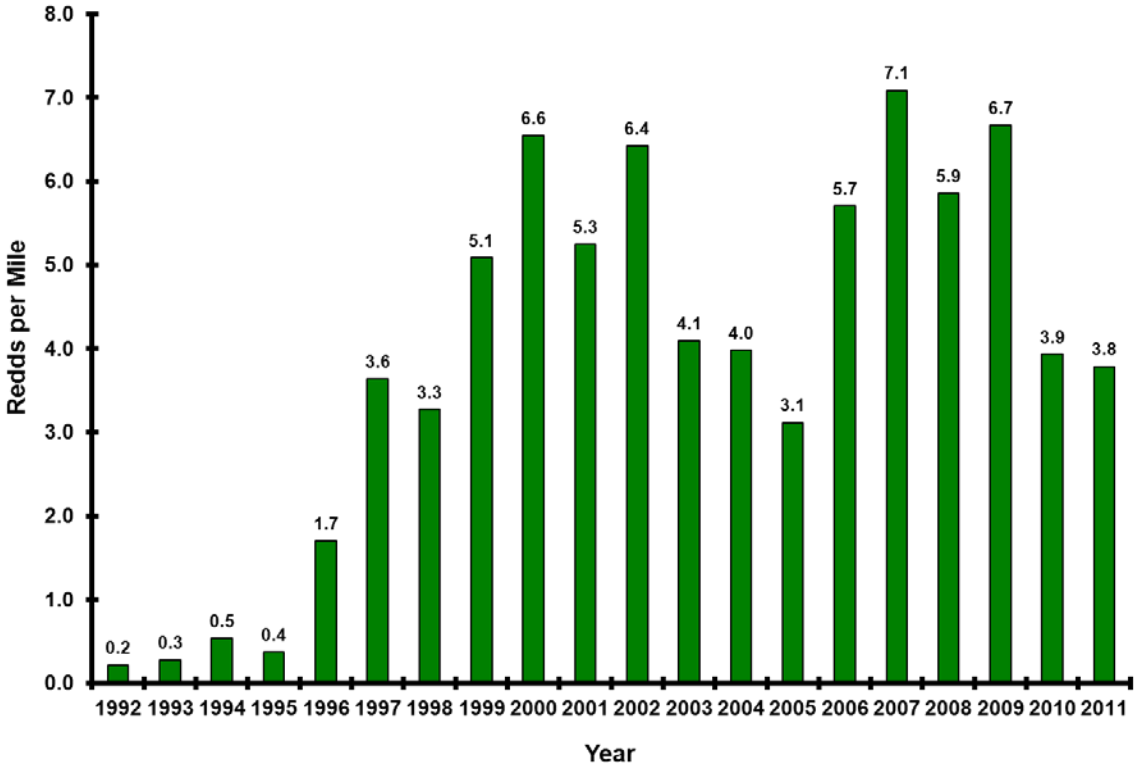

Figure A-2. The number bull trout redds per mile of stream observed in the North Fork Malheur River watershed from 1992-2011.

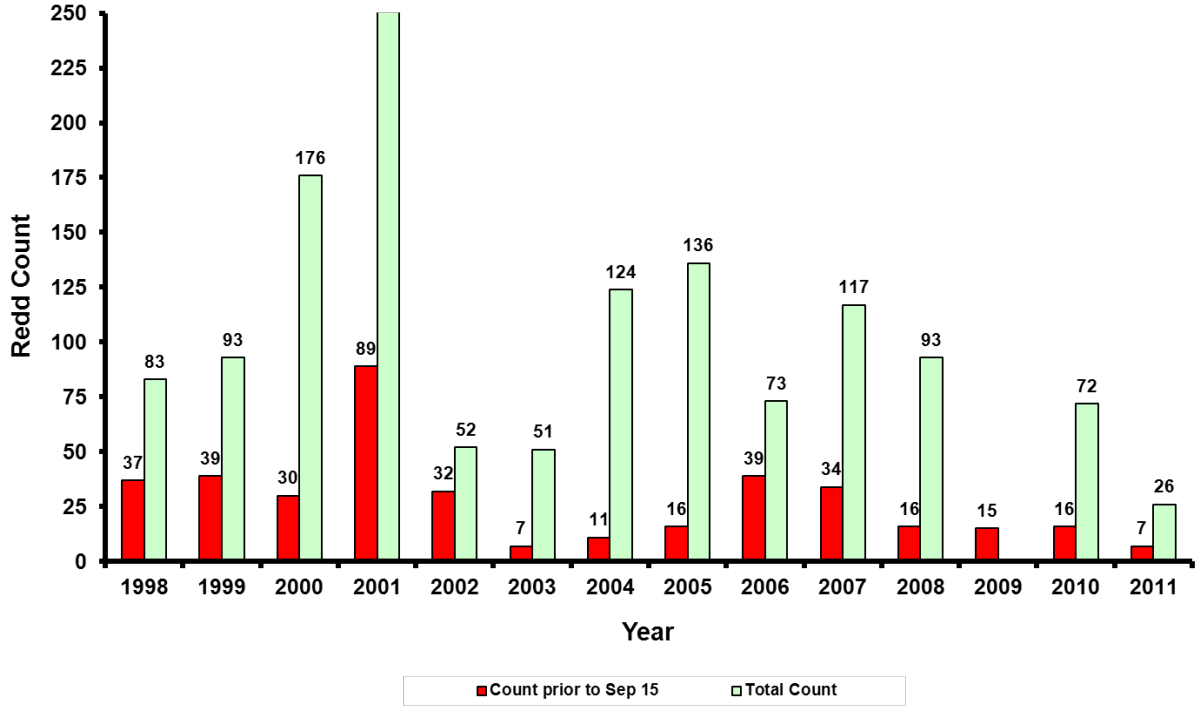

Figure A-3. The number of redds observed in the upper Malheur River watershed, 1998-2011. The red bars represent the number redds counted prior to September 15. The light green bars represent the total number of redds counted.

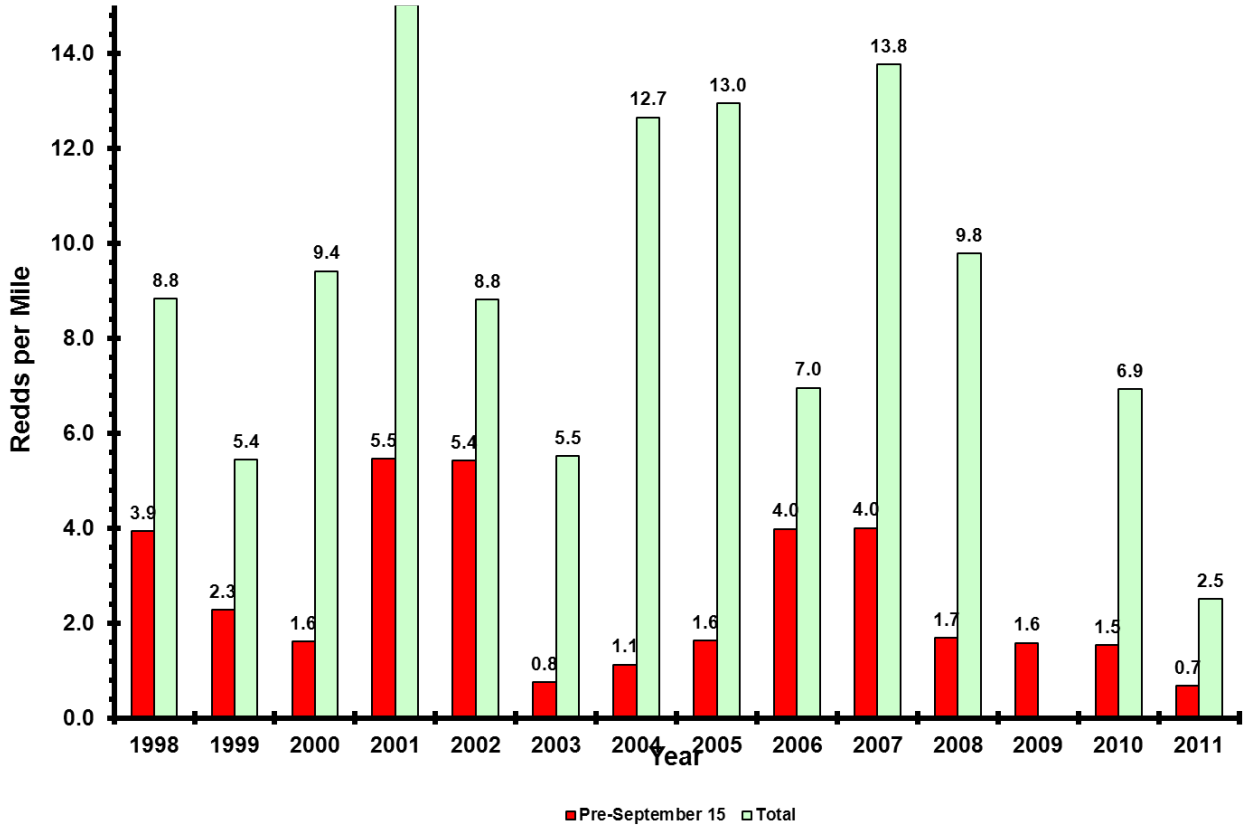

Figure A-4. The number of redds per mile in the upper Malheur River watershed, 1998-2011. Red bars represent the number redds per mile prior to September 15. Light Green bars represent the total number of redds per mile.

Chapter 2

Selective Removal of Brook Trout *Salvelinus fontinalis* in Lake Creek, Upper Malheur River, Oregon

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Contents

Selective Removal of Brook Trout *Salvelinus fontinalis* in Lake Creek, Upper Malheur River, Oregon

Drew Harper, Fish Biologist
Burns Paiute Tribe Department of Natural Resources

2.1 Introduction

Brook trout (*Salvelinus fontinalis*) have been introduced throughout the western United States. Though many of the introductions were originally intended to provide sport fishing opportunities, brook trout have been implicated in declines of native aquatic biota (Adams 1999). Due to the apparent increased dispersal ability in the downstream direction, the stocking of mountain lakes with brook trout can be especially detrimental (Adams 1999; Paul and Post 2001). Though the mechanism(s) through which brook trout affect native species may be variable, resource competition and hybridization are commonly cited factors (Dunham et al. 2002; Gunckel 2001; Ratliff and Howell 1992). In response to the identification of brook trout as a limiting factor to the recovery of ESA-listed bull trout (*S. confluentus*) in the Malheur River basin (USFWS 2002), the Burns Paiute Tribe Natural Resources Department began brook trout suppression efforts in 2010 (Poole and Harper 2011).

Nonnative brook trout exist in high numbers in the Upper Malheur River basin. Brook trout were introduced to the watershed in the 1930s by stocking in High Lake (Bowers et al. 1993), a naturally fishless lake which serves as the headwater source of Lake Creek. The reproductive success of brook trout in High Lake and, subsequently, Lake Creek and other tributaries has led to their dispersal into the majority of Upper Malheur tributaries that offer suitable habitat. This dispersal has resulted in competition between brook trout and native fish species as well as hybridization between brook and ESA-listed bull trout.

The presence of brook trout can pose serious threats to the bull trout population's long term viability because of its ability to outcompete and hybridize with the native. Indeed, resource competition and hybridization between the two species is documented in the Upper Malheur (Gunckel 2001; DeHaan et al. 2009). Brook trout threats, along with other environmental and anthropogenic factors, have imperiled bull trout in the Upper Malheur and led to the population being classified as having a "high risk" of extinction (Buchanan et al. 1997). Recovery Criteria for the Malheur Recovery Unit cite stable or increasing abundance trends in bull trout populations and the reestablishment of connectivity between the separated populations of the North Fork and Upper Malheur populations as actions necessary to achieve delisting (USFWS 2002). It has also been deemed necessary to achieve a reduction or elimination of threats from brook trout interaction in the Upper Malheur prior to restoration of passage (USFWS 2002). Full recovery of Malheur River bull trout is therefore contingent upon minimizing the threats posed by brook trout interactions in the basin.

In 2010, Burns Paiute Tribe (BPT) Natural Resources Department began implementation of a mechanical removal project aimed at eliminating brook trout from High Lake and associated headwater portions of its outlet stream, Lake Creek. High Lake and upper Lake Creek are high elevation sites in the Strawberry Mountains of eastern Oregon. Once naturally devoid of fish, this area now hosts populations of brook trout which may serve as source populations for the Upper Malheur watershed. In 2011, brook trout

suppression efforts were expanded to downstream reaches of Lake Creek, which has been determined to have the highest occurrence of bull trout/brook trout hybrids in the Upper Malheur (DeHaan 2009).

The operation of a seasonal weir and electrofishing removal of brook trout in Lake Creek were additional elements of brook trout suppression efforts in 2011. The objectives for these two methods in lower Lake Creek were to 1) selectively remove brook trout and 2) PIT tag native fishes to assess migration patterns. The following describes the methods and results of these additional brook trout suppression efforts.

Study Area

The study area is located on the southern flank of the Blue Mountains in eastern Oregon. A major headwater tributary to the Upper Malheur River, Lake Creek flows approximately 20 km from its source at High Lake to its confluence with Big Creek, where the two form the Upper Malheur River (Figure 1). Lake Creek Falls is located near river kilometer (RK) 16 and presents a complete barrier to upstream fish passage. Much of Lake Creek upstream of Lake Creek Falls is characterized by channel widths of 1-2 m and moderate gradients (2-5%) with intermittent steep reaches (15-20%) that may pose as barriers to upstream fish passage. Brook trout are the only fish species present above Lake Creek Falls. Below Lake Creek Falls, Lake Creek is characterized by moderate gradients (2-5%) and channel widths of 2-5 m. Summer stream temperature regimes in Lake Creek appear suitable for bull trout from Lake Creek Falls down to the weir location (BPT, unpublished data) however, between the weir and Forest Rd. 16 temperatures increase dramatically and often exceed bull trout thermal tolerances (Abel 2010). Fish species present in Lake Creek below Lake Creek Falls include brook trout, bull trout, brook trout/bull trout hybrids, redband trout (*Oncorhynchus mykiss gairdneri*), and sculpin (*Cottus sp.*).

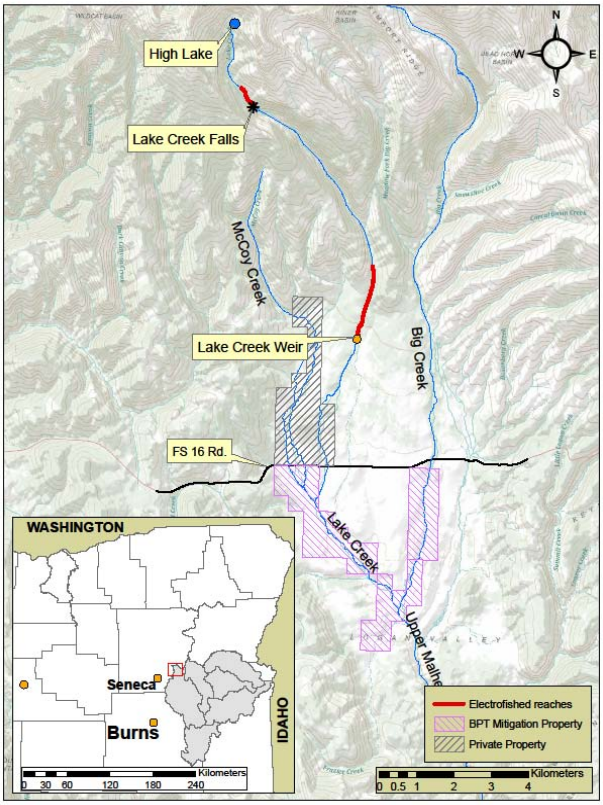

Figure 1. Map showing weir location, electrofished reaches, and property boundaries.

2.2 Methods

Weir

A rigid, picket-style weir with 1.4 cm spacing and fixed with upstream and downstream trap boxes was used to capture fish attempting to pass the weir's location. The weir was checked for fish at least once daily and cleared of debris as necessary. Captured fish were identified to species (except sculpins), measured for fork length (FL; mm), and weighed (g). The date of capture and trap location (up or downstream) were also recorded. Native species and hybrids were released in the direction of which they were trapped; brook trout were euthanized. Pectoral fin clips were collected from brook trout for

possible use in determining age. Bull trout and hybrids larger than 60 mm were anesthetized in 80 mg/L MS-222 (Finquel®) and implanted intraperitoneally with a 12 mm x 2.15 mm HDX PIT tag (Texas Instruments®). Tagged fish were then allowed to recover in freshwater before being released.

Electrofishing

A Smith-Root® model 12B backpack electrofisher was used to conduct single-pass removal and multiple-pass depletion estimates for brook trout in Lake Creek. The effort to remove brook trout via electrofishing began at the weir and proceeded upstream in 50 m increments. Locations for segment start and endpoints were recorded using a hand-held GPS unit. Single-pass sites consisted of a single upstream sweep with netters collecting as many fish as possible. Multiple-pass depletion estimates consisted of two or four passes, with an upstream and downstream sweep constituting one pass. If a 50% reduction in brook trout was obtained between pass one and two, the site was complete. If not, a second two passes were conducted. Depletion estimates were to be completed every 500 m in order to assess the effectiveness of the single-pass removal method. This was done by keeping fish captured in the first upstream sweep of a multiple-pass site separate from the downstream sweep so that the number of brook trout captured in the upstream sweep could be compared to the abundance estimate for that site. All fish captured were identified to species (except sculpins) and measured for fork length (mm). Native species were released in the section of their capture; brook trout were euthanized. Bull trout and hybrids were implanted with a PIT tag as described above prior to release.

Data Analysis

Data from the brook trout suppression efforts were analyzed for use in several applications: 1) to begin a baseline dataset to be used in comparisons with subsequent efforts, 2) to assess the efficacy of the single-pass removal method, and 3) to increase our understanding of brook trout population characteristics and dynamics in Lake Creek. For the baseline dataset, estimates of density were calculated for each salmonid species using the total number of a species captured divided by the total length of stream electrofished. Due to imperfect capture probabilities associated with our methodology, these estimates are considered to represent minimum densities. To assess the efficacy of the single-pass removal method, brook trout abundance was calculated using methods described by Seber and Le Cren (1967) for multiple-pass sites. The abundance estimate for a given multiple-pass site was then compared to the number of brook trout captured in the first upstream sweep at that site, resulting in a proportion of the total abundance captured in the first upstream sweep. To enhance our understanding of brook trout population dynamics in Lake Creek, weir data was assessed by month and direction of capture. Additionally, length-frequency histograms were constructed for brook trout, bull trout, and redband trout from data collected at the weir and through electrofishing.

2.3 Results

Weir

In 2011, the temporary weir on Lake Creek was installed on 24 May and removed for the winter season on 4 November. The weir was operational (i.e., trapping) for 149 days out of the 165 days between the aforementioned dates. The weir was modified to allow water and, consequently, fish passage due to ice build-up for one day (May 26) and because of a lack of personnel for one day (June 10). Furthermore, the weir was removed due to high flows and associated safety concerns from 20 June through 4 July. The weir operated continuously from 5 July through 4 November.

Five species of fish were captured over the 149 days the weir was operational, including brook trout (N=322), redband trout (N=16), bull trout (N=13), brook trout/bull trout hybrids (N=9), and sculpin (N=5). The majority of fishes were captured in the downstream trap; bull trout were the only species for which upstream captures outnumbered downstream captures (Table 1). The timing and directionality of peaks in captures (separated by month) varied among species; specifically, the peak of redband trout captures occurred in June in the downstream trap, bull trout captures peaked in July in the upstream trap, and peak brook trout captures occurred in October in the downstream trap (Figure 2).

Table 1. Upstream (US) and downstream (DS) weir captures by species.

Species	US	DS
Brook Trout	17	305
Bull Trout	9	4
Hybrid	2	7
Redband	0	16
Sculpin	0	5

Length-frequency histograms created from weir capture data reveal two potentially important insights into population characteristics in Lake Creek. For instance, brook trout as small as 124 mm were recognized to be sexually mature, indicating that the majority of brook trout captured at the weir were likely capable of reproducing. The second interesting suggestion from the length-frequency histograms was that the size classes of upstream and downstream migrating bull trout were separated by the fork length previously determined to separate mature from immature fish in the Malheur (226 mm; Schwabe 2006), with the smaller immature fish moving downstream. Length-frequency histograms from the weir captures are included in Appendix A.

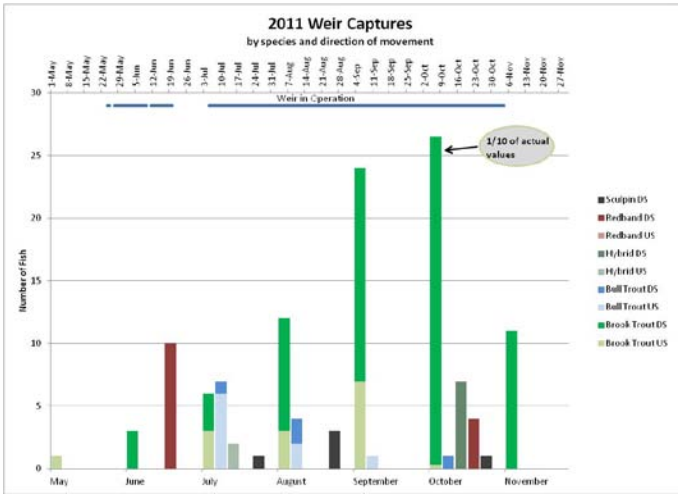

Figure 2. Monthly weir captures by species and direction of movement. Blue bar at top indicates periods of weir operation. Note that the October brook trout captures have been reduced by a factor of 10. US=upstream migrants; DS=downstream migrants.

The sample size of fish at the weir which were implanted with PIT tags was small. A delay in the release of the tags by the manufacturer resulted in a short tagging period. The tagging effort did not begin until 28 July, at which point the majority of bull trout and redband trout captures had already occurred. In all, eight tags were implanted into fish at the weir, including three in bull trout and five in brook trout/bull trout hybrids.

Electrofishing

Selective removal of brook trout via electrofishing in Lake Creek was conducted on eight days between 7 July and 18 August. Electrofishing in the reaches below Lake Creek Falls ended on 10 August in order to not disturb spawning bull trout. The effort included the sampling of a total of 3,193 m of Lake Creek (2,522 m below Lake Creek Falls, 671 m above). Four species of fish were captured below the falls; brook trout (N=221), bull trout (N=3), redband trout (N=51), and sculpin (N=324). All bull trout captured by electrofishing were implanted with PIT tags. Brook trout (N=45) were the only species captured above Lake Creek Falls. All 266 brook trout captured by electrofishing were euthanized and removed. Figure 3 shows the calculated densities of salmonid species in Lake Creek. Length-frequency histograms from electrofishing data are included in Appendix B.

Depletion methodology was completed at four sites and abundance estimates were subsequently calculated for those sites in order to estimate the efficacy of the single upstream sweep at capturing brook trout for removal. Though it was planned that depletion methodology would be conducted every 500 m, resources were limited and the desire to sample more stream with the intent of brook trout removal took precedence. At each of the four depletion sites, the first upstream sweep captured only 0-39% (mean=23%) of the total brook trout abundance estimate. However, if the first upstream and downstream sweep were combined the proportion of the total population captured at a site increased to 40-100% (mean=73%). These results would seem to indicate a substantial benefit by the addition of a downstream sweep to the current single upstream sweep methodology.

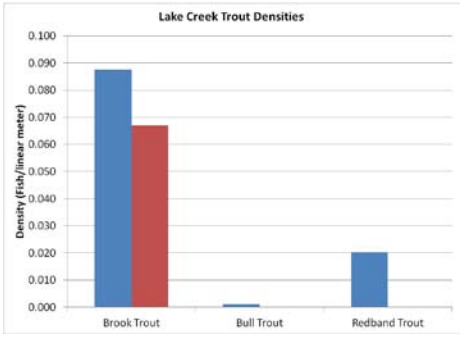

Figure 3. Respective densities of salmonid species in sampled reaches of Lake Creek. Blue bars indicate density below Lake Creek Falls; Red bar indicates density above Lake Creek Falls.

2.4 Discussion

Nonnative brook trout exist in high numbers in the Upper Malheur River basin. After having been introduced in the 1930s, brook trout appear to have dispersed to nearly all suitable habitat within the watershed. This dispersal has resulted in competition between brook trout and native fishes (Gunckel 2001) as well as hybridization between brook and ESA-listed bull trout (DeHaan 2009). In 2011, BPT brook trout suppression efforts were expanded to target downstream reaches of Lake Creek, which has been determined to have the highest occurrence of bull trout/brook trout hybrids in the Upper Malheur (DeHaan 2009). These suppression efforts included the operation of a seasonal weir and electrofishing. Though these methods were somewhat experimental in terms of implementation, suppression activities in Lake Creek permitted the capture and removal of brook trout, the establishment of some baseline data for comparison with future efforts, and provided a few insights into the characteristics and dynamics of fish populations there.

Weir

Operation of a seasonal weir on Lake Creek was useful in terms of capturing and removing brook trout, as well as highlighting a potential aspect of brook trout population dynamics that may be exploitable in terms of improving our efficiency. First, 322 brook trout were captured and removed via weir operation. Interestingly, of the 322 brook trout captured at the weir, 305 were captured attempting to move downstream. Furthermore, the vast majority (N=262) of those downstream migrating brook trout were captured in the span of one month (October). The cause(s) of such an apparent 'mass exodus' remains unclear at this time, though several hypotheses have been suggested:

1) A large number of brook trout moved upstream during June when the weir was not operational due to high flows. The downstream migration may then be an attempt to return to winter habitats.

2) Search for spawning habitat. This explanation seems to have some merit as many of the brook trout captured moving downstream in October were observed to be ripe for spawning. However, suitable spawning habitat is known to exist upstream of the weir location.

3) The density of brook trout upstream of the weir is driving the downstream migration. While this is possible, it is problematic. Considering that the electrofishing removal effort started at the weir,

reaches of Lake Creek directly above the weir should have had reduced densities in comparison to reaches that had not been sampled. This is not to imply that we removed every brook trout from the sampled area; certainly we did not. However, unless brook trout were, throughout the summer, filling the vacancies created by removal efforts (i.e., moving downstream), density should not have been a factor near the weir in the fall. Likewise, if density was the force persuading brook trout to move downstream we would not expect such an 'en masse' movement, but rather more of a 'trickle down' pattern.

Though the brook trout catch data from the weir provides plenty of food for thought, the data are from a single trapping season. Therefore, it is deemed prudent to operate the weir in subsequent years to determine whether the downstream migration is repeated. An effort to trap continuously through spring runoff should be made each year in an attempt to determine whether the simplest hypothesis (i.e., hypothesis 1 above; fish moved upstream when the weir was inoperable due to high water) should be rejected or not. If enough data can be collected to confidently reject that hypothesis, alternative hypotheses can be further explored. Whether the downstream migration represents a simple seasonal movement or complex density-dependant dispersal, if the pattern persists in subsequent years it could prove useful in planning future brook trout suppression efforts.

The weir data also raise questions about assumptions concerning our understanding of the native salmonid populations in Lake Creek. Not much is known about the population of bull trout in Lake Creek. The majority of past studies targeting bull trout were telemetry studies in which fish were captured in the Upper Malheur River. The majority of tagged fish were tracked into Big Creek, with only a few moving into Lake Creek (Fenton and Schwabe 2002; Fenton 2005). Those bull trout that did move into Lake Creek either turned around and subsequently moved into Big Creek (Fenton and Schwabe 2002) or stayed in lower Lake Creek (below the FS 16 Rd; see Figure 1) and were tracked to roughly the same location for five months (Fenton 2005). Lower Lake Creek is thought to pose a thermal barrier to bull trout migration due to water management practices on private property (Abel 2010). Therefore, the bull trout population in Lake Creek is thought to be largely, if not exclusively, comprised of resident fish. Whether the bull trout captured moving upstream at the weir were part of that resident population which overwintered in reaches of Lake Creek below the weir or represented a fluvial component of the Lake Creek bull trout population is unknown. Installation of a PIT tag detection array at the confluence of Big and Lake Creeks is planned for the spring of 2012 and could provide information to answer questions about connectivity between Big Creek and Lake Creek bull trout populations.

Operation of a seasonal weir on Lake Creek was useful in terms of capturing and removing brook trout as well as improving our understanding of the dynamics of salmonid populations there. However, the results of weir operation also raised a few questions that cannot be definitively answered at this time. One source of this uncertainty stems from the issue of inoperability during high flows. The Malheur River basin experienced very high snow pack in the winter of 2010-2011, resulting in a sustained period of high flows. During such flows, the weir could not be operated safely and was therefore removed for a period of time in which fish may have freely moved past the weir location. Seasonal operation of the weir in future years will continue to provide a point of capture that will allow selective removal of brook trout as well as to potentially provide important insights to improve our understanding of salmonid populations in Lake Creek and the Upper Malheur River.

Electrofishing

Electrofishing was conducted in Lake Creek in order to selectively remove brook trout, test the efficacy of the methodology (single upstream sweep) employed to reach that end, and establish some baseline

data for comparison with future efforts. Though we were able to successfully remove 266 brook trout via electrofishing, the first upstream sweep at calibration sites (depletion methodology) captured only 0-39% (mean=23%) of the total brook trout abundance estimate. However, if the first upstream and downstream sweeps were combined, the proportion of the total abundance captured at a site increased to 40-100% (mean=73%). Despite the fact that abundance estimates calculated from depletion methodology are biased and are more representative of a minimum estimate (White et al. 1982), the increase in efficiency by adding the downstream sweep to the single pass removal is likely real. Therefore, the current methodology should be modified to include a downstream sweep.

The most useful baseline data gathered is the relative densities of salmonid species in Lake Creek. Brook trout were 74 and 4 times more densely populated than bull trout and redband trout, respectively, in the reaches sampled within the native species' range. Again, though bias associated with the electrofishing methodology likely impacts these numbers, data from the weir as well as observations by field personnel support the notion that brook trout are much more abundant than native salmonids in Lake Creek.

A total of 588 brook trout were removed from Lake Creek through the combined efforts of weir operation and electrofishing. Unfortunately, it is not possible at this time to estimate what proportion of the Lake Creek brook trout population that number represents. Considering the relatively small portion of Lake Creek sampled (see Figure 1), it is likely that 588 brook trout represents a small portion of the total Lake Creek population. As suppression efforts move forward, a rigorous estimate of brook trout abundance in the targeted area will be vital to understanding the efficacy of such efforts. As of this writing, such an estimate is planned for the 2012 field season.

2.5 Acknowledgements

Special thanks to Bonneville Power Administration for funding this project.

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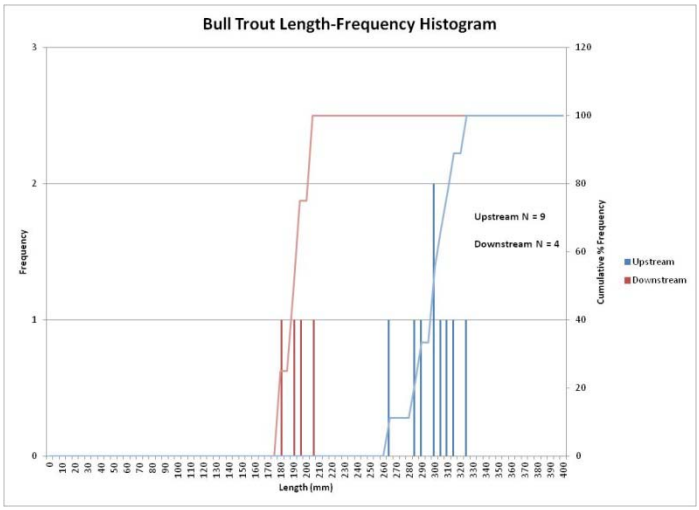
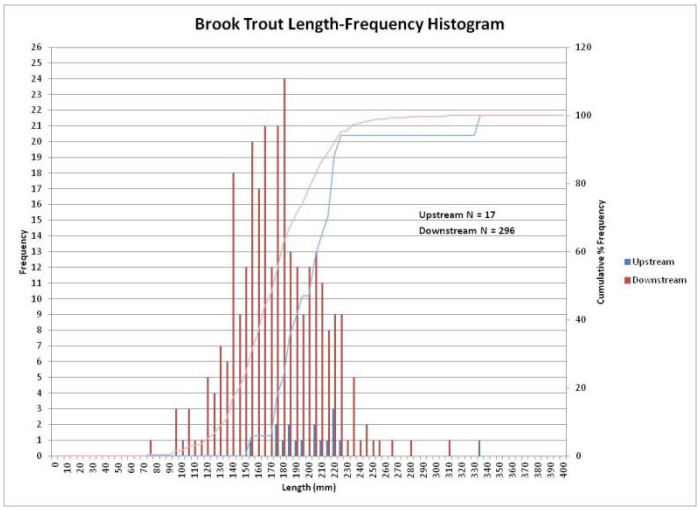
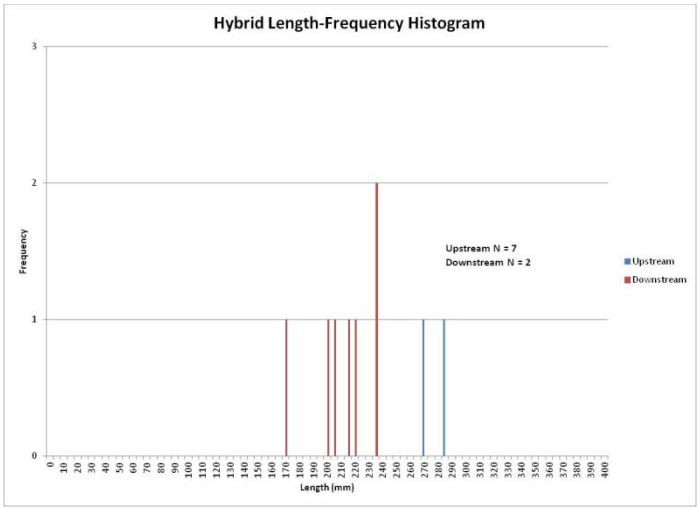
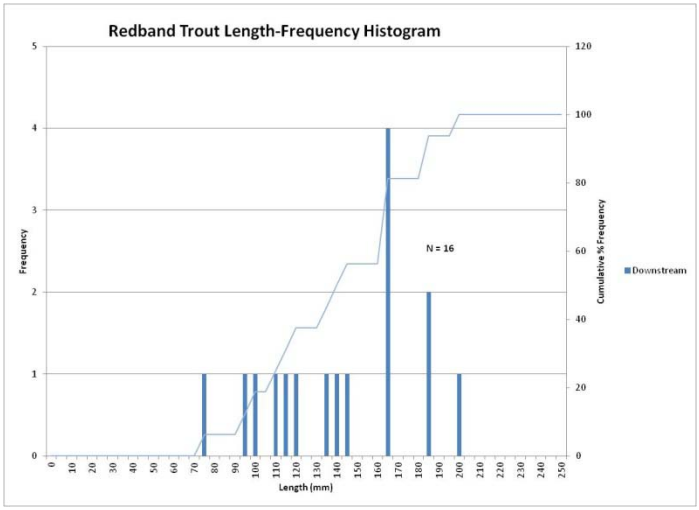
Appendix A. Length-Frequency histograms from the Lake Creek Weir (note differing scales on axes)

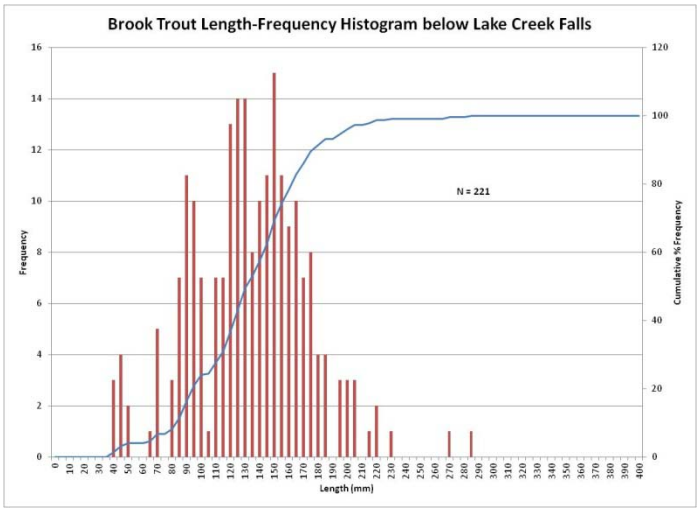


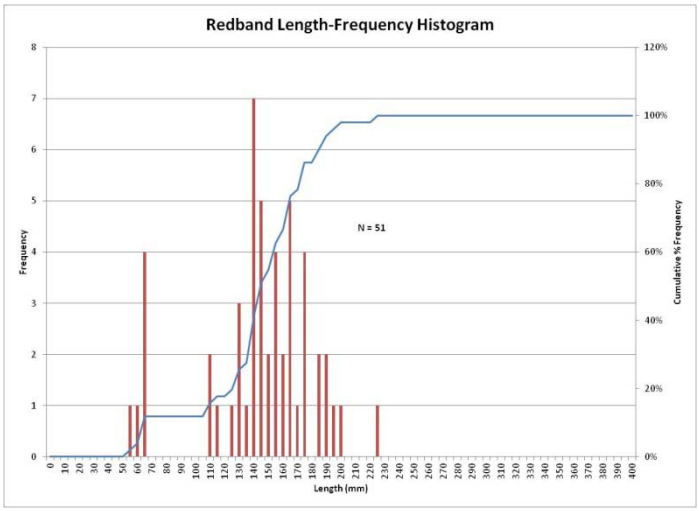


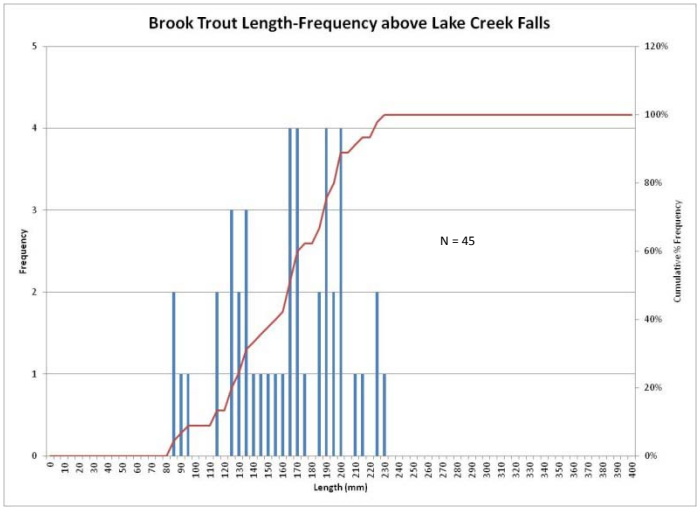




Appendix B. Length-Frequency histograms from Lake Creek electrofishing captures (note differing scales on axes)







Chapter 3

2011 Efforts to Trap and Haul Bull Trout *Salvelinus confluentus* Entrained Over Agency Valley Dam on the North Fork Malheur River, Oregon

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Contents

2011 Efforts to Trap and Haul Bull Trout *Salvelinus confluentus* Entrained Over Agency Valley Dam on the North Fork Malheur River, Oregon

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Abstract
Bull trout *Salvelinus confluentus* collection in the tailrace of Agency Valley Dam on the North Fork Malheur River was conducted by angling on 13 days between May 19, 2011 and June 16, 2011. All bull trout captured were transported upstream 3 miles where the North Fork Malheur River flows into the head of Beulah Reservoir. A total angling effort of 144.5 hours was expended yielding 5 bull trout. Other species angled in the tailrace include rainbow trout *Oncorhynchus mykiss*, large scale sucker *Catostomus macrocheilus*, mountain whitefish *Prosopium williamsoni*, and northern pike minnow *Ptychocheilus oregonensis*.

3.1 Introduction
Bull trout were listed as a threatened species under the Endangered Species Act in 1999 by the U.S. Fish and Wildlife Service. Bull trout in the Malheur River system have declined as a result of historical and current land use activities, the construction of dams, and fish eradication projects by poisoning (Bowers et al. 1993). One of the negative effects of dams on bull trout is entrainment. Bureau of Reclamation (BOR), Oregon Department of Fish and Wildlife (ODFW), and the Burns Paiute Tribe Natural Resources Department (BPT) have determined that entrainment of bull trout occurs over the Agency Valley Dam spillway on the North Fork Malheur River (Schwabe et al. 2001, 2007). Changes made in the 2000 irrigation season resulted in the release of water through the flow valves rather than over the spillway in an effort to reduce the number of entrained bull trout. Starting in 2001 and continuing until 2005, BPT did not angle any bull trout below the spillway. However, water levels were high in 2006 and resulted in water being released over the spillway. BPT continued the effort to monitor bull trout below the reservoir and collected 7 bull trout in the pool below the spillway in 2006 (Schwabe et al. 2007). Bull trout that are entrained below Agency Valley Dam are lost to the breeding population, and it is assumed entrained bull trout eventually perish due to poor habitat conditions below the reservoir. Therefore an effort must be made to return any entrained bull trout to the reservoir. The objective of this project is to reduce bull trout losses due to entrainment at Agency Valley Dam by angling in the tailrace and returning these fish to the North Fork Malheur River upstream of the dam.

3.2 Methods
BPT personnel angled below the reservoir in the spillway for an average of 28.9 hours each week from May 19 to June 16, 2011. Methods of angling included flies, artificial lures and bait. Tribal employees conformed to all state regulations on fishing. Data collection included total hours angled and total number and fork length of each fish species caught. Additional data was collected for bull trout including time caught, total length, weight, and presence/absence of Floy© tags related to ongoing BOR research in Beulah Reservoir. Non-target species of fish were

recorded and returned to the spillway pool. Upon capture, bull trout were transported in a five gallon bucket equipped with an aerator to a location on the North Fork Malheur River above Beulah Reservoir (UTM NAD83 Zone: 11 E 404488, N 4869510) and released.

3.3 Results

Angling began on May 19, 2011 and continued for an average of 28.9 hours a week until June 16, 2011. A total of 144.5 angling hours were recorded by BPT resulting in the collection of five bull trout in the tailrace. Fish species collected during the 2011 angling efforts include: rainbow trout *Oncorhynchus mykiss*, bull trout *Salvelinus confluentus*, large scale sucker *Catostomus macrocheilus*, mountain whitefish *Prosopium williamsoni*, and northern pike minnow *Ptychocheilus oregonensis* (Table 1). Fork lengths of fish were recorded and ranged from 168-520 mm for rainbow trout (AVG=357.8), 280-344 mm for bull trout (AVG=311.2), 300-525 mm for large scale sucker (AVG=405.7), 297-422 mm for mountain whitefish (AVG=340.3), and 200-326 mm for northern pike minnow (AVG=263) (Table 1). A summary of the daily fish angling effort is provided in Appendix A.

Table 1 Number and size of species angled in the tailrace of Agency Valley Dam in 2011

Species	150 mm+	200 mm+	250 mm+	300 mm+	350 mm+	400 mm+
Rainbow Trout *O. mykiss*	1	8	10	12	20	29
Bull Trout *S. confluentus*	-	-	1	4	-	-
Large Scale Sucker *C. macrocheilus*	-	-	2	13	16	-
Mountain Whitefish *P. williamsoni*	-	-	1	4	-	1
Northern Pike Minnow *P. oregonensis*	-	1	-	1	-	-

3.4 Discussion

High water yield for the water year 2011 necessitated the spilling of water from Beulah Reservoir over the spillway of Agency Valley Dam. Since the end of March 2011, Beulah Reservoir has been at or near 100% of the reservoir storage capacity which is 59,212 acre feet (http://www.usbr.gov, 2011). Releases remained high below the reservoir for the first two weeks of angling effort. The tailrace experienced flows between 650-1100cfs (http://waterdata.usgs.gov, 2011) on the first two days of angling, most likely attributing to the absence of fish capture in the 16 hours of effort. Catch rates greatly improved in the month of June as releases fluctuated from 400-650cfs (http://www.waterdata.usgs.gov, 2011). The Tribe's 2011 angling effort yielded five bull trout in the tailrace of Beulah Reservoir resulting in a catch rate of 0.03 bull trout per hour of angling effort. At least one captured bull trout had been Floy© tagged in the reservoir by BOR. One more individual appeared to have been Floy© tagged due to a circular puncture wound in the dorsal musculature, but had subsequently lost its tag (Figure 1). Scale samples were collected from three bull trout and one anal fin clip was obtained. Two bull trout were released without scale samples or fin clips taken due to stress related concerns. Bull trout were released upstream of the previous years release site due to restricted access from the full pool level of Beulah Reservoir. Fish were released at an accessible point on the North Fork Malheur River above private land holdings (Figure 2).

Figure 1. Bull trout with possible missing Floy© tag

Figure 2. Map of North Fork Malheur River and Bull Trout Release Site

Through 1999, release of water through the spillway was the predominant method of water release for downstream irrigation use at Agency Valley Dam. In 1999 anglers collected a total of

20 bull trout in the tailrace equating to one bull trout collected for every 20 angling hours (Table 2) (Schwabe et al. 2001). Since 2000, water releases have been primarily through the tubes at the base of Agency Valley Dam rather than over the spillway. Despite operational changes, BPT collected five bull trout from the tailrace in 2000 (Table 3) (Schwabe et al. 2001). Bull trout presence in the tailrace of Beulah Reservoir was also documented in 2006 (Table 3) when water practices were modified to spill water over the spillway due to a high water year which led to bull trout entrainment (Schwabe et al. 2007). Much like 2006, the high water year of 2011 required water releases over the spillway of Agency Valley Dam resulting in bull trout entrainment. It appears that modified water release operations during low water years significantly reduce but do not eliminate bull trout entrainment. However, high water years that require the release of water over the spillway provide a greater opportunity for the entrainment of bull trout.

Table 2 Bull trout and rainbow trout catch below Agency Valley Dam from 1999 to 2011

	# Fish Caught Spring		#Fish Caught Fall	
	Bull	Rainbow	Bull	Rainbow
1999†	20	150	NA*	NA*
2000	5	107	0	4
2001	0	13	0	34
2002	0	73	0	36
2003	0	7	NA*	NA*
2004	0	49	NA*	NA*
2005	0	6	NA*	NA*
2006†	7	106	NA*	NA*
2008	0	140	NA*	NA*
2011†	5	80	-	-
	Catch Rate (#/hour) Spring		Catch Rate (#/hour) Fall	
	Bull	Rainbow	Bull	Rainbow
1999†	0.05	0.34	NA*	NA*
2000	0.01	0.21	0.00	0.02
2001	0.00	0.08	0.00	0.59
2002	0.00	0.44	0.00	0.43
2003	0.00	0.35	NA*	NA*
2004	0.00	0.48	NA*	NA*
2005	0.00	0.08	NA*	NA*
2006†	0.04	0.67	NA*	NA*
2008	0.00	0.64	NA*	NA*
2011†	0.03	0.55	-	-

† Water released over spillway

* No creel in fall

3.5 Acknowledgements

A special thanks to the United States Bureau of Reclamation.

3.6 References

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Appendix A. Summary of daily angling effort below Agency Valley Dam in 2011

Date	Species	# Fish Captured	Hours Angled
5-19	-	0	9.75
5-20	-	0	6.25
6-2	Rainbow Trout	11	8
6-3	Rainbow Trout	13	9
	Bull Trout	1	
6-4	Rainbow Trout	8	17
	Bull Trout	3	
	Large Scale Sucker	5	
6-6	Rainbow Trout	5	9
	Large Scale Sucker	6	
6-7	Rainbow Trout	10	19
	Large Scale Sucker	9	
	Mountain Whitefish	2	
6-8	Rainbow Trout	5	9
	Bull Trout	1	
6-9	Rainbow Trout	3	12
	Mountain Whitefish	1	
6-12	Rainbow Trout	5	4.5
	Large Scale Sucker	1	
6-13	Rainbow Trout	8	13.5
	Large Scale Sucker	7	
	Mountain Whitefish	3	
6-15	Rainbow Trout	6	5
6-16	Rainbow Trout	6	22.5
	Large Scale Sucker	3	
	Northern Pike Minnow	2	
Total	**Rainbow Trout**	**80**	**144.5**
	Bull Trout	**5**	
	Large Scale Sucker	**31**	
	Mountain Whitefish	**6**	
	Northern Pike Minnow	**2**	

Chapter 4

Distribution and Abundance of Redband Trout *Oncorhynchus mykiss* in the Malheur River Basin, 2011

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Contents

Distribution and Abundance of Redband Trout *Oncorhynchus mykiss* in the Malheur River Basin

2011 Report of Continued Field Research

Derek Hawley, Fisheries Tech
Burns Paiute Tribe Natural Resources Department

4.1 Introduction

The Malheur River Basin supports resident populations of native redband trout *Oncorhynchus mykiss*. Though resident populations existed in the basin prior to large scale human activity, all of the basin's redband were forced to adopt a resident life history after dam construction projects in the twentieth century severed connectivity to marine environments. In addition to native populations of redband trout, recreational fisheries were historically supported by stocking of hatchery rainbow trout fingerlings (Hanson et al. 1990). Stocking occurred primarily in reaches of the mainstem and North Fork Malheur River.

Prior to 2007, surveys had been conducted to assess relative abundance and distribution of native trout in the Malheur Basin (Pribyl and Hosford 1985, Fenton et al. 2005), but no systematic, basin-wide survey of redband distribution and abundance had transpired before Oregon Department of Fish and Wildlife (ODFW) and Burns Paiute Tribe (BPT) undertook this study. Data collected from electrofishing in 2007 established a population estimate of 156,200 redband in the basin ±SE of 23,273 (Relative CI 29%) (Bangs et al. 2007).

While the 2007 study provided a basin-wide population estimate, it is exceedingly difficult to draw conclusions about the carrying capacity of the system or management goals from a one-year study that doesn't take into account year to year environmental variables. In fact, 2007 was an extremely low water year in the Malheur River Basin, which likely limited available redband trout habitat relative to years with higher stream flow (Bangs et al. 2007). BPT is repeating the study design over a five year cyclic schedule on public and tribal sites visited in 2007. The goal of this project is to expand the dataset that the 2007 study established, enabling BPT to better understand the Malheur redband population and potentially its response to resource management strategies. The exclusion of sites located on private holdings reduces the number of sites to be revisited to 108. This translates into a workload of 21-23 sites per year. Figure 1 maps survey sites visited in 2011 with associated redband densities recorded at each site.

4.2 Study Area

The Malheur River Basin is located in eastern Oregon. A detailed description of the basin including geographic location, river kilometers, elevation, climate, ownership, and native fishes can be found in the 2007 report produced by ODFW (Bangs et al. 2007). Briefly, 120 primary sampling sites and 80 oversample sites were randomly established in 2007 using the Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP). Roughly one-third of the basin is privately held and sites occurring in these areas have been excluded from further sampling. The remaining 108 sites are on state, federal or tribal lands.

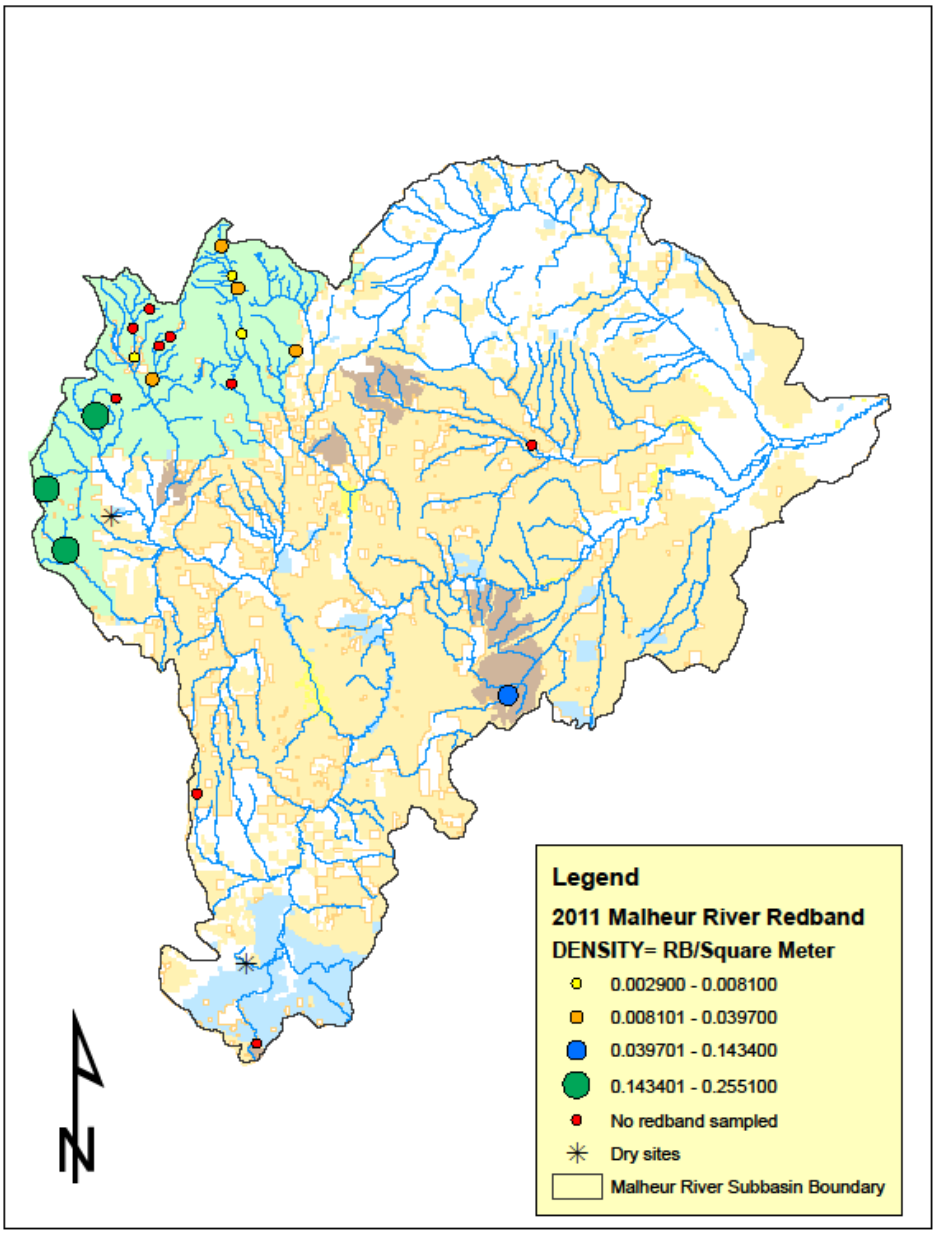

Figure 1. Redband densities (fish/m^2) associated with 2011 monitoring sites.

4.3 Methods

Methods in the field were derived from the *Redband Trout, Warner Sucker and Goose Lake Fishes Distribution and Abundance Survey Protocols* (Scheerer et al. 2007). Bangs et al. (2007) also summarizes sampling methods in good detail. The mark-recapture calibration methods used to estimate abundance and evaluate the accuracy of removal estimates is the only aspect of the 2007 effort to be excluded from the current sampling protocol. Two-pass depletion-removal estimates were conducted using backpack electro-shockers. Basic habitat data relating to stream dimensions, substrate composition, backwater and undercut bank measurements, and aquatic vegetation was collected as in 2007.

4.4 Results

Redband trout were observed at 11 of the 22 sites completed (*N*=138). Of the remaining 11 sites visited, 2 were dry and 9 sites had water but no redband present (Appendix A). Density estimates were calculated for each site that contained redband trout with fork lengths greater than 60 mm. Of the 11 sites where redband trout were present in the 2011 project year, all 11 sites yielded redband >60 mm. Densities ranged from 0.0055 fish/m^2 to 0.2551 fish/m^2 (Appendix A).

Number of redband at the 11 sites ranged from 1 individual at MalRiv0138 North Fork Malheur River to 45 individuals at MalRiv0127 Cottonwood Creek (Appendix A). Mean redband density at the 11 sites was 0.0907 fish/m^2, with a mean number of 13 redband per site.

Total biomass of redband at the 11 sites was calculated using the following length-weight regression in Excel:

Weight (g) = 10^ (2.9916* Log10 (Length (mm)-4.9055)

Redband biomass ranged from 11.29 grams to 822.78 grams at the 11 sites with redband (Appendix A). In reference to surface area at the sample sites, redband biomass ranged from 0.05 grams/m^2 to 5.3 grams/m^2 (Appendix A).

Fork lengths were measured on all redband trout captured. Fork lengths for all non-target species were collected for the first 20 individuals per site, with remaining fish tallied. Redband trout ranged in length from 60 mm to 290 mm, with a peak length frequency of 145mm (*N*=13; Figure 2). The 2007 report surmised that the peak in length frequency that year between 80 and 120 mm likely represented a mixture of age-1 and age-2 fish (Bangs et al. 2007). 8% (*N*=11) of redband encountered in the 2011 field season consisted of fish large enough for legal harvest in Oregon (≥200 mm).

4.5 Discussion

New redband population estimates in the Malheur River Basin were not calculated from the data collected in 2011. With only 11 confirmed redband sites of 22 sites visited in 2011, there is not enough data available to substantially change the population estimate of 156,200 redband in the basin ±SE of 23,273 from 2007. BPT plans to recalculate the population estimate after the 5 year cyclic site schedule is concluded in 2012. BPT will continue to report its annual findings from ongoing fieldwork throughout that timeframe.

4.6 Acknowledgements

BPT wishes to thank Bonneville Power Administration for their continued support of this research project.

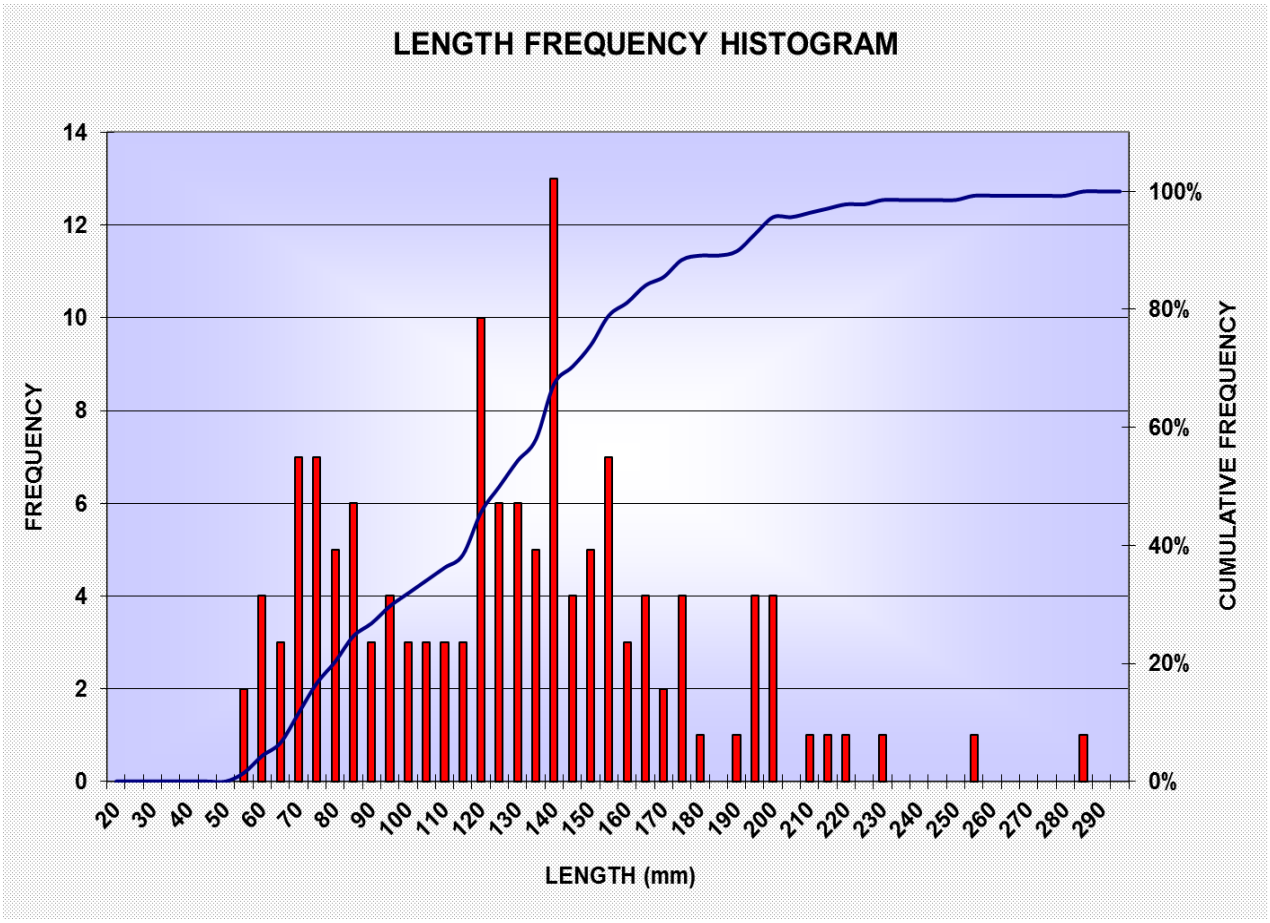

Figure 2. Length frequency histogram (5mm intervals) of redband trout captured by electrofishing in the Malheur River Basin, 2011 (*N*=138).

4.7 References

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SUMMARY TABLE OF SURVEY SITES
2011 REDBAND STUDY

SITE ID	STREAM	STATUS	LENGTH	MEAN WIDTH	AREA	# REDBAND	DENSITY	RB BIOMASS	BIOMASS (AREA)	TOTAL FISH	OTHER SPECIES
			meters	meters	meters²	*N*	fish/m²	grams	grams/m²	*N*	
MalRiv0118	Cottonwood Cr.	complete	31	1.42	44.02	0	0.0000	0	0.0000	0	
MalRiv0119	E. Fork Wolf Cr.	complete	42	1.32	55.44	0	0.0000	0	0.0000	0	
MalRiv0121	Pine Cr.	complete	30	1.04	31.2	7	0.2244	173.63	5.5651	17	SPD
MalRiv0122	Fopian Cr.	complete	30	2.02	60.6	2	0.0330	51.73	0.8536	16	COT
MalRiv0126	Summit Cr.	complete	81	3.39	274.59	0	0.0000	0	0.0000	49	BT, CSU, SPD
MalRiv0127	Cottonwood Cr.	complete	99	3.17	313.83	45	0.1434	1681.1	5.3567	392	CSU, SPD, RSS
MalRiv0129	Camp Cr.	complete	75	1.93	144.75	0	0.0000	0	0.0000	0	
MalRiv0130	Little Wolf Cr.	dry 9/6/11			0	0	0.0000	0	0.0000	0	
MalRiv0131	Deardorf Ditch	complete	60	1.82	109.2	0	0.0000	0	0.0000	0	
MalRiv0134	Bear Cr.	complete	51	3.92	199.92	4	0.0200	138.79	0.6942	90	SPD
MalRiv0135	Larch Cr.	complete	90	2.01	180.9	5	0.0276	132.96	0.7350	6	SPD
MalRiv0138	N. Fk. Malheur	complete	100	3.4	340	1	0.0029	7.1	0.0209	189	COT, SPD, WF
MalRiv0139	Summit Cr.	complete	87	3.15	274.05	0	0.0000	0	0.0000	48	BT, LND, SPD, CSU
MalRiv0141	Calamity Cr.	complete	51	1.23	62.73	16	0.2551	262.63	4.1867	16	
MalRiv0142	N. Fk. Malheur	complete	100	8.66	866	7	0.0081	461.62	0.5330	43	WF, COT, LND
MalRiv0144	Bully Cr.	complete	63	2.47	155.61	0	0.0000	0	0.0000	473	SPD, RSS, CSU
MalRiv0145	S. Fk. Malheur R.	dry 7/14/11			0	0	0.0000	0	0.0000	0	
MalRiv0147	Corral Basin Cr.	complete	30	0.86	25.8	0	0.0000	0	0.0000	0	
MalRiv0151	Big Cr.	complete	100	3.65	365	2	0.0055	229.72	0.6294	183	COT, BT, SPD, LND
MalRiv0153	Crane Cr (S.Fk.Trib.)	complete	30	1.98	59.4	0	0.0000	0	0.0000	0	
MalRiv0154	N. Fk. Malheur R.	complete	60	2.52	151.2	6	0.0397	295.4	1.9537	63	COT, BUT
MalRiv0155	E. Fork Wolf Cr.	complete	72	2.51	180.72	43	0.2379	1589.98	8.7980	66	COT

Code	Species
BT	Brook Trout
BUT	Bull Trout
COT	Sculpin
CSU	Largescale Sucker
LND	Longnose Dace
RSS	Redside Shiner
SPD	Speckled Dace

Chapter 5

2011 Stream Temperature Monitoring in the Upper Malheur

Logan Valley Wildlife Mitigation Property, 2011

Daniel Brown
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Contents

Tables continued

2011 Stream Temperature Monitoring in the Upper Malheur
Logan Valley Wildlife Mitigation Property

Daniel Brown, Fisheries Technician
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5.1 Introduction

The Burns Paiute Tribe Natural Resources Dept (BPT) has been monitoring stream temperatures in the headwaters of the Upper Malheur since acquiring the Logan Valley Wildlife Mitigation Property in April 2000. Data reveals that monitoring sites on Big and Lake Creeks continue to surpass the Department of Environmental Quality (DEQ) Stream Temperature Standard of 12° C for bull trout (*Salvelinus confluentus*) and 16° C for salmonids during the summer period. All sites on the Lake Creek drainage spent over 70% of the summer period with MWMT exceeding 12° C, and 3 of the 6 sites (sites 2, 7 and 9) spent over 80 days out of 120 in excess of 16° C. Lake Creek drainage sites on average spent 20% of the summer period above the Incipient Lethal Temperature for bull trout (20.9°C) whereas Big Creek sites never eclipsed such temperature maximums. Inflows from McCoy Creek are a major driver to Lake Creek thermal barriers that limit early summer bull trout migrants from contributing to the Lake Creek spawning population. Data indicates that the easternmost fork of Lake Creek as it enters the Tribe's Logan Valley property was again dewatered for a significant portion of the summer in 2011. Dry creek beds in Lake Creek resulting from upstream water withdrawals may be disrupting bull trout downstream movements after the autumn spawn event. These irregular, late season withdrawals occur on private property upstream of tribal land and may result in take of stranded bull trout.

5.2 Methods

5.2.1 Study Area

The Logan Valley Wildlife Mitigation Property is located south of the Strawberry Wilderness in Grant County, OR. The project consists of 1760 deeded acres in which Lake Creek, Big Creek, Crooked Creek and McCoy Creek combine to form the Upper Malheur River. BPT has maintained five temperature sites on the Upper Malheur since acquiring the property in April 2000 (Namitz 2000, Schwabe 2001, Schwabe 2002, Schwabe 2003, Schwabe 2004, Fenton and Schwabe 2005, Fenton 2006). Of these five original sites, two sites are stationed on Lake Creek, two sites are stationed on Big Creek and one site is stationed at their confluence (Table 1). In 2007 two more sites, with a focus on the Lake Creek drainage, were selected for temperature monitoring (Schwabe 2007) (Table 1). Another site (site 8) was added to Lake Creek in 2008 (Abel 2008) (Table 1). For the 2009 field season, two additional monitoring sites were added (Table 1), Site 9 (McCoy below 16 Road) and Site 10 (Lake Ck Ditch below 16 Road). Site locations are mapped in Figure 1.

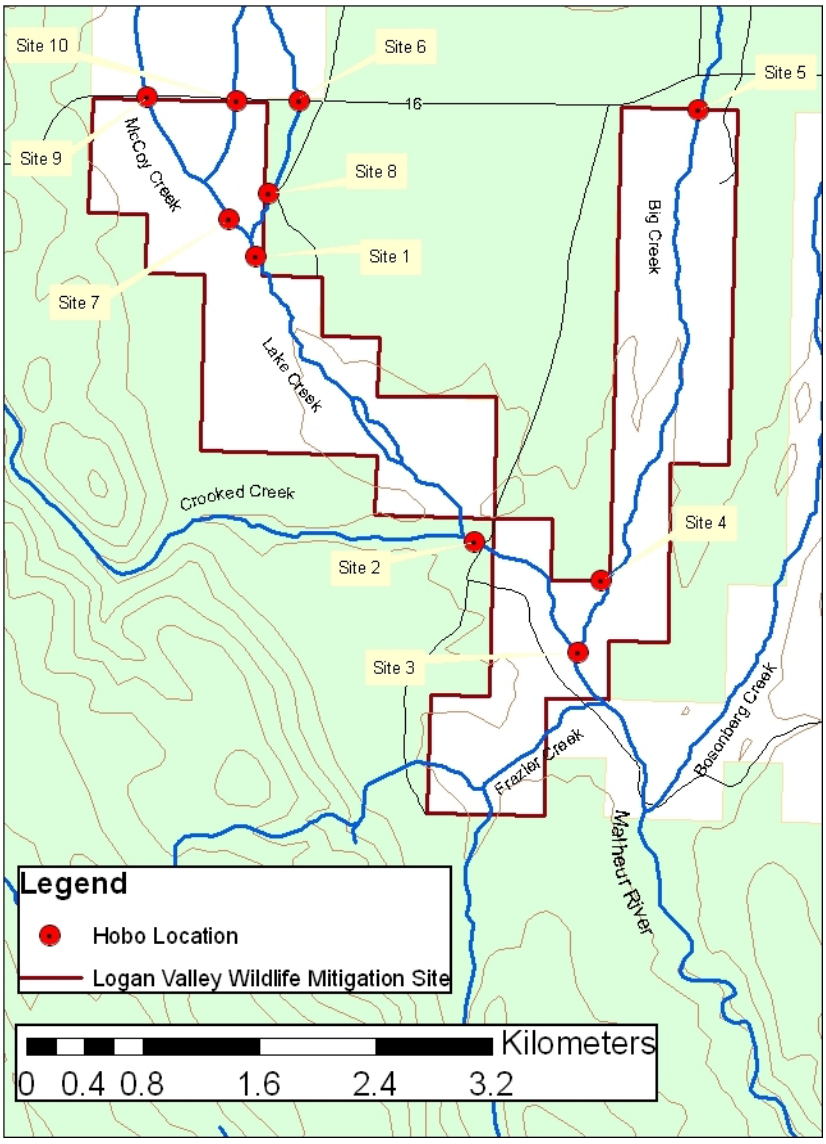

Figure 1: Stream temperature monitoring site locations.

Table 1: Stream Temperature Sites Monitored by BPT.

Site	Location	Year Initiated
1	**Lake Creek below McCoy Creek** "upper Lake"	**2000**
2	**Lake Creek below Crooked Creek** "lower Lake"	**2000**
3	**Malheur River below Big and Lake Creek** "Malheur"	**2000**
4	**Big Creek 1 Mile below 16 Road** "lower Big"	**2000**
5	**Big Creek below 16 Road** "upper Big"	**2000**
6	**Lake Creek below 16 Road**	**2007**
7	**McCoy Creek above Lake Creek**	**2007**
8	**Lake Creek at Cabin Bridge**	**2008**
9	**McCoy Creek below 16 Road**	**2009**
10	**Lake Creek Ditch below 16 Road**	**2009**

5.2.2 Field Techniques

In 2011, Tidbit v2 Temperature Loggers (hobos) manufactured by Onset Computer Corporation were deployed on the 25th of May and retrieved on the 5th of October. Hobos were subjected to accuracy checks prior to deployment using methods recommended by the Oregon Water Quality Monitoring Technical Guidebook (2001). Standards dictate that hobo readings can not vary from actual by more than ±0.5°C.

Eight pound anchors were used to secure hobos in stream. Timing of deployment has varied slightly from year to year, usually as a result of seasonal weather conditions. Having hobos deployed in Logan Valley by 1 May was the objective in 2011 and will continue to serve as the target date for deployment in the years ahead.

5.2.3 Data Analysis

Temperature data is often expressed in this report by the rolling daily maximum temperature averaged over a seven day period that is referred to as Mean Weekly Maximum Temperature (MWMT). This unit of measurement is also known as Seven Day Average Daily Maximum or 7DADM, and is synonymous with maximum rolling temperature calculations utilized in previous reports by BPT. Figures 1A – 10A in Appendix A plot the 2011 MWMT at each monitoring site against DEQ stream temperature standards. Figures 6A, 7A and 8A illustrate the periods that the respective streambeds were dewatered, necessitating the exclusion of data from these sites during this period.

The DEQ Stream Temperature Standard is 12° C MWMT for bull trout migration and juvenile rearing and is 16° C MWMT for salmonid core rearing areas (i.e. an area of moderate to high density use generally in a basin's middle to upper reaches) (OAR 2004). Sixteen centigrade has been cited as an important benchmark in relation to the thermal tolerance of bull trout as well; lab research found that bull trout feed consumption declined significantly at temperatures greater than 16° C (Selong et al. 2001). The same lab study identified 20.9° C as the Incipient Lethal Temperature (ILT) for bull trout. Temperatures listed above are thus important monitoring benchmarks utilized for comparative analysis throughout this report.

The timeframe 15 July – 15 August was outlined as the critical period for high stream temperatures in the Malheur by Perkins (1999) and has been used in previous BPT reports as an index for interpreting stream temperature data. Temporal occurrence of highest stream temperatures was identified to see if dates fell within the 32 day critical period (Table 2). Table 3 represents the average number of days and percent of total days in 2011 that MWMT eclipsed cited temperature benchmarks.

Daily Average Temperature (DAT) at each site in 2011 was calculated (Appendix X-B). Temperature data was compared to data on the movements of radio-collared bull trout through Logan Valley in 2000 and 2001 (Appendix C). The peak of bull trout migration through Logan Valley during both years was mid-June.

Table 2: Summary of Temperature Maximums at each Monitoring Site.

2011 Monitoring Period: May 25 - Oct 5 (°C)					
Stream Name	Site Number	Highest 7-day Max	Date of Occurrence	Absolute Maximum	Date of Occurrence
Lake Ck	1	21.60	8/6/2011	22.39	7/31/2011
Lake Ck	2	21.27	8/3/2011	22.37	7/31/2011
Malheur R.	3	19.38	8/3/2011	20.46	7/31/2011
Big Ck	4	18.39	8/3/2011	19.41	7/31/2011
Big Ck	5	15.79	8/6/2011	16.46	7/31/2011
Lake Ck*	6	20.76	7/30/2011	22.15	7/25/2011
McCoy Ck*	7	24.26	7/21/2011	26.52	7/18/2011
Lake Ck*	8	21.22	7/23/2011	22.30	7/18/2011
McCoy Ck	9	24.63	8/6/2011	25.67	8/5/2011
Lake Ditch	10	19.10	8/28/2011	19.27	8/28/2011

***Lake Creek sites #6 and #8 and McCoy Creek site #7 were dewatered for a significant portion of 2011 summer season. Data was excluded for this period.**

Table 3: Number of Days and Percent of Total Days in the 120 Day Summer Period that MWMT Eclipsed Temperature Benchmarks (2011).

Site	# Days > 12 ° C	# Days > 16 ° C	# Days > 20.9 ° C
1	120 (100%)	98 (82%)	10 (8%)
2	117 (98%)	96 (80%)	6 (5%)
3	114 (95%)	67 (56%)	0 (0%)
4	104 (87%)	45 (38%)	0 (0%)
5	78 (65%)	0 (0%)	0 (0%)
6[1]	***	***	***
7[1]	***	***	***
8[1]	***	***	***
9	120 (100%)	107 (89%)	41 (34%)
10	83 (69%)	65 (53%)	13 (11%)

[1] Lake Creek sites #6 and #8 and McCoy Creek #7 were dewatered for a significant portion of 2011 summer season.

5.3 Results

Logan Valley streams exceed temperature maximums based on DEQ temperature standards for bull trout migration and juvenile rearing habitat (12° C) as well as standards for salmonid core rearing habitat (16° C) (OAR 2004). Similar to peak temperatures in 2010, the date of highest stream temps in 2011 occurred during the Critical Stream Temperature Period (July 15 – August 15) at most sites (Table 2). The exception to this pattern was site 10, which was warmest in late August. Sites 6 and 8 were again dewatered for a significant portion of the summer monitoring period. Site 7 was dewatered due to the temperature logger mistakenly being placed in a side channel. All data collected post-dewater date has been excluded from this analysis. All sites on the Lake Creek drainage in 2011 spent the majority of the summer period with temperatures exceeding 12° C, and 3 of the 7 sites (Sites 1, 2 and 9) spent over 80 days out of 120 in excess of 16° C (Table 3). On Big Creek, Site 4 exceeded 16° C for 45 days, while site 5 did not surpass 16°C (Table 3). ILT was surpassed at all sites in the Lake and McCoy Creek drainages. 20.9° C was surpassed 10 of 120 days (8%) at site 1, 6 of 120 days (5%) at Site 2, 41 of 120 days (34%) at Site 9, and 13 of 120 days (11%) at Site 10 (Table 3). The confluence of Big and Lake Creek (Site 3) did not exceed the ILT of 20.9° C in 2011, though it narrowly missed that threshold (Table 3).

Analysis in 2009 found that late season irrigation on private land upstream of tribal property is dewatering Lake Creek when adult bull trout would be leaving spawning grounds for downstream overwintering areas (Abel 2009). Loss of regular flow during the fall migration could be stranding bull trout and leading to take of adult spawning participants. In 2011, Site 6 was dewatered on 6 August (Figure 2), Site 7 was dewatered on 23 July (Figure 3), and Site 8 was dewatered beginning 31 July (Figure 4).

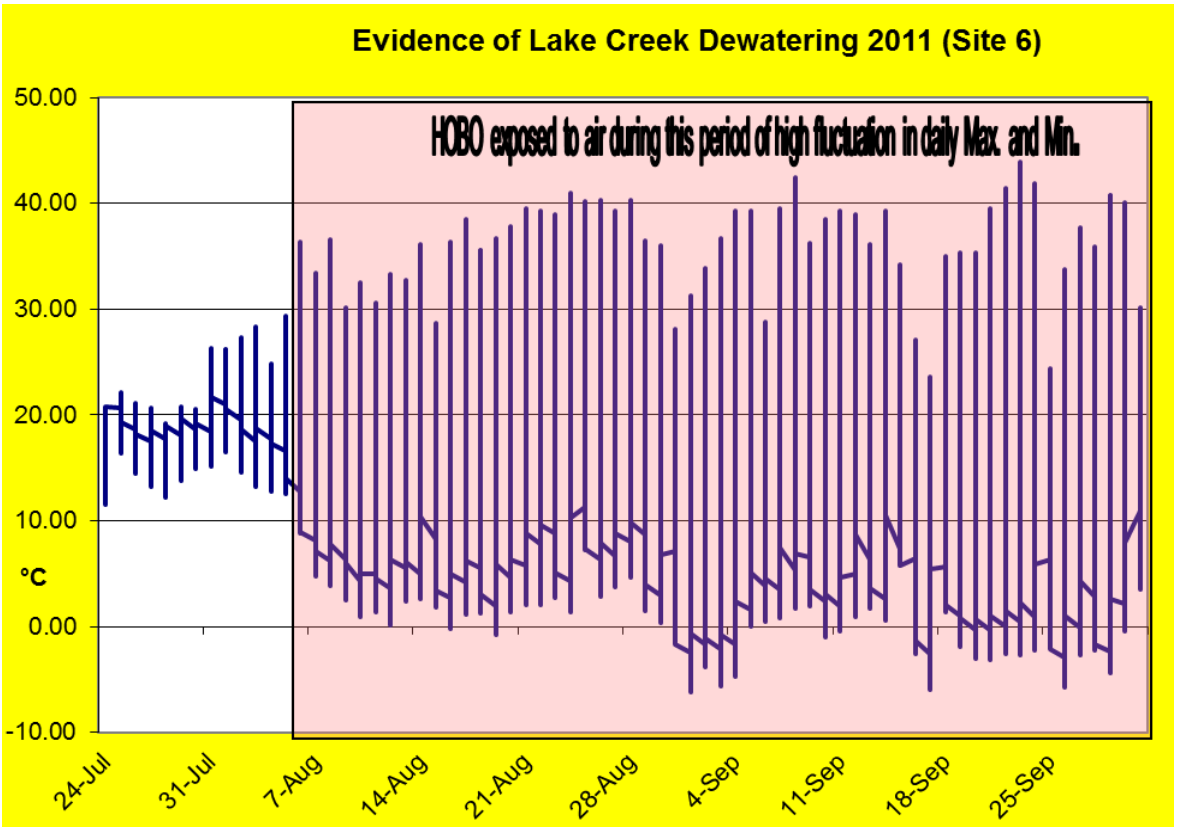

Figure 2. 2011 dewater period as expressed by hobo exposure to air.

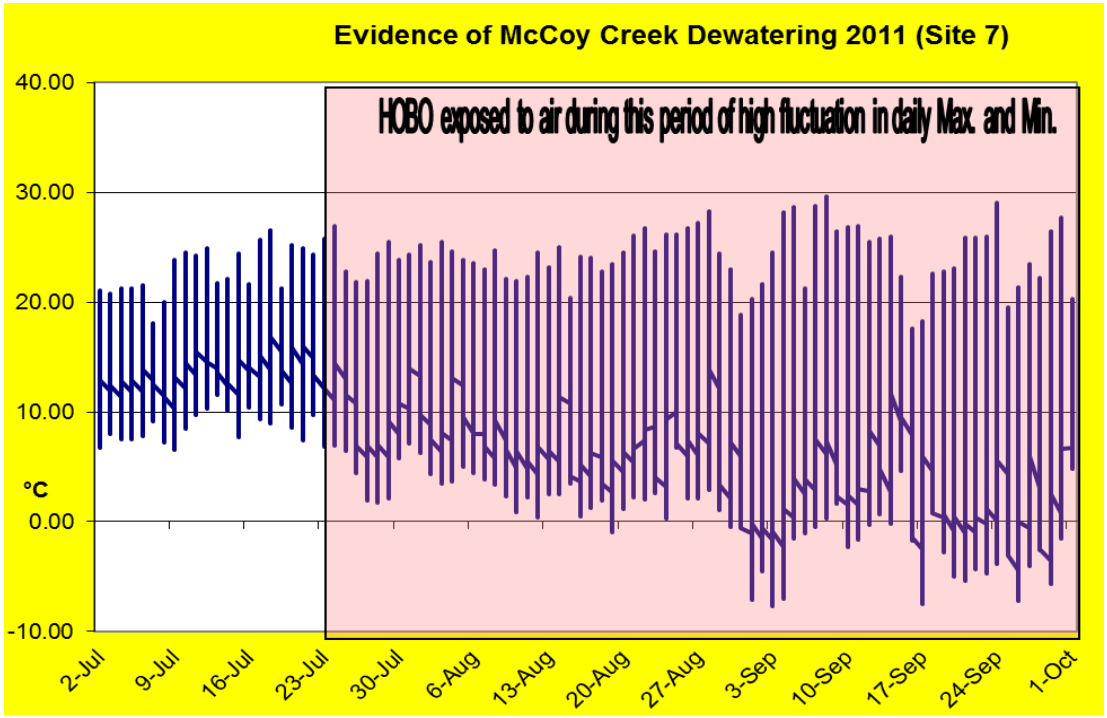

Figure 3. 2011 dewater period as expressed by hobo exposure to air.

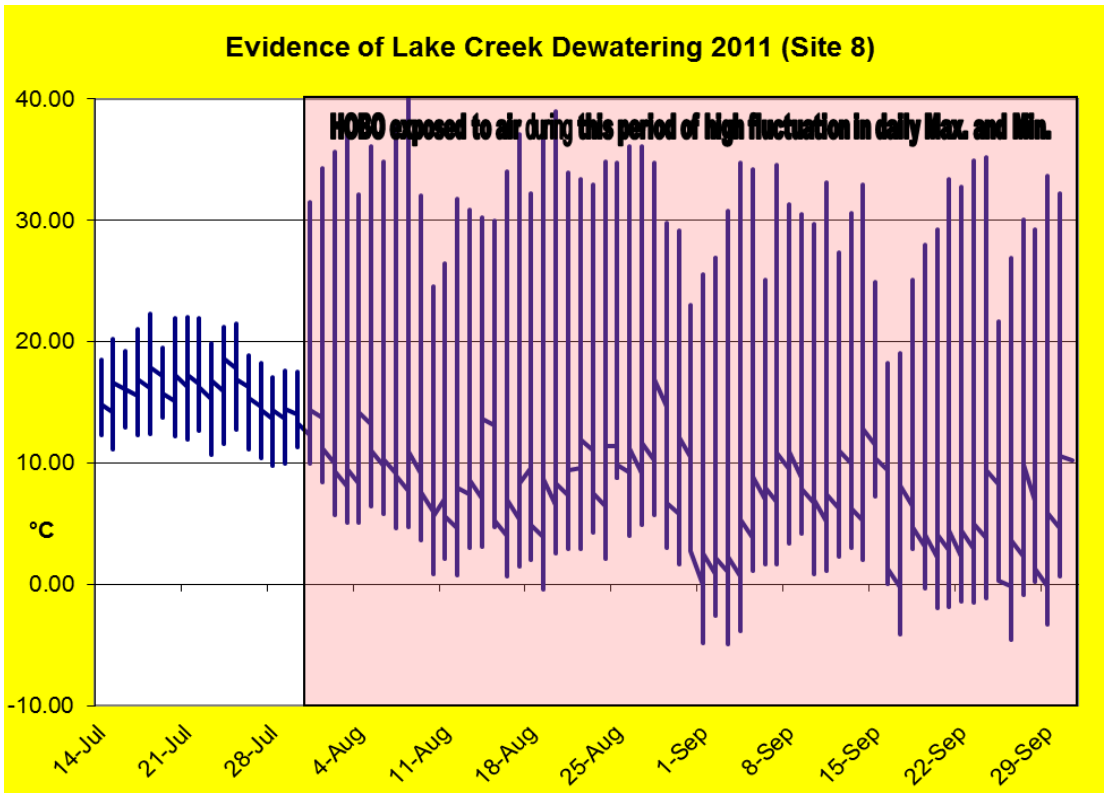

Figure 4. 2011 dewater period as expressed by hobo exposure to air.

5.4 Discussion

In 2000, Burns Paiute Tribe entered into a cooperative effort with the USDA Forest Service and the Oregon Department of Fish and Wildlife to document stream temperature trends in the Upper Malheur (Namitz 2000). The primary purpose of the monitoring effort was to utilize stream temperature data as an indicator of habitat suitability for the federally threatened bull trout (Namitz 2000). Bull trout are stenothermal, requiring a narrow range of cold water temperature conditions to rear and reproduce (Buchanan and Gregory 1997). In western North America, the bull trout is believed to be among the most thermally sensitive species in coldwater habitats (Buchanan and Gregory 1997; Haas 2001; Selong et al. 2001; Dunham et al. 2003), and maximum temperature has consistently been suggested as likely the most critical variable determining bull trout health and presence (Haas 2001).

Since BPT began stream temperature monitoring in Logan Valley, water temperatures have consistently surpassed the DEQ Bull Trout Temperature Standard of 12° C MWMT for a majority of the summer period at all monitoring sites (Namitz 2000, Schwabe 2001, Schwabe 2002, Schwabe 2003, Schwabe 2004, Schwabe and Fenton 2005, Fenton 2006, Schwabe 2007, Abel 2008, Abel 2009). 2010 MWMT data plotted against temperature benchmarks in Figures 1B – 10B (Appendix B) yield two observations. Those are: 1. Lake Creek sites warm earlier in the season, reached much higher maximum temperatures, and sustain critical temperatures for longer durations than in Big Creek sites. 2. McCoy Creek (Sites 7 and 9) is a major driver to high stream temperatures noted in Lake Creek.

From 2000 – 2008, Lake Creek sites 1 and 2 were already above 16° C, the temperature cited by Selong et al. (2001) as being associated with significant decreases in food consumption by bull trout in lab tests, on the very first day of the 120 day summer period beginning 7 June (Table 4). On average the bull trout ILT of 20.9° C was eclipsed on 2 July for Site 1 and 28 June for Site 2 (Table 4). By comparison, 16° C was not surpassed on the Upper Malheur (Site 3), lower Big Creek (Site 4) or upper Big Creek (Site 5) until 16 June, 19 June, and 4 July respectively (Table 4). From 2000 – 2009 Big Creek (Sites 4 and 5) never eclipsed the ILT during the summer period (Table 4), and stream temperatures recorded in 2010 were no exception (Table 3).

Table 4. Average Date of First Recorded MWMT over Cited Benchmarks for the 120 Day Period Beginning 7 June (2000 – 2008)

	Site 1 "upper Lake"	**Site 2** "lower Lake"	**Site 3** "Malheur"	**Site 4** "lower Big"	**Site 5** "upper Big"
>12°C	***no readings < 16° C***	***no readings < 16° C***	**7 June**	**7 June**	**14 June**
>16ºC	**7 June**	**7 June**	**16 June**	**19 June**	**4 July**
>20.9ºC	**2 July**	**28 June**	**13 July**	*no readings >20.9° C*	*no readings >20.9° C*

While ILT is an important surrogate for habitat utilization, the temporal variation between Big Creek and Lake Creek of when stream temperatures begin to reach critical maximums may trump mid-summer temperature extremes. Data collected from radio tagged bull trout in 2000 and 2001 suggests that, at least for fluvial bull trout populations, migration through the property occurs before the Critical Stream Temperature Period associated with annual temperature maximums (Appendix C *see also* Schwabe 2000

and Fenton and Schwabe 2001). In 2000, all radio tagged bull trout except one individual were above the Upper Malheur weir by 29 June (Map 1) and had successfully migrated upstream of the property by 6 July (Map 2). Similar movement patterns occurred in 2001 when all live individuals were above the weir by 29 June (Map 4), and all spawning participants were upstream of the property by 13 July (Map 5).

Exploring ways to maintain adequate stream temperatures for a longer duration of the spring could prove beneficial to migratory success of the breeding population, especially in regards to Lake Creek. Stream temperatures in Lake Creek during the Primary Migration Period reach critical thresholds sooner than in Big Creek (Table 4). The result is a thermal barrier that prevents upstream movements of fluvial bull trout migrants. A thermal barrier early in the Primary Migration Period would explain why no radio tagged bull trout used the Lake Creek corridor to access upstream spawning grounds in either 2000 or 2001. In 2001 bull trout 151224 attempted migration up Lake Creek (Map 3, Appendix C) but had retreated by 29 June to join Big Creek migrants (Map 4). Stream temperatures in Lake Creek had already surpassed bull trout ILT in 2001 when 151224 had attempted the Lake Creek migration (Schwabe 2001).

Current redd counts in the upper reaches of Lake Creek are low, and redd count data is muddled by indecipherable brook trout redds. In 2009, nine positively identified bull trout redds were counted at Lake Creek spawning grounds (Perkins 2009). It is likely from stream temperature data and past tracking efforts that the Lake Creek breeding population is comprised solely of a small resident, non-migratory population. The current status of the entire Upper Malheur bull trout metapopulation is considered to be at a high risk of extinction (Buchanan et al. 1997). If the small Lake Creek subpopulation truly is isolated and incapable of genetic drift due to thermal barriers, then the status of Lake Creek bull trout is indeed precarious.

Stream temperatures recorded on Big Creek are not ideal according to DEQ standards either, but temperatures may be adequate for the migratory population. Even if bull trout migration is largely completed before 15 July, the migratory population was still subjected to stream temperatures in excess of 16° C in 2011 (Appendix A, Figures 4 and 5).

Late season irrigation on private land upstream of tribal property is dewatering Lake Creek when adult bull trout would be leaving spawning grounds for downstream overwinter areas (Figure 2 and 4). Loss of regular flow during the fall migration could be stranding bull trout and leading to take of adult spawning participants. Our Department has been in contact with the landowner to seek a solution.

5.5 Acknowledgements

The Burns Paiute Tribe thanks Bonneville Power Administration for their continued financial support of this project.

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APPENDIX A
STREAM TEMPERATURES EXPRESSED BY MWMT

FIGURE 1A: LAKE CREEK BELOW McCOY CREEK (site 1)

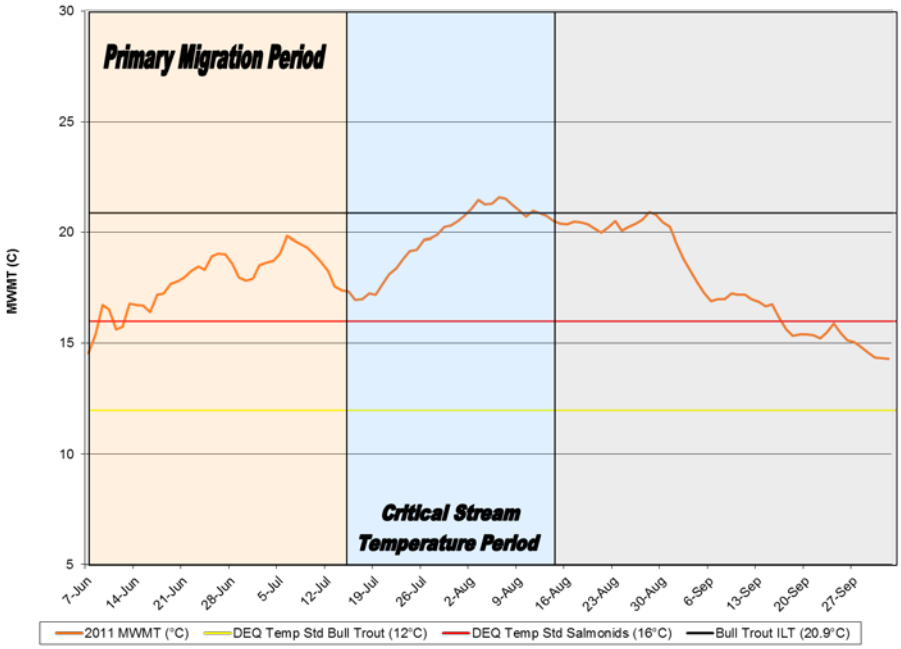


FIGURE 2A: LAKE CREEK BELOW CROOKED CREEK (site 2)

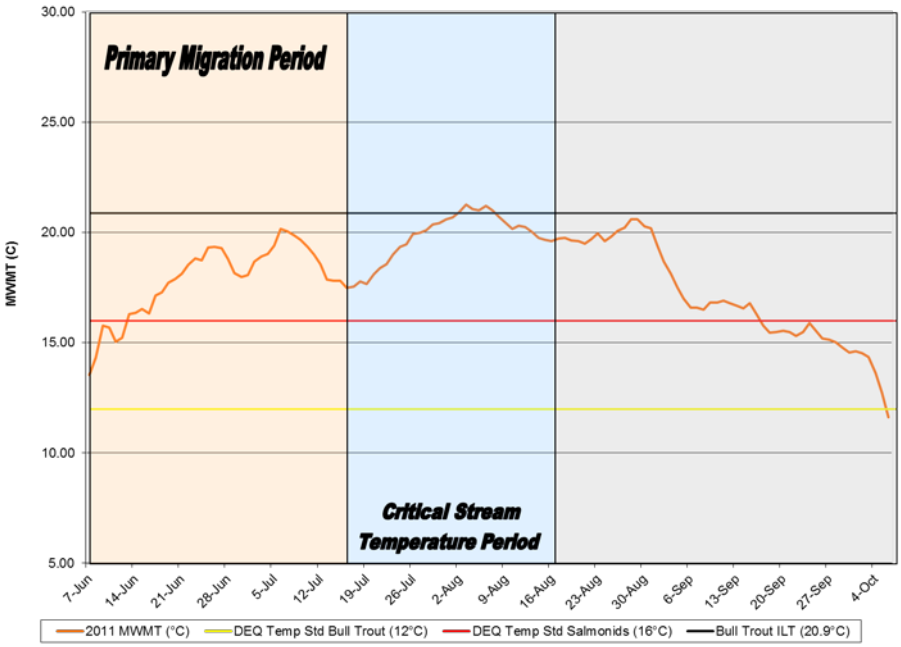


FIGURE 3A: MALHEUR BELOW BIG AND LAKE CREEKS (site 3)

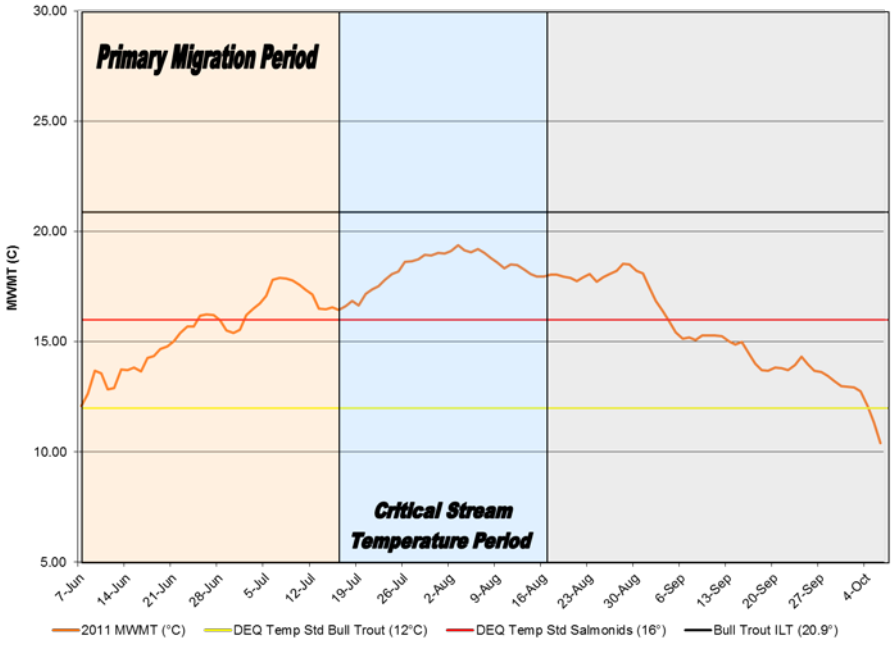


FIGURE 4A: BIG CREEK ONE MILE BELOW 16 ROAD (site 4)

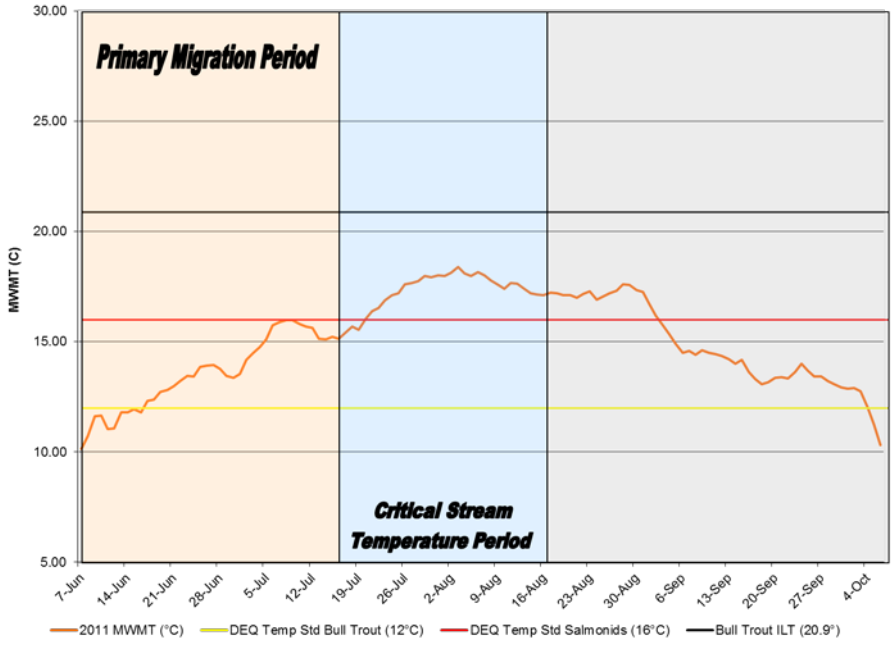


FIGURE 5A: BIG CREEK BELOW 16 ROAD (site 5)

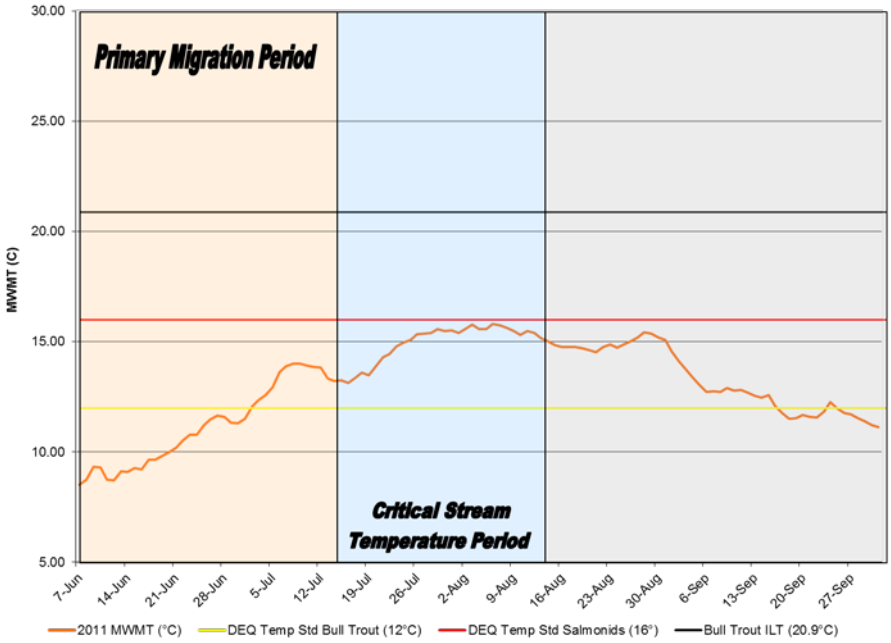


FIGURE 6A: LAKE CREEK BELOW 16 ROAD (site 6)

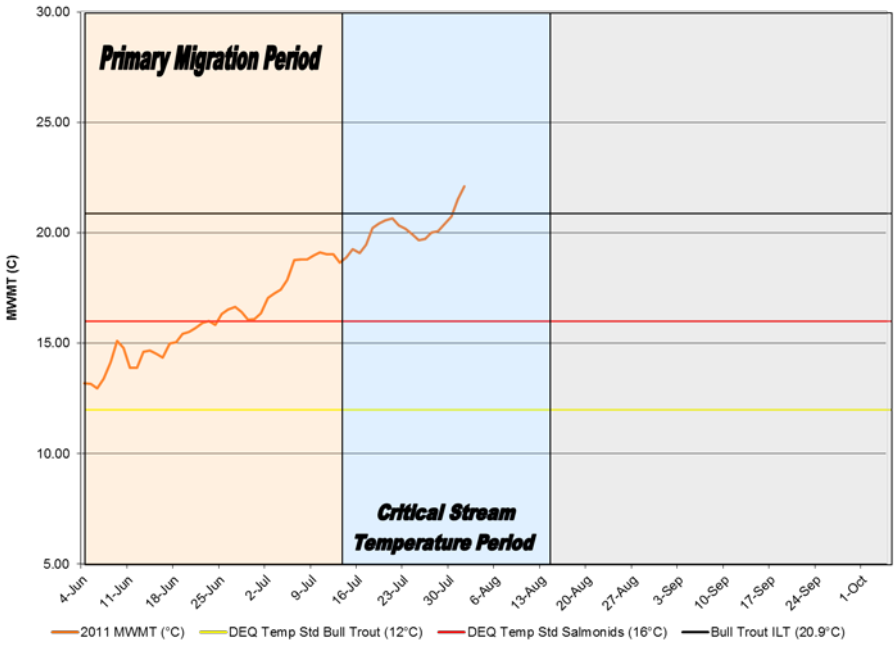


FIGURE 7A: McCOY CREEK ABOVE LAKE CREEK (site 7)

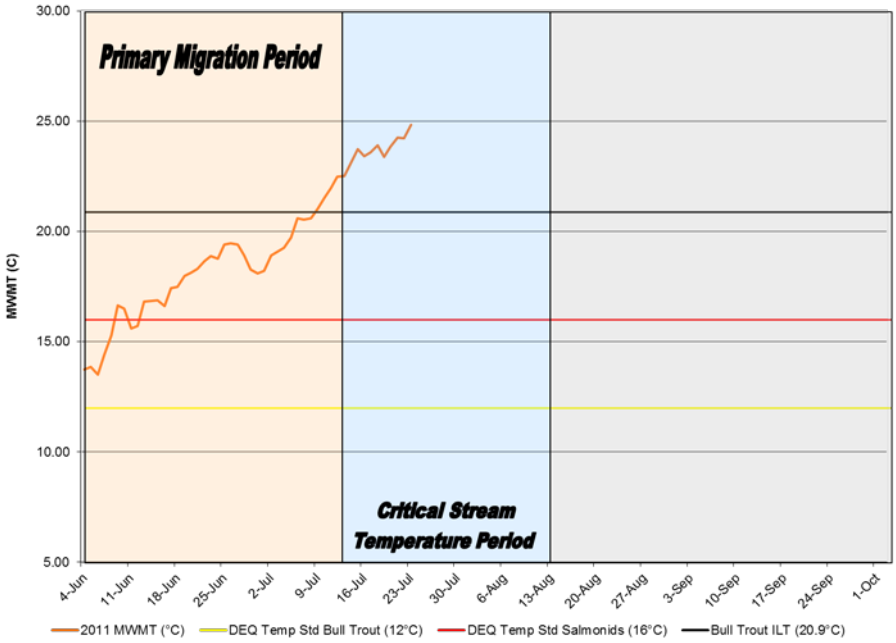


FIGURE 8A: LAKE CREEK AT BRIDGE (site 8)

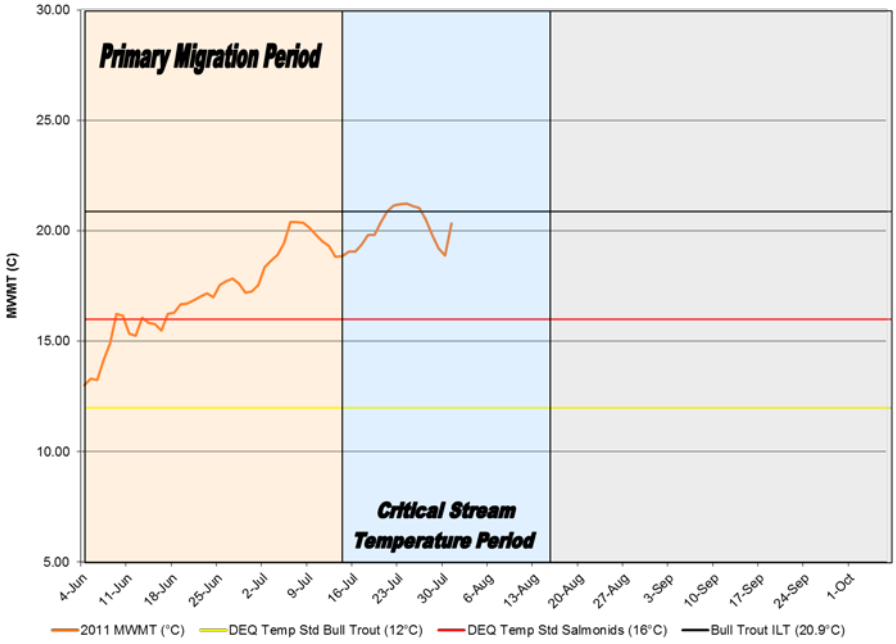


FIGURE 9A: McCOY CREEK AT 16 RD (site 9)

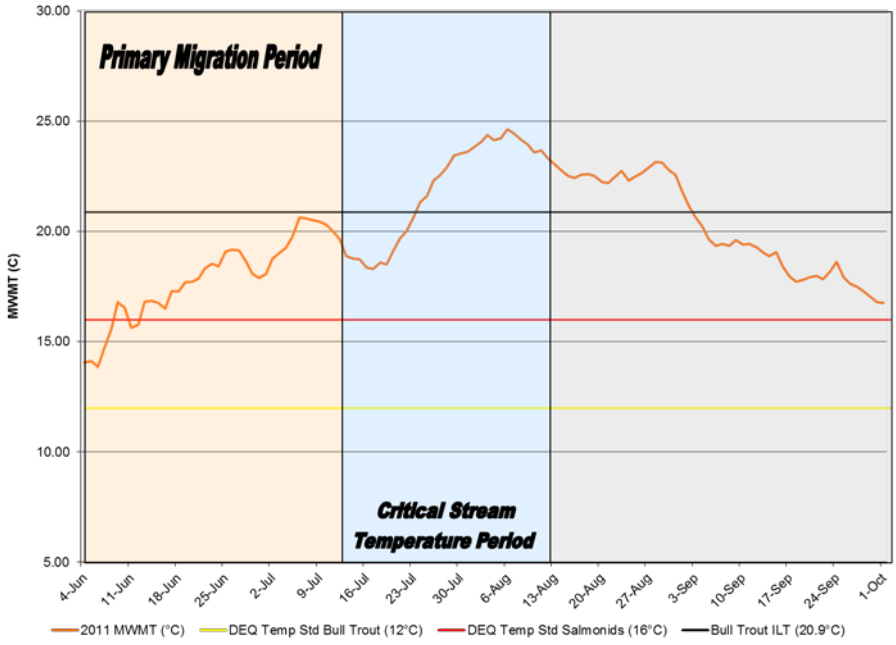
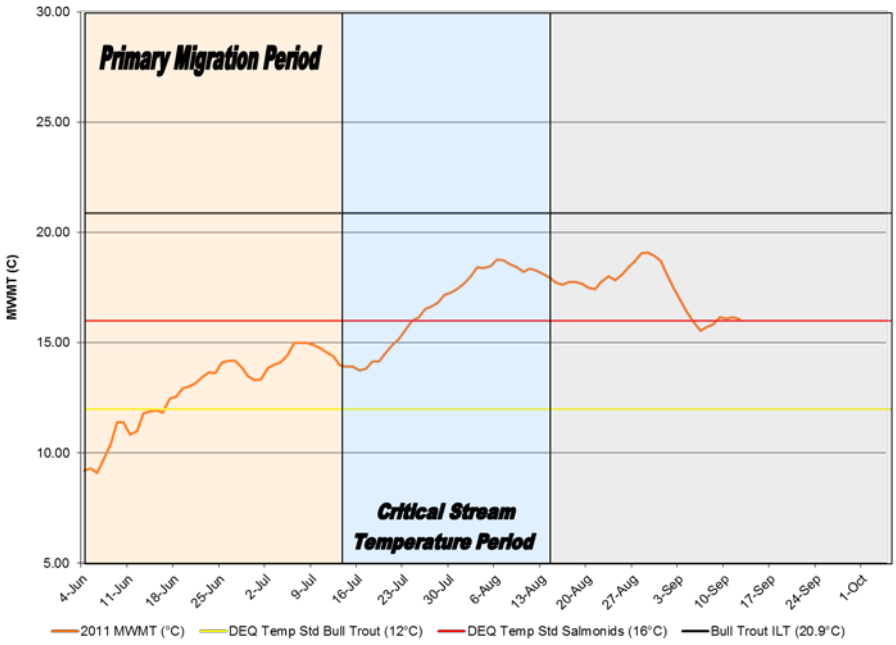
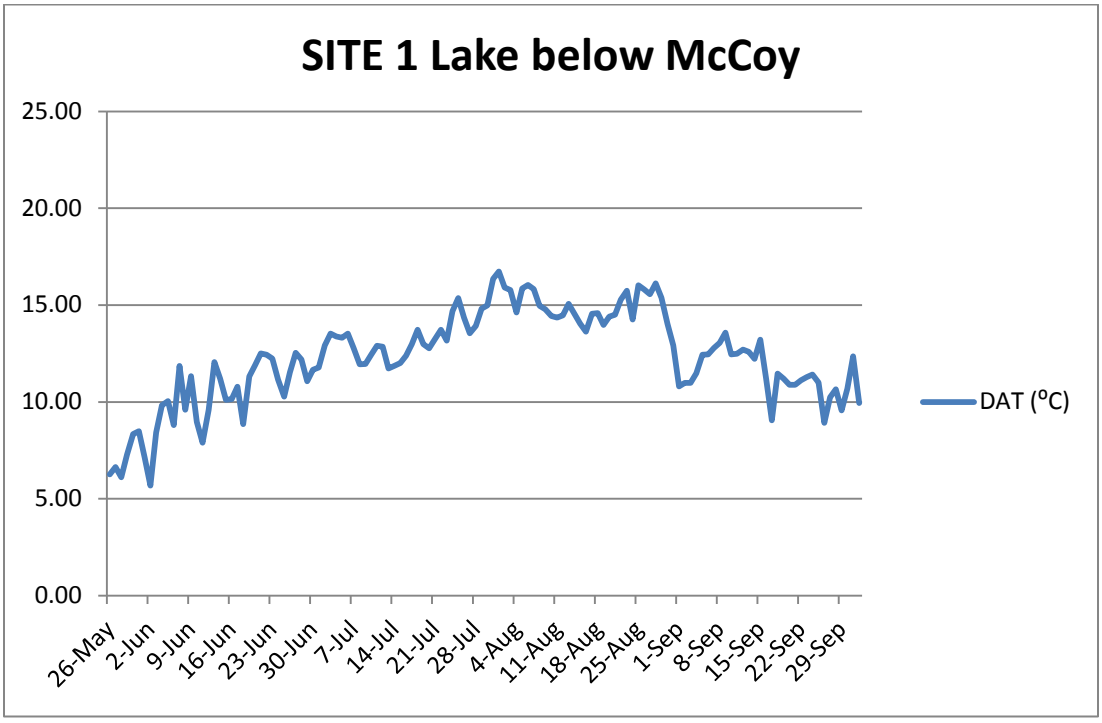
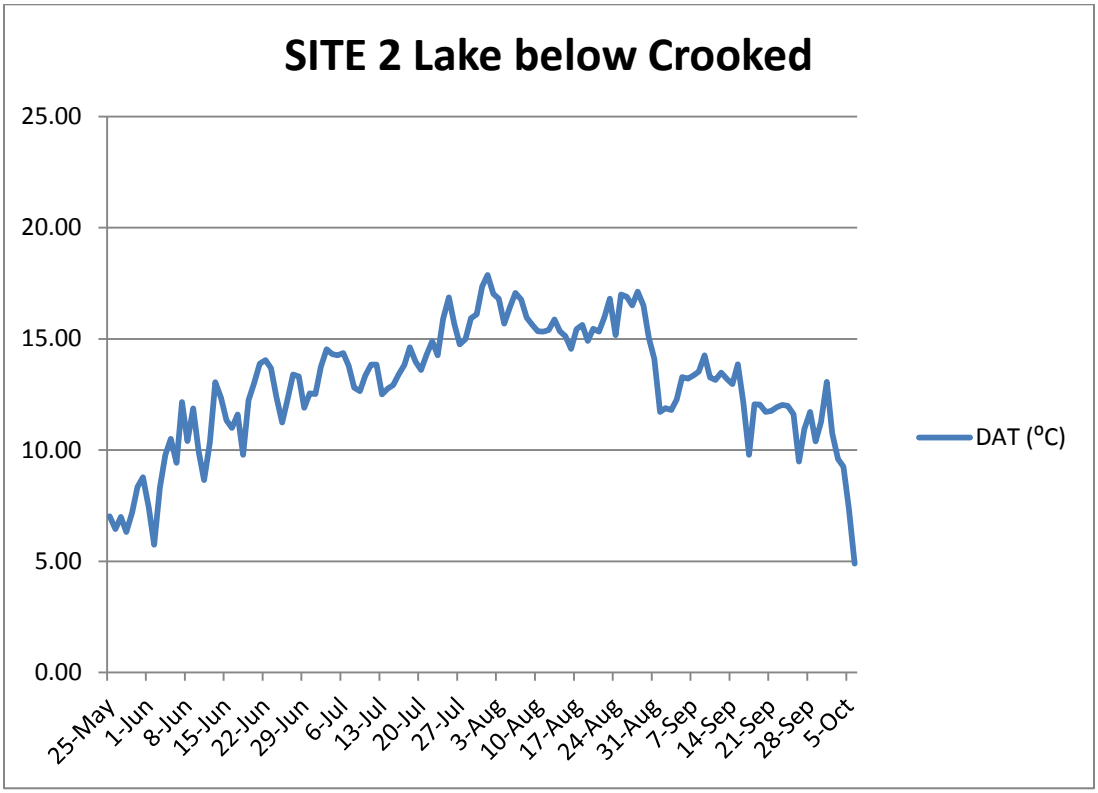


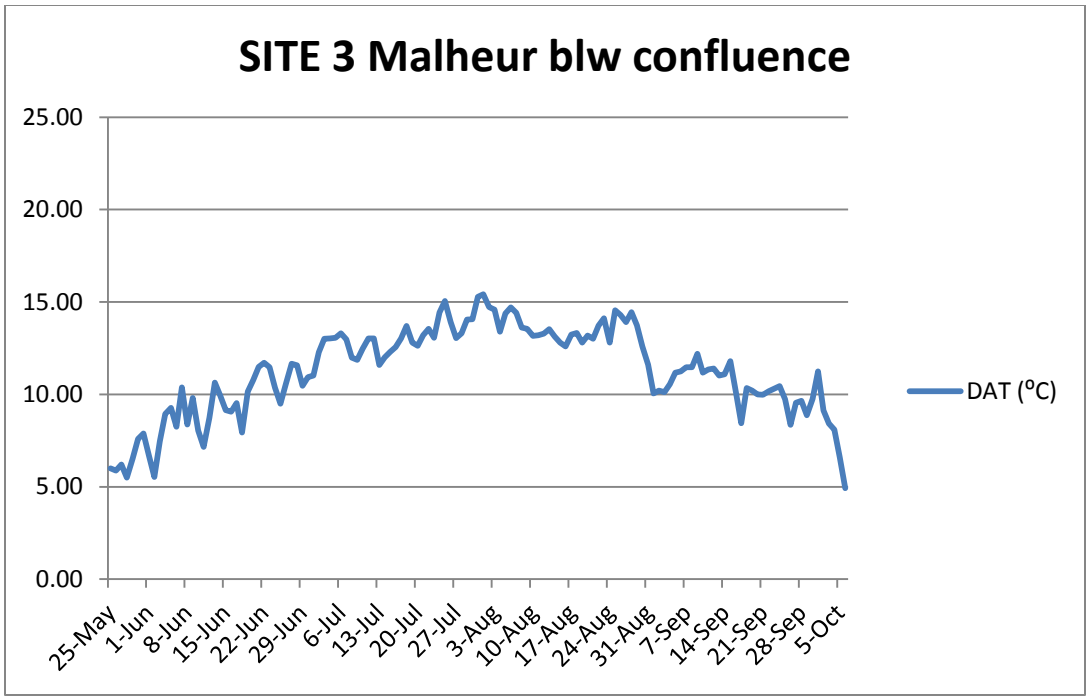
FIGURE 10A: LAKE CREEK DITCH AT 16 RD (site 10)

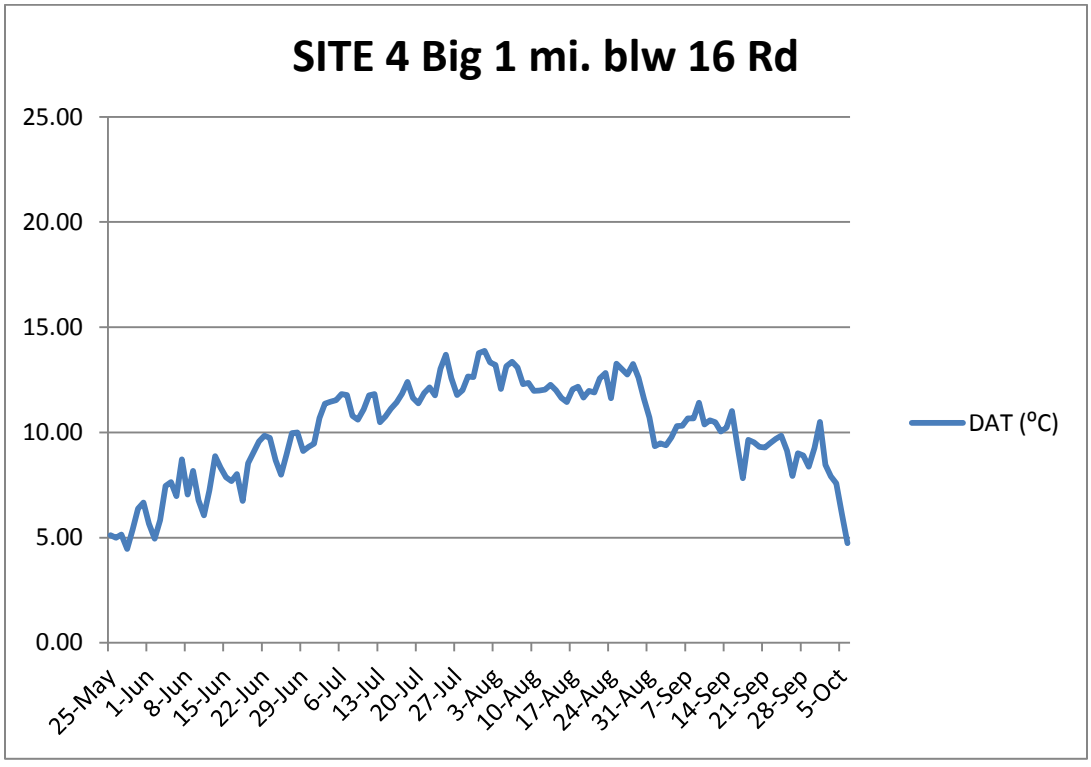


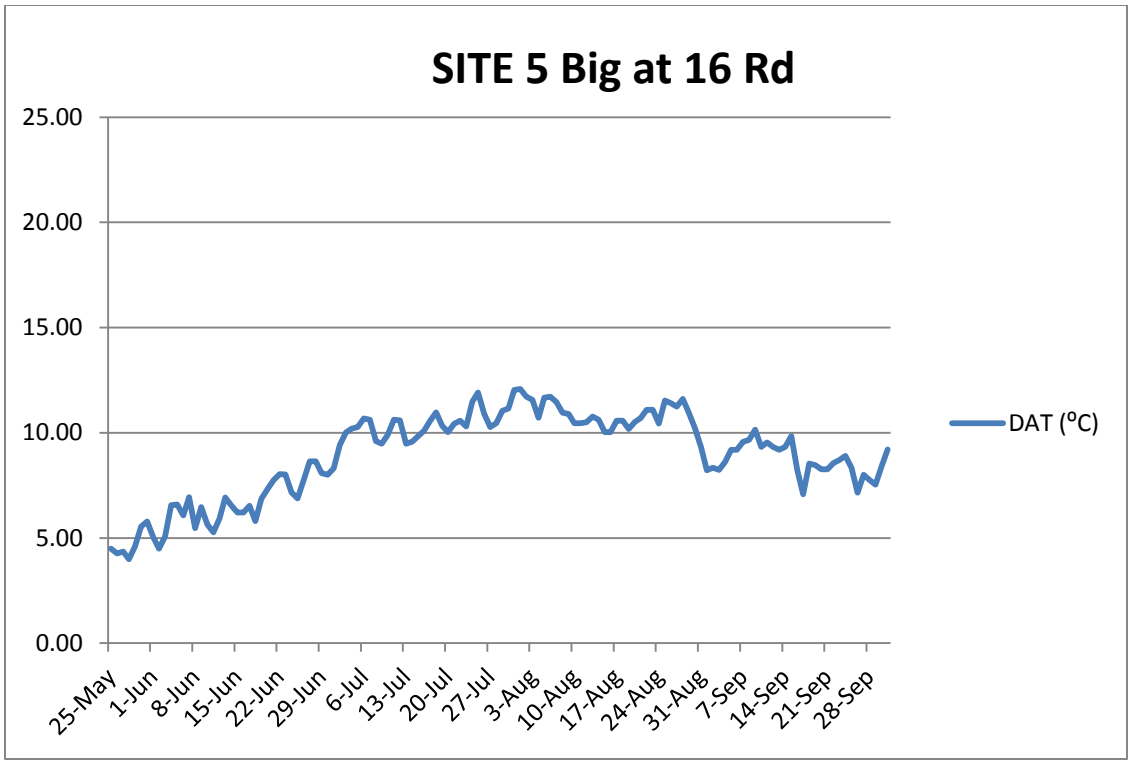
APPENDIX B
2011 DAILY
AVERAGE TEMPERATURE






SITE 3 Malheur blw confluence
25.00
20.00
15.00
10.00
5.00
0.00
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
10-Aug
17-Aug
24-Aug
31-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
DAT (°C)

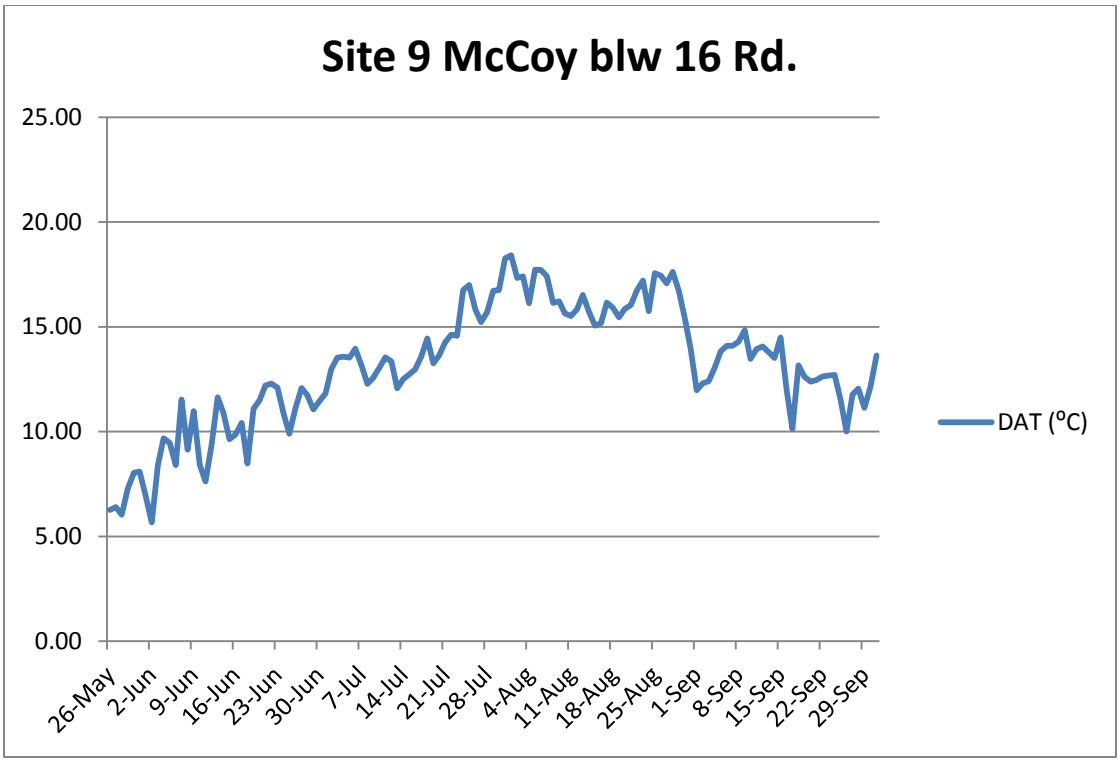

SITE 4 Big 1 mi. blw 16 Rd
25.00
20.00
15.00
10.00
5.00
0.00
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
10-Aug
17-Aug
24-Aug
31-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
DAT (°C)



**Site 6 Lake Cr Below 16 RD Dewatered 8/6/11 Data Not Useable

**Site 7 McCoy Cr Above Lake Cr Dewatered 7/23/11 Data Not Useable
(Data recorder mistakenly placed on side channel)

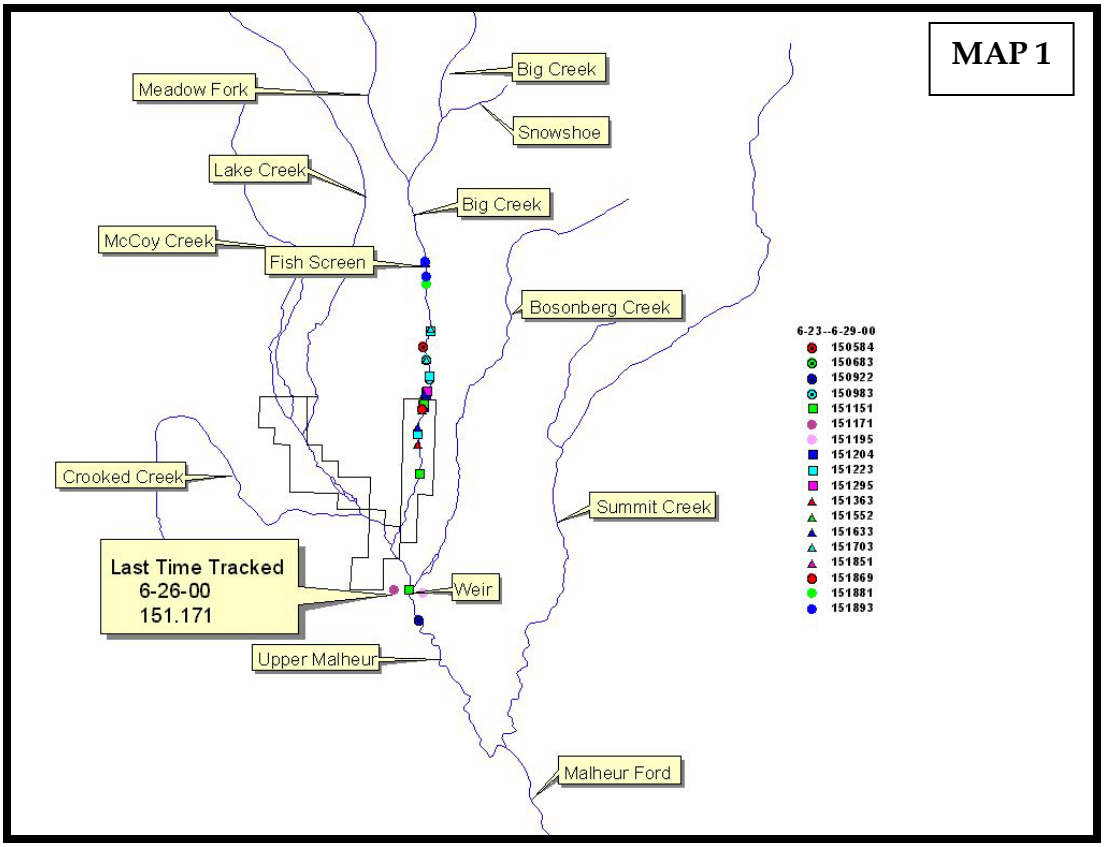
**Site 8 Lake Cr at Bridge Dewatered 7/31/11 Data Not Useable

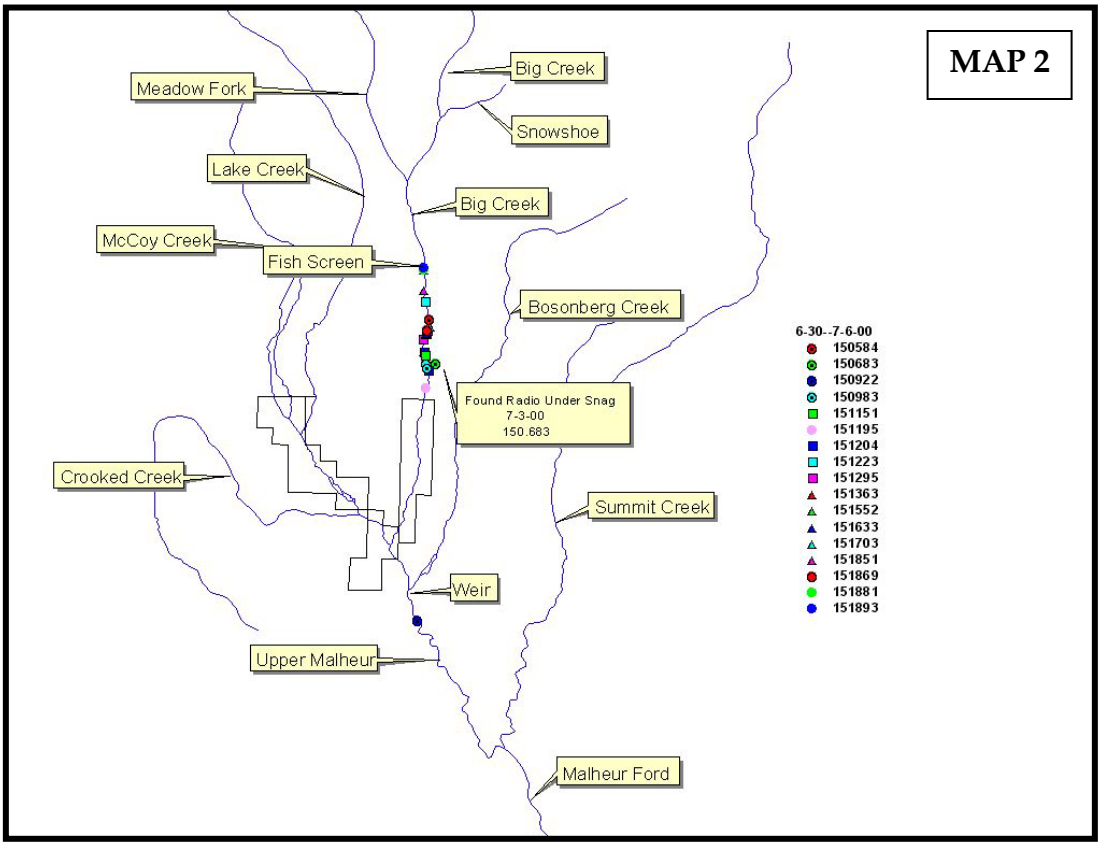


**Site 10 Lake Cr Ditch Blw 16 RD Dewatered 9/13/11 Data Not Useable

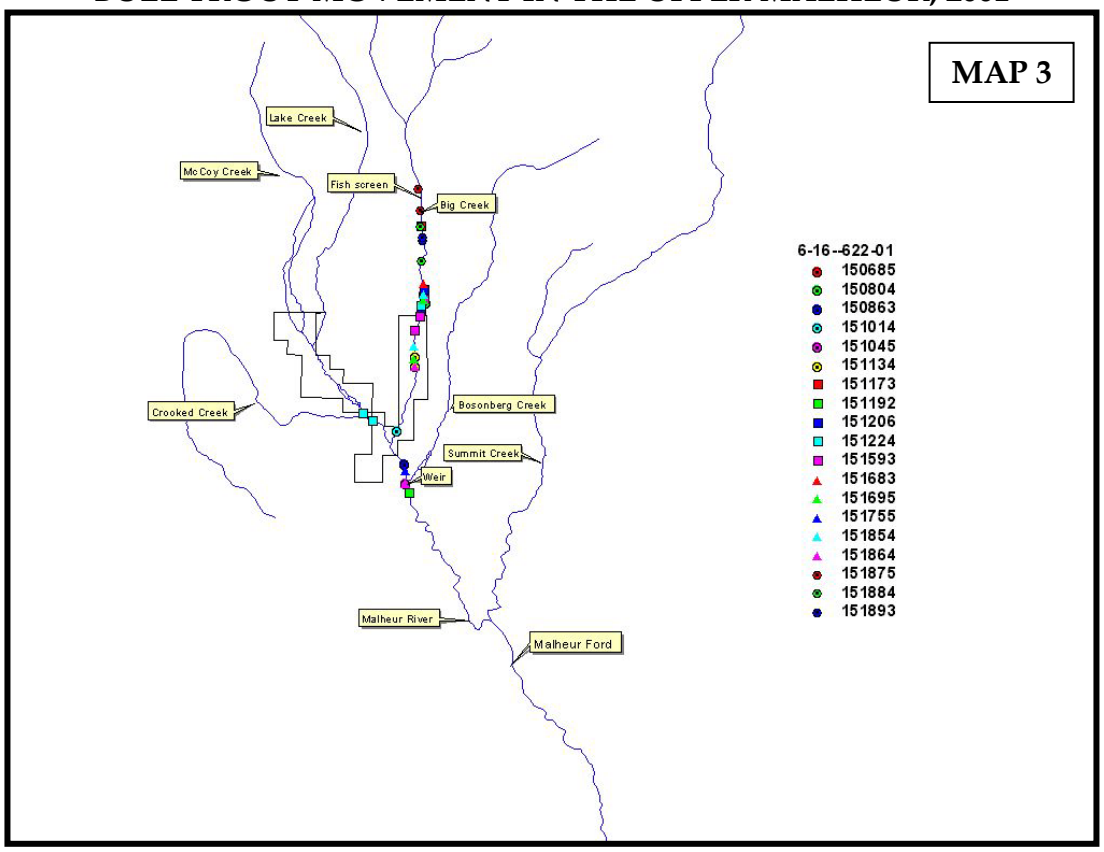
APPENDIX C

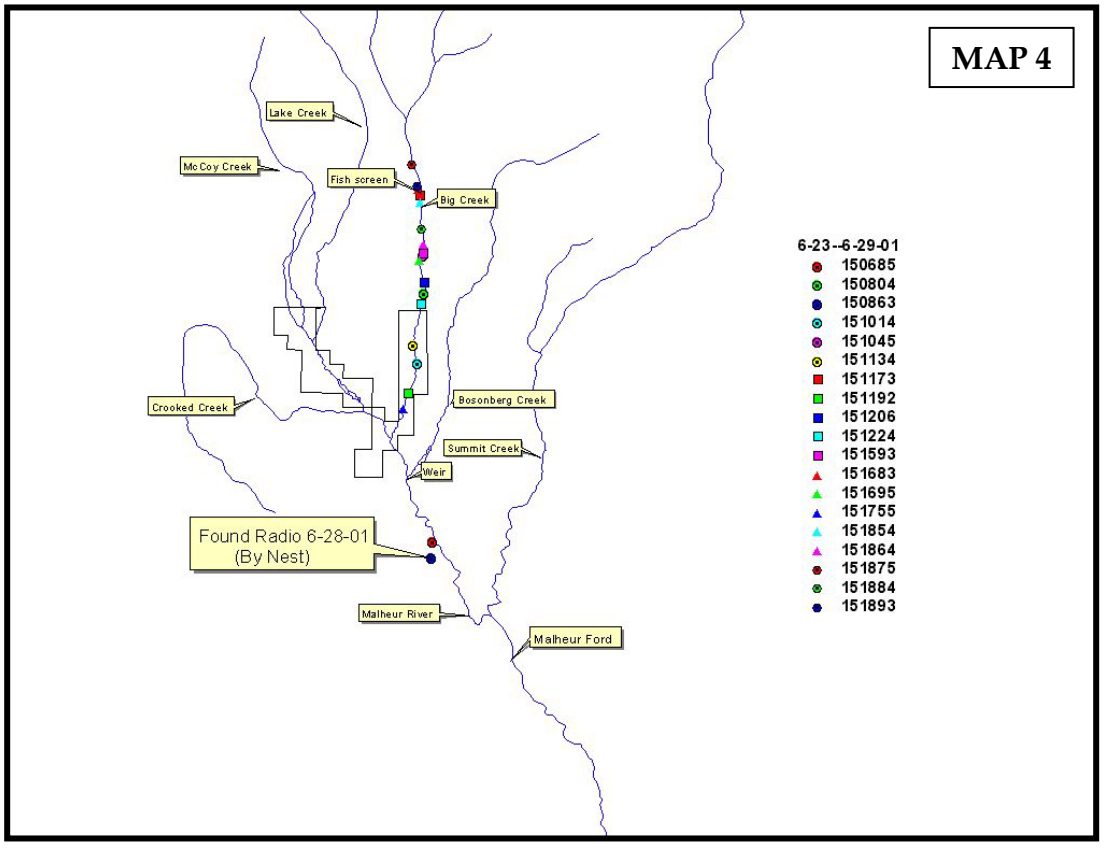
BULL TROUT MOVEMENT IN THE UPPER MALHEUR, 2000

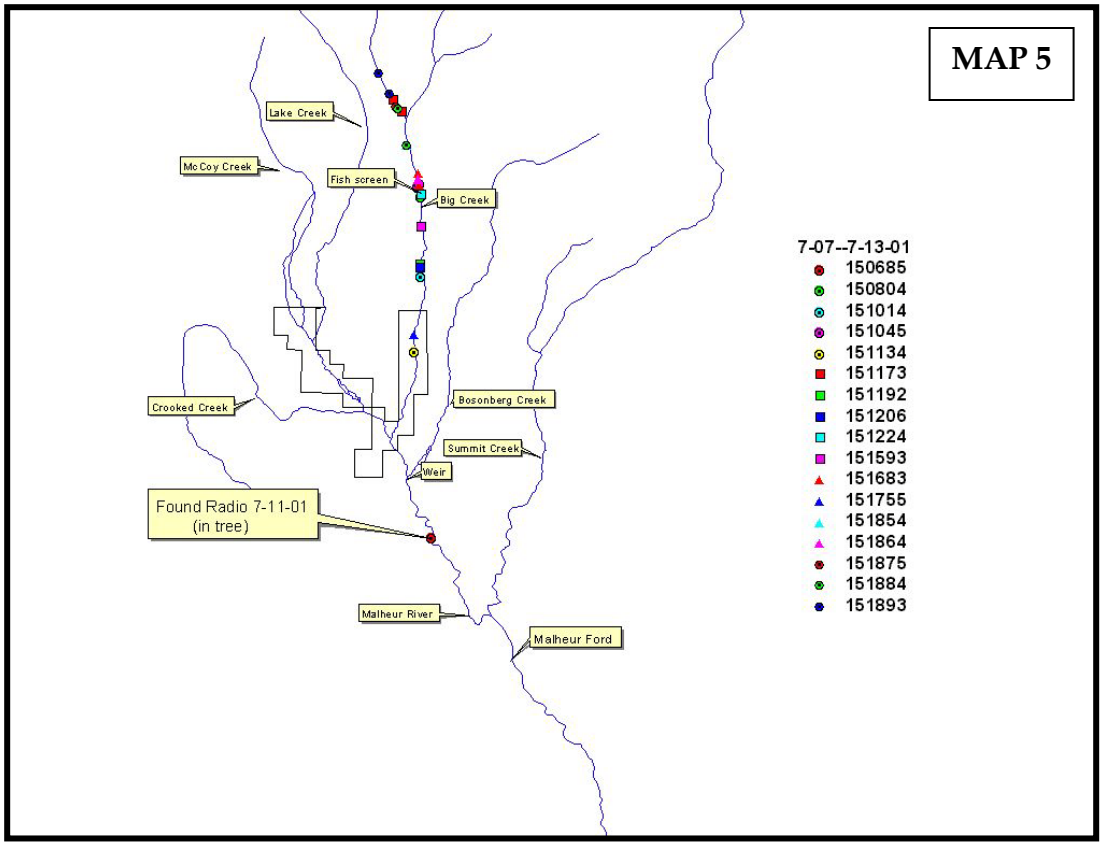




BULL TROUT MOVEMENT IN THE UPPER MALHEUR, 2001






MAP 5
Lake Creek
McCoy Creek
Fish screen
Big Creek
Crooked Creek
Bosonberg Creek
Summit Creek
Weir
Found Radio 7-11-01
(in tree)
Malheur River
Malheur Ford
7-07--7-13-01
150685
150804
151014
151045
151134
151173
151192
151206
151224
151593
151683
151755
151854
151864
151875
151884
151893

Chapter 6

Sampling and analysis to assess brook trout (*Salvelinus fontinalis*) population trends in High Lake (Oregon) using environmental DNA monitoring
2011 Report

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Figures continued

Sampling and analysis to assess brook trout (*Salvelinus fontinalis*) population trends in High Lake (Oregon) using environmental DNA monitoring

2011 Report

Brook trout (*Salvelinus fontinalis*) – Public domain image by Eric Engbretson (USFWS)

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Introduction

Recovery criteria for the Malheur Recovery Unit bull trout (*Salvelinus confluentus*) require stable or increased abundance of local populations and the reestablishment of connectivity between the separated populations (USFWS 2002). Other necessary actions include a reduction or elimination of threats from brook trout (*Salvelinus fontinalis*) interaction in the Upper Malheur River Basin (USFWS 2002). Full recovery of Malheur River bull trout is contingent upon minimizing the threats posed by brook trout interaction. Non-native brook trout introduced into High Lake threaten downstream bull trout populations in Lake Creek via hybridization (DeHaan 2010). Lake Creek supports spawning populations of the federally threatened native bull trout. Brook trout suppression and/or eradication activities throughout essential bull trout habitat in the Upper Malheur include brook trout removal in 5.8 acre High Lake, seasonal fish weir operations to prevent upstream brook trout movements, and a large-scale chemical treatment effort in streams lacking bull trout.

Cramer Fish Sciences (CFS) proposed the use of a DNA-based technique to monitor the abundance of brook trout, a non-native char in High Lake. The abundance of brook trout environmental DNA (*e*DNA) was investigated as a metric to track trends in the brook trout population. The use of *e*DNA as a tool for detection of species has been demonstrated in freshwater environments (Jerde et al. 2011, Teletchea 2009). While the tool has had greatest application in detecting "cryptic" (visually-evasive) species, we proposed to use *e*DNA as a means to track the abundance of a non-desirable species over the long-term. The primary objective of the pilot study was to validate the use of *e*DNA as a cost-effective means to monitor trends in a brook trout population.

Using the quantitative Polymerase Chain Reaction (qPCR) technique, we will estimate *e*DNA abundance in two successive years. Within each year, *e*DNA samples will be collected at two different time periods, in the summer when fish are distributed through High Lake and the fall when spawning congregation occurs (prior to removal effort). Total *e*DNA abundance will be estimated for the entire lake. Coincident with the first *e*DNA sampling event, the Burns Paiute Tribe (BPT) will be monitoring population abundance using traditional methods (e.g., hook and line mark-recapture). The brook trout population estimated by the BPT will be compared to the *e*DNA abundance estimates. We will test the hypothesis that trends observed by *e*DNA are comparable to those from traditional sampling. This interim report covers the year-1 summer and fall field sampling and qPCR.

Methods and Materials

The general approach CFS proposed included using a systematic sampling scheme to account for potential spatial variation of *e*DNA concentrations, with sampling occurring in two consecutive years. Within each year, sampling was to occur twice – once in the summer when the fish are dispersed throughout the lake and once in the fall when the fish are aggregated during spawning. At each sampling event, *e*DNA samples were to be collected on a vertical and horizontal grid. In the summer, *e*DNA samples will be collected before or after the mark-recapture sampling. In the fall, the *e*DNA samples will be collected prior to BPT's removal efforts. In the first sampling event (summer 2011), we applied a statistical rule of thumb and proposed to locate 20 samples systematically within the lake along 5 transects oriented at an azimuth of 45 degrees and approximately 125 feet apart. Along each transect, *e*DNA samples were collected at 100 foot intervals starting 50 feet from shore (Figure 1). At each location, BPT biologists collected composite grab sample from water strata. Strata depths were determined based on soundings taken at each sample location at each sample event. In the second sampling event (fall 2011), samples were located in potential spawning locations where we expected *e*DNA abundance may be higher. Three potential spawning areas in High Lake were identified by CFS and BPT biologists during the summer 2011 sampling event (Figure 3). In the second sampling event (fall 2011), we applied a statistical rule of thumb and proposed to systematically acquire at least 7 *e*DNA samples from each spawning area. At each location, BPT biologists collected one grab water sample from just above the spawning beds.

Soundings

At each station prior to collecting the water sample, sounding depths were recorded to the nearest $1/10^{th}$ foot precision using the Hawkeye H22PX Handheld Digital Sonar. As shoreline disturbance suspended large amounts of particles, we refrained from using the weighted measuring tape during water sampling so as not to disturb the substrate and suspend eDNA that would potentially misrepresent the water column's eDNA characteristics. On the following day (August 2, 2011) a tape measure weighted with a 6-inch vertical weight was used to measure depth alongside sonar measurements in random locations to compare the accuracy and precision of the digital sonar.

Staff Gauge Placement

A baked enamel style-C staff gauge was purchased from Ben Meadows, Inc. and installed at the south end of the lake (44°16'58.93"N, 118°41'3.92"W) on August 2, 2011, 12:30 PM. The gauge was mounted with 5 stainless steel nuts and bolted onto a 5.5 foot angle iron. With consultation from BPT biologists, the top elevation of the staff was positioned adequately to handle high water level encounters. The staff gauge was placed approximately 10 feet from shore to discourage disturbance by waders or hikers and the face of the gauge was positioned obliquely to minimize unwanted attraction, yet remained readable.

GPS Accuracy

Both GPS handhelds are capable of sub 3-meter accuracy but they do not fulfill the proposal criteria of the sub-meter x,y and x,y,z coordinate requested; although the GPS Map 76S approaches mapping grade GPS criteria. The eTrex legend GPS was used on transects for ease of use by technicians and quicker, although less accurate, averaging for marking points**.**

The locations from Garmin eTrex direct downloads of UTM and Lat/Long readings were inconsistent at times along transects during water sampling. Final positions were determined by heads-up edits of what would be expected with a greater accuracy, but not necessarily to sub-meter precision. Yet, the sample sites remain repeatable, because rope tie-off areas for transects can be identified, with sample points relocated based on rope and tape measurements. The data from GPS handhelds did not achieve sub-meter accuracy, but this would not dramatically effect grid re-sampling, given physical distances along ropes would enhance positioning. As such, the use of transect ropes will be essential for repeatability of grid sampling and in maintaining position when multiple samples are collected at a location. Additionally, the use of two sets of ropes would help speed up collection efforts and aid with maintaining straight sampling lines when anchoring is not an option.

Water Samples

We adapted sampling and analysis techniques in response to logistical considerations and sample results. Composite 1 L water samples were collected during the summer sampling period instead of 2 L discrete-depth samples. This reduced the overall sample volume—a major consideration when transporting water samples from a remote location—while providing representative samples in which brook trout eDNA could be detected. During the fall sampling, one 1 L grab sample was collected from just above the spawning bed at each sample location instead of multiple 2 L discrete-depth surveys. This adjustment reduced overall water volume sampled, but more importantly, increased the probability that eDNA would be detected. This choice was practical—improving confidence in results—and provided an opportunity to test the performance of two different sampling strategies.

Summer Event – Water samples were collected at 19 locations, with sites located systematically along five NE – SW oriented transects (Figure 1). A taut rope was tied from one side of the lake to the other to locate sample sites 50 feet from the shore and subsequently at 100 foot intervals marked with ribbon or a small clamp. An additional sample was collected off-transect (i.e., 4.5aux) at the SE portion of the lake to capture the shallower area of the lake outlet. Although not part of the systematic sample grid, field biologists felt the auxiliary sample might characterize the shallow nearshore area that was not included in the transect. The auxiliary point location near the lake outlet also provided a supplemental depth measurement for contour estimates around the outlet area. A float tube and 2-man inflatable raft was used for accessing the sampling locations. To collect samples, a 2.2 liter Wildco® Van Dorn Vertical Opaque PVC water sampler was used with 25 feet of nylon cord and a 250 gr solid bronze messenger (Figure 2). For locations less than 6 feet in depth, composite water samples were collected from two points in the water column, 0-3 feet above the lake bed and from 0-3 feet below the lake surface. For locations in excess of 6 feet in depth composite water samples were collected from three points in the water column, 0-3 feet above the lake bed, the midpoint, and 0-3 feet below the lake

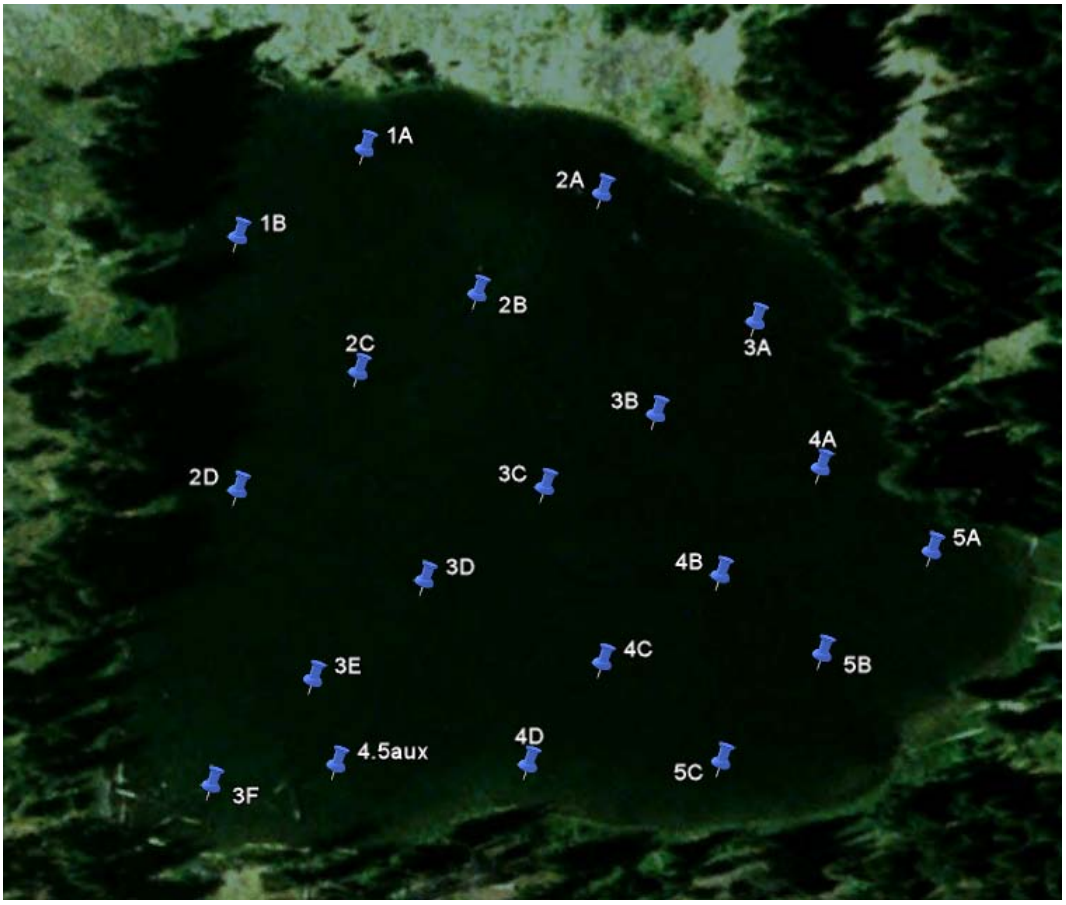

Figure 1. Representations of sample locations based on GPS readings, edge coordinates, landscape features, and field spacing of transects.

Figure 2. Van Dorn Vertical water sampler. Please note an acrylic version is shown, but an opaque PVC version was used (www.wildco.com).

surface. The general water sampling scheme maintained an acceptable protocol for un-stratified (mixed without thermocline) lakes, where each composite sample included equal portions of water from the surface, mid-depth, and within 1 meter from the bottom.

Brook Trout Spawning Areas - On August 2, 2011 BPT biologists and technicians provided input on areas where spawning activity or spawning redds have been observed. Mr. Dominguez subsequently paddled the shoreline areas noting gravelly characteristics and feeder tributaries that are gravel sources. The predominant spawning areas appeared to be the northeast (Area 1) and northwest (Area 2) areas. Observations of recently emerged fry concentrated in the southeast in mid-September along with a small gravel delta formed by a tributary suggested that the southeast portion (Area 3) was also utilized for spawning. Wave action from the northeast to southeast create conditions that provide the mixing conditions that could oxygenate shoreline redds.

Figure 3. Locations of potential brook trout spawning areas in High Lake, Oregon.

Fall Event – Sampling points were located within each spawning area at uniform intervals parallel to the shore and along the midpoint of the extent of spawning (Figure 3). BPT biologists determined the spacing by first measuring the length of the spawning area along the shore and then dividing that length by eight, with 100 meters, 82 meters, and 94 meters being the total length of spawning areas 1, 2, and 3

respectively. The first sample (#21) was taken at the western edge of spawning area 1, with subsequent sampling proceeding in an easterly manner.

All collections from the fall period were obtained on October 14th, 2011. Each collection was a 1 liter water sample obtained from 0 to 3 feet above the substrate. A Swing Sampler was used to collect the water samples (Figure 4). The end of the sampler swings for collecting samples at different angles (up to a maximum of 90°) and the fiberglass pole extends to 12 feet. A large snapper holds bottles in the 1,000 ml range. BPT biologists attached a new bottle for each water sample and extended the pole to a length that enabled them to reach the midpoint of the spawning bed. Once the Swing Sampler was setup, the BPT biologist inserted the bottle upside down into the lake, maintaining an airlock in the bottled, until they reached an appropriate depth above the spawning area. Once at desired depth, the pole was rotated so that water filled the bottle. Once filled, the sample bottle was returned above the water surface as quickly as possible.

Figure 4. The Swing Sampler is specially designed for collecting a sample from a flowing stream or river. (www.enasco.com).

In addition to composite grab samples, two control samples were submitted at each sample event. There were two negative controls, a "sampling control" and a "cooler control". A single 1 liter sampling control should be collected from a water source in the field that is known to be free of brook trout using the same equipment used for sampling composite grab samples. When in route to the study area for summer collections, it was determined that a "sampling control" prepared in the field could not guarantee absence of brook trout DNA. There was too much uncertainty in the region about brook trout distribution. Upon consultation with Gregg Schumer, field staff disinfected the sampling device by soaking it in 10% bleach solution, rinsed thoroughly, and filled the sampler with well water upon our return to the field office. This procedure was followed for the fall sampling control as well. For the summer collection event, a 1 liter cooler control of de-ionized water was maintained alongside the other water samples through the handling and shipping process. The cooler control was intended to be filled prior to the sampling event, but was not filled until we left the field. One of the bagged set of bottles from the manufacturer did not contain a lid and we wanted to ensure the capability of carrying out 20 samples. In retrospect, the solution was to re-sequence the bottle numbers, begin sampling and send

someone on the 1.5 hour round trip hike to secure another bottle for later in the afternoon for 20 samples. De-ionized water was not available during the fall sampling for a cooler control, so lake water was used instead.

Water Sample Processing and Shipping

All water samples were placed in 1 liter bottles and either held in the lake water for a short duration (10 minutes) or directly shuttled to the bank where the samples were buried in a patch of snow for the duration of the day. Upon exiting the study areas, water samples were packed in backpacks with dry ice, snow, or block ice fragments, placed in a cooler and transported 1.5 hours back to Burns, OR. Final labeling and packaging occurred and the samples were placed in a freezer. For sample bottles completely full, a limited amount of water was poured out to prevent damage to the bottle or seal from expansion. Upon return from the field samples were placed in plastic bags and frozen. The samples were packed in insulated boxes with dry ice and chemical cold packs. Packages were mailed from Burns, OR using FedEx and arrived the next day at around 11 A.M in Auburn, CA.

Based on the condition of the eDNA water samples shipped overnight and costs, the field staff suggested that alternative shipping options be considered, such as dry or chemical ice within a standard cooler. The difficulties with managing 1-liter bottles of water in the field is appreciated, and Cramer Fish Sciences is currently designing an unpowered field water filtration device, which may dramatically lower field effort, given water would not have to be packed out from the field. Additionally, if the development of the filtration device is delay, alternative shipping methods will be considered.

Fin Clips

We collected 29 right pelvic fins of brook trout primarily according to the protocol described in the sample method primer titled “Fish DNA Sample Collection Protocol” (Appendix 1). The fins were placed loosely in an envelope and allowed to dry before flattening. Filter paper was not available to dry out the fin clips, so fins were placed loosely on paper partially within an envelope to air dry prior to closing. In evaluating the collected tissue sample upon collection from biologists it was apparent that some samples may not have been completely dry prior to closing the coin envelopes. This was likely a field adjustment so as not to leave them out in the windy environment and the need to relocate their angling positions during the catch-and-release population estimate. Samples were mailed to Gregg Schumer on August 5, 2011. Approximately 30 additional tissue samples were sent to the BPT fishery manager to provide additional samples from the region if necessary. Genomic DNA was extracted from a fin clip of each sample using the Qiagen DNeasy tissue kit.

qPCR

To design a species-specific 5′ exonuclease assay, a 485 bp segment of the mitochondrial cytochrome b gene (cyt-b) was sequenced for six brook trout using conserved animal primers, H15149 and L14724 (Irwin et al. 1991; Kocher et al. 1989). Cyt-b sequences were aligned using Sequencher software version 4.8 (Gene Codes) and Primer Express® Oligo Design software (Applied Biosystems) was used for primer and probe design. A BLAST search was conducted using the NCBI nucleotide database to ensure that the cyt-b DNA template for the designed primers and probe had no known homology with other identified nucleotides strings. From this conserved region of the cyt-b gene, a forward primer, reverse primer, and a species-specific probe were designed to perform a 5′ exonuclease (TaqMan™) assay (Table 1).

Table 1 Brook Trout Primer Probe set. All sequences are shown in the 5′ to 3″ direction.

Primer	Sequence (5′ – 3′)
FWD	TGGCCAACCTCCGAAAAAC
REV	AGGTCGACTAGTGCGTCATTAGC
Probe	CCCACTCCTAAAAAT (BHQ1a-6FAM)

For samples used to validate the species-specific assay, PCR was performed in a 5 µl total volume containing: 1 µl DNA template, 1X QuantiTect Multiplex PCR NoROX kit (Qiagen), 1.8 µM final concentration of both forward and reverse primers and 0.06 µM final concentration for the probe. Thermal cycling occurred with Bio-Rad's Chromo4™ real-time detector under the following conditions: initial enzyme activation of 10 min at 95 ºC, 40 cycles of 15 s denaturation at 95 ºC, 1 min annealing/extension at 60 ºC. C(t) values were quantified using Opticon Monitor software (ver 3.1; Bio-Rad). The assay was also validated for specificity by testing for cross reactivity with a panel of common fish species including: *Oncorhynchus mykiss* (rainbow trout), *Alosa sapidissima* (american Shad), *Parcina macrolepida* (bigscale logperch), *Pomoxis nigromaculatus* (black crappie), *Lepomis machrochirus* (Bluegill), *Cyprinus carpio* (carp), *Ictalarus punctatus* (channel catfish), *Micropterus salmodoides* (largemouth bass), *Spirinchus thaleichthys* (longfn smelt), *Menidia beryllina* (mississippi silverside), *gambusia affinis* (mosquito fish), *Clupea pallasii* (pacific herring), *Cottus asper* (prickly sculpin), *Lepomis gibbosus* (pumpkinseed), *Catostomus occidentalis* (sacramento sucker), *Tridentiger bifaciatus* (shimofuri goby), *Pogonichthys maerolepidotus* (sacramento splittail), *Morone saxatilis* (Striped bass), *Dorosa petenense* (threadfin shad), *Hypomesus nipponensis* (wakasagi smelt), *Ameiurus catus* (white catfish), *Pomoxis annularis* (white crappie), and *Acanthogobius flavimanus* (yellowfin goby). Eight no template controls were included per plate and the threshold was set above background fluorescence for each reporter dye. Samples were considered positive with a cycle threshold (Ct) value ≤ 38.

Results

Lake Level

A baked enamel style-C staff gauge angle iron was driven solidly into the lake bed with a final reading of 1.96' relative lake level. High Lake water elevation at the time of water sampling was determined to be 7475.6 feet. This determination was made using two hand held GPS situated side by side, a Garmin eTrex Legend Cx and a Garmin GPSMap 76S chartplotting receiver. After initializing, the GPSMap 76S was placed alongside the Garmin eTrex in an open area at the water's edge. The GPS Map76S indicated a 3D differential location receiver status, which indicated the use of WAAS differential data and at least four satellites. The 12 parallel-channel receiver continuously tracks up to 12 satellites, and satellite coverage was eight or more most of the day. Both receivers took readings for 15 minutes and the average reading for both was recorded. The GPSMap 76S appeared to be communicating with more satellites during this 15 minute period, offering a more reliable position estimate. In general, the site offered a very good view to the sky, but to a lesser extent at the northern and western edge.

On August 1, 2011, depths were recorded to the nearest 1/10th foot precision using the Hawkeye H22PX Handheld Digital Sonar at each sounding. Lake depths were recorded at each location during systematic grid sampling (Table 3). On the following day, a tape measure weighted with a 6-inch vertical weight was used to measure depth alongside sonar. We did not test in the middle of the lake or deepest parts where wind-caused boat drift and low depths could change the angle of the lowered tape. Visibility was just beyond 10 feet and for all the readings the weight was observable until it reached the substrate. The differences in measurements between the two methods were consistent with the type of substrate encountered (i.e. the weighted tape measurement was slightly longer if it penetrated into organic substrates) (Table 2).

Table 2 Depth comparison of handheld sonar and weighted tape. All depths are in feet.

Sonar	Weighted tape
10.9	11.2
11.3	11.6
11	11
10.2	10.3
8.5	8.6
6.3	6.4

qPCR

All brook trout known templates were detected with the brook trout species ID assay developed for a mitochondria cytochrome b gene locus. There were no observed cross reactions of the brook trout assay when other common fish species were used as templates.

A brook trout DNA standard curve was constructed to relate the detection level (i.e., number of qPCR cycles to a) with quantity of DNA present within each water sample (Figure 5). This curve was used to calculate the milligrams of DNA per liter of water sampled.

In the summer sampling period, water samples were collected in a systematic grid pattern on August 1, 2011, with the location and depth recorded for each site (Table 3). Each water sample was a composite composed of collections made from multiple depths in the water column. Results from water samples analyzed for the presence of brook trout DNA were provided by Cramer Fish Sciences laboratory staff on September 20, 2011. Brook trout DNA was detected at three grid locations, 1A, 3D, and 4C, with quantities of DNA being 0.00016, 0.00003, and 0.00010 mg/L, respectively (Table 3). Detection of brook trout DNA was not observed in any negative control, while a strong signal was observed in the positive brook trout control.

In the fall sampling period, seven water samples were collected in each of three putative spawning areas used by brook trout on October 14, 2011 (Figure 3). Water samples were obtained every 12.5, 10.25, and 11.75 meters within spawning areas 1, 2, and 3, respectively (Table 4). Results from water samples analyzed for the presence of brook trout DNA were provided by Cramer Fish Sciences laboratory staff on November 9, 2011. Brook trout DNA was detected within five water samples, the 2nd site from spawning area 2, and the 1st, 2nd, 3rd, and 5th sites from spawning area 3 (Table 4). DNA concentrations for each detection are shown in Table 4.

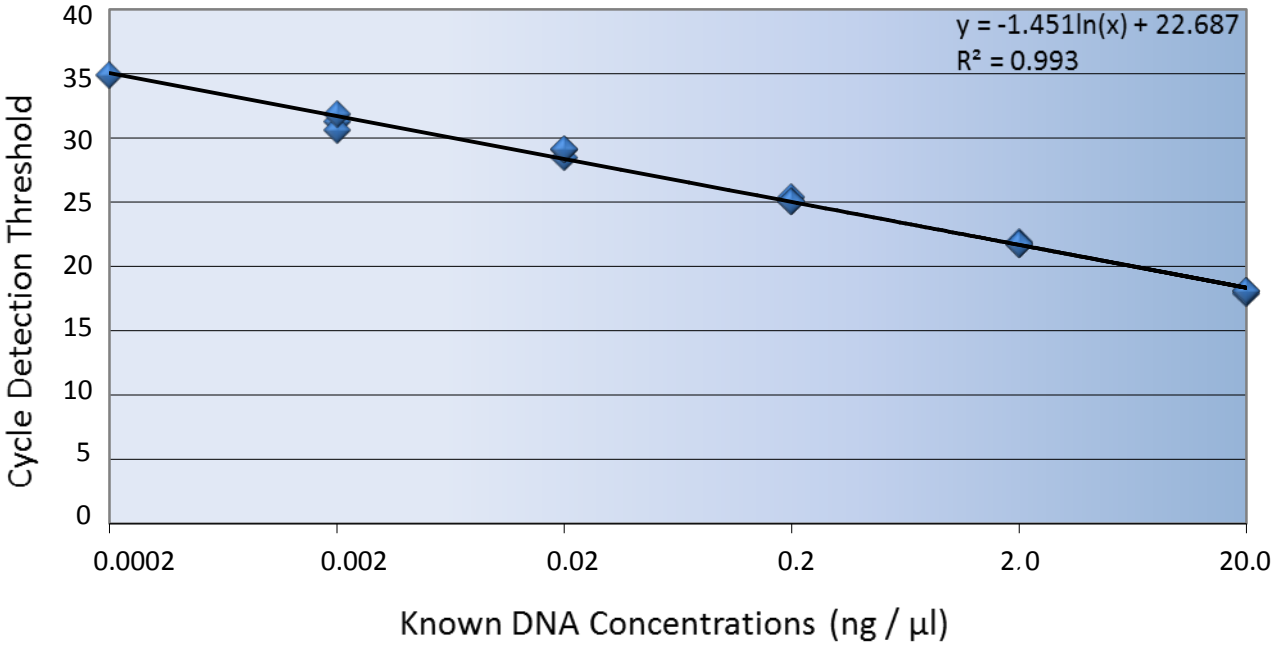

Figure 5. Standard curve relating cycling threshold with known concentrations of brook trout DNA.

Table 3 Summer systematic sampling, August 1-2, 2011. Transect ID are grid locations shown in Figure 1. Latitude and Longitude are spatially referenced as described in methods. Depth is relative to lake water level at staff gauge (7475.6 feet). C(t) is cycler threshold value for qPCR reaction, with any value ≤ 38 being a positive detection of brook trout DNA and smaller values representing greater DNA concentration. DNA is quantity of brook trout DNA contained within 1-L water samples.

	Transect	Mid-depth Composite							
Sample No.	ID	Sampling Depth (ft)			Latitude	Longitude	Lake Depth (ft)	C(t)	DNA [mg/L]
1	1A	0-3	4	8	44°17'3.58"N	118°41'2.59"W	8.6	35.37	0.00016
2	1B	0-3	3.75	6.5	44°17'2.98"N	118°41'3.81"W	7.5	No Detection	
3	2A	0-3	6.6	12.3	44°17'3.27"N	118°41'0.30"W	13.3	No Detection	
4	2B	0-3	4.5	8	44°17'2.58"N	118°41'1.50"W	9.1	No Detection	
5	2C	0-3	5.5	10	44°17'2.04"N	118°41'2.65"W	10.9	No Detection	
6	2D	0-3	5.1	9	44°17'1.22"N	118°41'3.83"W	10.3	No Detection	
7	3A	0-3	5	9	44°17'2.38"N	118°40'58.82"W	10	No Detection	
8	3B	0-3	6.6	10	44°17'1.75"N	118°40'59.78"W	11.3	No Detection	
9	3C	0-3	6.5	10	44°17'1.10"N	118°41'0.96"W	11	No Detection	
10	3D	0-3	6.6	10	44°17'0.48"N	118°41'2.06"W	11.3	37.75	0.00003
11	3E	0-3	5.3	9.5	44°16'59.88"N	44°16'59.88"N	10.6	No Detection	
12	3F	0-3	4		44°16'59.26"N	118°41'4.22"W	5	No Detection	
13	4A	0-3	4.5	8	44°17'1.38"N	118°40'58.19"	9.1	No Detection	
14	4B	0-3	5.2	9.5	44°17'0.69"N	118°40'59.07"W	10.4	No Detection	
15	4C	0-3	5.3	9.5	44°17'0.08"N	118°40'59.97"W	10.6	36.12	0.00010
16	4D	0-3	4		44°16'59.39"N	118°41'0.91"W	5	No Detection	
17	5A	0-3	3.5	6	44°17'0.80"N	118°40'57.13"W	7	No Detection	
18	5B	0-3	4.4	7.8	44°17'0.10"N	118°40'58.18"W	8.8	No Detection	
19	5C	0-3	3.1	5.2	44°16'59.36"N	118°40'59.15"W	6.2	No Detection	
20	4.5aux	0-3	6.3	5.7	44°16'59.31"N	118°41'2.88"W	6.7	No Detection	
Cooler Control	-	-	-	-	-	-	-	No Detection	
Sample Control	-	-	-	-	-	-	-	No Detection	
Elution Control	-	-	-	-	-	-	-	No Detection	
Filtration control	-	-	-	-	-	-	-	No Detection	
Positive Control	-	-	-	-	-	-	-	15	

Table 4 Fall spawning area sampling, October 14, 2011. Spawning area locations are shown in Figure 3. Transect distance is interval between collections in meters, from west to east direction. C(t) is cycler threshold value for qPCR reaction, with any value ≤ 38 being a positive detection of brook trout DNA and smaller values representing greater DNA concentration. DNA is quantity of brook trout DNA contained within 1-L water samples.

Sample No.	Spawning Area	Transect Distance (m)	C(t)	DNA [mg/L]
21	1	12.50	No detection	
20	1	25.00	No detection	
19	1	37.50	No detection	
18	1	50.00	No detection	
17	1	62.50	No detection	
16	1	75.00	No detection	
15	1	87.50	No detection	
14	2	10.25	No detection	
13	2	20.50	34.09	0.00039
12	2	30.75	No detection	
11	2	41.00	No detection	
10	2	51.25	No detection	
9	2	61.50	No detection	
8	2	71.75	No detection	
7	3	11.75	36.96	0.00005
6	3	23.50	35.37	0.00016
5	3	35.25	36.06	0.00010
4	3	47.00	No detection	
3	3	58.75	38.03	0.00003
2	3	70.50	No detection	
1	3	82.85	No detection	
Cooler Control	-	-	No detection	
Sample Control	-	-	No detection	
Elution Control	-	-	No detection	
Filtration Control	-	-	No detection	
Negative Control	-	-	No detection	
Positive Control	-	-	14.24	

Discussion

During this reporting period, a novel species-specific molecular assay was developed for brook trout and this assay was validated by testing for positive reactivity on known brook trout samples and the absence of reactivity on other common fish species. A molecular diagnostic assay useful for qPCR did not exist for brook trout, which establishes a reliable and sensitive surveillance tool that unambiguously detects brook trout. We also show that this method detects the presence of brook trout DNA in water samples, which expands the capabilities for investigating the presence, distribution, and containment of an invasive species beyond that of more traditional survey approaches (e.g., electrofishing, netting). Overall, we show this method provides a mechanism for rapid reconnaissance and statistically defensible trend analysis for an invasive char species. There are some limitations to eDNA analysis however, and we discuss below what our observations suggest about using the current eDNA study design for quantifying brook trout abundance and make recommendations for analysis improvements. Another beneficial project refinement would be to test the brook trout species ID assay against bull trout tissues. While it is unlikely that the brook trout assay cross reacts with other fish species, it would be beneficial to verify that is the case.

We employed two sampling strategies which give us the opportunity to test the relative effectiveness of each in comparison to hook-and-line sampling completed by BPT biologists. Between the first and second eDNA water sampling events, BPT biologists conducted a hook-and-line capture-recapture survey to estimate brook trout abundance (N=1486 +/- 533). During our summer sampling event, a systematic sampling design was implemented to obtain water samples from High Lake. During our fall sampling, the High Lake shoreline was surveyed at putative brook trout spawning areas. At a minimum, we intended to use these water samples to test whether brook trout DNA could be detected from water samples themselves without physically sampling fish, which was indeed the case. We also intended to use these samples to quantify eDNA abundance. Several key discoveries were made in doing so.

Development of the standard curve relating cycling threshold with known concentrations of brook trout DNA (Figure 5) was based on replicated analyses at each known concentration. From this analysis, we can interpret the variances of estimated DNA concentration to determine a quantitation limit. Several rules could be used (e.g., Gibbons and Coleman 2001). We chose to use measurements that have a limited relative standard deviation (RSD) of (10%). This definition is also used by the American Chemical Society to limit the span of the 99% confidence interval to about 30% of the concentration value. We also looked for the point along the standard curve about which the prediction error was not biased. On our standard curve, the RSD and prediction criteria occurred at a known concentration of 0.2 mg/L. Analytical results below this value (and above 2×10^{-5} (concentration when Ct = 38)) should be considered reliable for detection of brook trout eDNA; results above this value can be considered reliable for quantification of brook trout eDNA.

All positive laboratory results from the summer and fall sampling should be considered reliable for detection of brook trout eDNA, but not reliable for quantification. Therefore, total eDNA abundance in this lake was not calculated as originally intended. Instead, we will interpret data based on a frequency of detections among samples collected. Both sampling strategies employ randomization processes that

support statistical testing of differences of these proportions—between sampling methods and between events. Contingency table analysis (see Sheskin 1997) is an appropriate inferential statistical test for two independent samples of categorical data (e.g., detect v. non-detect). Proportions can also be used as indices for comparing trends found in associated hook-and-line sampling conducted by BPT biologists.

From sampling completed in 2011, the proportion of samples collected using a systematic sampling strategy with brook trout DNA detected was 15.0%. In comparison, the detection rate in samples collected at brook trout spawning areas was 23.8%. These rates are not statistically different ($P>0.05$). This result suggests that there was no difference between the two sampling strategies; however, review of the spatial pattern of detections suggests otherwise. Laboratory results from the spawning ground samples suggest that spawning area 3 may be a higher use area, with 4 of 7 collections having reliable detections of brook trout DNA (a 57% detection rate). Tribal biologist noted the presence of grass offshore from spawning area 3 and some sites were less than a foot in depth. Two of 3 detections of brook trout DNA observed from the grid sampling were in the vicinity of spawning area 3 (Figure 6). The detection rate at spawning area 3 is significantly greater than that among the samples collected using systematic sampling ($P<0.5$).

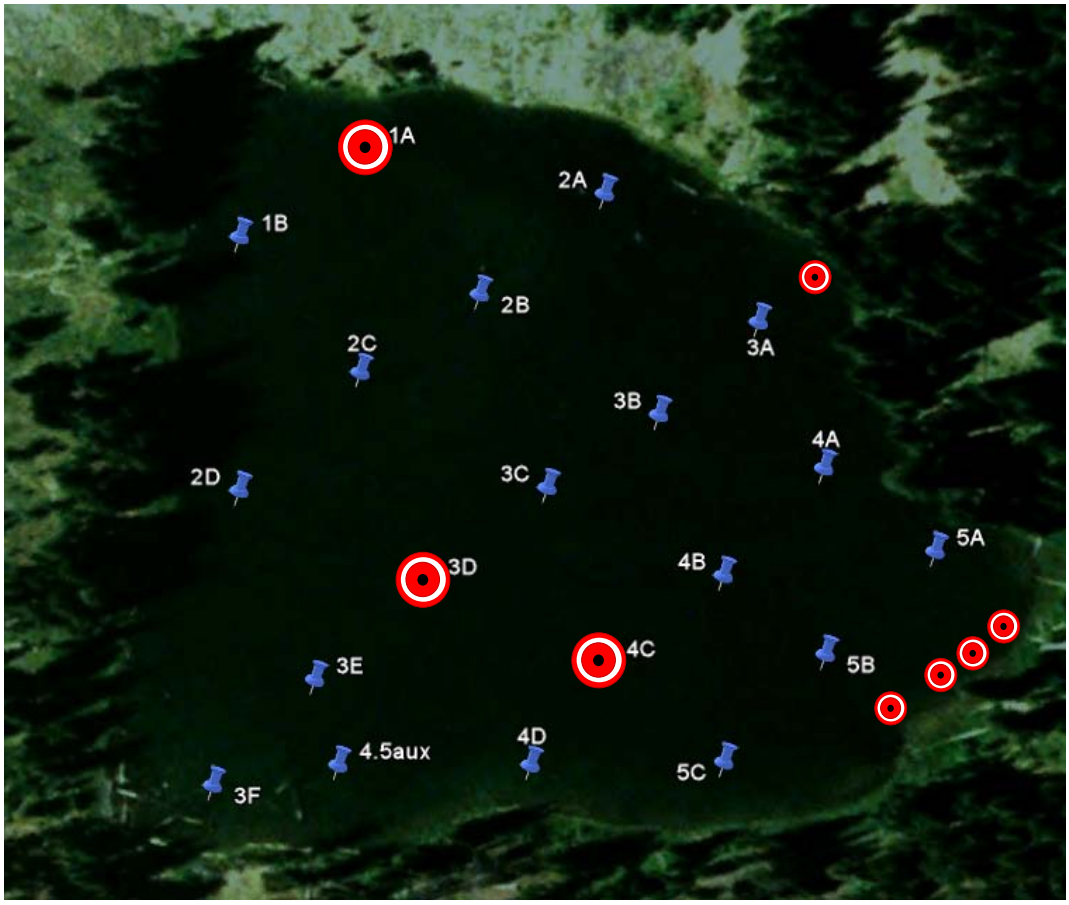

Figure 6. Approximate locations of water samples that tested positive for brook trout DNA.

These results provide the basis for recommendations for future sampling and analysis. In 2012, we recommend that BPT continue sampling following the field and lab protocols established during 2011. Comparable methods will yield comparable analytical results. Minimization of method effects will be of value when comparing results between years. Contingency table analysis (see Sheskin 1997) will be an appropriate inferential statistical test for two independent samples of categorical data (e.g., detect and non-detect categories). These results will be evaluated along with results of hook-and-line capture-recapture surveys conducted by BPT biologists. If possible, the 2012 population survey should be conducted between the first and second eDNA water sampling events as they were in 2011. Population trends will help us interpret the extent to which any differences in the frequency of eDNA detections may be attributable to sampling or laboratory methods or to real changes in the brook trout population.

Our findings also provide a basis for general recommendations for use of brook trout eDNA sampling and analysis. Population estimates from the hook-and-line sampling suggest that brook trout are present at self-sustaining levels. Yet, eDNA was only detected at levels below the quantitation limit. This limited our ability to treat results as continuous variables and calculate total eDNA abundance. The detection threshold is very sensitive for the qPCR brook trout DNA assay, but refinements could be made to the qPCR laboratory methods for brook trout DNA to further increase sensitivity (i.e., $C(t) \leq 40$). Yet, this course of action would not necessarily improve the precision of abundance estimates. Precision could be improved by increasing the total volume in each sample. Although eDNA would still be at low concentrations, more total eDNA would be filtered per collection, thereby lowering the number of cycles needed to detect brook trout eDNA. As we see in Figure 5, few cycles yields more reliable results. Practically, however, this is prohibitive for the concentrations encountered in our samples. Sample volume needs to increase about 1,000x in order to approach the quantitation limit, which is impractical at High Lake.

Therefore, it appears that the greatest use of brook trout eDNA sampling and analysis is for detecting the presence of eDNA. Based on our findings, we do not recommend using these techniques for determining abundances, a conclusion corroborated by other eDNA studies (Ficetola et al. 2008; Jerde et al. 2011). Given enough samples, detection monitoring can be accomplished reliably. Detection monitoring can also support the evaluation of trends from sample event to sample event. Of interest would be testing the hypothesis that the proportion of samples with detectable brook trout eDNA differs among sample events and comparing eDNA detection rate with CPUE at time of brook trout removal. These tests could be conducted using contingency table analysis (see Sheskin 1997). Current sampling intensities and volumes at High Lake are “rule-of-thumb” levels that support such analyses. Greater statistical power could be gained through higher sampling rates, but that is not necessarily recommended at this time. After the 2012 sampling, we will have more information to better to inform such guidance for High Lake, and more generally for similar lentic environments.

Acknowledgements

We would like to thank the Burns Paiute Tribe for information on High Lake brook trout, fall water collections, and invaluable help in the field.

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Appendix 1

FISH DNA SAMPLE COLLECTION PROTOCOL

Genomic Variation Laboratory

Updated 5/2007

1) **Collecting tissue from the fish:** Cut a small piece of fin tissue from the caudal (preferred) or adipose fin of a live fish using clean scissors or a scalpel blade. Hands of the collector should be cleaned of mucus and scales between handling fish, and scissors/knife should be rinsed between samples. Tissue size should be at a minimum 5 sq. mm. (see below †), which is about the size of a hole punch. If the fin is too small to collect this size sample, take a portion of a pelvic fin.

2) **Transferring tissue to storage container:** Each tissue sample is stored separately in individual containers: coin envelopes for dry fin clips, or cryotubes for wet tissues or fin clips.

a. ***Dry fin clips***: It is critical that samples be completely dry in order for DNA extraction in the lab to be successful.

(1) Label a standard scale envelope (unbleached kraft paper) with all relevant details (date, water body, location (latitude and longitude or UTMs if available), species, individual fish identification number, length, weight, etc.)

(2) Air dry the samples on filter paper until all mucus and moisture in the fin has evaporated and the tissue is dry to the touch. Place the fin clip in the envelope and loosely close the envelope. Do not seal the envelope, as air and moisture should be allowed to escape to help the fin sample dry out. Do not rubber-band envelopes together until samples inside are **completely dry**.

b. ***Wet tissue***: Alternatively, collected tissues may be deposited into a preservative-filled (typically DMSO or 95% ethanol) cryotube. It is crucial that wet tissue samples be completely immersed and not exposed to air (vial should be filled to the top). Exposure of alcohol-stored tissue to air can cause cell wall fracturing and loss of DNA into the liquid buffer. A minimum 10:1 ratio of preservative to tissue is desired.

(1) Place the fin clip into a small glass or plastic vial containing high strength (80% to 95%) ethanol. The ethanol will preserve the tissue and the DNA at room temperature, so does not need to be refrigerated.

(2) Label each vial with a permanent (Sharpie) marker. Ensure each sample can be identified later by including the following information on each label: locality, sample number, collection date, and species. (see below example §).

3) **Recording data:** The date of collection, detailed locality information (accurate description of locality is critical – include GPS info if possible), collector(s) name, species, subspecies, type of collection (e.g. fin clip), fork length, and sex, should be written on data sheets. Use the following abbreviations for species identity: CAGT = California golden trout, LKGT = Little Kern golden trout, KRRT = Kern River rainbow trout, and RBT = rainbow trout. Use "CAGT/RBT" format to indicate fish that clearly appear to be hybridized with rainbow trout.

4) **Storing samples:** Samples must be kept out of extreme sun/heat (e.g. dashboards, hot warehouses), especially those in ethanol, as this may damage the DNA.

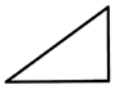
5) **Shipping samples:** Repackage dried fin clips separately and attach field notes for shipping. Dry samples can be sent surface mail with no special packaging.

† *approximate* size of fin clip:

§ sample cryovial label:

S. Fork Kern R. @Kennedy Mdw. #12 7/26/2004 CAGT

n clip:



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7/26/2004 CAGT