Evaluate the Life History of Native Salmonids in the Malheur Subbasin

Burns Paiute Tribe, Natural Resources Department, Fisheries Program

Project # 1997-019-00, Contract # 56472

For work completed 04/12-03/13

FY 2012 Annual Report

Prepared for

Bonneville Power Administration
Northwest Power and Conservation Council

Background

In 2012 the Burns Paiute Tribe Natural Resources Department Fisheries Program completed multiple complementary statements of work via funding through four separate federal contracts. In this report we summarize and interpret the work funded through Bonneville Power Administration Project No. 1997-019-00 and through one additional Bureau of Reclamation Native Affairs Program contract. Analysis of data collected through additional contracts will be included in future annual reports. These additional contracts include a second Bureau of Reclamation Native Affairs contract and an award from the National Marine Fisheries Service Pacific Coastal Salmon Recovery Fund.

The Bonneville Power Administration Statement of Work for FY2012 included the fifth and final year of electrofishing surveys for redband trout abundance and distribution throughout the basin; continuous temperature monitoring on the Logan Valley Wildlife Mitigation Property; completion of brook trout population estimates and removal via electrofishing at random sites in Lake Creek; and the second year of operation of a rigid weir on Lake Creek above Lake Creek Youth Camp.

Through a separate contract with the Bureau of Reclamation Native Affairs Program, BPT completed the second year of evaluating the experimental use of environmental DNA (eDNA) to monitor brook trout populations in High Lake (FY2012-2014 award). This project occurred in partnership with Cramer Fish Sciences in Gresham, OR.

2012 field data collection not reported in FY2012 Annual Report:

There was no spring bull trout trap and haul in the tailrace of Agency Valley Dam in 2012. The contract is conditional and occurs only when water flow occurs over the spillway. This did not occur in 2012 as water stored at Beulah Reservoir did not reach full capacity prior to release of water through flow valves for downstream irrigation.

An additional Bureau of Reclamation (Native Affairs program) was utilized to complete the third year of a 2010 project to evaluate the use of mechanical methods to remove nonnative brook trout in High Lake, the historic seed source for Upper Malheur brook trout populations. In July-August 2012, the Burns Paiute Tribe removed brook trout in High Lake via gillnetting, angling, and electrofishing. The results of the third year will be reported in future annual reports as a cumulative, multi-year analysis and will include recommendations for future removal efforts.

A FY2011 grant was awarded through National Marine Fisheries Service Pacific Coastal Salmon Recovery Fund (PCSRF) to continue to evaluate the suitability of habitat in the Malheur River for Chinook salmon reintroduction. These funds were used in 2012 to collect habitat data using USDA Forest Service Level II stream survey protocol and utilizing local Forest Service staff trainings. The intent of this type of data collection was to isolate putative suitable spawning and holding areas for adult Chinook salmon.

FY2010-FY2012 efforts that have occurred under PCSRF will be cumulatively reported in a future annual report to Bonneville Power Administration and will include recommendations on completing anadromous outplantings in the Malheur River. This grant was not awarded to the Burns Paiute Tribe for FY2012.

The 2012 Burns Paiute Tribe Fisheries Program staff 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), Keith Kennedy (Seasonal Fisheries Technician), and Robert Wilson (Seasonal Biological Technician).

Erica Maltz, Fisheries Program Manager
22 June 2013

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Chapter 1

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

Drew Harper
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Burns, OR

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

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

1.1 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. 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 isolated 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 to the Upper Malheur watershed. In 2011, brook trout suppression efforts, in the form of electrofishing removal and seasonal weir operation, 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 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 (Harper 2012).

The elements of brook trout suppression in Lake Creek were essentially the same in 2012 as 2011, with a few adjustments. In 2012, the objective of estimating abundance of brook trout via electrofishing in Lake Creek was added. To that end, removal of brook trout took something of a subservient role. Also, because of an observed spike in captures of downstream migrating brook trout in October 2011 (Harper 2012), the decision was made to PIT tag brook trout exhibiting similar behavior in 2012. The following describes the methods and results of the Lake Creek brook trout suppression efforts of 2012.

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 barriers to upstream fish passage (Harper, personal observation). Brook trout are the only fish species present above Lake Creek Falls (Fenn 2003). Below Lake Creek Falls, Lake Creek is characterized by moderate gradients (2-5%) (Harper, personal observation) 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). Therefore, brook trout suppression efforts are currently being focused on the Lake Creek watershed from the weir upstream to High Lake. Fish species present in Lake Creek below Lake Creek Falls include brook trout, bull trout, brook trout x bull trout hybrids, redband trout (*Oncorhynchus mykiss gairdneri*), and sculpin (*Cottus sp.*).

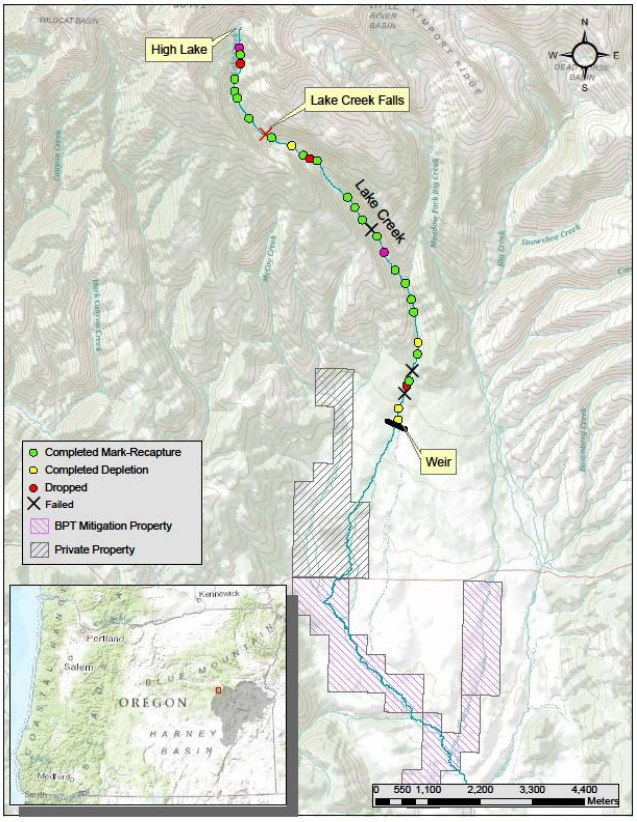

Figure 1. Map showing weir location, electrofishing sites, and property boundaries.

1.2 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 future genetics investigations. Bull trout and hybrids larger than 60 mm were anesthetized in 50 mg/L MS-222 (Finquel®) and implanted intraperitoneally with a 12 mm x 2.15 mm HDX PIT tag (Texas Instruments®). Additionally, some downstream migrant brook trout were PIT tagged. Those brook trout were anesthetized using clove oil. Tagged fish were then allowed to recover in freshwater before being released.

Electrofishing

Smith-Root® models 12B and LR24 backpack electrofishers were used to conduct mark-recapture and multiple-pass depletion methodologies at randomly selected sites in Lake Creek. Sites were selected using GIS and the *spsurvey* package (Kincaid and Olsen 2012) for use in R software (R Development Core Team 2011), which generates a random, spatially-balanced site draw within a sample frame using the Generalized Random Tessellation Stratifed (GRTS) design described by Stevens and Olsen (2004). The sample frame was established as the 14.5 km length of Lake Creek from the location of the seasonal weir to High Lake (Figure 1). Thirty sites were selected for completion in an attempt to obtain as accurate abundance estimate as possible when extrapolating site estimates to the sample frame.

Crews navigated to sites using a hand-held GPS unit. Site lengths were calculated by multiplying the average wetted width by 30 and UTM locations for start and endpoints were recorded. Thirty meters and 100 meters were established as the minimum and maximum site lengths, respectively. Block nets were set at upstream and downstream boundaries. Mark-recapture methodologies were attempted at each site, however, if a block net failed overnight, it was reset and multiple-pass depletion methodologies were conducted. For both methods, a single pass was comprised of a slow, deliberate upstream sweep from the lower block net to the upper and a ½-effort sweep back downstream to the lower block net with one electrofisher and one to three netters. All fish captured via electrofishing were measured (fork length; FL), enumerated, and identified to species (except sculpins). Native species were released outside of the site. Bull trout were inspected for PIT tags and/or a secondary mark (adipose clip). If the number of total fish captured was low, bull trout were retained and PIT tagged. When captures were high, bull trout were immediately released at the nearest block net and their length was estimated when possible in order to reduce stress on those fish.

Mark-recapture methodologies were conducted over two days, with a marking-run on the first day and a recapture-run the second day. The marking-run consisted of a single pass. Brook trout captured on the marking-run were marked by a right pelvic fin clip. The recapture-run consisted of a three-pass depletion survey. Four passes were made if brook trout captures were not reduced by at least 50% in each successive pass for the first three passes. At sites where mark-recapture failed, depletion surveys were conducted in the same manner as the recapture-run.

Data Analysis

Data from the brook trout suppression efforts were analyzed for use in several applications: 1) to establish a baseline dataset to be used in comparisons with subsequent efforts, 2) to assess the efficacy of the electrofishing removal method, 3) to estimate total brook trout abundance between the weir and High Lake, and 4) 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 at each electrofishing site using the total number of a species captured divided by the length of the site. Due to imperfect capture probabilities associated with our methodology, these estimates are considered to represent minimum densities.

Capture efficiency of brook trout, defined here as the proportion of the total number of brook trout present that are captured in a sample, was calculated to assess the efficacy of the electrofishing removal method. Capture efficiency for a site was calculated as the number of brook trout caught divided by the abundance estimate for that site. Abundance estimates for mark-recapture sites were calculated using a variation of the Chapman modification of the Peterson equation described by Ricker (1975). Captures from the recapture-run were pooled to serve as the second sample data. The generalized removal method described by White et al. (1982) was used for depletion estimates. These estimates were calculated using scripts written for the *fishmethods* package (Nelson 2011) in R (R Development Core Team 2011). Also, in order to promote maximum efficiency in future removal efforts, capture efficiency was calculated for each pass. Capture efficiency per pass was calculated as

$$CE_{Pi} = P_{ni}/(N-P_j)$$

where CE_{Pi} = capture efficiency for Pass *i*, P_{ni} = brook trout captured in Pass *i*, N = estimated abundance, and P_j = total removals prior to Pass *i*.

Total brook trout abundance in Lake Creek between the weir and High Lake was estimated by extrapolating individual site densities. This was accomplished using scripts written for the *spsurvey* package (Kincaid and Olsen 2012), which takes advantage of the Local Neighborhood Estimator of variance described by Stevens and Olsen (2004).

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 trout species, from data collected at the weir and through electrofishing. Where applicable, these data were compared to data from 2011.

1.3 Results

Weir

In 2012, the temporary weir on Lake Creek was installed on 18 May and removed for the winter season on 8 November. The weir was operational (i.e., trapping) for 162 of the 175 days between the aforementioned dates. The weir was modified to allow water and, consequently, fish passage due to high flows and associated safety concerns from 22 May through 27 May and because of a wildfire from 31 August through 7 September.

Five species of fish were captured over the 162 days the weir was operational, including brook trout (n=155; 139 removed, 16 downstream migrants PIT tagged), redband trout (n=20), bull trout (n=9), brook trout/bull trout hybrids (n=2), and sculpin (n=8). The majority of fishes were captured in the downstream trap (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; brook trout captures peaked in October in the downstream trap. These patterns of redband and brook trout captures are similar to those observed in 2011 (Harper 2012). Bull trout captures did not show a defined peak, although all bull trout captures occurred between June and July (Figure 2).

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

Species	US	DS
Brook Trout	59	96
Bull Trout	5	4
Hybrid Brook/Bull	1	1
Redband Trout	5	15
Sculpin	0	8

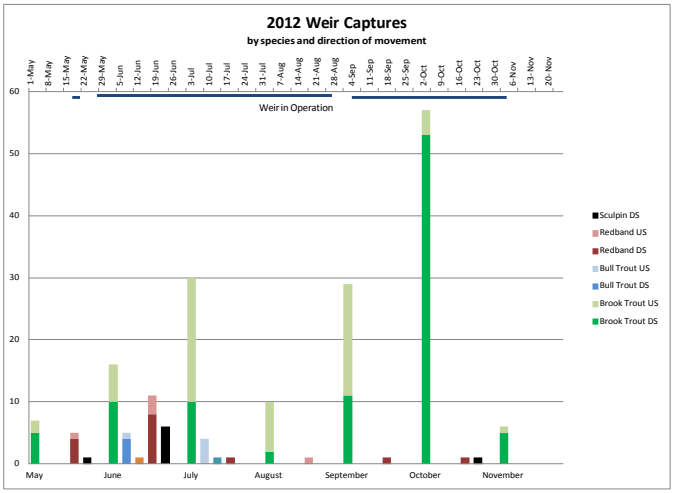

Figure 2. Monthly weir captures by species and direction of movement. Blue bar at top indicates periods of weir operation. US=upstream migrants; DS=downstream migrants.

Length-frequency histograms were created from weir capture data for brook trout and bull trout. Length-frequency histograms from the weir captures are included in Appendix A.

Electrofishing

Electrofishing in Lake Creek began on 26 June and ended on 2 October. Sites below Lake Creek Falls were completed prior to August 15 to avoid interruption of bull trout spawning. Twenty-five sites were visited; 18 of which were completed as mark-recapture, four were completed as depletion, and three sites were failed due to problems maintaining closure of the population or equal effort (Figure 1). The remaining five sites from the original sample of 30 were dropped because they were located within the boundaries of another site (four) or were unable to be sampled (one; flow was subsurface).

Five species of fish were captured in the electrofishing effort, including brook trout (n=1,386), bull trout (n=57), bull x brook hybrid (n=34), redband trout (n=58), and sculpin (n=443). Of the 1,386 brook trout captured by electrofishing in 2012, 1,093 were removed from the system. The remaining 293 were marked but not recaptured.

The density of each salmonid species was calculated for each electrofishing site. To facilitate comparisons between species, the densities were calculated from raw catch data (not abundance estimates) because abundance estimates were not calculated for species other than brook trout. Brook trout densities were the highest of all species at all sites. However, the magnitude by which brook trout dominated the catch varied by site (Figure 3). Electrofishing captures are summarized in Appendix C.

Electrofishing capture efficiency of brook trout was calculated at each site in order to help guide future brook trout removal efforts. Cumulative capture efficiency, calculated using the total brook trout captures at a site divided by the estimated abundance at that site, ranged from 0.39 to 1.0.

The analysis of capture efficiency per pass revealed a stark contrast in the trend of the calculated values for depletion versus mark-recapture methodology. Specifically, calculated capture efficiency increased with each pass for depletion sites, while it decreased for mark-recapture sites (Figure 4). It appears this difference stems from the fact that the depletion estimates were equal to (or very close to) the total number of brook trout captured (i.e., sample size = abundance estimate). Consequently, capture efficiency, as calculated here, would tend toward 1.0 with each successive pass because most, if not all, of the abundance estimate would be caught by the end of Pass 2. The decrease in capture efficiency with each successive pass apparent from the mark-recapture data (Figure 4), which is thought to be less biased than the depletion data (Rosenberger and Dunham 2005), has implications for developing an effective approach to electrofishing removal efforts.

Brook trout abundance estimates were calculated from electrofishing data for each site, which were used to calculate an extrapolated abundance estimate for the entire sample frame. Estimates at individual sites ranged from 16.2 (95% CI = 13.1 to 19.2) to 261.3 (95% CI = 199.6 to 323.1) (Figure 5). The extrapolated abundance estimate is 11,797 (95% CI = 9,362 to 14,232) brook trout between the weir and High Lake.

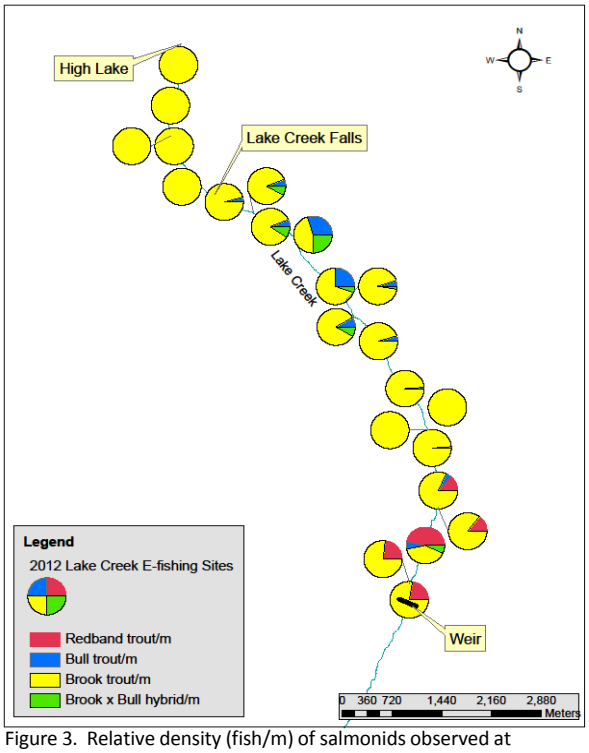

Figure 3. Relative density (fish/m) of salmonids observed at electrofished sites in Lake Creek, 2012.

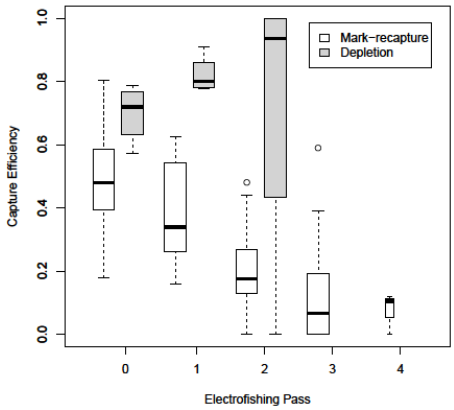

Figure 4. Capture efficiency of brook trout per electrofishing pass, calculated as the number of brook trout captured in a pass divided by the abundance estimate minus total removals from previous pass(es). Mark-recapture: n = 17, Depletion: n = 4

Length-frequency histograms created from electrofishing data revealed a multimodal distribution of lengths in the brook trout population (Appendix B). This multimodal length distribution is likely related to age class distribution. As such, the majority of brook trout captured appear to have been between the ages of 1 and 3 years old and, not surprisingly, few age-0 brook trout were captured, which is likely a factor of decreased capturability of fish <60 mm in length.

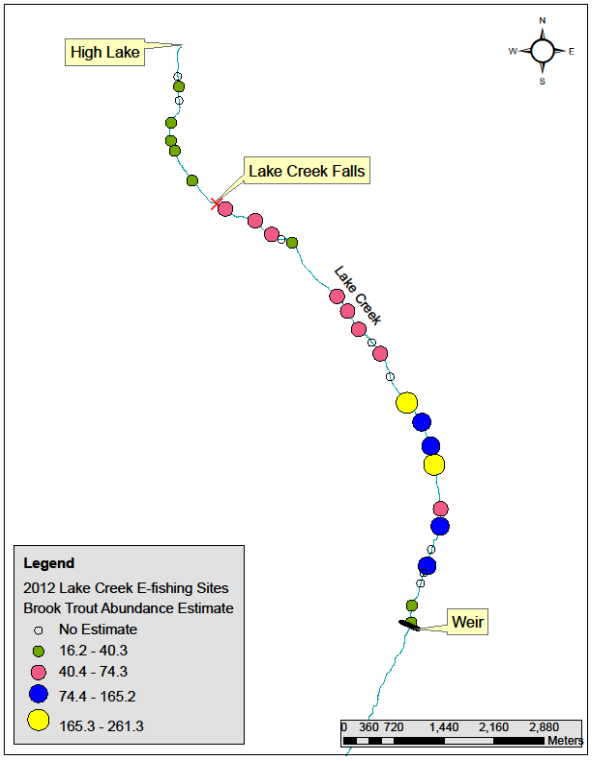

Figure 5. Estimated abundance of brook trout at electrofished sites in Lake Creek, 2012.

PIT Tagging

Six bull trout and 16 brook trout were PIT tagged at the weir in 2012. Two of the bull trout were tagged after being captured in the upstream trap and four were tagged after capture in the downstream box. All 16 brook trout that were captured in the downstream trap in October were tagged. In order to minimize the stress associated with capture and handling, only three fish were PIT tagged after being captured by electrofishing. Two of those fish tagged were bull trout and the third was a brook x bull trout hybrid. Between the 2011 and 2012 field seasons 21 bull trout, 16 brook trout, and six hybrids have been implanted with PIT tags in Lake Creek.

1.4 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 reaches downstream of Lake Creek Falls, which has been determined to have the highest occurrence of bull trout/brook trout hybrids in the Upper Malheur (DeHaan 2009). The suppression efforts conducted in 2012 included the operation of a seasonal weir and electrofishing. Though these methods remain 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 provide a few insights into the demographics and dynamics of fish populations there. Furthermore, the analysis of electrofishing capture efficiency provided information that should prove valuable in maximizing the efficiency of future brook trout removal efforts.

Through the combined efforts of operating a weir and electrofishing, 1,541 brook trout were captured in Lake Creek in 2012. Of those captured, 1,232 were euthanized and removed from the system. Brook trout that were captured but not removed were either PIT-tagged (n = 16) or were captured in the mark-run at an electrofishing site where they were marked, released, and not recaptured (n = 293). Though the number of brook trout removed is small relative to the estimated 11,797, it is anticipated that removals will increase substantially in 2013 as we focus our efforts strictly on removing brook trout. To that end, the analysis of capture efficiency offers some insights that will help to shape future implementation in order to maximize the efficiency of ongoing brook trout removal efforts.

Analysis of capture efficiency at mark-recapture electrofishing sites in Lake Creek in 2012 indicated that the first pass at a site had the most 'bang-for-the-buck' in terms of the proportion of the number of brook trout captured to the number of brook trout present (Figure 4). Sites that failed mark-recapture

and, thus, necessitated depletion estimates, showed the opposite trend. The source of the difference was discussed previously (Section 1.3) and is likely a function of underestimation of abundance, which has been widely documented as a shortcoming of depletion methodologies (Peterson et al. 2004). Because mark-recapture estimates are thought to be less biased (Rosenberger and Dunham 2005), results of the mark-recapture analysis will be used to plan future removal efforts.

Baseline data collected in 2012 will permit assessment of the efficacy of brook trout suppression efforts. Such assessments will be possible through comparison of metrics such as estimated brook trout abundance in Lake Creek, density of various species at specific locations, the extent of a given species' distribution, and the frequency of sites occupied by a given species. Decreases in brook trout metrics accompanied by increases in metrics concerning native species would indicate that the brook trout suppression efforts were having desired effects. Though some metrics will likely be compared on an annual basis (e.g., the number of brook trout removed annually or length-frequency of captured fishes), it is expected that multiple years of suppression efforts will be required before a response is detectable in metrics such as species abundance/density. As such, the effort to repeat brook trout abundance estimation is not planned until 2017.

With 2012 being the second consecutive year of weir operation in Lake Creek, some comparisons to 2011 data are possible. First, the number of captures decreased for each brook trout and bull trout, but increased for redband trout and sculpin. Second, the patterns (timing and directionality) of captures for brook trout and redband trout in 2012 were quite similar to those observed in 2011. Namely, the majority of brook trout captures were in the downstream trap in the month of October and the majority of redband trout captures were in the downstream trap in June. The pattern in redband trout captures is likely explained by a post-spawn return to preferred habitats downstream. In contrast, the probable cause of the brook trout pattern is much less clear. An understanding of the mechanism driving the pattern of brook trout captures could be valuable, considering the large proportion of total captures accounted for by downstream captures in October. Harper (2012) suggested a few working hypotheses, however, teasing out the true driver of the apparent downstream migration of brook trout is likely infeasible when one considers the changes to the population resulting from electrofishing suppression activities.

Suppression of the brook trout population in Lake Creek has, thus far, been focused on the removal of individuals captured through mechanical methods. However, in these early stages of the suppression effort, the removal of brook trout has been to some extent impeded by attempts to gather important background information pertaining to the population. For instance, although we were able to remove brook trout via electrofishing, obtaining data for a reliable abundance estimate took precedence in 2012. Acquiring such data by mark-recapture surveys reduced our ability to remove as many brook trout as possible in two ways: 1) Some brook trout that were captured, marked, and released were not recaptured, and 2) Mark-recapture surveys are labor-intensive and time consuming, which limited the length of stream that could be sampled. Nonetheless, the objective of suppressing the brook trout population in Lake Creek through mechanical methods, especially where electrofishing is concerned, should be achieved more efficiently by applying the information gained through this year's efforts in planning future implementation.

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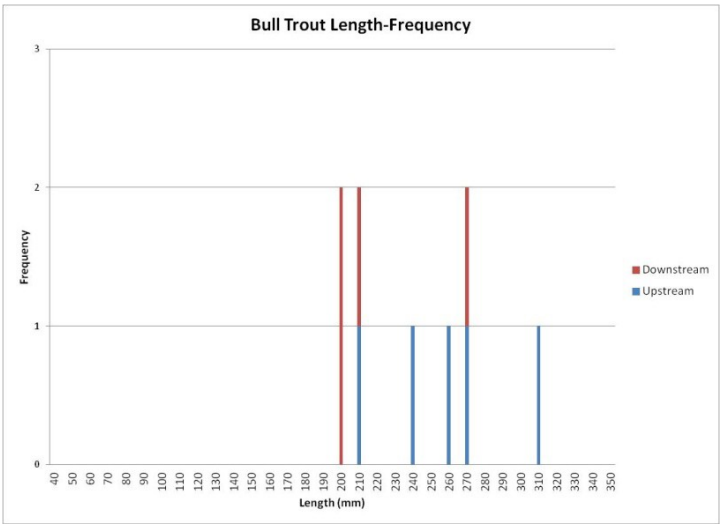
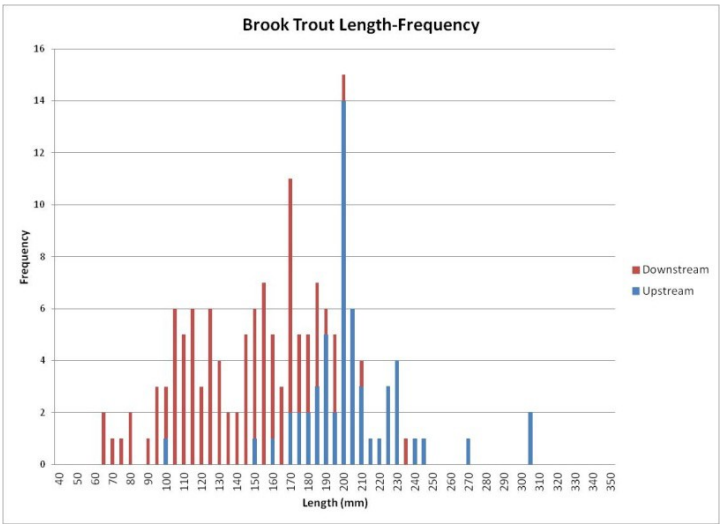
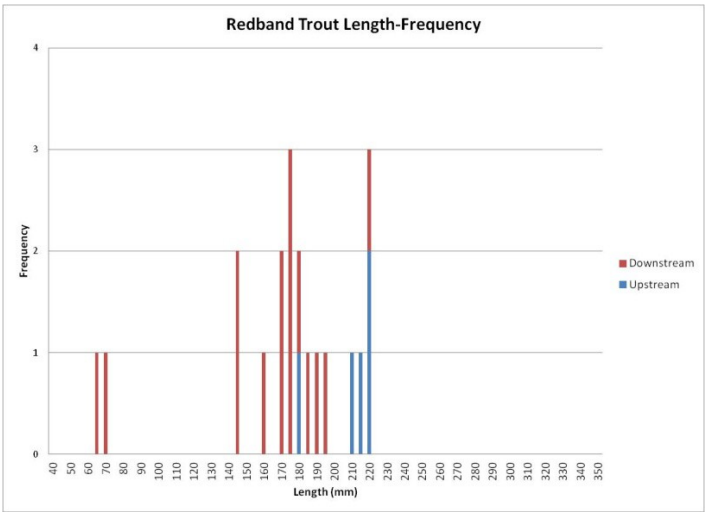
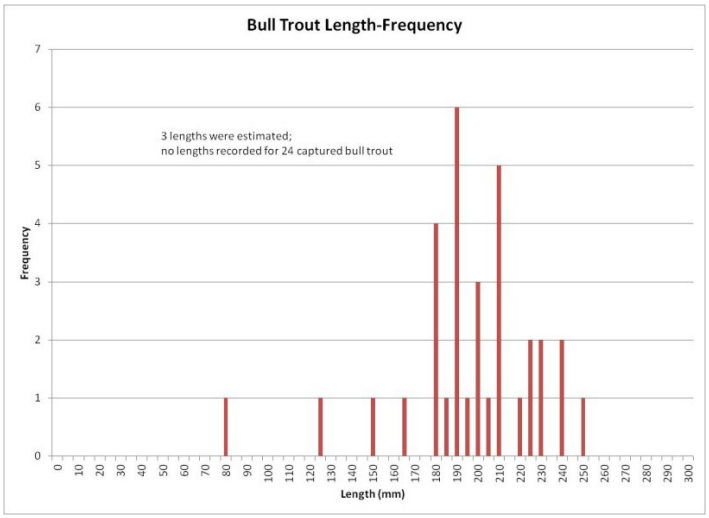
Appendix A. Length-Frequency histograms from weir data.

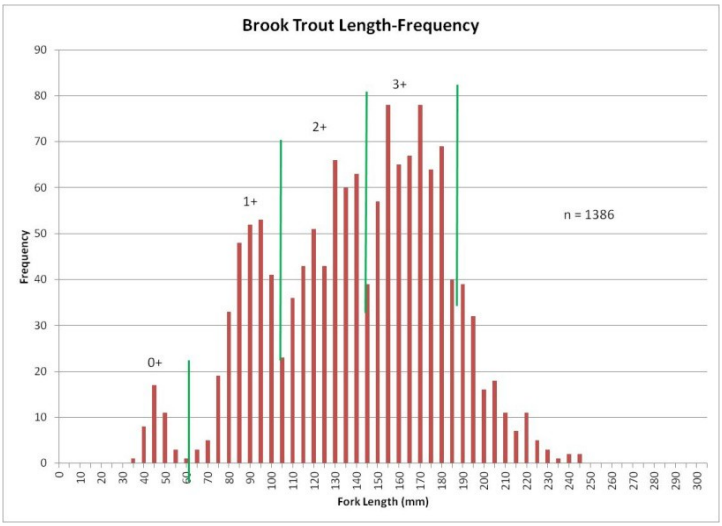


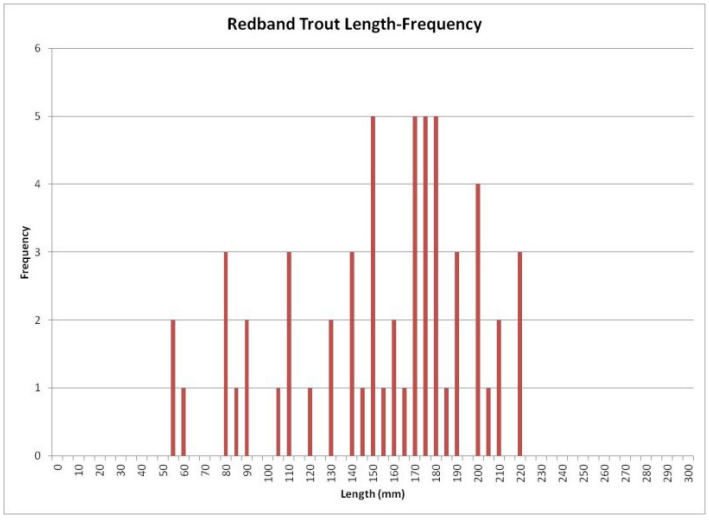




Appendix B. Length-Frequency histograms from electrofishing data. *Note: Brook trout age classes are estimated on that histogram.*







Appendix C. Electrofishing captures summary table.

Species Codes: BT = Brook Trout, BUT = Bull Trout, RB = Redband Trout, HYB = Bull x Brook Trout

Site ID	BUT	BT Not Removed	BT Removed	HYB	RB	BT/m	BUT/m	HYB/m	RB/m
19	2	12	24	1	0	0.360	0.020	0.010	0.000
195	0	2	21	0	0	0.652	0.000	0.000	0.000
291	0	17	31	0	8	0.436	0.000	0.000	0.073
547	0	0	40	0	12	0.400	0.000	0.000	0.120
963	14	7	31	3	0	0.380	0.140	0.030	0.000
1059	0	56	122	1	2	1.835	0.000	0.010	0.021
1571	0	9	12	0	6	0.221	0.000	0.000	0.063
1923	4	7	46	6	0	0.558	0.042	0.063	0.000
1987	4	11	29	4	0	0.426	0.043	0.043	0.000
2339	1	4	43	0	7	0.480	0.010	0.000	0.071
2691	2	8	38	0	0	0.613	0.027	0.000	0.000
2755	0	15	153	0	0	1.527	0.000	0.000	0.000
2947	0	1	14	0	0	0.150	0.000	0.000	0.000
3011	1	11	0	0	0	0.110	0.010	0.000	0.000
3107	3	0	63	0	11	0.612	0.029	0.000	0.107
3363	1	27	33	1	7	0.667	0.011	0.011	0.078
3715	17	6	20	14	0	0.224	0.147	0.121	0.000
3779	2	48	116	0	0	1.640	0.020	0.000	0.000
3971	0	6	22	0	0	0.280	0.000	0.000	0.000
4035	2	14	26	0	0	0.400	0.020	0.000	0.000
4302	0	15	88	0	1	1.212	0.000	0.000	0.012
4451	0	4	12	0	0	0.258	0.000	0.000	0.000
4881	3	0	43	4	0	0.430	0.030	0.040	0.000
4955	0	4	32	0	0	0.422	0.000	0.000	0.000
5077	1	9	34	0	4	0.377	0.009	0.000	0.035
Totals	57	293	1093	34	58				

depletion

incomplete

Chapter 2

Distribution and Abundance of Redband Trout *Oncorhynchus mykiss* and Brook Trout *Salvelinus fontinalis* in Summit Creek, Malheur River Basin, Oregon

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

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Distribution and Abundance of Redband Trout *Oncorhynchus mykiss* and Brook Trout *Salvelinus fontinalis* in Summit Creek, Malheur River Basin, Oregon

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

2.1 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 ± 45,615 redband in the basin (Bangs et al. 2007). After the 2007 estimate, an effort was made to assess trends in the redband population by annually revisiting the sites sampled in 2007. However, a resources shortfall resulted in an inability to resample the entire sample frame on an annual basis. Instead, from 2008-2011, a smaller number of sites were revisited each year in an attempt at gathering population trend data. Unfortunately, the desired trend data was not attainable due to high interannual and among-site variability. The high costs associated with completing work at the level of effort required by even the small annual sample size combined with the low precision of estimates resulted in a change of approach to redband assessments in 2012.

In order to facilitate purveyance of useful information to co-managers, the approach to assessing the redband trout population in the Malheur River Basin was changed in 2012 to focus on a more local scale and produce valuable data in a shorter time frame. This was accomplished by narrowing the focus of redband population assessment to Summit Creek, a watershed that has been a focus of Forest Service restoration efforts. Furthermore, BPT conducted population assessments for redband trout and brook trout in Summit Creek in 2001 (Fenton 2003), which provide a point of reference for comparison with species distributions and density values. Because brook trout have been implicated in declines of native aquatic biota (Adams 1999) and the suppression of their populations in the Upper Malheur watershed is a major focus of the BPT fisheries program, assessment of the brook trout population in Summit Creek was added as a secondary objective of this project.

2.2 Study Area

The Summit Creek watershed drains 57,725 m² on the southern flank of the Blue Mountain Province. Summit Creek flows approximately 29 km from its headwaters in the Strawberry Mountains to its confluence with the Upper Malheur River at river kilometer 298 (Figure 1). Several small tributaries join

Summit Creek as it meanders through dense-canopy forest and broad, open meadow habitats. Stream flows in the Summit Creek watershed are highly dependent on snowpack. With the exception of a small parcel of private property in Summit Prairie (Figure 1), the entire watershed is publicly owned and managed by US Forest Service Prairie City Ranger District.

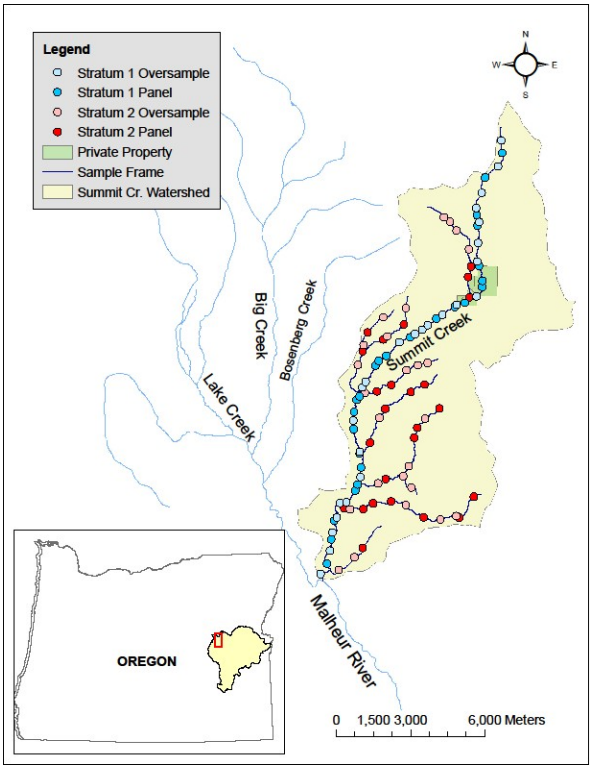

Figure 1. Location map showing Summit Creek watershed and sampling site draw.

2.3 Methods

To assess the redband population in Summit Creek, Smith-Root® models 12B and LR24 backpack electrofishers were used to conduct multiple-pass depletion methodologies at randomly selected sites. Sites were selected using GIS software and the *spsurvey* package (Kincaid and Olsen 2012) for use in R software (R Development Core Team 2011), which generates a random, spatially-balanced site draw within a sample frame using the Generalized Random Tessellation Stratifed (GRTS) design described by Stevens and Olsen (2004). The sample frame included all streams considered to potentially support redband trout within the Summit Creek watershed (Figure 1) and was stratified into two strata. Stratum 1 consisted of the mainstem of Summit Creek (28.968 km of stream); Stratum 2 included tributary streams (27.790 km of stream). Fifty sites were drawn in each stratum, with the intention that 25 sites would be completed in each. Panel sites for each stratum (Figure 1) were to be completed first. However, in the event that a Panel site was dry or could not be accessed (e.g., private property) the other 25 sites in a stratum (Oversample sites) provided replacement sites that maintained spatial balance.

Crews navigated to sites using a hand-held GPS unit. Site lengths were calculated by multiplying the average wetted width by 30 and UTM locations for start and endpoints were recorded. Thirty meters and 100 meters were established as the minimum and maximum site lengths, respectively. Block nets were set at upstream and downstream boundaries. Two/Four-pass depletion methodology was conducted at all sites. That is, two passes were conducted if the number of redband captured in pass 2 was 50% or less of the number captured in pass 1. If pass 2 captures exceeded 50% of pass 1 captures, another two passes (i.e., passes 3 and 4) were conducted and the captures from passes 1 and 2 were pooled to represent pass 1 and passes 3 and 4 were pooled to represent pass 2. A single pass was comprised of a slow, deliberate upstream sweep from the lower block net to the upper and a ½-effort sweep back downstream to the lower block net with one electrofisher and one to three netters. After each pass, all fish captured via electrofishing were measured (fork length; FL), enumerated, and identified to species before being released below the downstream block net. Depletion methodologies also targeted brook trout; however, failure to meet the 50% criteria in the first two passes was not a trigger to conduct passes 3 and 4. Brook trout captured in Summit Creek were euthanized and removed from the system.

Data Analysis

The generalized removal method described by White et al. (1982) was used to obtain depletion estimates of abundance for each trout species at each site. These estimates were calculated using scripts written for the *fishmethods* package (Nelson 2011) in R (R Development Core Team 2011). Abundance estimates were divided by the length of the survey to obtain density values (fish/m). Density values were then used to calculate extrapolated redband trout and brook trout abundance estimates for Stratum 1. This was accomplished using scripts written for the *spsurvey* package (Kincaid and Olsen 2012), which takes advantage of the Local Neighborhood Estimator of variance described by Stevens and Olsen (2004). The length of stream in Stratum 1 was modified to fit the observed upper extent of fish habitat.

Species distributions were estimated by extrapolating from sample sites using GIS. Starting at the mouth of Summit Creek, adjacent sites where a species was present were connected by a line on the sample frame shapefile. The initial point of absence was considered to be halfway between a sample site where presence was observed and the adjacent site upstream where presence was not observed. The species was then considered to be absent for the length of stream up to the halfway point between the next upstream site where presence was again observed and the adjacent downstream site where presence was not observed.

2.4 Results

Forty-eight sites were visited in the 2012 Summit Creek effort (Figure 2). Twenty-six of those sites were in Stratum 1, 22 were in Stratum 2. Trout were present at 25 sites in Stratum 1, the single site where trout were not present was determined to be upstream of the upper limits of fish (Figure 2). Two sites in Stratum 1 had only a single species of trout present (one with only brook trout and one with only redband trout) (Figure 2). Nongame species, including bridgelip sucker, largescale sucker, redside shiner, and speckled dace, were present at 23 sites in Stratum 1. In Stratum 2, seven sites had water but no fish, 13 were dry, and two sites had trout (one with brook trout, one with redband trout) (Figure 2). Nongame fish were not observed in Stratum 2.

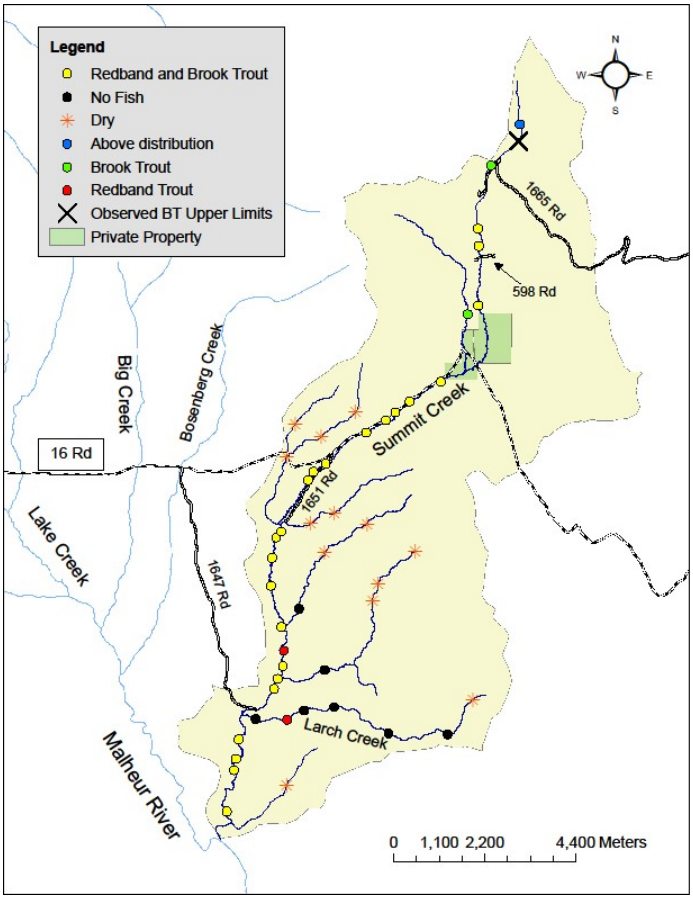

Figure 2. Locations and response values of sites visited in the Summit Creek watershed in 2012.

Depletion estimates of abundance could be calculated for redband trout at 24 of the 25 sites where they were present. One site with redband failed depletion (Summit-023), so the number of captures was used as a minimum abundance estimate. Depletion estimates for brook trout were

calculated for all 25 sites at which they were present. Failure to detect a species at a site resulted in an abundance estimate of zero.

In Stratum 1, densities (fish/m) calculated from abundance estimates ranged from 0 to 1.63 for redband trout and 0 to 2.00 for brook trout. In Stratum 2, densities ranged from 0 to 0.06 for redband trout and 0 to 0.94 for brook trout. Nine sites did not have length values recorded (7 in Stratum 1; 2 in Stratum 2). In such cases, the average width was multiplied by 30 to provide an estimate of site length for calculating density.

Density values were then extrapolated to the sample frame for Stratum 1 to obtain overall average density and abundance estimates for Summit Creek. Table 1 gives the extrapolated estimates. Estimates from the 2001 assessment of Summit Creek salmonid populations (Fenton 2003) are also included in Table 1 for comparison.

Table 1. Redband trout and brook trout abundance and density estimates for 2012 and 2001. Data for 2001 was taken from Fenton (2003).

	2012		2001	
Species	Abundance (±95% C.I.)	Density (fish/m)	Abundance (±95% C.I.)	Density (fish/m)
Redband Trout	12462 (±3261)	0.45 (±.12)	7756 (±1702)	0.353
Brook Trout	11996 (±2980)	0.43 (±.11)	2186 (±1716)	0.099

2.5 Discussion

Assessment of the redband trout population in the Malheur River Subbasin was pared down in 2012 in comparison to the previous four years. This was done because, despite the large amount of effort put forth in previous years, the resulting information was not of a quality useful to fishery managers. The study design employed in 2012 was intended to provide managers with information on abundance and distribution of salmonid species in Summit Creek, a watershed where BPT has conducted population assessments in the past (Fenton 2001) and USFS has ongoing riparian restoration activities. The current assessment of salmonid populations in Summit Creek provides information on how the fishery has changed over 11 years. Furthermore, Summit Creek has been identified as a potential target for chemical treatment to remove brook trout and restore native fisheries. An up-to-date evaluation of the trout populations will permit managers to make better informed decisions concerning the future of this watershed and its fisheries.

Although differences in study design between 2001 and 2012 require caution when comparing abundance and density estimates, changes in relative abundance and distribution of redband trout and brook trout are evident. Though 2/4 pass 50% depletion methodologies were applied in both years, the methods for selecting sample sites and extrapolating data from the sample sites to the length of Summit Creek differed. In 2001, sampling began at the confluence; sample sites were 50 meters in length and separated by 500 meter intervals (Fenton 2003). In 2012, sample sites were randomly selected and the length of a sample site was dependent upon the wetted width at each site, as described previously in the Methods section of this report. The methods for estimating overall abundance and average density in Summit Creek in 2001 were not described by Fenton (2003). Therefore, caution must be used in comparing those estimated values between years. However, after inspection of Table 1, one conclusion that can be arrived at with confidence is that brook trout have increased in abundance relative to redband trout. This conclusion is further validated by the change in distribution of brook trout between 2001 and 2012.

The distribution of brook trout in Summit Creek has increased dramatically since 2001. In the 2001 study, only one brook trout was found between the mouth of Summit Creek and site 17 (Fenton 2003), which by doing the simple math, would have been around river kilometer 8.5. The brook trout that was captured was in site 2 (Fenton 2003), which would have been around river kilometer 0.6. Therefore, in 2001, there was an approximately 8 km section of Summit Creek where only native species were present (Figure 3). This has shrunk to approximately 0.7 km in 2012 (Figure 3). Redband trout distribution, on the other hand, appears largely unchanged. This species was detected at all but one site in both 2001 (Fenton 2003) and 2012. In both years, the only site where redband were not detected was the uppermost site sampled.

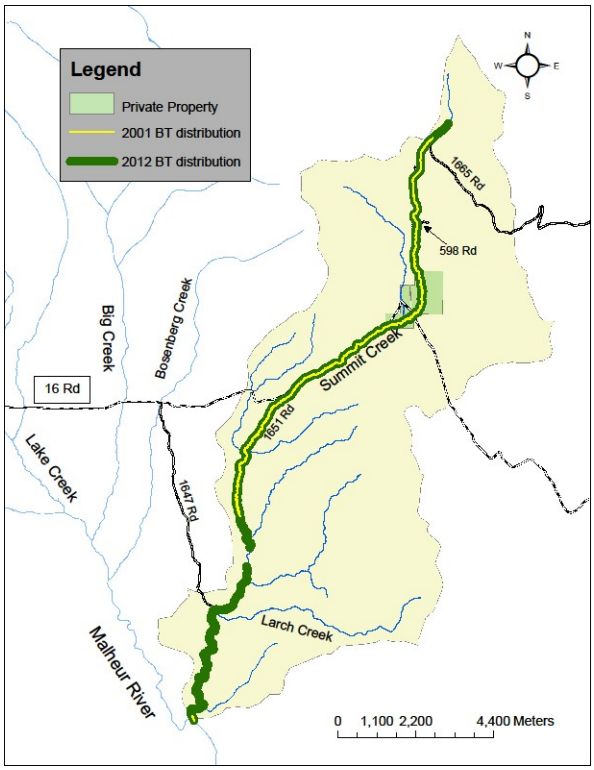

Figure 3. Comparison of brook trout distribution in 2001 and 2012.

The assessment of salmonid populations in Summit Creek has highlighted some important changes that have taken place in the fishery. The apparent expansions in range and abundance of brook trout in Summit Creek should be of great interest to fishery managers. Although redband trout appear to remain the most abundant salmonid species to date, it is by a small degree and if current trends continue brook trout may begin to depress the redband population. Brook trout have been widely implicated in declines of native aquatic biota (Adams 1999) and were observed in this study to prey on redband (one brook trout captured had a redband trout in its mouth). As such, in order to preserve the redband population in Summit Creek, fishery managers should strongly consider implementing management practices to suppress or eradicate brook trout in Summit Creek.

2.6 References

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Chapter 3

2012 Stream Temperature Monitoring in the Upper Malheur

Logan Valley Wildlife Mitigation Property, 2012

Daniel Brown
Burns Paiute Tribe Natural Resources Department
Burns, OR

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2012 Stream Temperature Monitoring in the Upper Malheur
Logan Valley Wildlife Mitigation Property

Daniel Brown, Fisheries Technician
Burns Paiute Tribe Natural Resources Department

3.1 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 where useable data was collected spent over 90% of the summer period with MWMT exceeding 12° C, and spent over 70 days out of 120 in excess of 16° C. Lake Creek drainage sites on average spent 38% 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 channel of Lake Creek as it enters the Tribe's Logan Valley property was again dewatered for a significant portion of the summer in 2012. 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.

3.2 Methods

3.2.1 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.

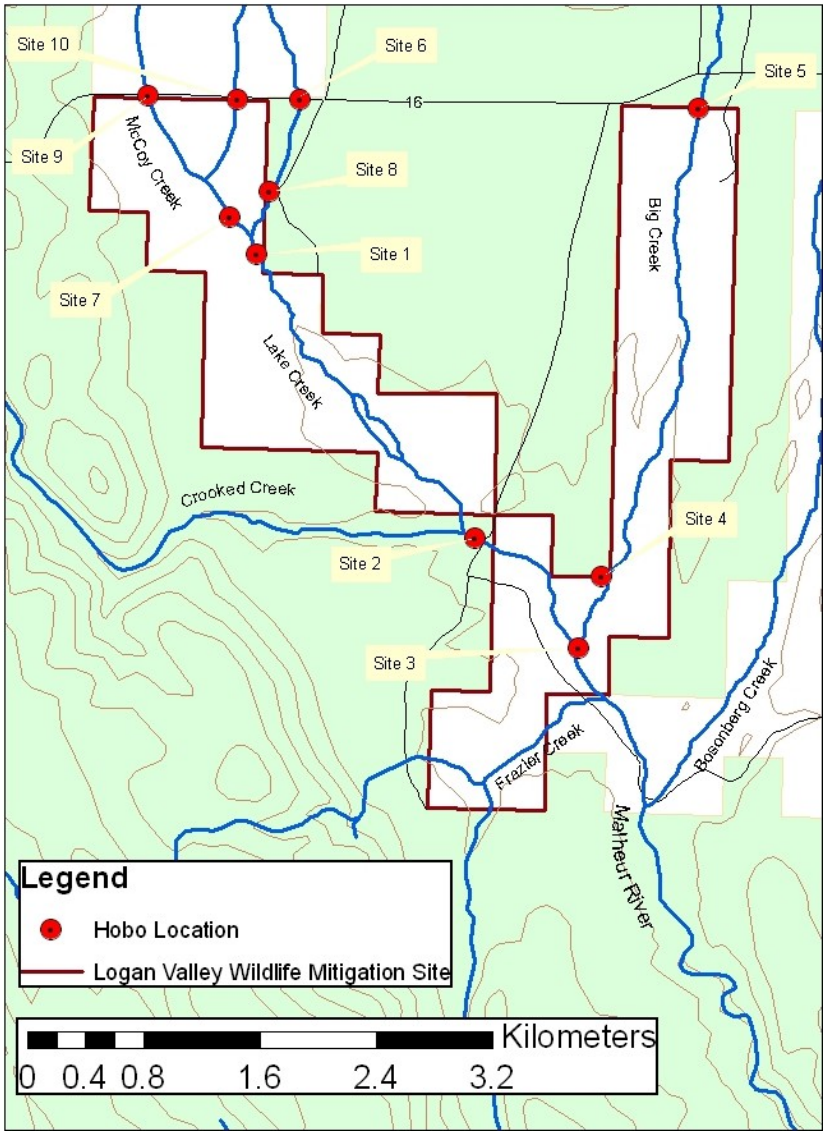

Figure 1: Stream temperature monitoring site locations.

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**

3.2.2 Field Techniques

In 2012, Tidbit v2 Temperature Loggers (hobos) manufactured by Onset Computer Corporation were deployed on the 8th of May and retrieved between the 23rd-29th 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 2012 and will continue to serve as the target date for deployment in the years ahead.

3.2.3 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 2012 MWMT at each monitoring site against DEQ stream temperature standards. Figures 6Aand 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 2012 was calculated (Appendix B). Temperature data was compared to data on the movements of radio-tagged 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.

2012 Monitoring Period: May 8 - Oct 23 (°C)					
Stream Name	Site Number	Highest 7-day Max	Date of Occurrence	Absolute Maximum	Date of Occurrence
Lake Ck	1	23.95	8/1/2012	24.90	8/6/2012
Lake Ck	2	23.72	8/1/2012	25.07	8/6/2012
Malheur R.	3	21.22	7/13/2012	22.20	7/11/2012
Big Ck	4	19.56	7/13/2012	20.51	7/11/2012
Big Ck	5	17.53	7/13/2012	18.41	7/10/2012
Lake Ck*	6	*	*	*	*
McCoy Ck	7	23.53	8/9/2012	24.65	8/6/2012
Lake Ck*	8	*	*	*	*
McCoy Ck*	9	28.70	7/13/2012	29.77	7/11/2012
Lake Ditch	10	22.06	8/10/2012	23.21	8/6/2012

***Lake Creek sites #6 and #8 were dewatered beginning in early July 2012 rendering data not useable.**

Table 3: Number of Days and Percent of Total Days in the 120 Day Summer Period that MWMT Eclipsed Temperature Benchmarks (2012).

Site	# Days > 12 ° C	# Days > 16 ° C	# Days > 20.9 ° C
1	120 (100%)	96 (80%)	46 (38%)
2	120 (100%)	110(92%)	51 (43%)
3	113 (94%)	71 (59%)	4 (3%)
4	108 (90%)	56 (47%)	0 (0%)
5	100 (83%)	43 (36%)	0 (0%)
6[1]	***	***	***
7	120 (100%)	94 (78%)	46 (38%)
8[1]	***	***	***
9	120 (100%)	104 (87%)	61 (51%)
10	110 (92%)	71 (59%)	25 (21%)

[1] Lake Creek sites #6 and #8 were dewatered for a significant portion of 2012 summer season.

3.3 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). The date of highest stream temperatures in 2012 occurred during the Critical Stream Temperature Period (July 15 – August 15) at 4 of the 8 sites (Table 2). Malheur River Site 3, Big Creek sites 4 and 5 and McCoy Creek site 9 all reached their absolute maximum temperatures on July 13. Sites 6 and 8 were again dewatered for a significant portion of the summer monitoring period. All data collected post-dewater date has been excluded from this analysis. All sites on the Lake Creek drainage in 2012 spent the vast majority of the summer period with temperatures exceeding 12° C, and 4 of the 7 sites (Sites 1, 2, 7 and 9) spent over 90 days out of 120 in excess of 16° C (Table 3). On Big Creek, Site 4 exceeded 16° C for 56 days, and site 5 exceeded 16°C for 43 days (Table 3). ILT was surpassed at all sites in the Lake and McCoy Creek drainages in 2012. 20.9° C was surpassed 46 of 120 days (38%) at site 1, 51 of 120 days (43%) at Site 2, 46 of 120 days (38%) at Site 7, 61 of 120 days (51%) at site 9, and 25 of 120 days (21%) at Site 10 (Table 3). The confluence of Big and Lake Creek (Site 3) exceed the ILT of 20.9° C for 4 days (3%) in 2012, in 2011 it narrowly missed surpassing 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 2012, Site 6 was dewatered on 3 July (Figure 2) and Site 8 was dewatered beginning 6 July (Figure3).

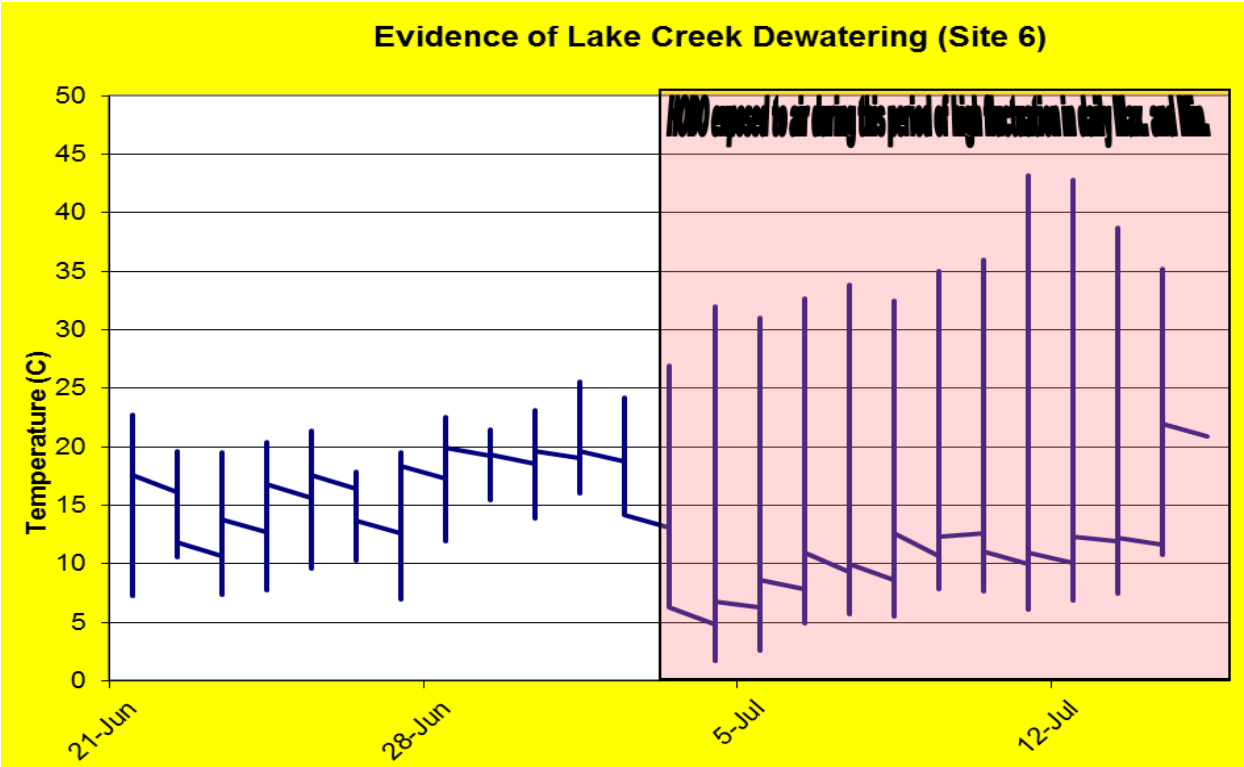

Figure 2. 2012 dewater period as expressed by hobo exposure to air.

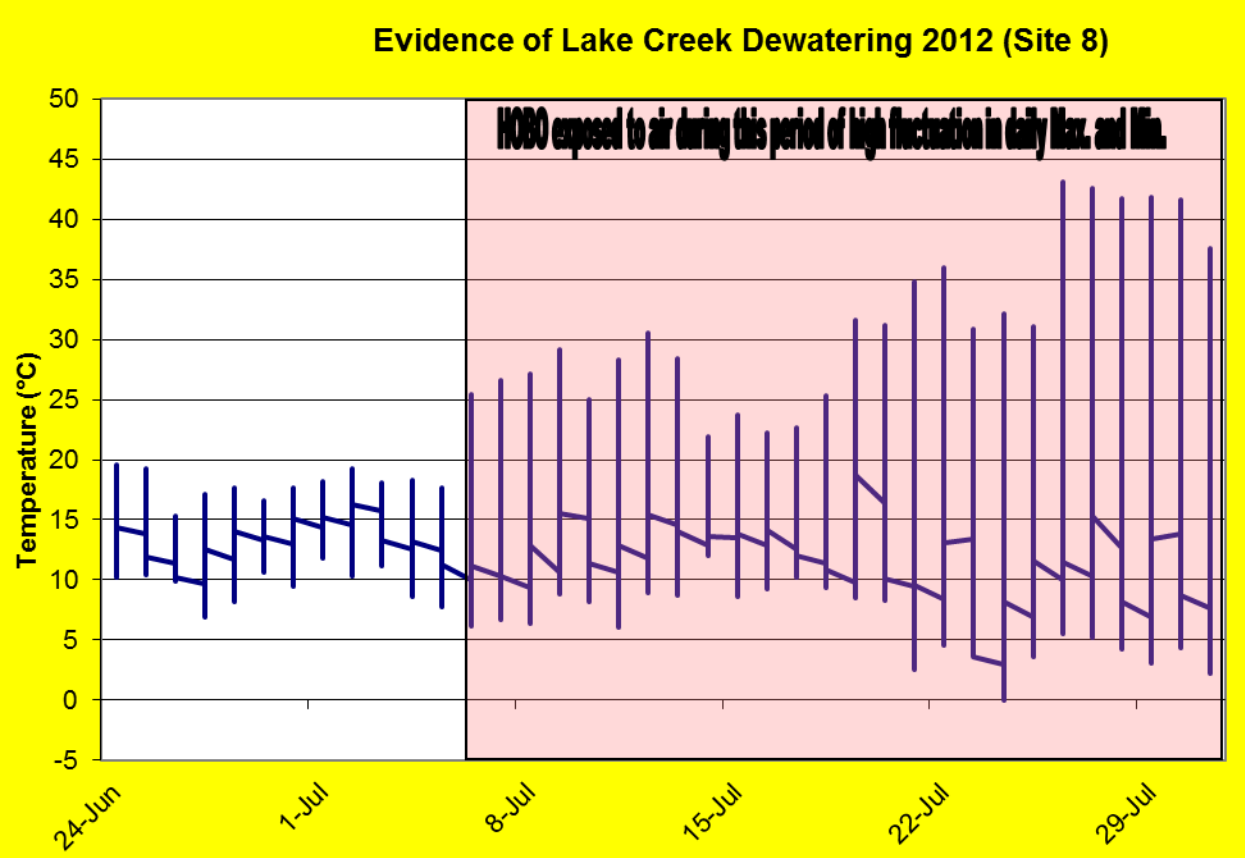

Figure 3. 2012 dewater period as expressed by hobo exposure to air.

3.4 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 2012 (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 3). 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.

3.5 Acknowledgements

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

3.6 References

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APPENDIX A
STREAM TEMPERATURES EXPRESSED BY MWMT

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

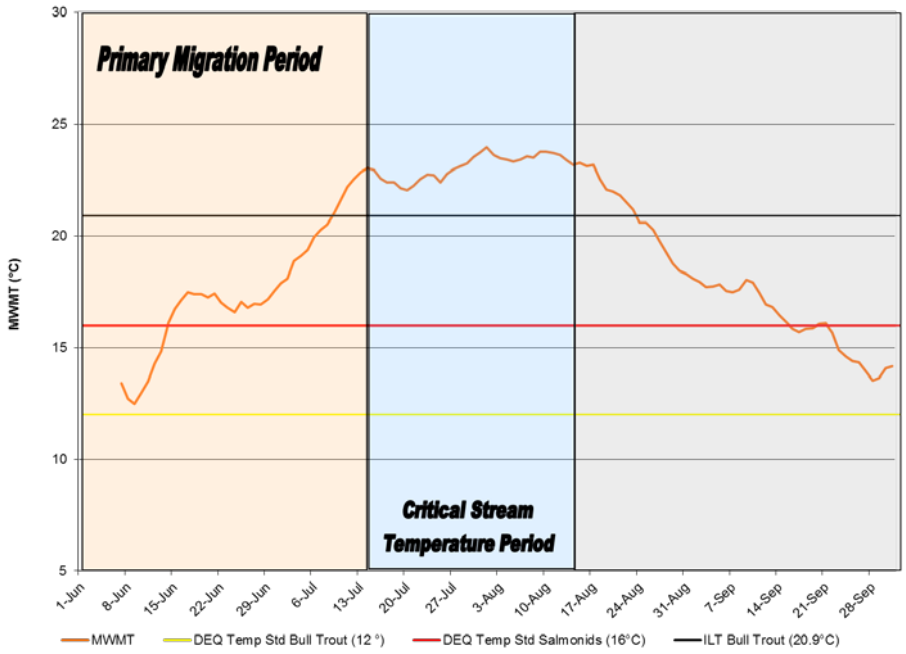


FIGURE 2A: LAKE CREEK BELOW CROOKED CREEK (site 2)

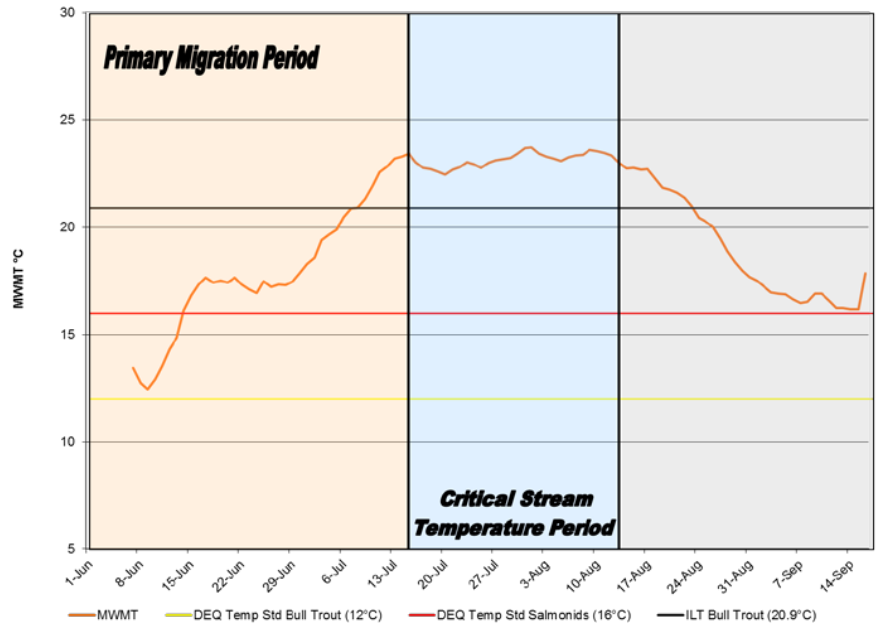


FIGURE 3A: MALHEUR BELOW BIG AND LAKE CREEKS (site 3)

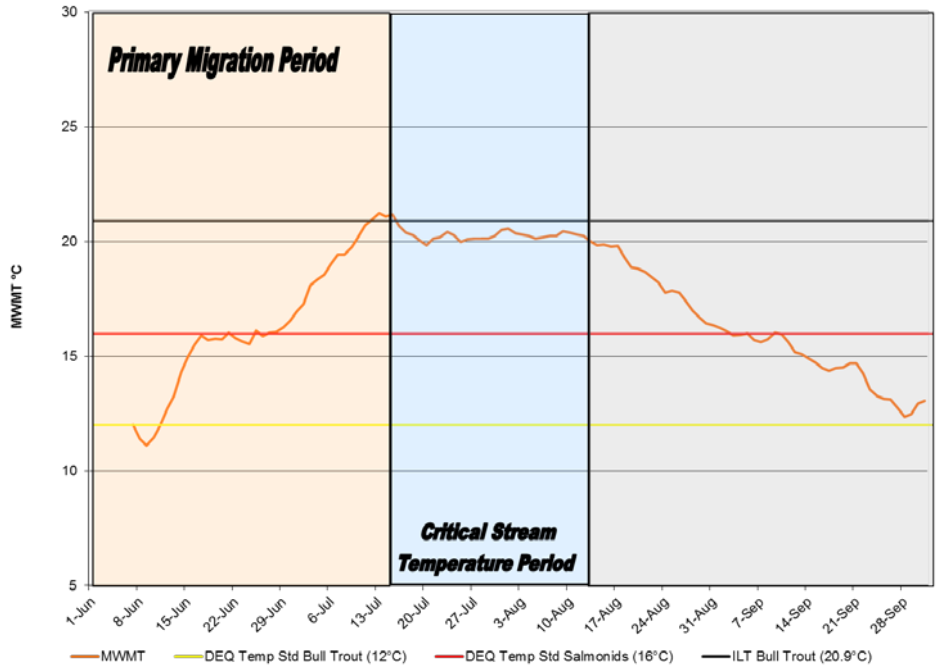


FIGURE 4A: BIG CREEK ONE MILE BELOW 16 ROAD (site 4)

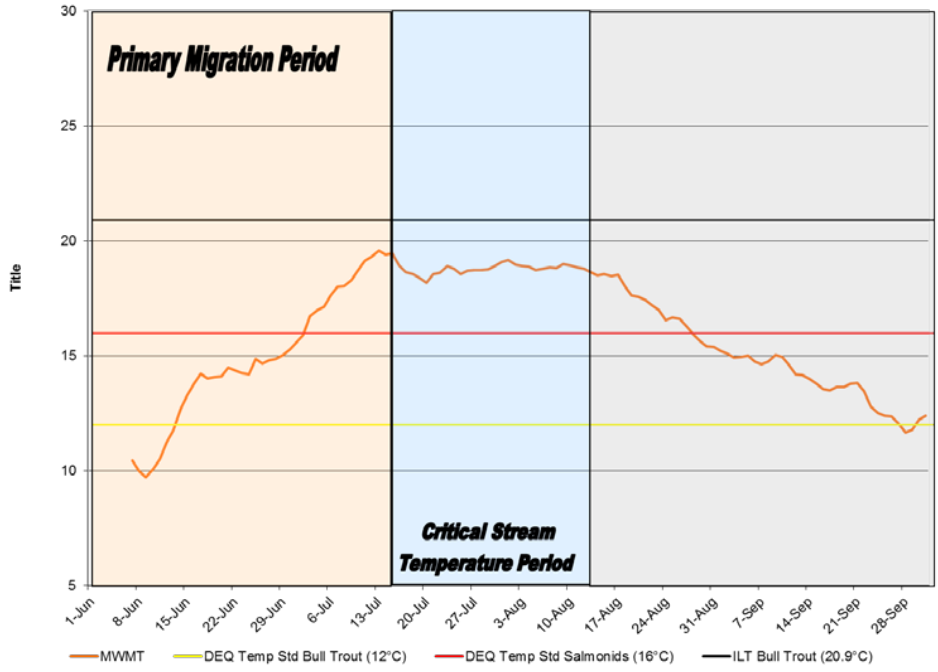


FIGURE 5A: BIG CREEK BELOW 16 ROAD (site 5)

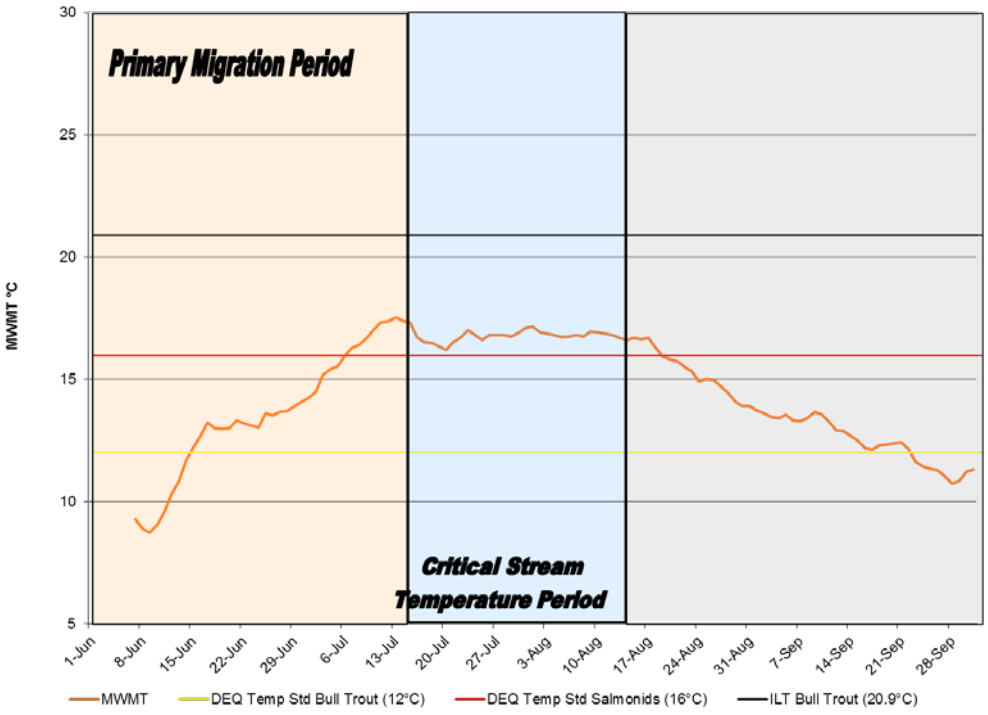


FIGURE 6A: LAKE CREEK BELOW 16 ROAD (site 6)

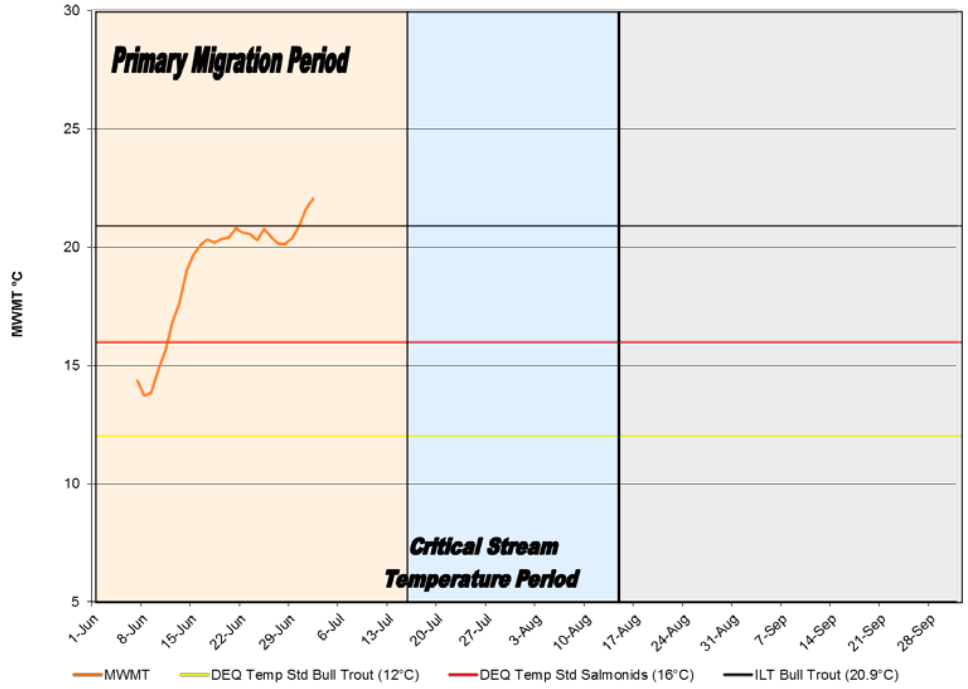


FIGURE 7A: McCOY CREEK ABOVE LAKE CREEK (site 7)

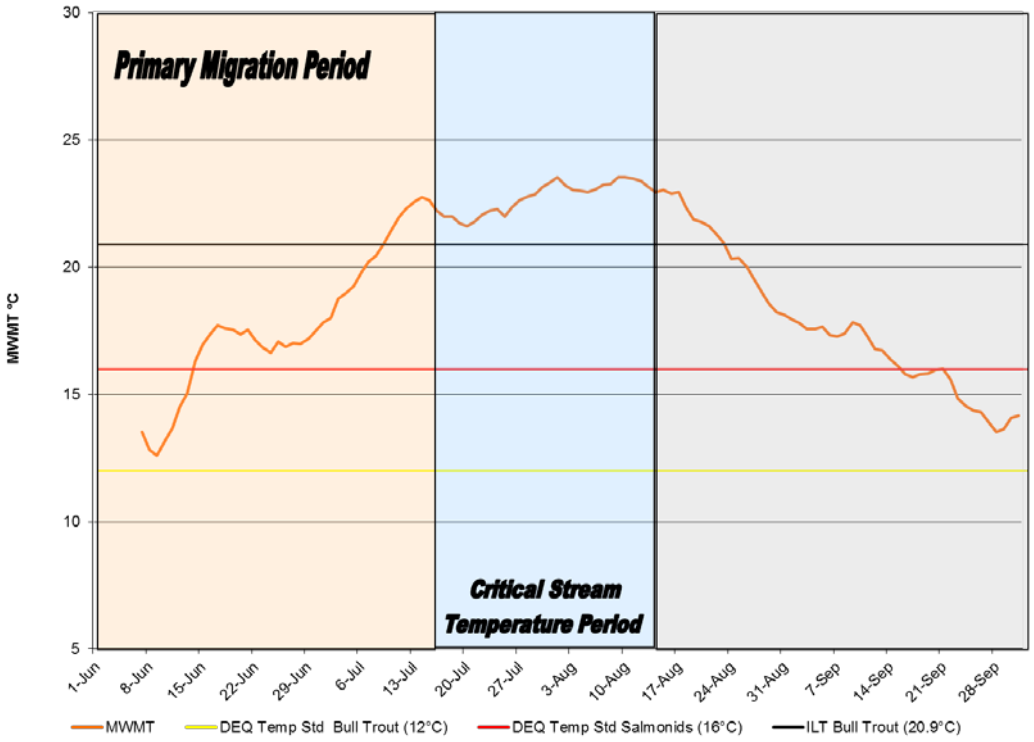


FIGURE 8A: LAKE CREEK AT BRIDGE (site 8)

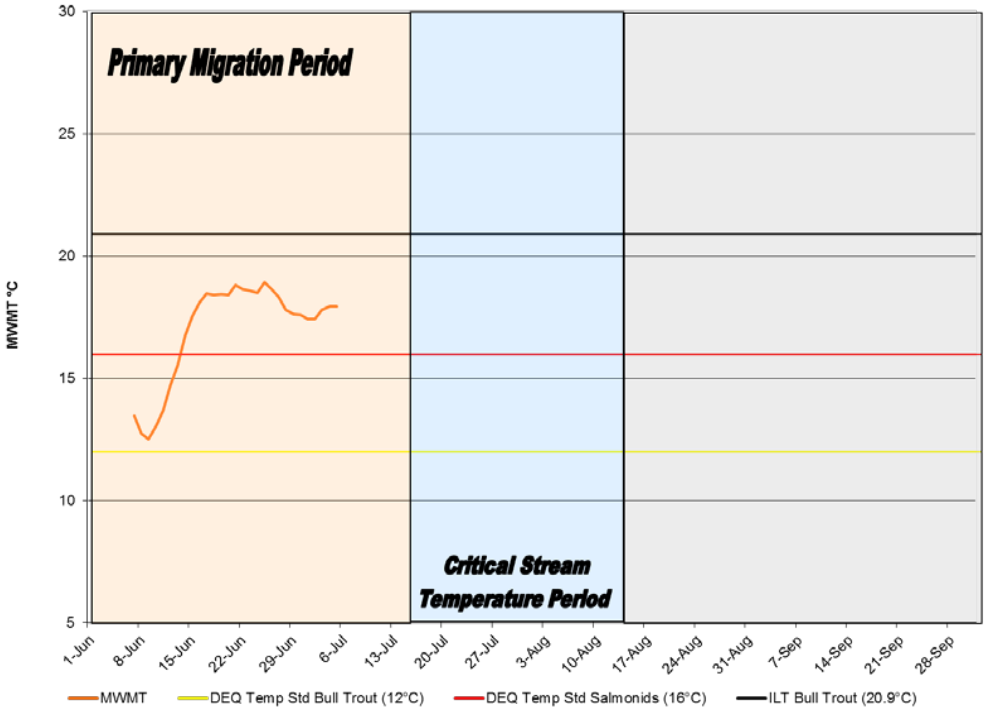


FIGURE 9A: McCOY CREEK AT 16 RD (site 9)

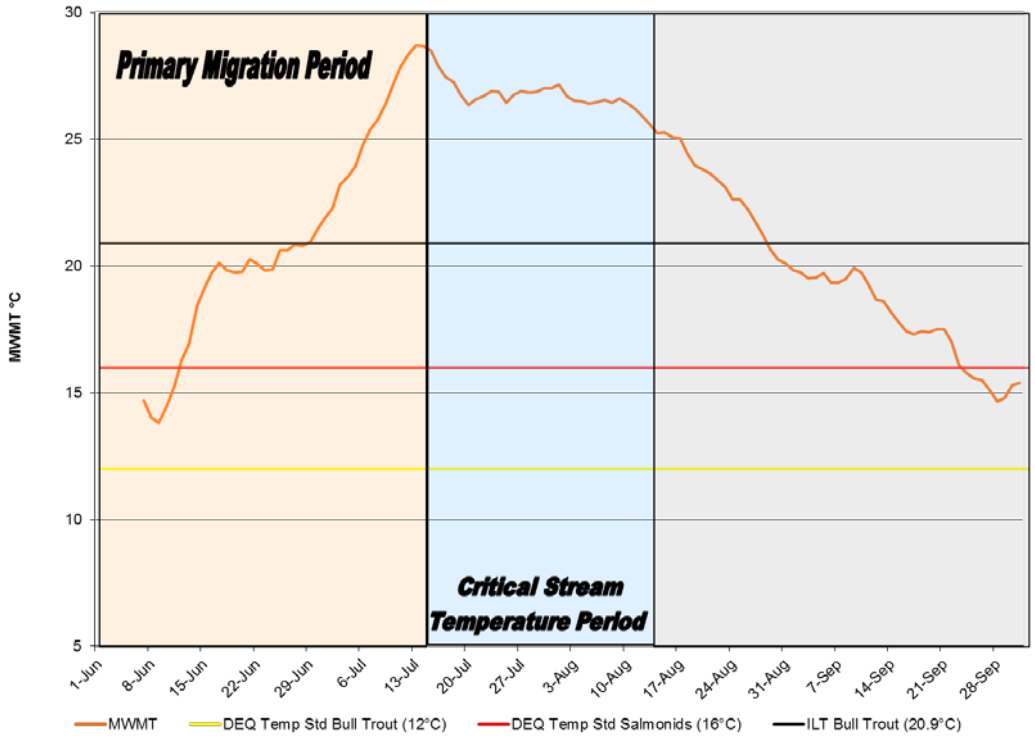
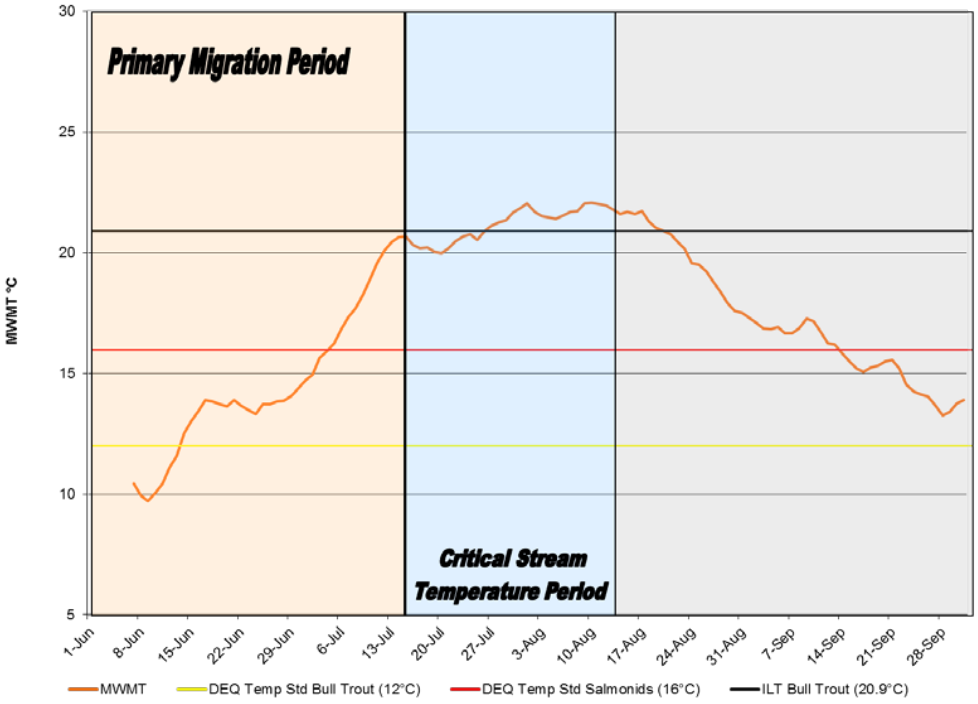


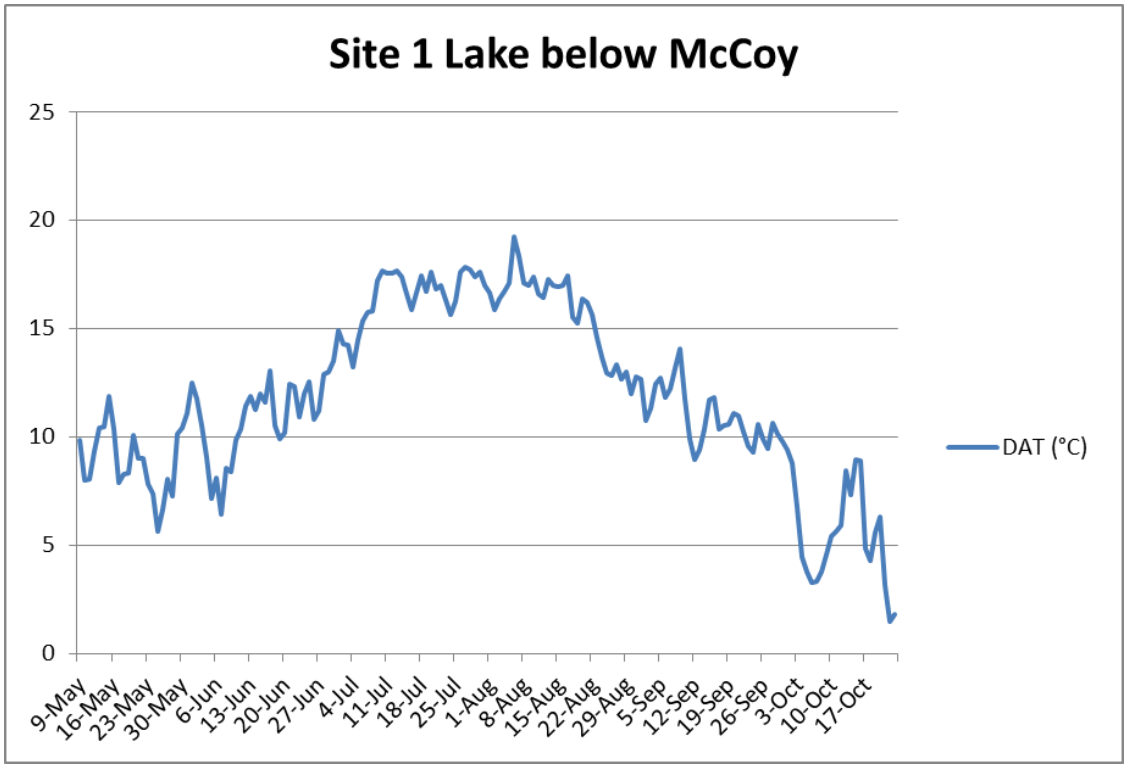
FIGURE 10A: LAKE CREEK DITCH AT 16 RD (site 10)

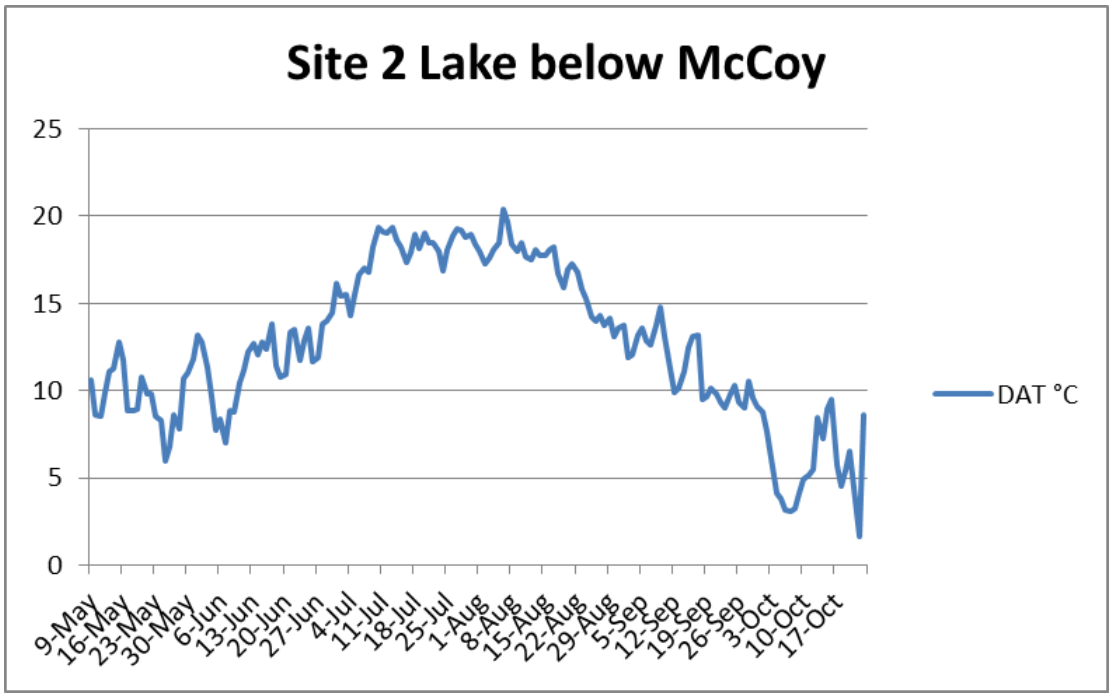


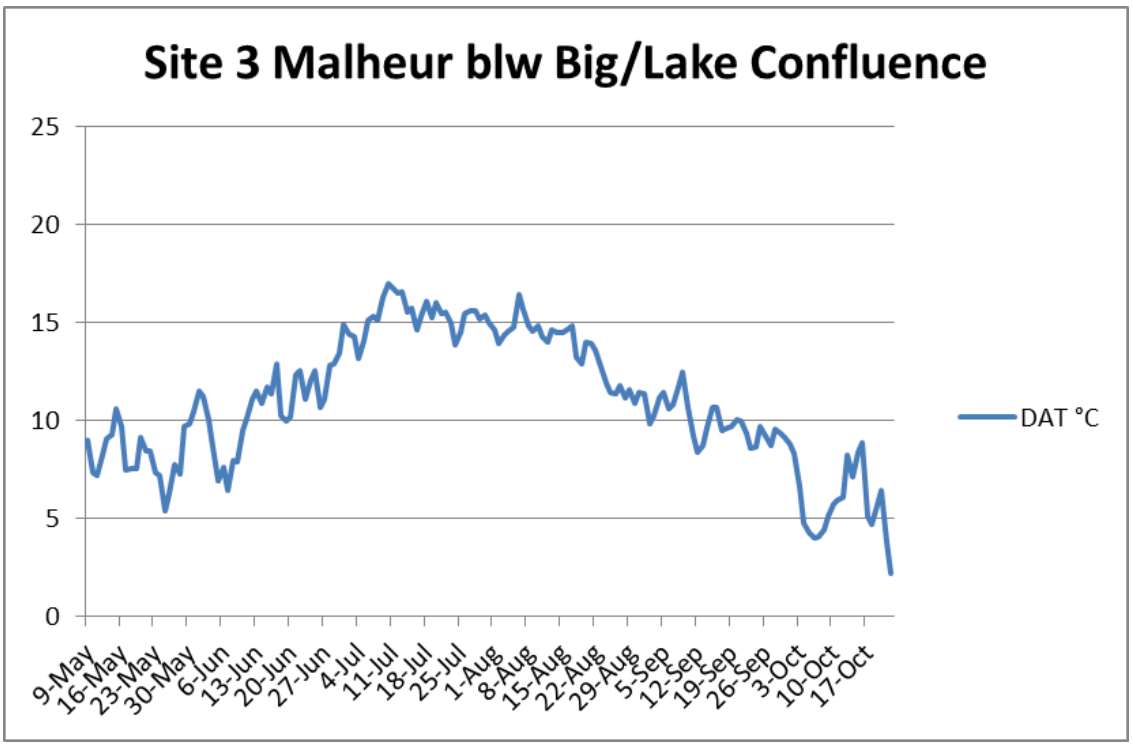
APPENDIX B

2012 DAILY

AVERAGE TEMPERATURE






Site 3 Malheur blw Big/Lake Confluence
25
20
15
10
5
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9-May
16-May
23-May
30-May
6-Jun
13-Jun
20-Jun
27-Jun
4-Jul
11-Jul
18-Jul
25-Jul
1-Aug
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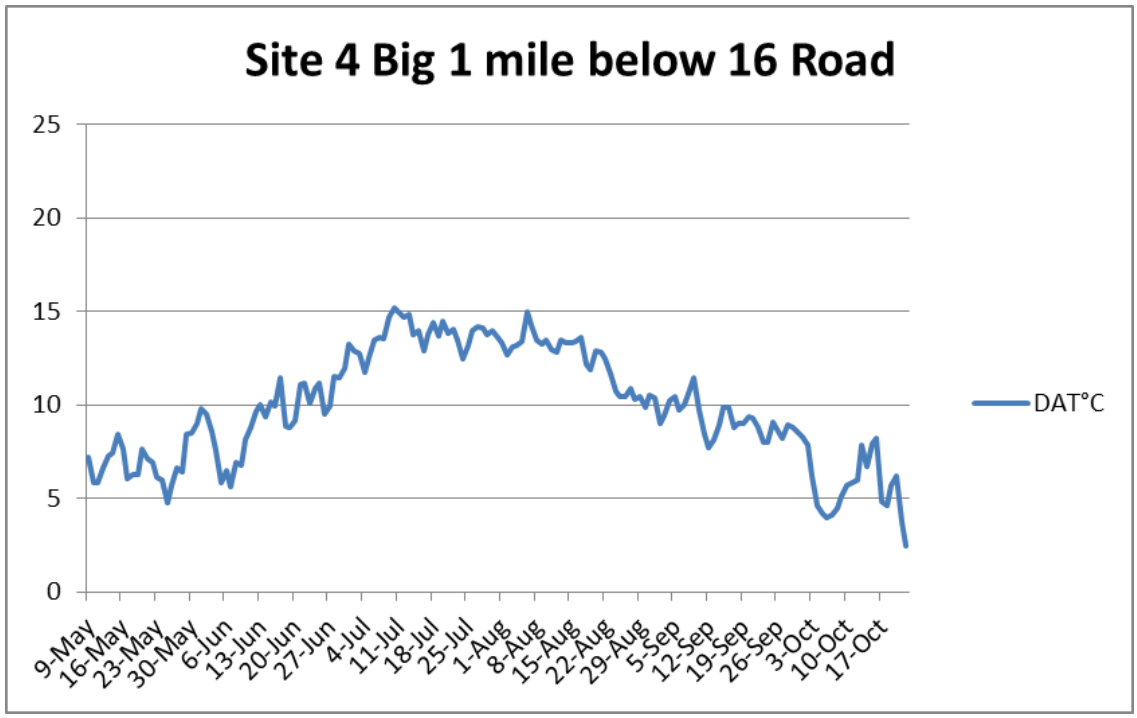
26-Sep
3-Oct
10-Oct
17-Oct
DAT °C


Site 4 Big 1 mile below 16 Road
25
20
15
10
5
0
9-May
16-May
23-May
30-May
6-Jun
13-Jun
20-Jun
27-Jun
4-Jul
11-Jul
18-Jul
25-Jul
1-Aug
8-Aug
15-Aug
22-Aug
29-Aug
5-Sep
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26-Sep
3-Oct
10-Oct
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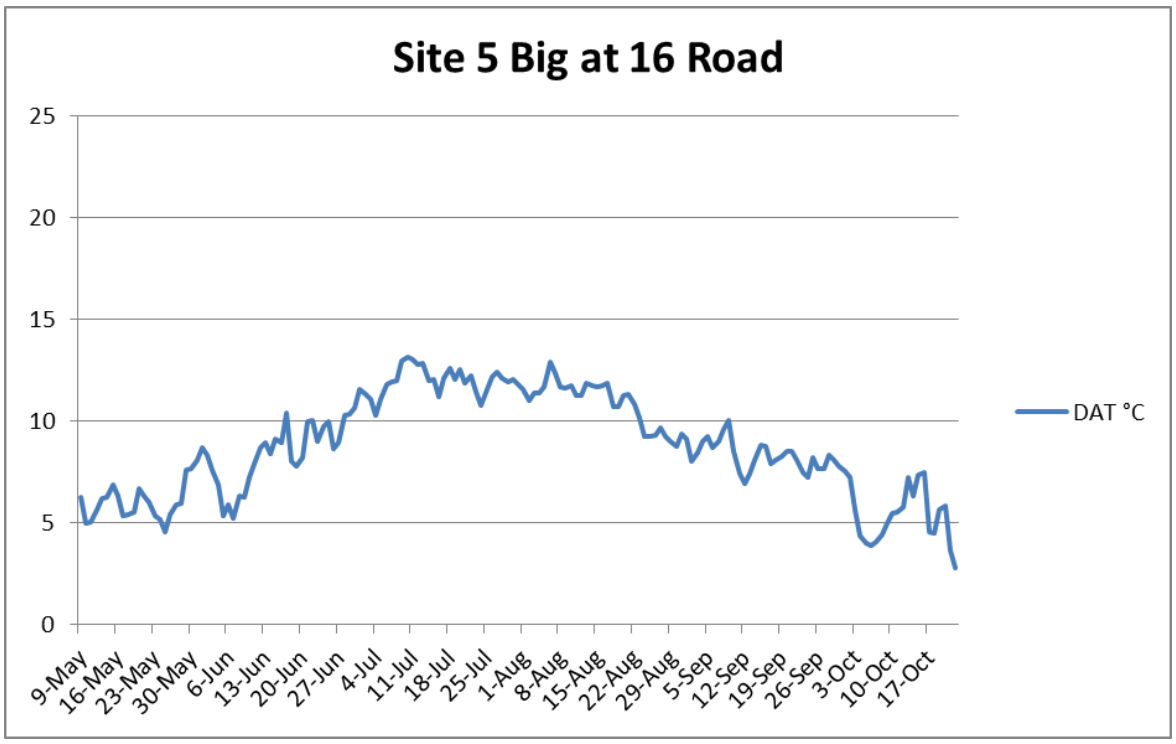
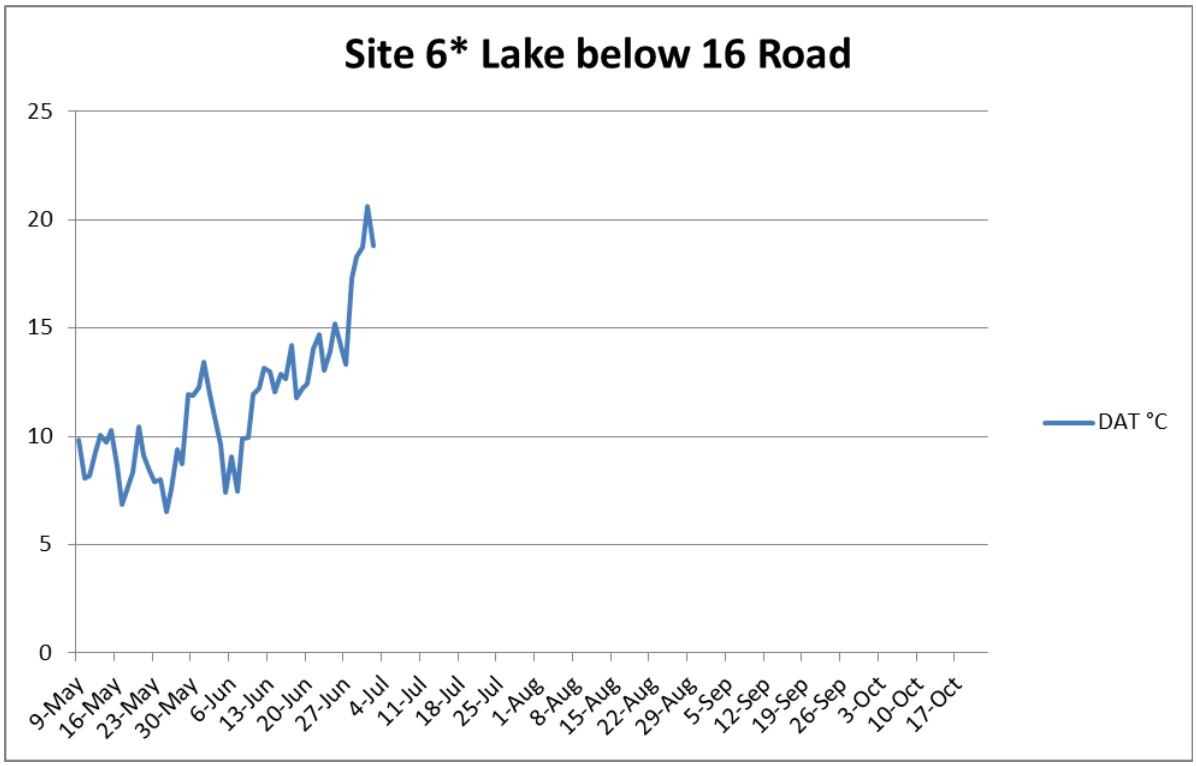
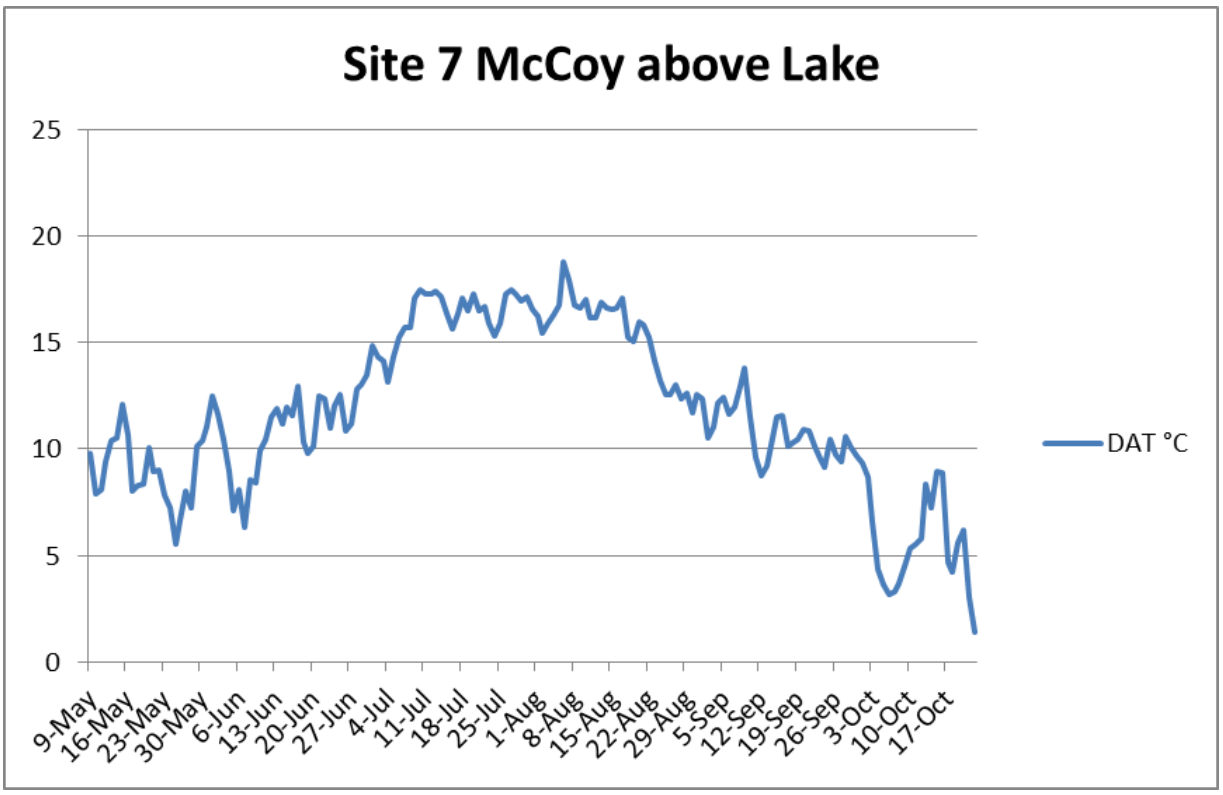
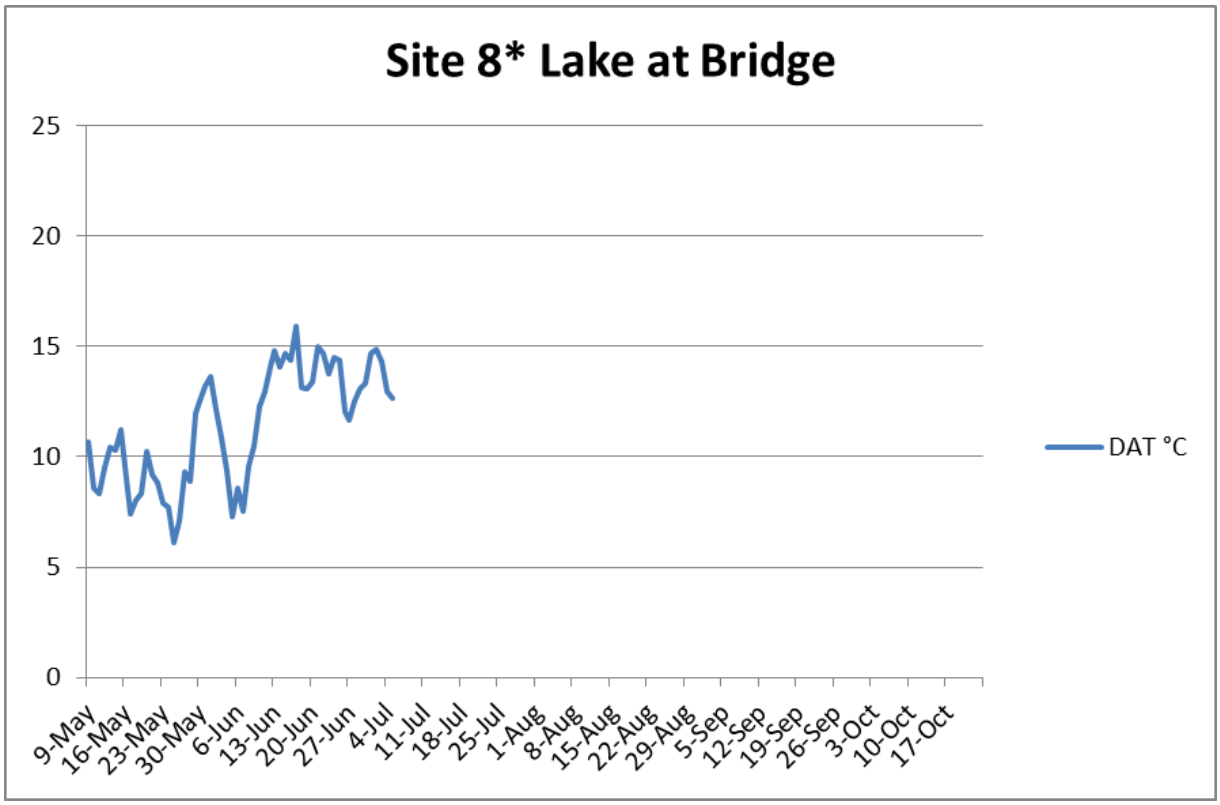
DAT°C



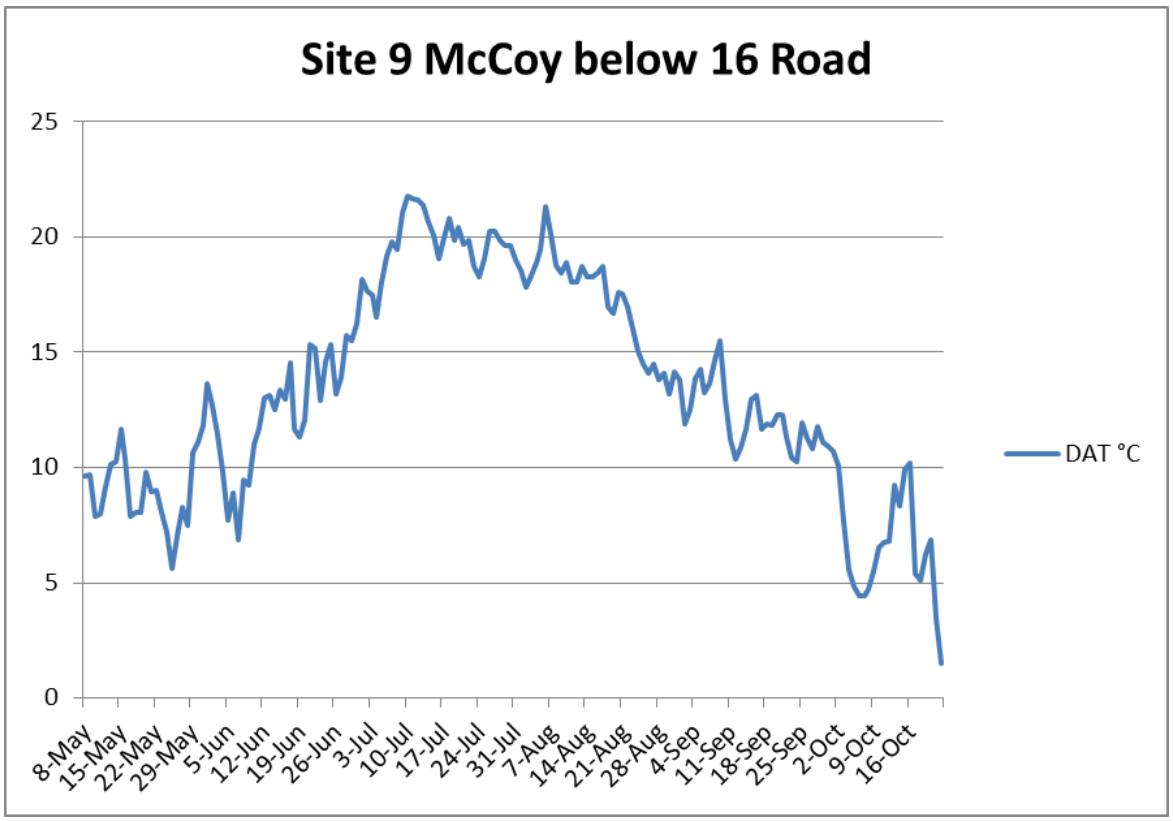


*Site 6 Lake Cr Below 16 RD Dewatered 7/3/12 Data not useable after this date.





*Site 8 Lake Cr at Bridge Dewatered 7/6/12 Data not useable after this date.


Site 9 McCoy below 16 Road
25
20
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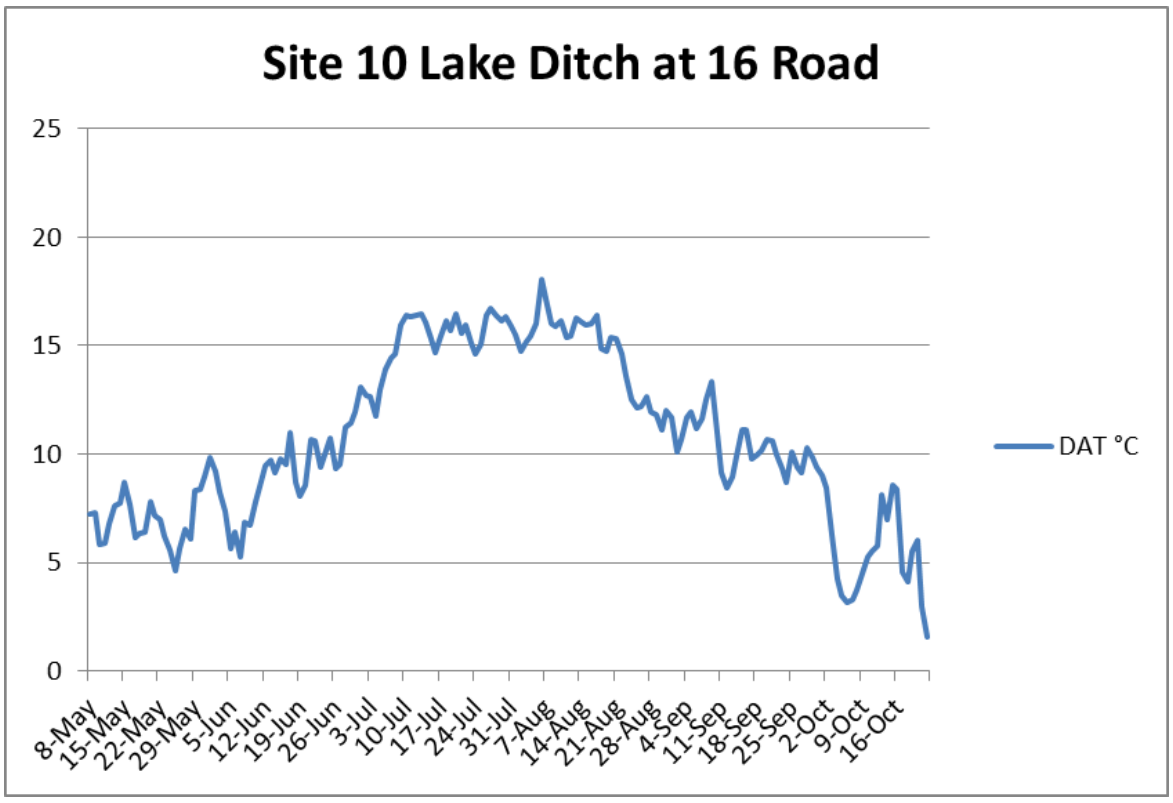
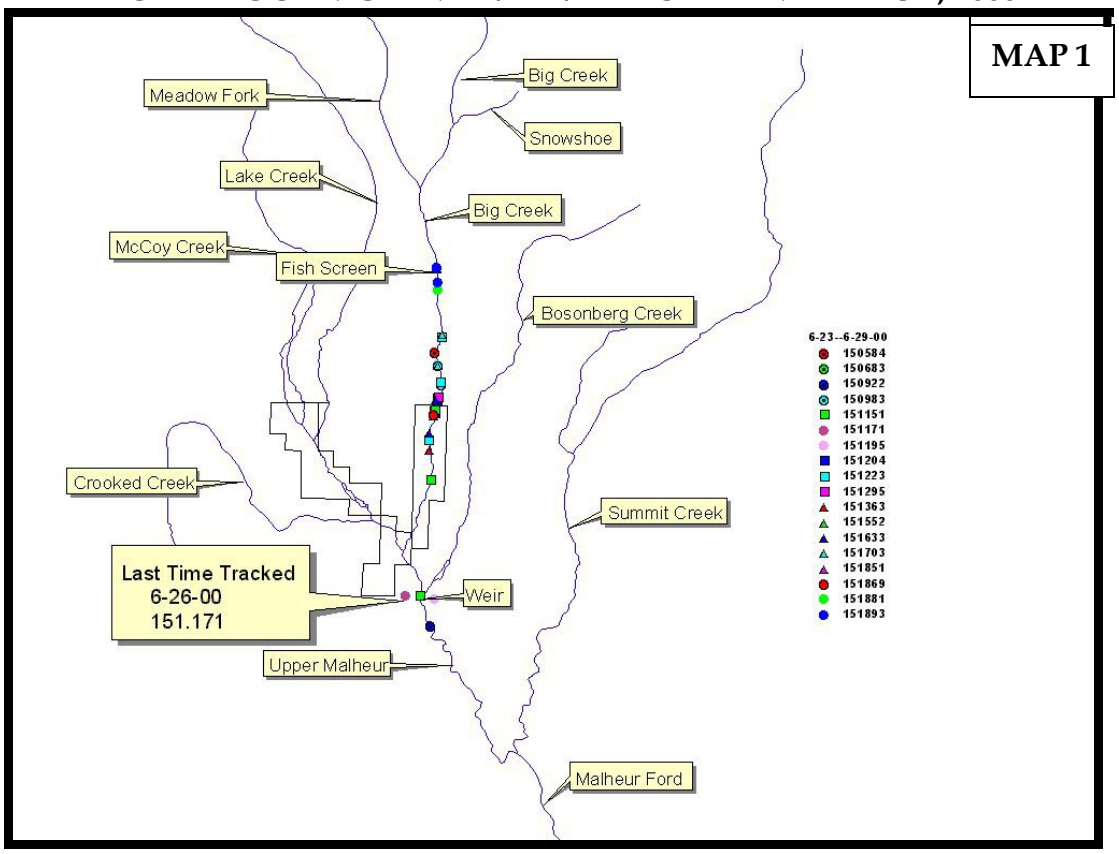
8-May
15-May
22-May
29-May
5-Jun
12-Jun
19-Jun
26-Jun
3-Jul
10-Jul
17-Jul
24-Jul
31-Jul
7-Aug
14-Aug
21-Aug
28-Aug
4-Sep
11-Sep
18-Sep
25-Sep
2-Oct
9-Oct
16-Oct
DAT °C


Site 10 Lake Ditch at 16 Road
25
20
15
10
5
0
8-May
15-May
22-May
29-May
5-Jun
12-Jun
19-Jun
26-Jun
3-Jul
10-Jul
17-Jul
24-Jul
31-Jul
7-Aug
14-Aug
21-Aug
28-Aug
4-Sep
11-Sep
18-Sep
25-Sep
2-Oct
9-Oct
16-Oct
DAT °C

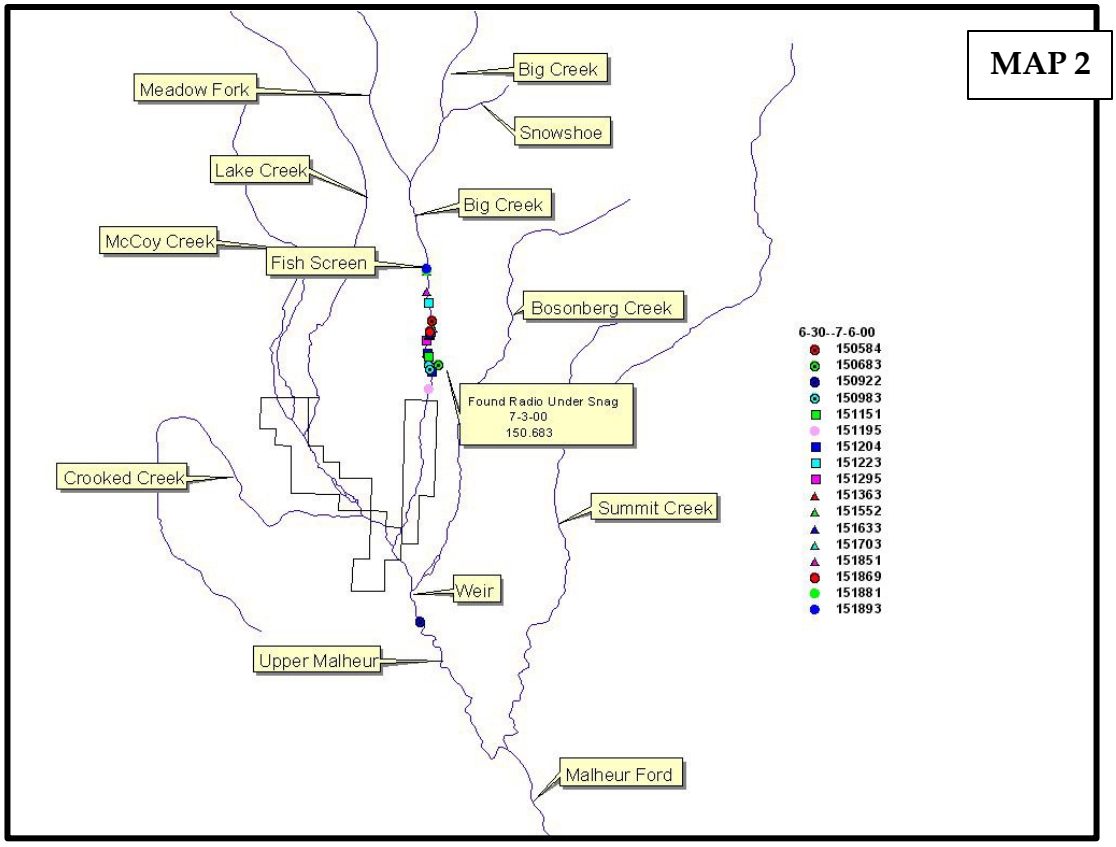
APPENDIX C

BULL TROUT MOVEMENT IN THE UPPER MALHEUR, 2000

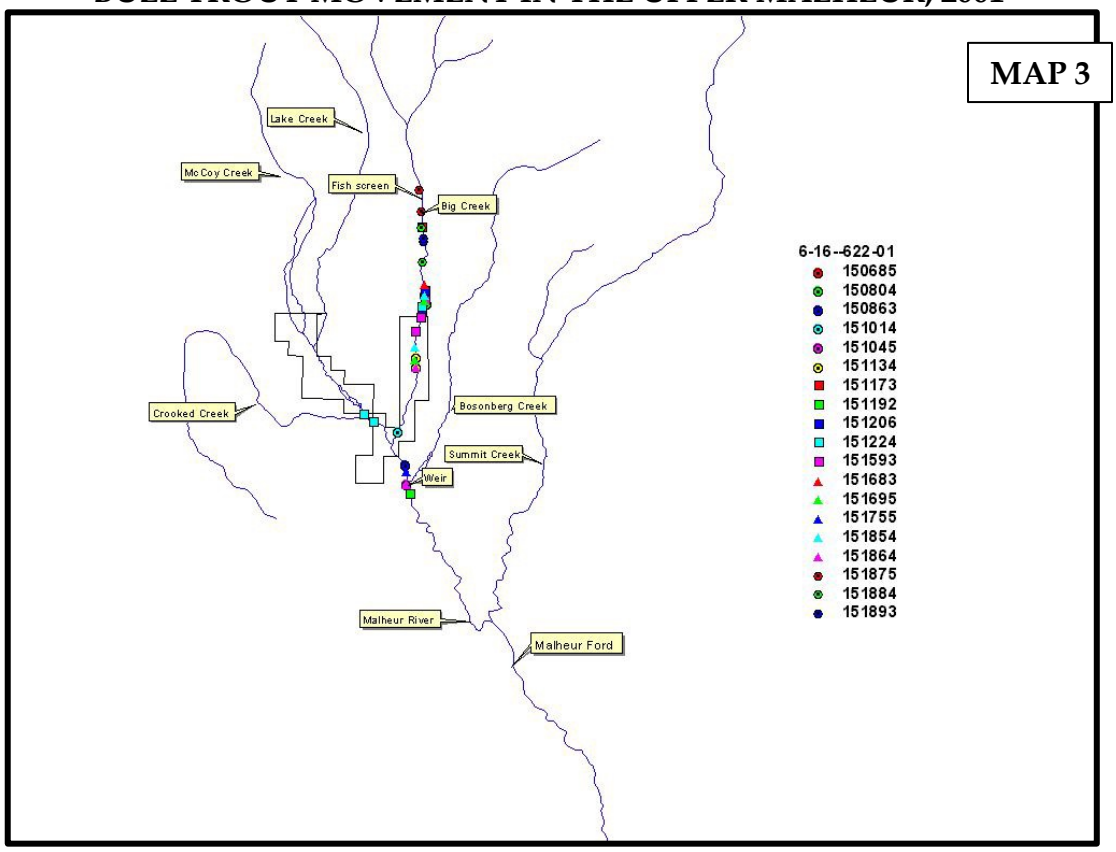
MAP 1

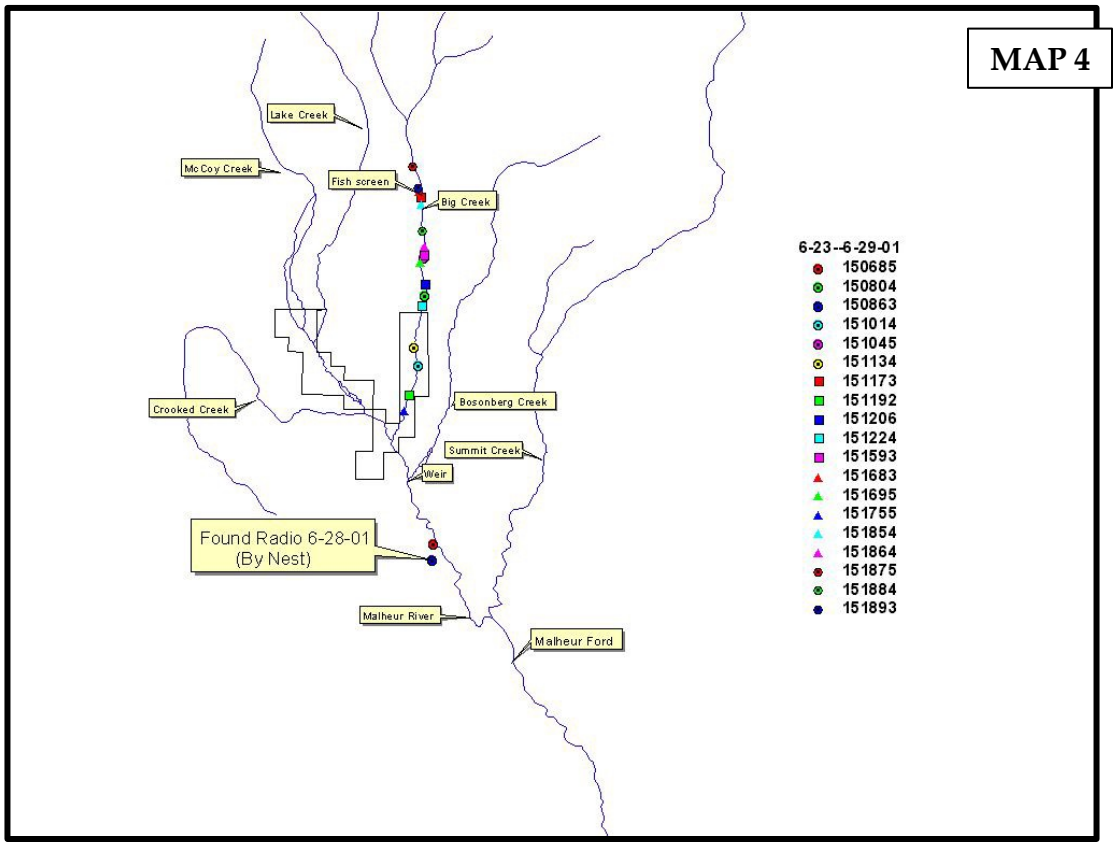


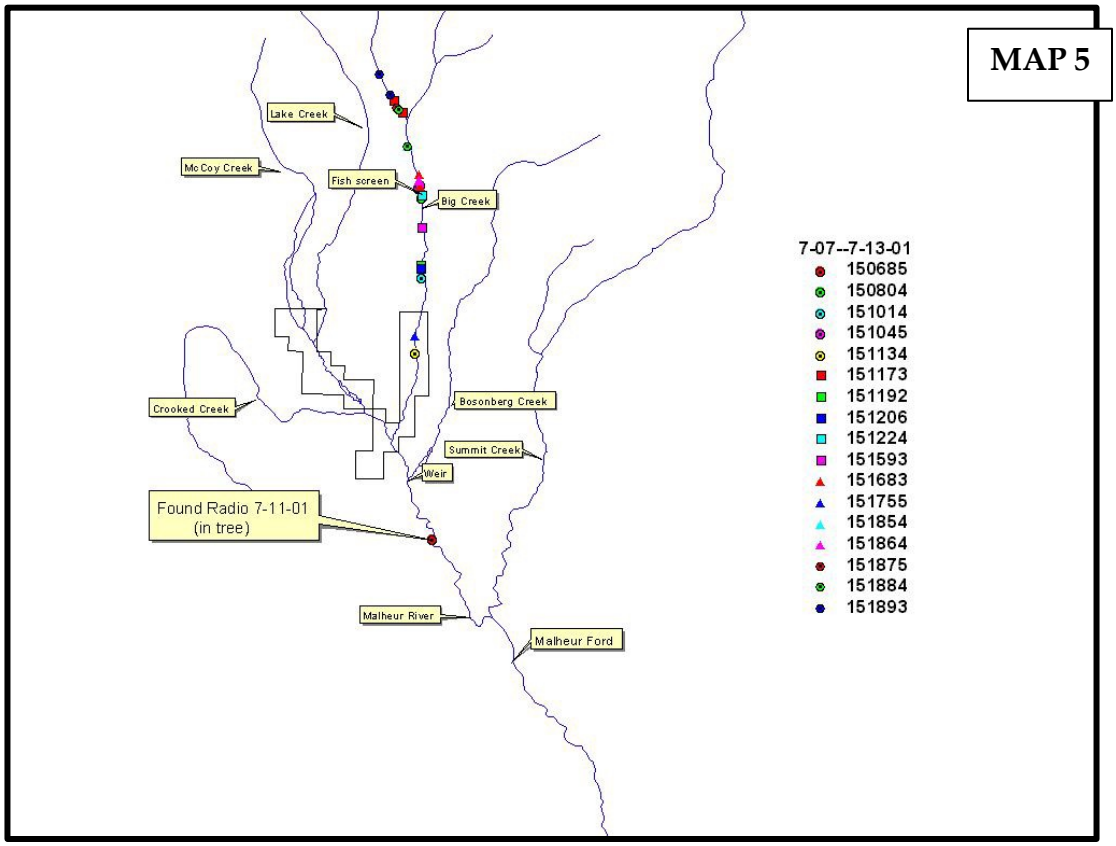
MAP 2



BULL TROUT MOVEMENT IN THE UPPER MALHEUR, 2001




MAP 4
Lake Creek
McCoy Creek
Fish screen
Big Creek
Crooked Creek
Bosonberg Creek
Summit Creek
Weir
Found Radio 6-28-01
(By Nest)
Malheur River
Malheur Ford
6-23--6-29-01
150685
150804
150863
151014
151045
151134
151173
151192
151206
151224
151593
151683
151695
151755
151854
151864
151875
151884
151893


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 4

Sampling and analysis to assess brook trout (*Salvelinus fontinalis*) population trends in High Lake (Oregon) using environmental DNA monitoring
2012 Report

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

2011-12 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; summer 2012), 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; fall 2012), 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; fall 2012), 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 - 2011

At each station prior to collecting the water sample, sounding depths were recorded to the nearest 1/10th 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 - 2011

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 - 2011

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 were 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 was essential for repeatability of grid sampling and in maintaining position when multiple samples were 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 (2011) – 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 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.

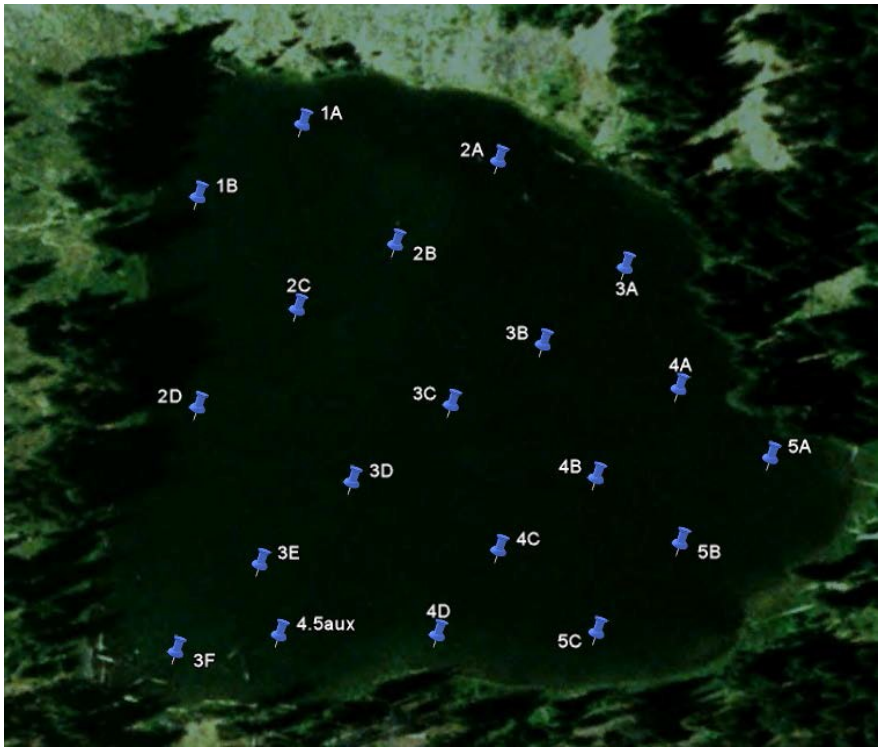

Figure 1. Representations of 2011 summer sample locations based on GPS readings, edge coordinates, landscape features, and field spacing of transects. 2012 summer samples were collected along the transect lines established in 2011.

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

Summer Event (2012) – Water samples were collected from 18 locations, with sites located systematically along the transect lines developed in 2011 (Figure 1); although exact coordinates were not recorded at the position each water sample was collected. For 2012 event, one fewer sample was collected along transect line 3 and the auxiliary site (i.e., 2011 – 4.5aux) was also omitted. All samples were stored in lake until daily completion of sampling. A cooler control was taken on both sampling days of lake surface water, which was stored and transported in same manner as water samples.

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 were 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.

Fall Event (2011) – 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 (#2011-21) 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.

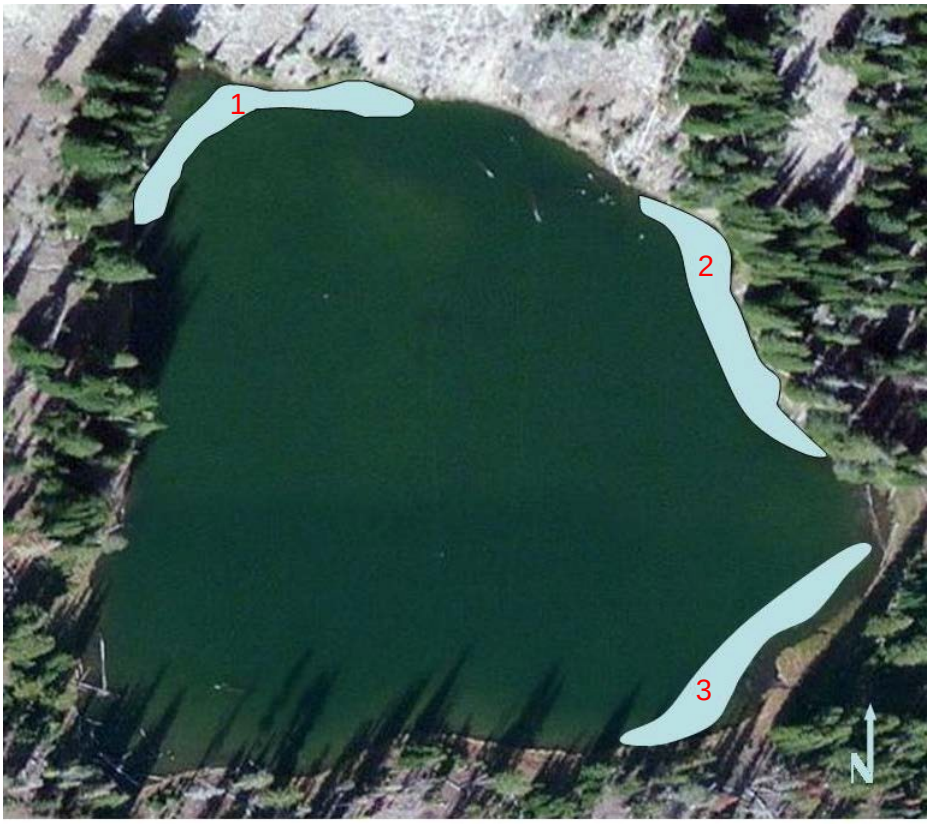

Figure 3. Locations of potential brook trout spawning areas in High Lake, Oregon.

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.

Fall Event (2012) – Sampling points were approximately located within each spawning area designated in 2011 (Figure 3), with collections taken in a manner described for 2011. 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 112 meters, 90 meters, and 94 meters being the total length of spawning areas 1, 2, and 3 respectively. The first sample (#2012-19) was taken at the western edge of spawning area 1, with subsequent sampling proceeding in an easterly manner. A cooler control was taken from lake surface water after sample #2012-19 was taken. A sample control was taken using the grab sampler in Silvies River, OR multiple hours after completion of water sampling in High Lake. The Silvies River is highly suspected to be free of brook trout as the mainstem is not conducive to supporting trout populations.

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 (or llama paniers) with dry ice, snow, or block ice fragments, placed in a cooler and transported 1.5 – 2 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 in 2011, 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

In 2011, 29 right pelvic fins of brook trout were collected 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

In 2011, 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).

A staff gauge was not installed for 2012 sampling events.

Table 2 Depth comparison of handheld sonar and weighted tape in 2011. 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) with quantity of DNA present within each water sample (Figure 5). This curve was used to calculate the milligrams of DNA per liter contained within all water samples.

Summer 2011 – 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.

Fall 2011 – 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.

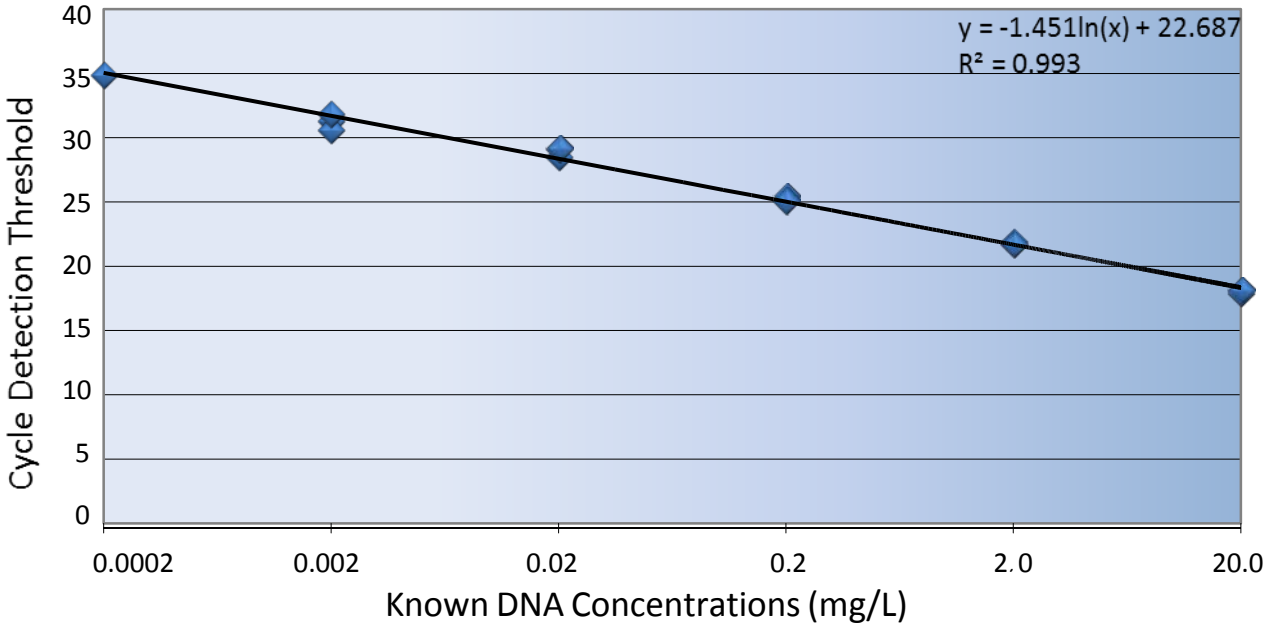

Figure 5. Standard curve relating cycling threshold with known concentrations of brook trout DNA.

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]
2011-1	1A	0-3	4	8	44°17'3.58"N	118°41'2.59"W	8.6	35.37	0.00016
2011-2	1B	0-3	3.75	6.5	44°17'2.98"N	118°41'3.81"W	7.5	No Detection	
2011-3	2A	0-3	6.6	12.3	44°17'3.27"N	118°41'0.30"W	13.3	No Detection	
2011-4	2B	0-3	4.5	8	44°17'2.58"N	118°41'1.50"W	9.1	No Detection	
2011-5	2C	0-3	5.5	10	44°17'2.04"N	118°41'2.65"W	10.9	No Detection	
2011-6	2D	0-3	5.1	9	44°17'1.22"N	118°41'3.83"W	10.3	No Detection	
2011-7	3A	0-3	5	9	44°17'2.38"N	118°40'58.82"W	10	No Detection	
2011-8	3B	0-3	6.6	10	44°17'1.75"N	118°40'59.78"W	11.3	No Detection	
2011-9	3C	0-3	6.5	10	44°17'1.10"N	118°41'0.96"W	11	No Detection	
2011-10	3D	0-3	6.6	10	44°17'0.48"N	118°41'2.06"W	11.3	37.75	0.00003
2011-11	3E	0-3	5.3	9.5	44°16'59.88"N	44°16'59.88"N	10.6	No Detection	
2011-12	3F	0-3	4		44°16'59.26"N	118°41'4.22"W	5	No Detection	
2011-13	4A	0-3	4.5	8	44°17'1.38"N	118°40'58.19"	9.1	No Detection	
2011-14	4B	0-3	5.2	9.5	44°17'0.69"N	118°40'59.07"W	10.4	No Detection	
2011-15	4C	0-3	5.3	9.5	44°17'0.08"N	118°40'59.97"W	10.6	36.12	0.00010
2011-16	4D	0-3	4		44°16'59.39"N	118°41'0.91"W	5	No Detection	
2011-17	5A	0-3	3.5	6	44°17'0.80"N	118°40'57.13"W	7	No Detection	
2011-18	5B	0-3	4.4	7.8	44°17'0.10"N	118°40'58.18"W	8.8	No Detection	
2011-19	5C	0-3	3.1	5.2	44°16'59.36"N	118°40'59.15"W	6.2	No Detection	
2011-20	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	200.0

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]
2011-21	1	12.50	No detection	
2011-20	1	25.00	No detection	
2011-19	1	37.50	No detection	
2011-18	1	50.00	No detection	
2011-17	1	62.50	No detection	
2011-16	1	75.00	No detection	
2011-15	1	87.50	No detection	
2011-14	2	10.25	No detection	
2011-13	2	20.50	34.09	0.00039
2011-12	2	30.75	No detection	
2011-11	2	41.00	No detection	
2011-10	2	51.25	No detection	
2011-9	2	61.50	No detection	
2011-8	2	71.75	No detection	
2011-7	3	11.75	36.96	0.00005
2011-6	3	23.50	35.37	0.00016
2011-5	3	35.25	36.06	0.00010
2011-4	3	47.00	No detection	
2011-3	3	58.75	38.03	0.00003
2011-2	3	70.50	No detection	
2011-1	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	337.7

Summer 2012 – Water samples were collected in a systematic grid pattern on July 23, 2012 and July 24, 2012, with the collection points situated along established project transect lines (Figure 1) and lake depth recorded for each location (Table 5). Each water sample was a composite composed of collections made from multiple depths in the water column. Water samples were analyzed in triplicate, with brook trout DNA not detected in any water samples. Detection of brook trout DNA was not observed in any negative control, while a strong signal was observed in the positive brook trout control (Table 5).

Fall 2012 – In the fall sampling period, eight water samples were collected on October 18, 2012 in each of the three putative spawning areas described in 2011 (Figure 3). Water samples were obtained every 14, 11.25, and 11.75 meters within spawning areas 1, 2, and 3, respectively (Table 6). Water samples were analyzed in triplicate, with brook trout DNA not detected in any spawning ground water samples. Detection of brook trout DNA was not observed in any negative control, while a strong signal was observed in the positive brook trout control (Table 6).

Table 5 Summer systematic sampling, July 23, 2012 and July 24, 2012. Transect lines on which samples were collected were described in 2011 sampling (Figure 1). Lake depth is relative to lake surface water level and was recorded at time of sampling by field personnel. 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.	Transect ID	Sampling Depth (ft)	Latitude	Longitude	Lake Depth (ft)	C(t)	DNA [mg/L]
2012-1	1-1	-	-	-	6.3	no detection	
2012-2	1-2	-	-	-	8.5	no detection	
2012-3	2-1	-	-	-	9.2	no detection	
2012-4	2-2	-	-	-	10.6	no detection	
2012-5	2-3	-	-	-	9.7	no detection	
2012-6	2-4	-	-	-	10.3	no detection	
2012-7	3-1	-	-	-	4.5	no detection	
2012-8	3-2	-	-	-	10.6	no detection	
2012-9	3-3	-	-	-	10.9	no detection	
2012-10	3-4	-	-	-	10.9	no detection	
2012-11	3-5	-	-	-	11.2	no detection	
2012-12	4-1	-	-	-	6.6	no detection	
2012-13	4-2	-	-	-	10.7	no detection	
2012-14	4-3	-	-	-	10.8	no detection	
2012-15	4-4	-	-	-	9.1	no detection	
2012-16	5-1	-	-	-	6.5	no detection	
2012-17	5-2	-	-	-	7.6	no detection	
2012-18	5-3	-	-	-	3.7	no detection	
Cooler Control						no detection	
Filter Control						no detection	
Elution Control						no detection	
No Template Control						no detection	
Positive Control						14.16	356.9

Table 6 Fall spawning area sampling, October 18, 2012. Spawning area locations are approximately 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.

Sample No.	Spawning Area	Transect Distance (m)	C(t)	DNA [mg/L]
2012-19	1	7	No detection	
2012-20	1	21	No detection	
2012-21	1	35	No detection	
2012-22	1	49	No detection	
2012-23	1	63	No detection	
2012-24	1	77	No detection	
2012-25	1	91	No detection	
2012-26	1	105	No detection	
2012-27	2	3.4	No detection	
2012-28	2	14.6	No detection	
2012-29	2	25.87	No detection	
2012-30	2	37.12	No detection	
2012-31	2	48.37	No detection	
2012-32	2	59.62	No detection	
2012-33	2	70.87	No detection	
2012-34	2	85.4	No detection	
2012-35	3	9.4	No detection	
2012-36	3	21.15	No detection	
2012-37	3	32.9	No detection	
2012-38	3	44.65	No detection	
2012-39	3	56.4	No detection	
2012-40	3	68.15	No detection	
2012-41	3	79.9	No detection	
2012-42	3	91.65	No detection	
Cooler/ Sample Controls			No detection	
Filter/Elution Controls			No detection	
No Template Control			No detection	
Positive Control			14.55	272.8

Discussion

Under this contract, 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 traditional survey approaches (e.g., electrofishing, netting). We show this method can provide a mechanism for rapid reconnaissance and statistically defensible trend analysis for an invasive char species. Yet, we observed some limitations to eDNA analysis and 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.

Two sampling strategies were employed to provide the opportunity to test the relative effectiveness of each method and to compare water sampling with 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. Further, mechanical removal of brook trout was conducted between sampling events. During the summer sampling event (2011 and 2012), a systematic sampling design was implemented to obtain water samples from High Lake. During the fall sampling (2011 and 2012), 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 (200 parts per billion). 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 2011 summer and 2011 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) compared with 0% (area 1) and 14% (area 2). 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). In 2011, the detection rate at spawning area 3 was significantly greater than for other spawning areas and greater than among the samples collected using systematic sampling ($P<0.5$).

The results from 2011 provided a basis for 2012 sampling and analysis recommendations. In 2012, we recommended that BPT continue sampling following the field and lab protocols established during 2011, as comparable methods would yield comparable analytical results. Minimization of method effects would be of value when comparing results between years. In 2012, no positive detections of brook trout DNA were observed in water samples, which means that essentially no brook trout DNA was present in samples analyzed in the lab. Given the same sampling protocols were followed both years; there are two interpretations of these results. Brook trout DNA could have been present in High Lake and sampled during water collection, but degraded prior to testing. This will be considered further below. Another explanation could be that brook trout DNA was present at low levels in the lake making it unlikely to occur within water collections at current sampling rate (i.e., ~ 20 samples per event). Given that brook trout abundance was estimated in 2011 as N=1486 (+/- 533) and N=1458 (+/- 1134) for 2012, with up to 800 individuals mechanically removed from the lake between fall 2011 and fall 2012, the lowered density could have influenced detection of DNA. Takahara et al. (2012) reported a correlation between concentration of eDNA and biomass suggesting that eDNA copy number (per liter) may reflects biomass (mg per liter). This observation corroborated results by Ficetola et al. (2008) showing eDNA failed to detect target species in natural ponds when the relative abundance was low. Furthermore, Taberlet et al. (2012) showed that eDNA degrades in the water column on the order of days, suggesting that removal of individuals from the lake prior to water collection would contribute to fewer DNA copies present at time of sampling. Nevertheless, brook trout were likely still present in High Lake at time of water sampling, so methods to decrease false negative rates need to be considered.

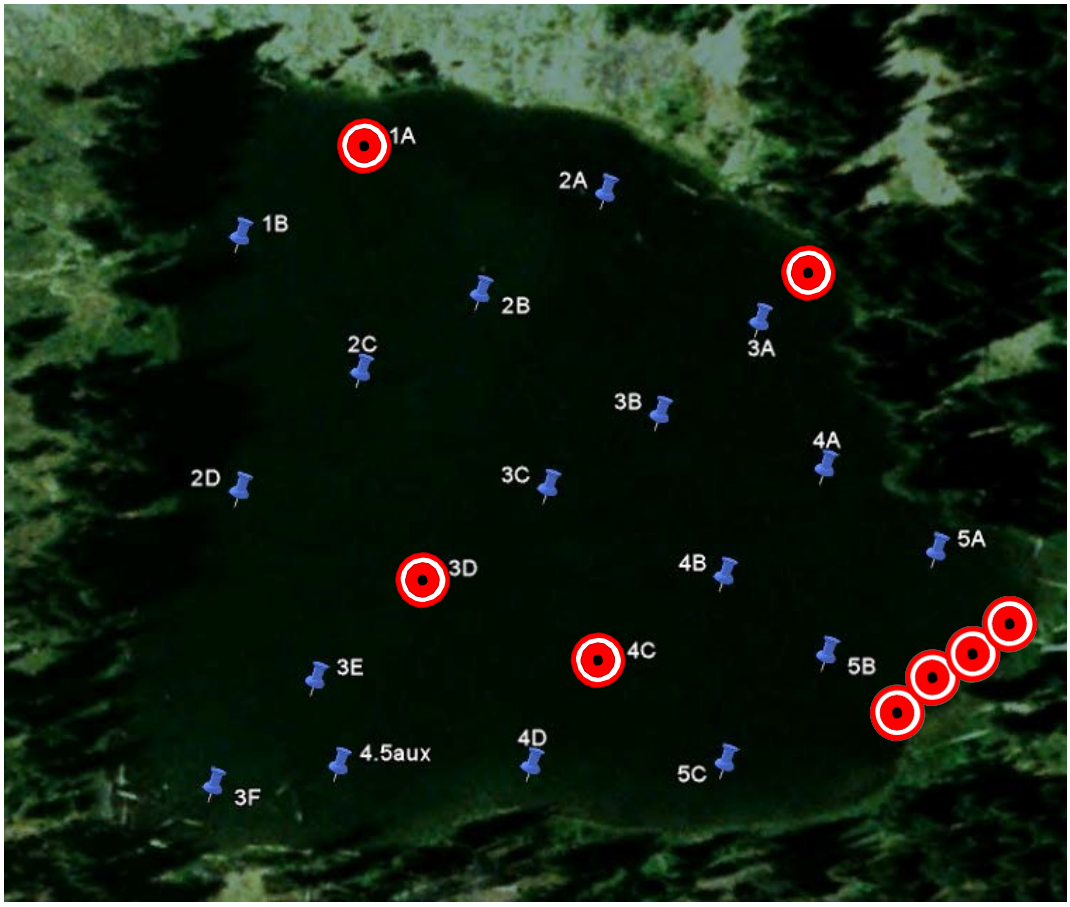

Figure 6. Approximate locations of water samples that tested positive for brook trout DNA.

Further considerations for use of brook trout eDNA in sampling and analysis relate to sample rate (i.e., volume) and filtration methods. 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 (only detection). 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, fewer 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 using current methods. Adoption of new filtration technology would need to occur to achieve suggested volume target increases per sample.

While filtration technology would enable increased volumes of water to be sampled, a more critical aspect likely is decreasing the time from water collection to extraction of DNA, which would decrease the opportunity for DNA degradation. Published accounts of using eDNA vary considerably in the amount of water sampled. For example, 1000 2-L samples (Jerde et al. 2011), 20 1-L samples (Takahara et al. 2012), or one 10-L sample (Goldberg et al. 2011). Yet, all methods process water quite rapidly following collection. In controlled trials of ingested tissue for target species, detection rate degrades rapidly after about 12 hours. For DNA analysis that uses scat as collection medium, after about 12 hours samples appear to have markedly reduced detectable DNA. For eDNA specific tests, the most detailed publication using mesocosms showed that concentrations of a few hundred copies of DNA present within stocked aquaria disappeared on the order of 5 days after animal removal. If High Lake has at equilibrium on the order of ~1000s of copies of DNA to sample at any given time, then the DNA molecules actually sampled may degrade after a month or two from collection to extraction. For fall sampling events, collection to extraction was approximately 40 days in 2011 and 50 days in 2012. While adopting new filtration methods would allow for larger volumes to be sampled, the critical benefit in our view would be reducing the time to extraction (i.e., 5 – 7 days), which would likely increase detection rate.

In the near term, the greatest use of brook trout eDNA sampling and analysis appears to be 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). Yet, the eDNA to biomass relationship defined by Takahara et al. (2012) (i.e., 1000 copies of eDNA per gram biomass) should be considered further. Given enough samples, detection monitoring appears to be technically feasible. Detection monitoring also appears to support the evaluation of trends from sample event to sample event. Of interest would be comparing eDNA detection rate with CPUE at time of brook trout removal, which 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 (or volumes), which is recommended, as it is a by-product of employing filtration methods to decrease time to extraction.

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.

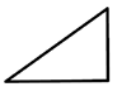
5) **Shipping samples:** Repackage dried fin clips separately and attach field notes for shipping. Dry samples can be sent surface mail with no special packaging.

† *approximate* size of fin clip:

§ sample cryovial label:

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

n clip:



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