Evaluate the Life History of Salmonids in the Malheur Subbasin

Burns Paiute Tribe Natural Resources Department, Fisheries Program
Burns, Oregon

FY 2019 Annual Report
Project 1997-019-00
Contract #67693

Prepared for Bonneville Power Administration and Northwest Power and Conservation Council

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For work completed January 2019-December 2019

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Background and Context for FY2019 Annual Report

The Bonneville Power Administration (BPA) has supported fisheries research and management conducted by the Burns Paiute Tribe (BPT) Natural Resources Department in the Burns Paiute ancestral homeland since 1997. This report summarizes work completed by the BPT Fisheries Program in 2019. Field work conducted, data collected, objectives accomplished, and management activities fulfilled were approved by the Northwest Power and Conservation Council during the 2011-12 Categorical Review of Resident Fish Projects.

The primary focus of BPT Fisheries in 2019 was to continue mechanical efforts to suppress invasive brook trout. Brook trout (native to eastern United States) were introduced into the Upper Malheur around the 1930's, and brook trout remain the primary limiting factor (as identified by U.S. Fish and Wildlife Service Endangered Species Act recovery plans) to bull trout recovery. Until regulatory processes can be completed to allow progress toward brook trout eradication via chemical treatment, mechanical suppression will be conducted by BPT Fisheries. In 2019, BPT Fisheries electroshocked high-density sites in lower Lake Creek, electroshocked the entire reach of upper Lake Creek, and set gillnets in High Lake.

BPT Fisheries continued monitoring the ten-annual temperature sites on the BPT Logan Valley Mitigation Property. 2019 was a high-water year which resulted in the loss of one of the hobos located on McCoy Creek on the Logan Valley Mitigation Property. BPT also continued monitoring efforts at locations in the Upper Malheur and over in the North Fork of the Malheur. 2019 temperature results support past trends. 1) Lake Creek in Logan Valley continues to have high temperatures which can act as thermal barriers to bull trout. 2) The North Fork of the Malheur temperature sites are consistently cooler compared to the Upper Malheur sites.

The BPT Fisheries program applied eDNA sampling and backpack electrofishing to investigate the suspected presence of invasive brook trout in the Little Malheur. The Upper North Fork of the Malheur River is disconnected from the Malheur basin by Agency Valley Dam. Brook trout were assumed to be absent from this system. In 2018, BPT Fisheries paired environmental DNA (eDNA) samples with electroshocking in the Little Malheur River. Confounding 2018 results required BPT Fisheries to return to the Little Malheur, summer of 2019, and conduct a broad eDNA sampling and electroshocking effort. BPT took 34 eDNA samples on the Little Malheur (mainstem and tributaries). Six sites were positive for brook trout eDNA presence, and BPT will work with the TAC to address confirmed presence of brook trout in the Little Malheur.

BPT continued baseline data collection and outreach for future piscicide (rotenone) treatments in Lake Creek. BPT Fisheries participated in multiple outreach events for the Help Native Fish program (www.helpnativefish.com) to educate public on local fisheries management and Eastern Oregon native fish species. 2019 did not include the annual ODFW North Fork Malheur bull trout spawning surveys (in which BPT assists) due to the Cow Fire (wildfire). The 2019 BPT Fisheries Staff included: Brandon D. Haslick (Fish Project Manager), Rebecca Fritz (Fish Biologist), Gabe First Raised (Seasonal Fisheries Technician), and Truston Snapp (Seasonal Fisheries Intern).

Chapter 1: Selective Removal of Brook Trout (*Salvelinus fontinalis)* in Lake Creek, Upper Malheur River, Oregon

Rebecca J. Fritz and Brandon Haslick
BPT Natural Resource Department, Fisheries Program

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Chapter 1:

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

Rebecca J. Fritz and Brandon D. Haslick
BPT Natural Resource Department, Fisheries Program

1.1 Introduction

Malheur River Bull Trout (*Salvelinus confluentus)* were listed as threatened under the Endangered Species Act in 1998 (USFWS 2015). The Bull Trout Recovery Plan (USFWS 2015) identifies the key threats to bull trout within geographically broad Recovery Units and their associated local Core Areas. 2019 BPT Fisheries management for bull trout recovery falls within the Upper Snake River Recovery Unit and the Upper Malheur River Core Area. Specifically, this year's management actions were implemented in Lake Creek focusing on the removal of invasive brook trout (*Salvelinus fontinalis).* Brook trout have been determined the primary threat to Upper Malheur Bull Trout recovery (USFWS 2002, 2015).

Invasive Brook Trout in the Upper Malheur

Brook trout occur in abundance in the Upper Malheur Subbasin as a result of authorized and unauthorized stockings. Around the 1930's brook trout were stocked in Lake Creek's source, High Lake (Bowers et al. 1993). Invasive brook trout in the Upper Malheur Subbasin outcompete (Gunckel et al. 2002) and hybridize with threatened bull trout (Dehaan et al. 2009). The growing

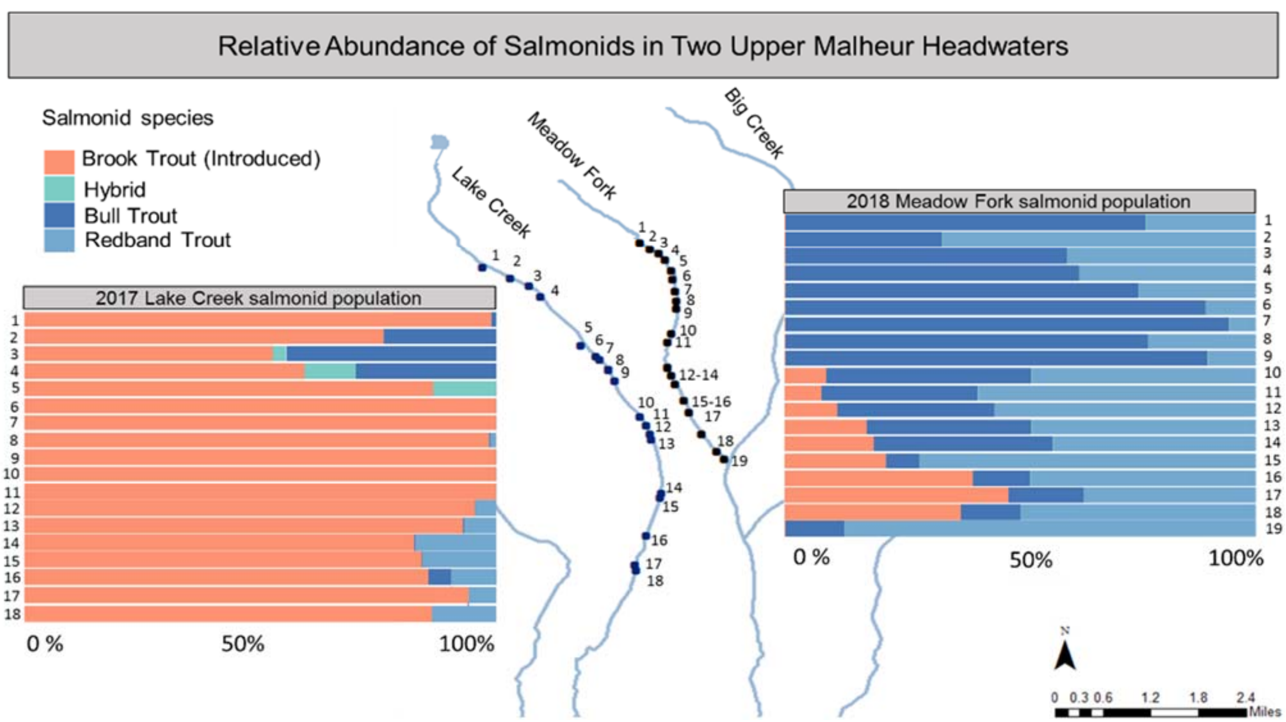

Figure 1.1 Relative abundance of 2017 and 2018 BPT population estimates**.**

competition for resources, along with hybridization, has been directly contributing to bull trout population decline in the Upper Malheur.

The two major tributaries which form the Upper Malheur and are the focus of the BPT's management are Lake Creek and Big Creek. A tributary of Big Creek, Meadow Fork Big Creek, is dominated by native trout species despite the presence of brook trout (Crowley 2018). Neighboring Lake Creek has the opposite trend as brook trout significantly outnumber bull trout (Crowley 2017) (Figure 1.1). Due to a natural fish barrier, the uppermost three km of Lake Creek and High Lake contain only invasive brook trout. This allows them to reproduce without competition for resources- thus providing a 'seed source' population to invade downstream bull trout Critical Habitat. Therefore, High Lake and upper Lake Creek are of immediate management concern. The overarching focus of 2019 BPT field work was to continue brook trout suppression efforts in Lake Creek. Brook trout were removed using mechanical methods from multiple sites in the lower reach of Lake Creek and from the entire reach of upper Lake Creek to provide relief to the native salmonids.

1.2 Methods

The 2019 BPT Fisheries Program focused efforts on continuing the mechanical removal of brook trout from Lake Creek and High Lake. Mechanical methods included: backpack electrofishing efforts in Lake Creek, gill-netting efforts in High Lake, and angling in High Lake. Lake Creek Falls separates upper Lake Creek (inhabited only by brook trout) from lower Lake Creek (habitat to multiple salmonid species: brook trout, bull trout and redband trout (*Oncorhynchus mykiss gairdneri)*). The falls create a division in the Lake Creek fishery as well as in the following brook trout suppression methods.

Electrofishing lower Lake Creek

Fisheries used a LR24 Smith-Root backpack electrofisher to mechanically remove brook trout from Lake Creek. Brook trout removal occurred at specific sites below Lake Creek Falls (lower Lake Creek) (Figure 1.2 (A)). The selected sites had been surveyed in previous years and were considered high-density brook trout sites. Electrofishing took place beginning the 28^{th} of June and continued through the 11^{th} of July. At the start of each site a crew of two/three people performed a single pass survey working upstream. Electroshocker settings were maintained at 400 volts, 40 Hz, and at a 40% duty cycle. Brook trout captured were measured for length (fork length) and euthanized. Subsets of brook trout were weighed throughout sampling until weight data had been collected from 100 individuals. Trout fry (salmonid fry < 50 mm) were not directly targeted for capture in lower Lake Creek.

Non-target species were encountered at sites in lower Lake Creek. Any non-target species captured were taken downstream and revived. These captures were counted but, no other data were taken. Captured (brook x bull) hybrids (referred to hereafter as, hybrids) were measured and euthanized. Unless otherwise specified, data on the three individual hybrids removed during sampling season were combined with brook trout data.

Electrofishing upper Lake Creek

Upper Lake Creek was treated as a single site. A crew of two people began upstream of Lake Creek Falls and electroshocked the entire reach until High Lake. Shocking this section involved multiple efforts (a total of five) which took place from mid-July until the end of August. The final section was shocked coinciding with a gill netting effort in High Lake. All captured trout fry were counted and euthanized from upper Lake Creek.

Gill-netting and angling in High Lake

2019 suppression efforts ended with a final removal event in High Lake using two ¾ inch gill nets. BPT Fisheries spent a week in August 2019, setting gill-nets and angling. Two gill nets were set in High Lake and left to soak for 24 hours. Brook trout were pulled from nets and euthanized. All trout captured were measured (fork length in mm) and a subset of weights was taken (~100 randomly selected fish). Nets were cleaned are deployed again in a different section of High Lake. While nets were soaking BPT crew members angled for brook trout in High Lake.

Data Analysis

All 2019 data were analyzed using R studio (R version 3.6.0) and maps were created in ArcMap 10.5. Condition factor (K) was calculated for every brook trout that was both measured and weighed (100 individuals) in lower Lake Creek. The mean (K) is reported and was calculated in R studio where W = weight in grams and L= length in mm. $K = \frac{10^5(W)}{(L)^3}$ (Ricker 1975). Reports can be found on www.cbfish.org under project number 1997-01900. Data from the hybrids captured were incorporated in with brook trout analysis unless specified otherwise.

1.3 Results

In total, 2,257 brook trout were removed from Lake Creek and High Lake using various mechanical methods (Table 1). Three hybrids were also removed from Lower Lake Creek (2,260 total salmonids removed).

Table 1 Total brook trout removed in 2019 using mechanical methods

	Electrofishing	*Gill-netting*	*Angling*
Lower Lake Creek	777	—	—
Upper Lake Creek	448	—	—
High Lake	—	1,022	10
# Removed / Method	1,225	1,022	10
Total # Brook Trout Removed	**2,257**		

Lower Lake Creek electroshocking

Stream temperatures ranged from (7 - 12 °C) throughout lower Lake Creek shocking sites. Four fish species (brook trout (Table 1), bull trout (29 individuals captured), redband trout (5 individuals captured), sculpin (*Cottus spp*), and unidentified 'trout fry' (defined as salmonid fry < 50 mm) were encountered during lower Lake Creek electrofishing surveys. No bull trout or redband trout mortalities resulted from the year's sampling effort.

Brook trout (and hybrids) made up the greatest proportion (~93%) of the overall population captured in the 2019 lower Lake Creek Sites (Figure 1.2 (B)). Lengths ranged from 60-250 mm (Figure 1.2 (C)) (with the highest frequency occurring in the 100-150 mm sizes (Appendix Figure 1.3). Combing the length data with collected weights resulted in the average condition factor for (100) fish sampled was K= 1.196 (or, 1.2). This value places the physical body condition of lower Lake Creek brook trout as being considered relatively fair (Appendix Figure 1.4) (Barnham & Baxter, 1998).

Upper Lake Creek and High Lake

Brook trout are the only fish species to occur above Lake Creek Falls and in High Lake. 448 brook trout were removed from upper Lake Creek by electroshocking the entire reach from Lake Creek Falls to High Lake (Figure 1.2 (A)). The final portion of upper Lake Creek was shocked concurrently with the High Lake gill-netting. A total of 1,032 brook trout were removed from High Lake with two ¾ inch gill nets and angling. High Lake brook trout had a slightly lower mean condition factor (K= 1.18) than the brook trout sampled in lower Lake Creek.

1.4 Discussion

Mechanical methods unable to effectively eradicate Lake Creek brook trout

The focus of the BPT fishery program is to protect, restore, and enhance native fish assemblages in the Malheur River with an emphasis on ESA-listed bull trout. The unencumbered recruitment of brook trout in upper Lake Creek and High Lake which then populates lower Lake Creek Critical Habitat has driven almost a decade of BPT brook trout suppression efforts using mechanical methods (Poole and Harper 2011). BPT Fisheries established a baseline population estimate for Lake Creek brook trout in 2012 and compared it with the estimate resulting from a replicated study conducted in 2017. The intention of this research was to examine the effectiveness of five years of brook trout suppression efforts using mechanical methods (Harper 2013; Crowley 2017) addressing multiple questions. What impact did removal have physically on the brook trout population? Do removal efforts effectively remove a significant proportion of brook trout? Were there lasting impacts?

The five-year BPT study looked at the effectiveness of mechanical suppression and resulted in three main conclusions which were further supported by the 2019 data. **1)** Mechanical suppression efforts have not resulted in a significant change in brook trout body size or condition. After 2012, BPT saw a higher frequency of captures shifting to a slightly smaller size class but, when combined with a similar condition factor throughout the study, changes were considered minimal (Crowley 2017). The 2019 condition factor followed this trend (Appendix 1.4). **2)** Although by the end of the Lake Creek study BPT reduced the brook trout population by

~30%, there was no increase in native salmonid populations (Crowley 2017) and brook trout still made up the majority of the salmonid population.

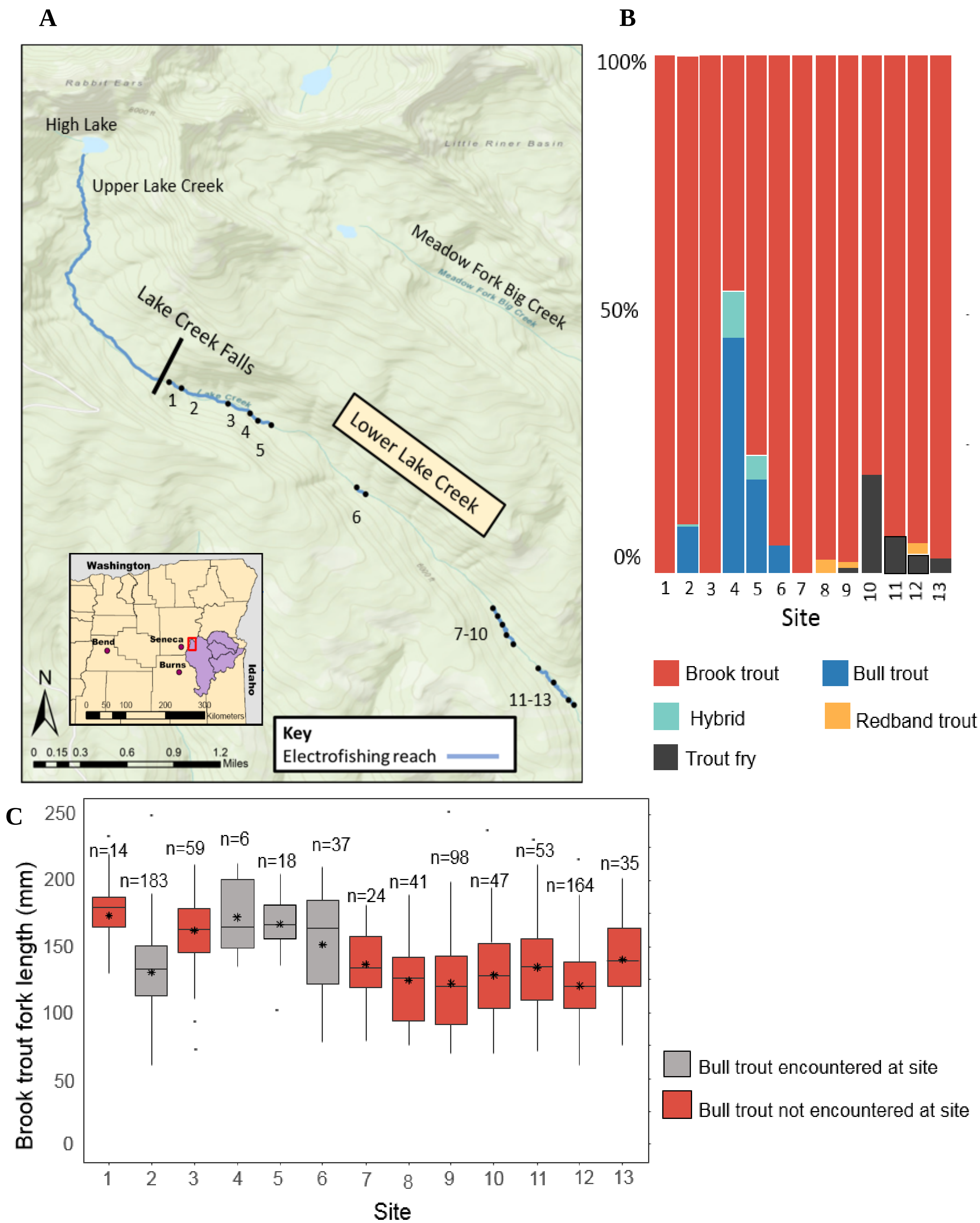

Figure 1.2 (A) Map of Lake Creek 2019 electrofishing sites (B) 2019 relative abundance of salmonids at each electrofishing site in lower Lake Creek (C) Lengths of lower Lake Creek brook trout and the three hybrids**.** N= 779. (*) denotes the mean for the site

Continuing the trend, 2019 brook trout dominated the lower Lake Creek salmonid relative abundance (Figure 1.2 B). **3)** The Lake Creek brook trout population is resilient and rebounds despite the removal efforts. The Lake Creek brook trout population can almost completely recover to pre-suppression numbers within a year. Wildfires in 2013 and 2015 prevented High Lake removal efforts and the Lake Creek population strongly rebounded (Crowley 2015). 2019 removal totals were no lower than previous years. In total, 2019 mechanical suppression efforts removed over 2,000 brook trout from Lake Creek, and ~800 of which were directly removed from habitat shared by native salmonids.

Lack of success in eradication efforts using mechanical methods has been demonstrated outside of the BPT's efforts in Lake Creek. Various studies in multiple streams have scrutinized the inability of backpack electrofishing to fully eradicate invasive trout (Thompson &Rahel 1996; Meyer et al. 2006) as well as its higher cost in effort and resources when compared to a piscicide treatment (Buktenica et al. 2013). A collaborative management effort using electrofishing to target brook trout in Idaho streams ended with several conclusions mirroring BPT's own findings. The conclusions: electrofishing removal efforts failed to eradicate 100% of the population, saw a large increase in age-0 abundance after removal efforts, and did not result in a significant increase in native fish populations (Meyer et al. 2006).

Further limitations with using electrofishing to eradicate brook trout are emphasized by outside studies and also experienced by BPT. For instance, 1) electrofishing is size selective (Reynolds 1996). A common pattern among projects is the inability to effectively capture all fry (Thompson &Rahel 1996; Meyer et al. 2006). This problem is compounded in the BPT efforts in lower Lake Creek. Due to the desire to protect struggling populations of native salmonids, BPT does not capture or remove unidentified fry in lower Lake Creek. 2) Complete eradication using mechanical methods may be an effective option for small streams and/or simple habitat. Habitat complexity (log jams, pools, beaver dams) limits the ability to completely capture all targeted trout during electrofishing. Lake Creek has complex habitat throughout the entire reach. Log jams, pools, subterranean flow, marshes, side springs, pools are all examples of locations where BPT removal efforts likely fail to remove all brook trout. The ineffectiveness of mechanical methods to completely eradicate brook trout is supported by outside studies, and further restricted by Lake Creek's complex habitat as well as a limited field season.

1.5 Recommendations

Throughout removal efforts, BPT has formulated a plan to fully eradicate brook trout from Lake Creek using rotenone. While BPT works with agency partners to implement such a treatment, suppression efforts aim to continue in Lake Creek and High Lake to provide relief to native salmonids. In 2019, BPT had continued collaboration with the Malheur River Bull Trout Technical Assessment Committee (TAC) which formed and created the Upper Malheur Watershed Bull Trout Conservation Strategy in 2017 (TAC 2017). BPT will continue mechanical suppression in Lake Creek until the implementation of an anticipated, large scale, interagency rotenone treatment in the Upper Malheur.

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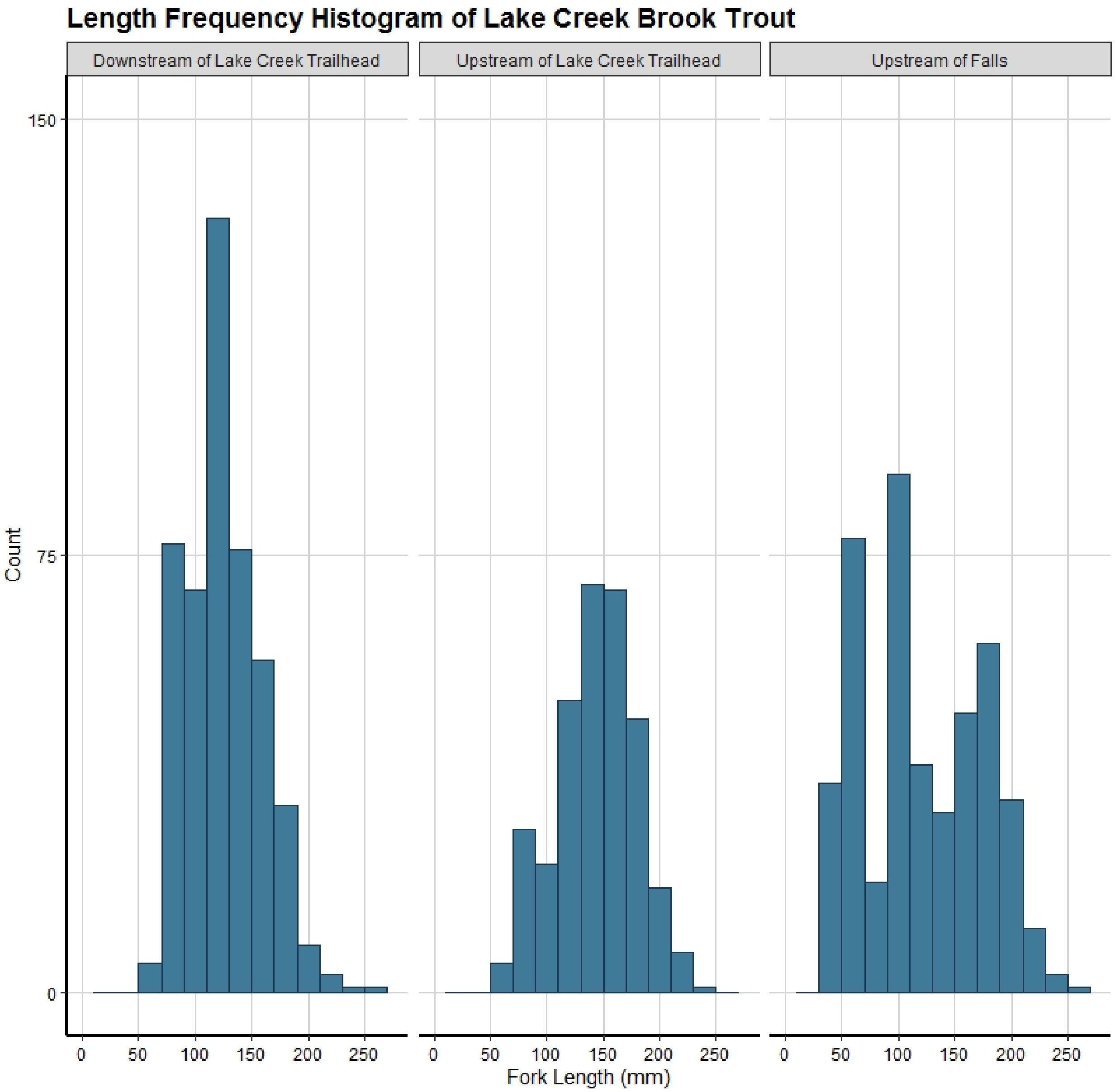
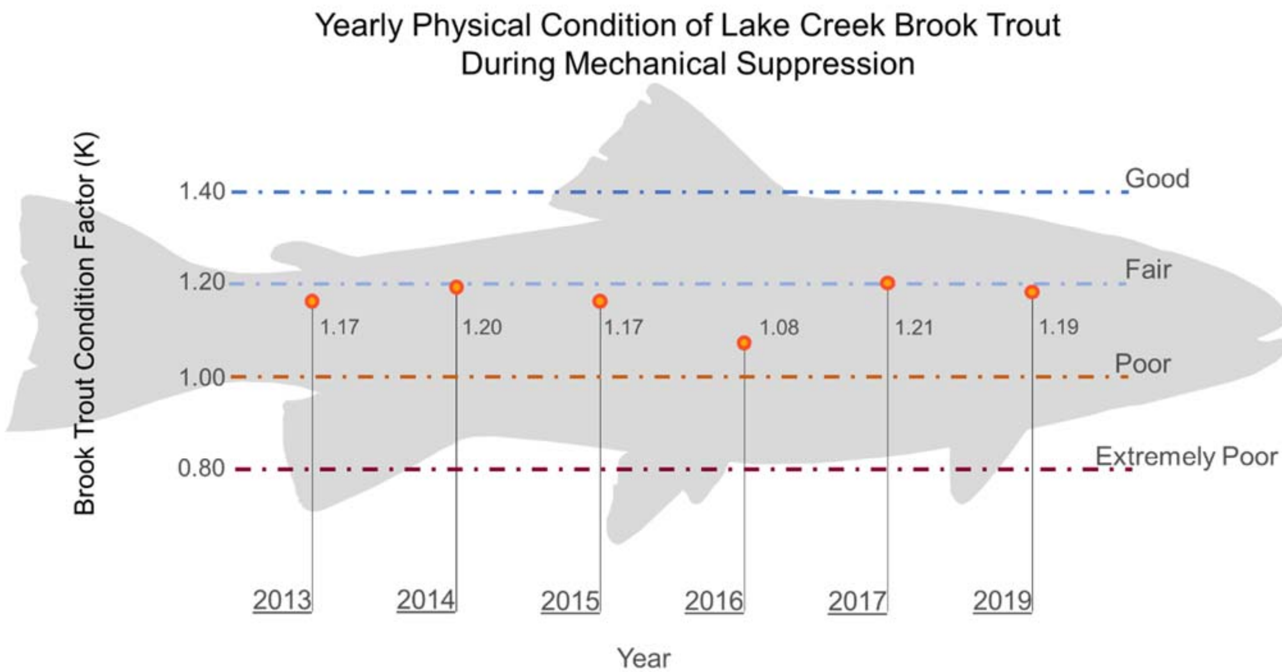
Appendices



Appendix Figure 1.3 2019 histogram detailing brook trout lengths (mm). Lengths are measured as fork length. Relative to habitat shared with native salmonids, Downstream of Trailhead (downstream of site #7) = redband trout encountered; Upstream of Trailhead (upstream of site 7) = bull trout encountered; Upstream of falls = single species (brook trout)



Appendix Figure 1.4 Mean condition factor (K) calculated for the lower Lake Creek brook trout throughout BPT suppression efforts

Chapter 2: Stream Temperature Monitoring in the Upper Malheur Subbasin, the *Logan Valley Wildlife Mitigation Property*, and the North Fork of the Malheur Subbasin

Burns Paiute Tribe Natural Resources Department, Burns OR 97720

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Chapter 2

Stream Temperature Monitoring in the Upper Malheur Subbasin, the Logan Valley Wildlife Mitigation Property, and in the North Fork of the Malheur Subbasin

Rebecca J. Fritz and Brandon D. Haslick
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2.1 Introduction

Stream temperature directly impacts native fish populations. Three of the native Malheur River salmonids (bull trout, redband trout, and (reintroduced for a put-and-take fishery) Chinook salmon (*Oncorhynchus tshawytscha)*) are considered vulnerable to climate change (Halofsky and Peterson 2017). Of these three, bull trout are a current management focus for the BPT Fisheries Program and are considered the most sensitive to high stream temperatures (Buchanan and Gregory 1997; Haas 2001; Selong et al. 2001; Dunham et al. 2003). Stream temperatures are an important component in understanding habitat quality and fish distribution, particularly in respect to bull trout populations. High stream temperatures create thermal barriers, threaten spawning success/early stage survival, and decrease resiliency to wildfire or environmental disturbances (Rieman et al. 2007; Halofsky and Peterson, 2017).

The Burns Paiute Tribe began monitoring stream temperatures in the Upper Malheur Subbasin after the purchase of the Logan Valley Wildlife Mitigation Property (LVWMP) in the spring of 2000. This property includes the confluence of the headwater tributaries which form the Upper Malheur River (also referred to as the Middle Fork of the Malheur). A series of ten stream temperature sites have been monitored annually to track the effects of habitat improvement projects on the property (Figure 2.1 A). Since the establishment of the annual sites, BPT's stream temperature monitoring has expanded to include various sites in the Upper Malheur as well as in the North Fork of the Malheur.

The BPT temperature monitoring program has grown since it started in 2000, and currently incorporates multiple objectives. 1) BPT continues to monitor thermal barriers to bull trout on the LVWMP. 2) BPT monitors the temperatures of the Upper Malheur headwaters to inform future bull trout management efforts. 3) BPT monitors temperatures throughout bull trout habitat in the North Fork of the Malheur and 4) collaborates with partner agencies to place loggers in locations which will contribute to the interagency monitoring effort as well as potentially provide temperature data for significant temperature modeling efforts.

2.2 Methods

Study Area

The Burns Paiute Tribe Fisheries Program monitors temperatures in the Malheur River Watershed in Eastern Oregon. The sites selected for temperature monitoring fall into two subbasins: Upper Malheur and North Fork of the Malheur. BPT temperature sites in the Upper

Malheur are further grouped by 1) the ten annual sites located on the BPT Logan Valley Mitigation Property and 2) sites on the major Upper Malheur tributaries.

Logan Valley Mitigation Property

The Logan Valley Wildlife Mitigation Property is located south of the Strawberry Mountains, located in the Strawberry Mountain Wilderness in eastern Oregon. This property spans 1,760 acres and includes the confluence of McCoy Creek, Lake Creek, and Big Creek which form the Malheur River (or Middle Fork of the Malheur). These headwater tributaries come together approximately 200 river miles upstream from where the Malheur joins the Snake River. In 2000, the Tribe began collecting seasonal (spring-fall) data on stream temperatures at five sites of the LVWMP. These sites have been maintained in the same locations and five more have been added within the property boundaries over time (Table 2.1) (Namitz 2000; Schwabe 2001, 2002, 2003, 2004, 2007; Fenton and Schwabe 2005, 2007; Fenton 2006; Abel 2008, 2009; Brown 2010, 2011, 2012; Haslick 2014, 2015, 2016, 2017, 2018).

Table 2.1 Burns Paiute Tribe ten annual temperature sites on the Logan Valley Wildlife Mitigation Property**.** (*) denotes the loggers exposed to air temperature during the 2019 monitoring period

Site #	Location	Year Initiated	2019 Hobo Retrieved	Year Initiated Reference
1	Lake Creek below McCoy Creek	2000	Yes	Namitz 2000
2	Lake Creek below Crooked Creek	2000	Yes	Namitz 2000
3	Malheur River below Big/Lake Creek	2000	Yes	Namitz 2000
4	Big Creek 1-mile south FS-16 Road	2000	Yes	Namitz 2000
5	Big Creek below FS-16 Road	2000	Yes	Namitz 2000
6	Lake Creek below FS-16 Road	2007	Yes *	Schwabe 2007
7	McCoy Creek above Lake Creek	2007	No	Schwabe 2007
8	Lake Creek at Cabin Bridge	2008	Yes *	Abel 2008
9	McCoy Creek below FS-16 Road	2009	Yes	Abel 2009
10	Lake Creek Ditch below FS-16 Road	2009	Yes	Abel 2009

Upper Malheur River

The ten annual stream temperature sites in Logan Valley are the overarching focus of the BPT monitoring effort. However, the tribe has expanded the program to include loggers upstream (North) of the LVMP. These sites are on Lake Creek (including the High Lake outlet), Big Creek, and McCoy Creek (seven sites to date in 2019) (Figure Appendix 2.5).

North Fork of the Malheur River

BPT has also expanded the temperature monitoring effort to include tributaries in the neighboring North Fork Subbasin. This involves nine monitoring sites on the North Fork of the Malheur and its tributaries (Figure Appendix 2.5). These locations are on streams in USFS managed forests. In total, for the 2019 field year BPT was actively monitoring stream temperatures in both Grant and Baker County- Eastern Oregon.

Field Techniques

Pre/Post Deployment:

All stream temperatures were monitored using Tidbit v2 Temperature Loggers (hereafter referred to as, loggers) which are a product of the Onset Computer Corporation. Prior to stream deployment, the battery life and memory storage were checked, and all loggers were set to take a temperature reading at the start of every hour.

Field Deployment:

All temperature loggers at the Logan Valley Mitigation Property were set in the field either May 16th or May 20th, 2019. Most Upper Malheur loggers and North Fork loggers were also set within this time frame. Due to snow limiting road access, Corral Basin (#12) and Swamp Creek RM3 (#26) loggers were placed on June 7th. The High Lake logger (#17) was placed July 18th (Appendix Figure 2.5). At the stream site, each logger was directly attached to an eight-pound anchor and placed in the thalweg of the stream. Anchors were secured by cable and tied off on a tree or staked into the bank. Loggers were collected within the last week of October-first week of November. Once gathered from the field, loggers were required to pass post-deployment accuracy tests.

Data Analysis

The BPT monitors temperatures starting in late spring through late fall. Due to the yearly differences in logger deployment, BPT reports temperatures from June 1st – September 30th. This establishes a standard 122-day monitoring period for most loggers (road access and snow level can alter individual deployment dates).

Data are analyzed using the same methodology as previous years summarizing temperature data using mean weekly maximum temperature (MWMT) in °C (as summarized in Haslick 2018). MWMT (the average of a rolling 7-day temperature maximum) is used due to its accuracy as a biological parameter describing stream temperatures. Specific temperature benchmarks are recognized as standard parameters and used in this report. The first two Stream Temperature Standards established through the Department of Environmental Quality (DEQ) are 12 °C MWMT (optimal temperature for rearing juvenile bull trout and considered the maximum temperature for bull trout migration) and 16 °C is the ideal temperature for core salmonid rearing areas (OAR 340-04102004). The final temperature standard highlighted in this report is the Incipient Lethal Temperature (ILT) in which stream temperatures ≥ 20.9 °C are harmful to ESA listed bull trout (Selong et al. 2001). Evidence for the two dewatered sites (Logan Valley annual sites #6 and #8) in which, the loggers were exposed to air, is provided(Appendix Figure 2.7). The point of air exposure is identified, and further temperature data are excluded. 2019 data were analyzed using R Studio version 3.6.0, “Planting of a Tree” (R Studio 2019) and maps of were created using ArcMap 10.5. Raw data can be obtained by contacting Brandon Haslick (brandon.haslick@burnspaiute-nsn.gov).

2.3 Results

Oregon Department of Fish and Wildlife (ODFW) defines the, 'critical period' for high stream temperatures in the Malheur watershed as, the summer timeframe which falls within the dates, July 15th thru August 15th (Perkins 1999). Peak high stream temperatures occur within or near this critical period (Figure 2.2 A) and the critical periods has been used as a base index for comparing yearly stream temperatures in the Upper Malheur (Namitz 2000; Schwabe 2001, 2002, 2003, 2004, 2007; Fenton and Schwabe 2005, 2007; Fenton 2006; Abel 2008, 2009; Brown 2010, 2011, 2012; Haslick 2013, 2014, 2015, 2016, 2018).

Logan Valley Mitigation Property ten annual sites

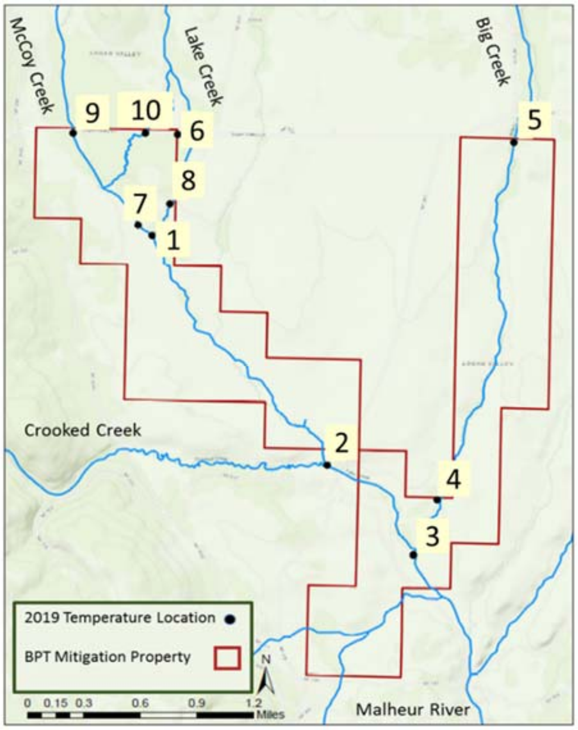
The 2019 BPT Logan Valley temperature sites (Figure 2.1 **A**) all had MWMT temperatures peak within the critical period (Figure 2.1 **B**). When comparing only the complete datasets, all but three sites exceed the ILT threshold for bull trout (20.9 C) (Selong et al. 2001). Of these three locations, the two sites on Big Creek (Site 4 and 5) have shown a pattern of not exceeding the ILT temperature standard (Figure 2.2 **B**).

When comparing the highest recorded daily temperatures for the last three years (2017-2019), 2019 had relatively cooler peak temperatures (Figure 2.2 B). Though the MWMT highs fall within the expected timeframe, four of the nine sites analyzed had peak daily average temperature occur July 12th (just days prior to the defined critical period). Of 122 sample days, most sites monitored had greater than 90 sampling days exceeding a MWMT of over 12°C: the DEQ Temperature standards for bull trout habitat. When comparing the two years, site #5 and site #10 show relatively cooler temperatures in 2019. Three sites (3,4,5) did not have a MWMT exceed the ILT limit for Bull Trout (20.9 °C) (although site 3 was close). All 2019 sites had a lower % of days compared to the previous year in MMWT > 20.9 °C.

Table 2.2 The total number of days (and %) for the last two seasons in which MWMT exceeded specified temperature benchmarks at the LVWMP. Sites 6 and 8 excluded due to air exposure. Only sites with temperature data for both years included.

	DEQ: Bull Trout ***Days >12 °C***		**DEQ: Salmonids** ***Days >16 °C***		**ILT: Bull Trout** ***Days >20.9°C***	
Site #	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**
1	119 days 98%	106 days 87%	92 days 75%	94 days 77%	29 days 24%	30 days 25%
2	120 days 98%	122 days 100%	95 days 78%	104 days 85%	44 days 36%	58 days 48%
3	112 days 91%	117 days 96%	75 days 61%	84 days 69%	0	3 days 2%
4	111 days 91%	107 days 88%	67 days 55%	66 days 54%	0	0
5	89 days 73%	104 days 90%	14 days 11%	32 days 38%	0	0
9	122 days 100%	116 days 100%	104 days 85%	106 days 91%	54 days 44%	62 days 53%
10	105 days 86%	116 days 100%	68 days 56%	84 days 72%	4 days 3%	39 days 34%

A



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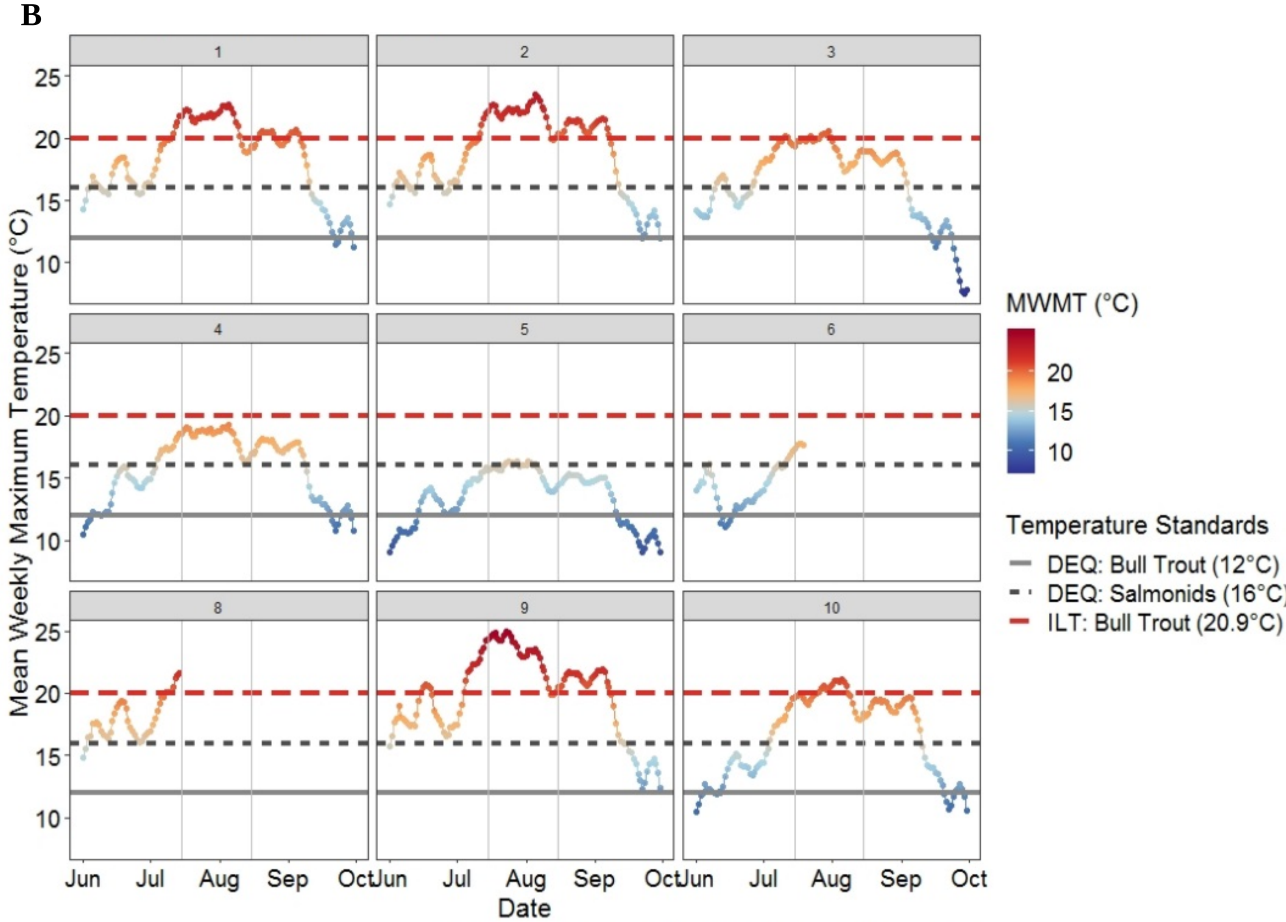
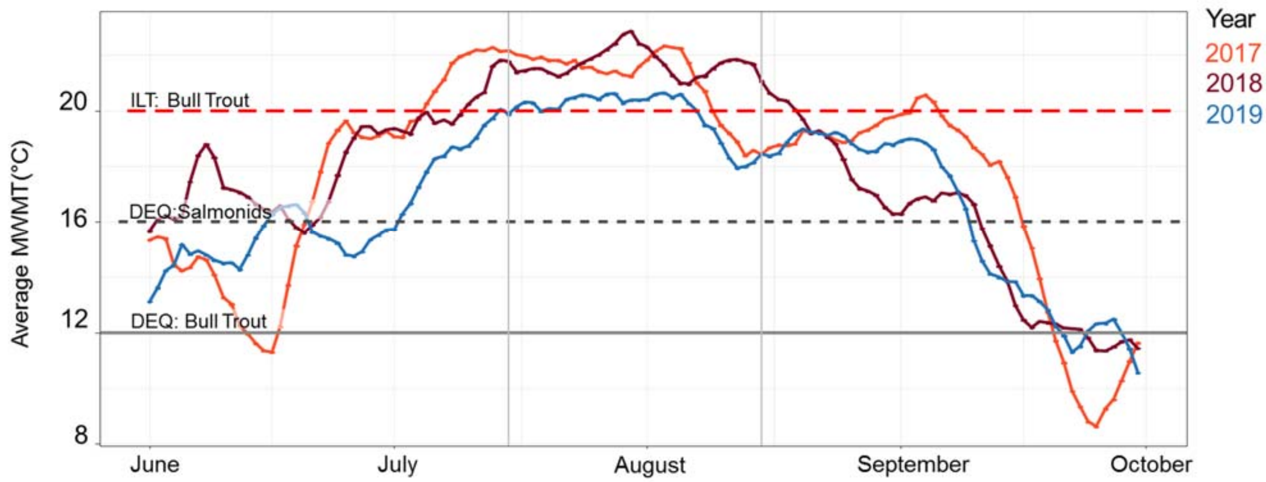


Figure 2.1 (A) BPT ten annual temperatures sites on the LVWMP **(B)** BPT 2019 MWMT (°C) values for the nine 2019 annual sites. Vertical lines denote the 'critical time period' for bull trout migration. Site 6 and 8 had an incomplete dataset due to air exposure. Site 7 was not retrieved in 2019.

A



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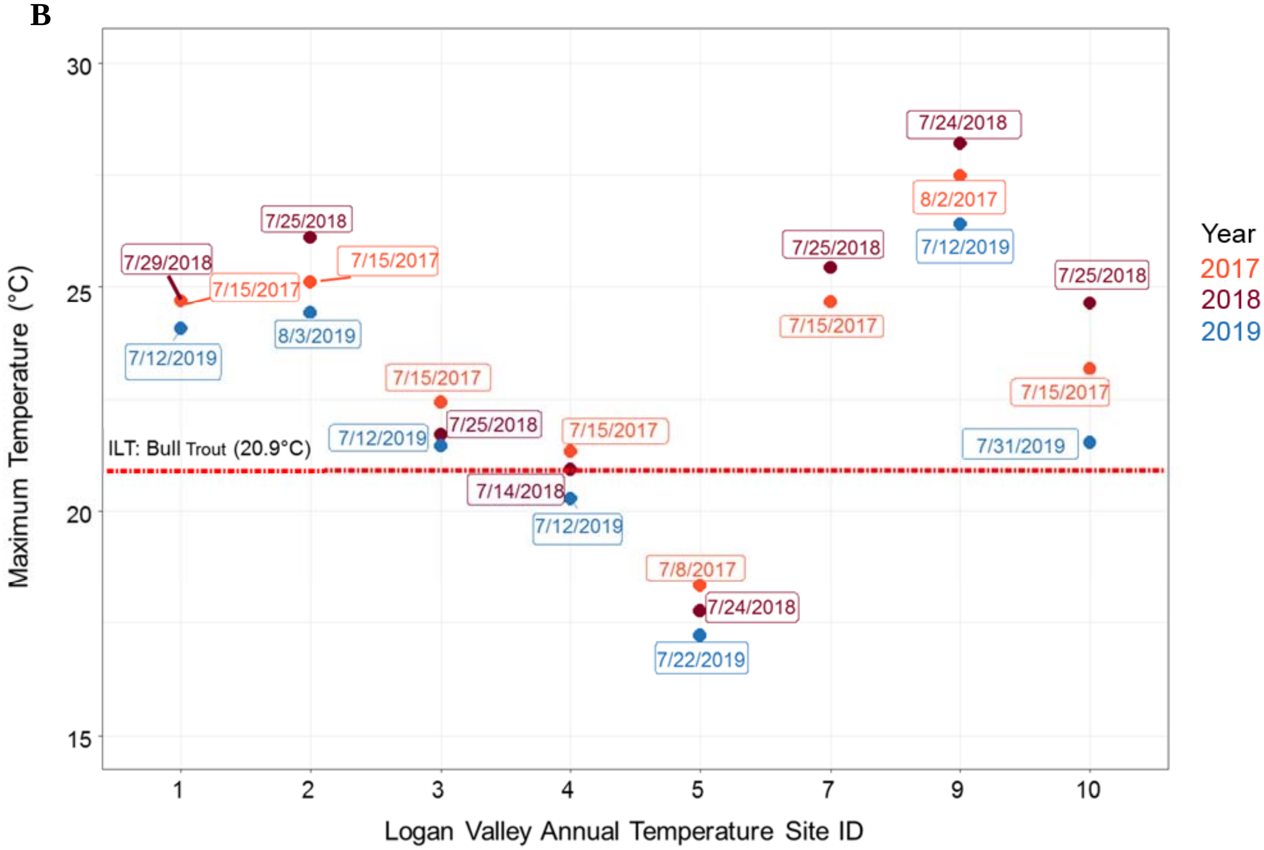

Figure 2.2 (A) Average MWMT (°C) BPT LVWMP ten annual temperature sites for the past three years. Temperatures are recorded for the BPT monitoring period: June 1st- September 30th. Vertical lines denote the 'critical time period' for bull trout migration. **(B)** The highest temperatures recorded for each site (excluding site 6 and 8) for the BPT LVWMP for the past three years (data found in **Appendix Table 2.3**)

Upper Malheur and North Fork Locations

BPT temperature monitoring has expanded to encompass multiple locations upstream of the LVWMP in the Upper Malheur tributaries as well as throughout the neighboring North Fork Malheur. The North Fork Malheur provides valuable habitat to a distinct population of bull trout (MW Council 2004). Comparatively, North Fork tributaries have temperatures which remain cooler throughout the summer critical period for bull trout (Figure 2.3). Of the BPT monitoring locations the ten annual sites (sites 1-10) in Logan Valley result in consistent thermal barriers to bull trout migration (Figure 2.3; 2.4).

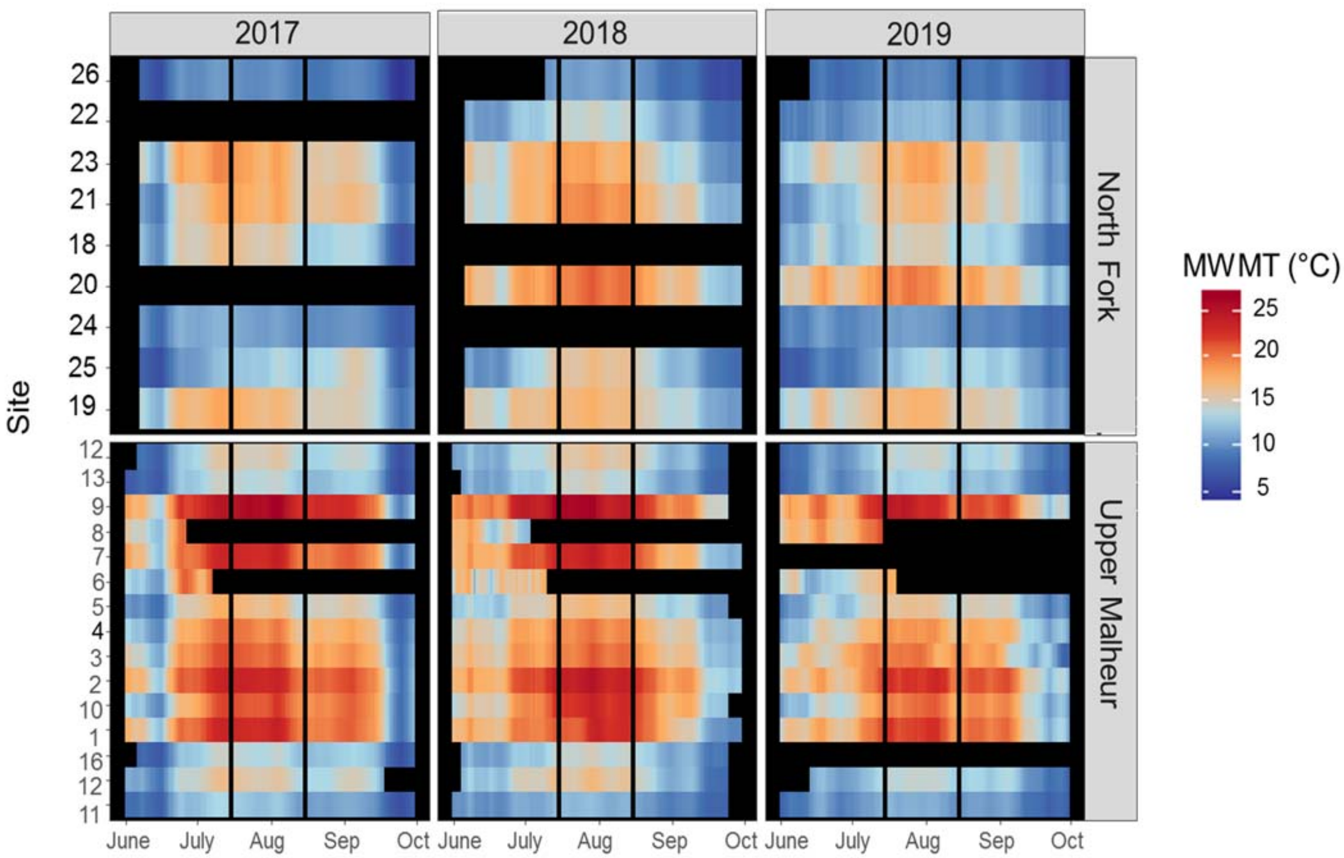

Figure 2.3 The past three years MWMT (°C) for North Fork and Upper Malheur BPT temperature logger locations**.** Sites 1-10 are the LVWMP ten annual sites. Vertical lines denote the critical period for bull trout (July 15th - August 15th). Sites (y-axis) correspond to the map: ***Appendix Figure 2.5***

2.4 Discussion

The Burns Paiute Tribe Fisheries Program entered a cooperative effort with the USDA Forest Service and ODFW to document stream temperature trends in the Upper Malheur (Namitz 2000). The BPT has been actively monitoring some temperatures in Logan Valley for nearly two decades (Namitz 2000) and this effort has grown to include over twenty locations in two different subbasins of the Malheur Watershed (the Upper Malheur and the North Fork of the

Malheur) which flow into the Malheur River (Haslick 2018). The purpose of collecting temperature data is to monitor stream habitat suitability for ESA listed bull trout. 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 the most thermally sensitive species; requiring cold water 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 presence (Haas 2001; Dunham et al. 2003). The ten annual monitoring sites in Logan Valley occur in U.S. Fish and Wildlife Service designated bull trout Critical Habitat (75 FR 63897 2010).

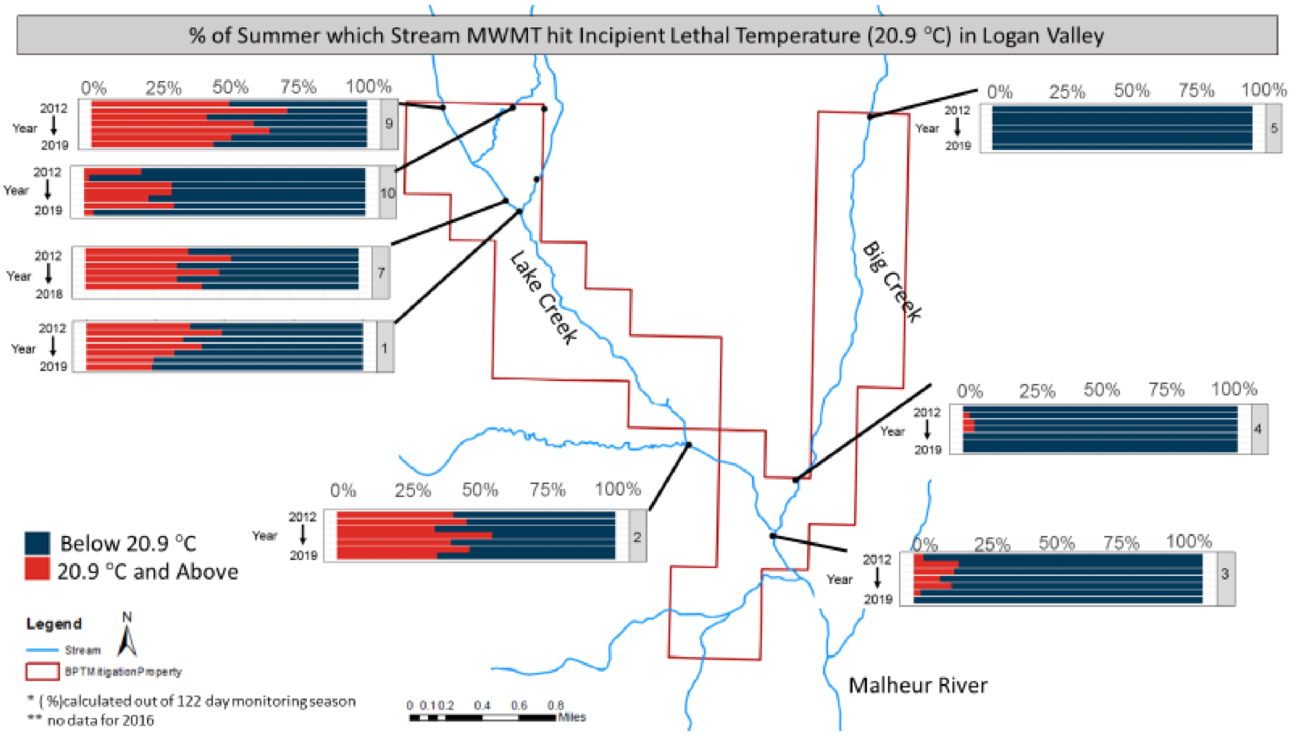

Figure 2.4 Percent of the days during the summer monitoring season (June 1st- October 1st) in which temperatures reach or exceed ILT at ten annual sites. (%) calculated out of a 122-day monitoring season. No data available for 2016 or for sites 6 and 8 (dewatered annually)

Logan Valley Mitigation Property sites consistently reveal thermal barriers to bull trout

Upstream of the BPT Logan Valley property, the tributaries forming the Upper Malheur run through forested National Forest and designated wilderness. Groundwater inputs create cool water temperatures in these headwaters, making them valuable bull trout habitat. The temperatures of these tributaries rise as they enter Logan Valley becoming restrictive to bull trout at most sites throughout the summer months (Figure 2.4). Several trends have been observed over time regarding temperatures on the LVWMP. 1) Big Creek lowers the temperature of the Malheur River (site 3). 2) McCoy Creek (sites 7 and 9) is a driver of the hot stream temperatures in Lake Creek. 3) Finally, lack of continuous flow throughout the summer (sites 6 and 8) presents barriers to migrating fish and could potentially lead to entrainment (Figure 2.3, Appendix Figure 2.7) (Haslick 2018). Continual monitoring of the LVWMP annual temperature sites has provided, and will continue to provide, important information regarding land use practices thought Logan Valley. The ten LVWMP loggers will provide valuable information regarding status of present and future restoration efforts.

BPT monitors temperatures upstream of the LVWMP on the Upper Malheur headwaters to inform current and future bull trout recovery efforts. Loggers located on Lake Creek provide temperature data in habitat where bull trout populations are facing competition and hybridization from invasive brook trout. Invasive brook trout are identified as the primary threat to Upper Malheur bull trout recovery, and monitoring stream temperatures in the headwater streams informs future management actions. Tracking temperature trends will provide important habitat information for planned brook trout eradication efforts. For instance, Lake Creek, particularly upper Lake Creek and High Lake, are the first locations for proposed rotenone efforts.

When compared to the Upper Malheur sites (namely, LVWMP), the upper North Fork Malheur has experienced fewer lasting effects of anthropogenic pressures (logging and livestock grazing) (Haslick 2016). North Fork stream temperatures maintain a pattern of being cooler as compared to Upper Malheur (Figure 2.3). North Fork logger locations are in reaches with active bull trout spawning, rearing, and migration (Perkins 2009, Haslick 2016) and therefore are providing data on valuable bull trout habitat. BPT collaborates with agency partners on logger locations and data are made available to provide a large picture of temperatures in the North Fork system.

BPT Fisheries will continue monitoring temperatures in the locations reported for the foreseeable future. Stream temperature data collected in the Upper Malheur and the North Fork Malheur by the BPT helps guide understanding regarding future climate impacts on bull trout. Using temperature data from watersheds throughout the Columbia Basin, scientists are effectively modeling future climate change scenarios. These models provide guidance for habitat restoration, bull trout recovery, and focused management efforts. BPT collaborates with USFS and the U.S. Geological Survey NorWeST to provide stream temperature data which can further develop and fine tune models (Haslick, 2018). BPT will continue future collaboration with partner agencies to collect important temperature data throughout the Upper Malheur and the North Fork Malheur.

2019 challenges and changes

2019 temperature monitoring experienced some challenges. One of the Logan Valley annual temperature loggers was not collected. The logger was not attached to the anchor upon fall retrieval. Two of the Upper Malheur loggers (McCoy Creek and Lake Creek) could not be located during the fall collection event. BPT suspects the loggers at this location were stolen due to proximity to a road and trailhead. Different locations will be considered for the 2020 monitoring effort.

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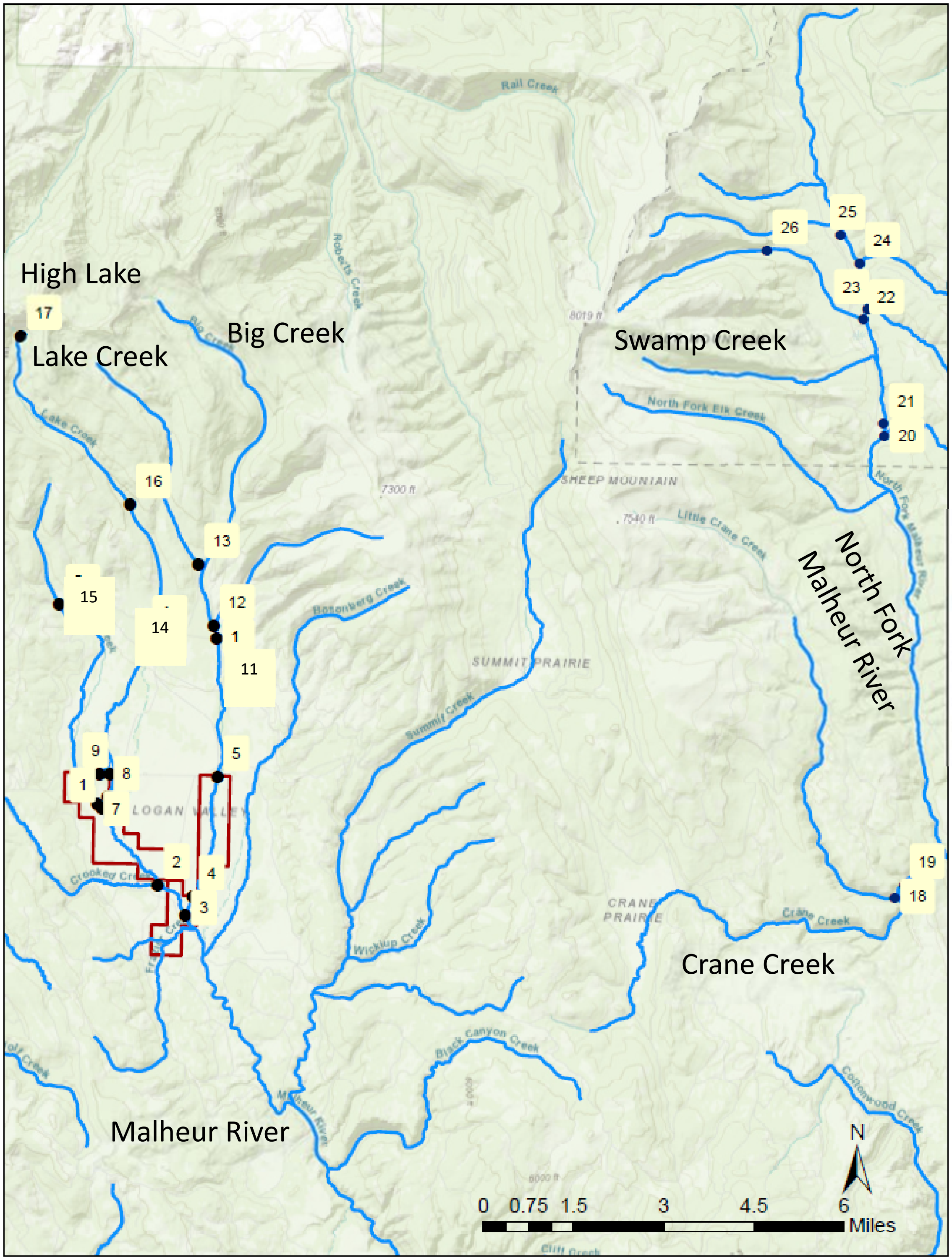
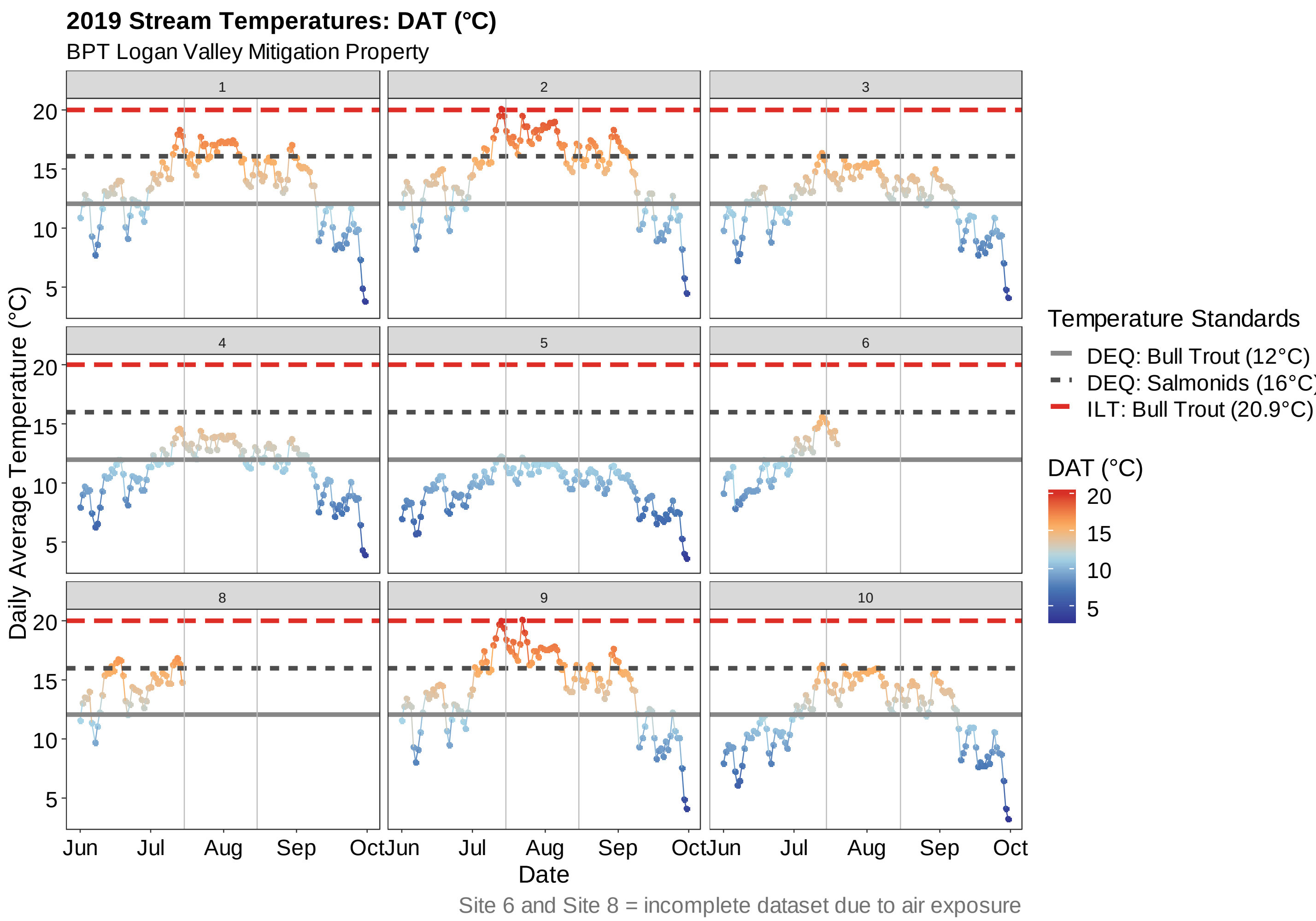
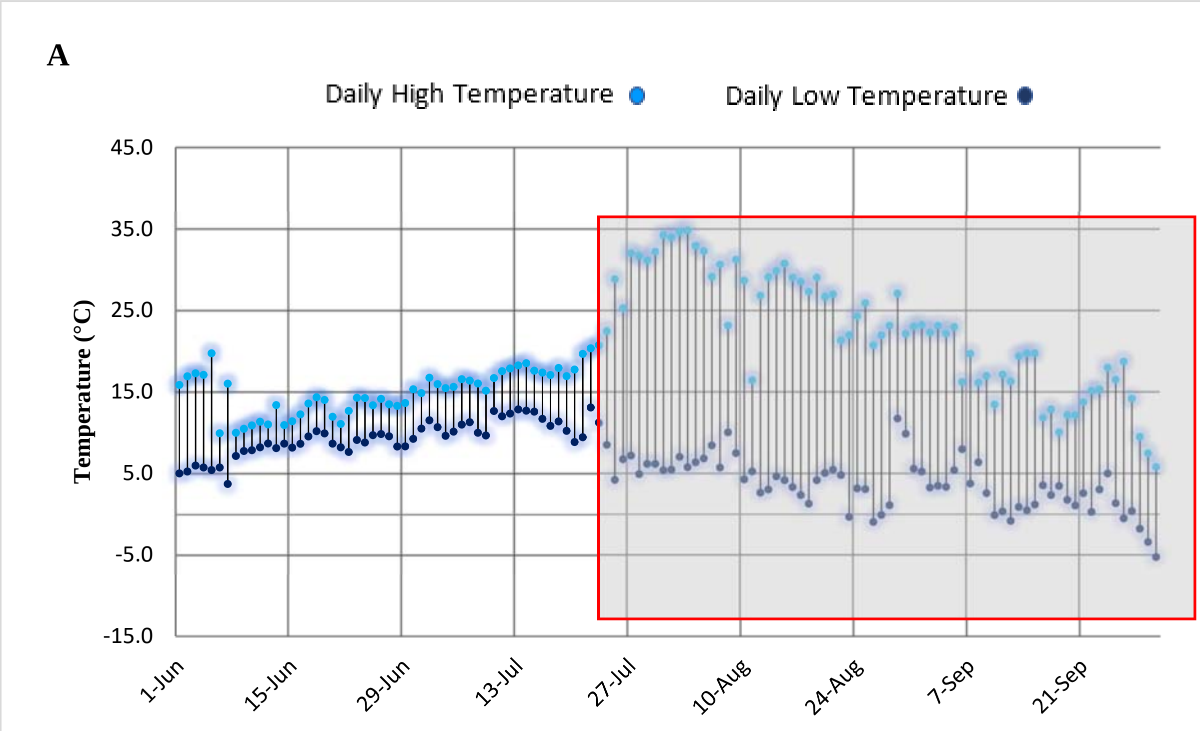
Appendices

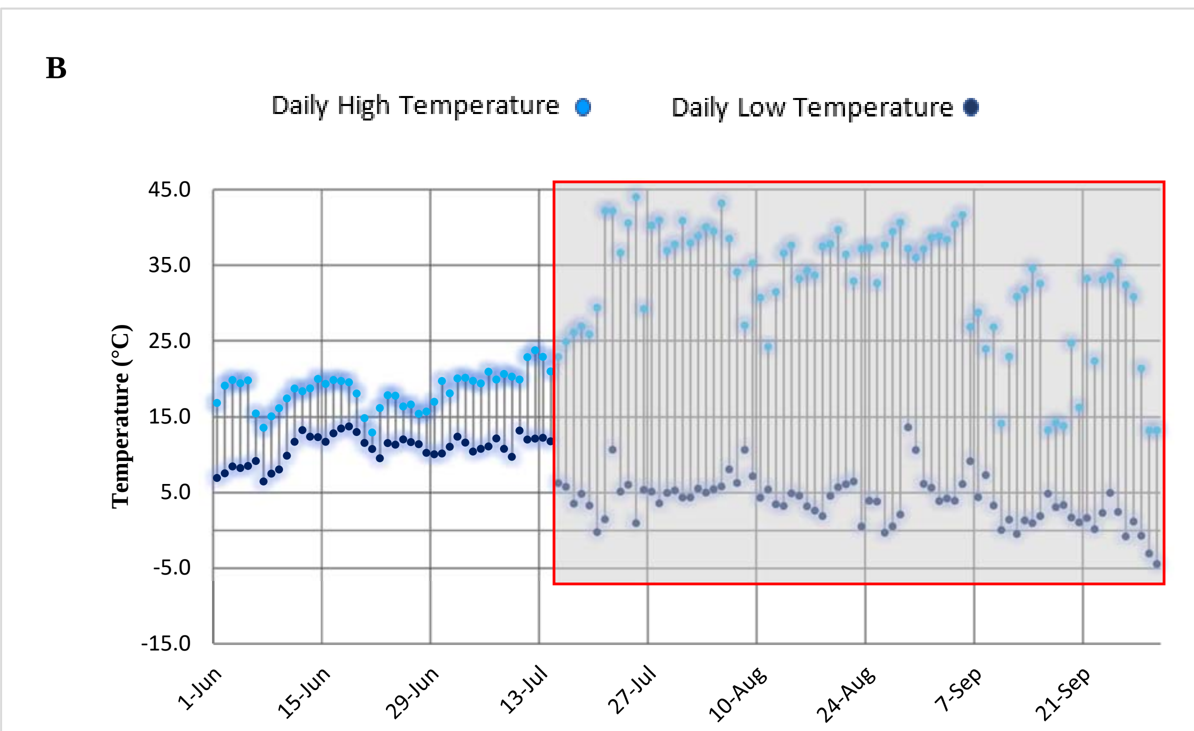


Appendix Figure 2.5: Map of all 2019 BPT temperature loggers



Appendix Figure 2.6 Daily Average Temperature (DAT) for the Ten Annual Logan Valley Wildlife Mitigation sites. Vertical lines at July 15th-August 15th are the Critical Period for bull trout





Appendix Figure 2.7 Daily High and Low Temperatures indicate dewatering a**t (A**) LVWMP site #6 Lake Creek below the 16 Road and **(B)** LVWMP site #8 Lake Creek at Cabin Bridge.

Appendix Table 2.3: Summary of Temperature Maximums at annual Logan Valley Wildlife Mitigation Property BPT Temperature Sites. Dewatered sites (6 & 8) not included 2019. Temperature Monitoring Period: June 1st- September 30th

Site	Year	Highest MWMT (°C)	MWMT Date	Absolute Maximum(°C)	Maximum Date
1	2017	23.96	8/3/2017	24.68	7/15/2017
	2018	23.84	8/1/2018	24.68	7/29/2018
	2019	22.72	8/5/2019	24.07	7/12/2019
2	2017	23.88	7/15/2017	25.11	7/15/2017
	2018	25.3	7/30/2018	26.11	7/25/2018
	2019	23.5	8/5/2019	24.41	8/3/2019
3	2017	21.65	7/13/2017	22.42	7/15/2017
	2018	21.24	7/30/2018	21.7	7/25/2018
	2019	20.52	7/30/2019	21.44	7/12/2019
4	2017	20.56	7/13/2017	21.32	7/15/2017
	2018	20.33	7/30/2018	20.91	7/14/2018
	2019	19.24	8/5/2019	20.27	7/12/2019
5	2017	17.77	7/13/2017	18.34	7/8/2017
	2018	17.31	7/29/2018	17.75	7/24/2018
	2019	16.32	8/2/2019	17.2	7/22/2019
7	2017	24.06	8/3/2017	24.65	7/15/2017
	2018	24.53	7/30/2018	25.43	7/25/2018
9	2017	26.69	8/2/2017	27.48	8/2/2017
	2018	26.64	7/29/2018	28.2	7/24/2018
	2019	24.95	7/23/2019	26.4	7/12/2019
10	2017	22.18	8/3/2017	23.16	7/15/2017
	2018	24.01	7/30/2018	24.63	7/25/2018
	2019	21.16	8/5/2019	21.51	7/31/2019

Chapter 3: Applying eDNA Methods to Elucidate the Suspected Presence of Invasive Brook Trout in the Little Malheur River

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Chapter 3

Applying eDNA Methods to Elucidate the Suspected Presence of Invasive Brook Trout in the Little Malheur River

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3.1 Introduction

Environmental DNA (eDNA) has become a powerful tool in fisheries management (Teletchea, 2009; Jerde et al. 2011). Sampling for eDNA is a non-invasive method when compared to other fishery sampling methods (gill netting or electroshocking) which is important when working in habitat used by endangered or threatened species. EDNA has been successfully applied to survey fish species composition (Minamoto et al. 2012), detect invasive fish species (Tahahara et al. 2013; Keskin 2014), and determine the completeness of eradication efforts (Carim et al. 2020). Although, the presence an organism's DNA reveals that the species exists in the sampled environment, DNA does not provide accurate data regarding the health, age, population, or even if the organism was alive at the time the sample was collected. Despite these limitations, using eDNA to detect the presence of a rare/cryptic species is an exciting application in fisheries management, as current survey methods are largely unable to sample all individual fish inhabiting a site.

The Burns Paiute Tribe (BPT) Fisheries Program has developed an eDNA protocol to determine the presence of invasive brook trout in streams (Crowley, 2017). The protocol has been developed with Cramer Fishery Sciences and involved multiple experiments over years to test different field methods for collecting brook trout DNA from various freshwater environments. The intention is to use eDNA as a monitoring method to assess the effectiveness of complete brook trout eradication post planned future rotenone treatments in the Upper Malheur (Schumer et al. 2019).

The BPT expanded the role of eDNA in the Fishery Program in 2018 when a small eDNA sampling event was paired with a large electrofishing effort to investigate the potential illegal introduction of brook trout in the Little Malheur River. When the 2018 eDNA results (positive for brook trout DNA) contradicted electrofishing efforts (no brook trout captured), BPT returned to the Little Malheur River for an expanded 2019 eDNA/electrofishing effort. BPT aimed to 1) find concrete proof of brook trout presence in the Little Malheur and 2) understand the extent of the invasion in order to 3) inform agency partners for future management decisions.

Problem: A potential brook trout introduction in the Little Malheur River

The North Fork of the Malheur has been historically blocked from the growing invasion of brook trout in the Malheur River Watershed. The Agency Valley Dam on the North Fork Malheur is impassible, as it prevents upstream fish migration effectively separating the North Fork drainage from the Upper Malheur (Figure: 3.1). Agency Valley Dam construction ended in the 1930's (corresponding with the first introductions of brook trout in the Upper Malheur) (Schwabe et al. 2004) , so brook trout have heavily populated the Middle Fork but are not known to occur in the North Fork drainage (Buckman et al. 1992).

The belief of a 'brook trout free' North Fork Subbasin was upended when in 2018, partner agencies in the Technical Assessment Committee (TAC), a bull trout recovery working group, had concerns regarding a potential illegal brook trout introduction into the Little Malheur River (a tributary of the North Fork Malheur River). Despite zero brook trout encountered during the 2018 electrofishing efforts, the small-scale eDNA sampling effort (three eDNA samples from a single location (FS-16 road crossing) (BPT 2018)), tested positive for brook trout DNA. No management decisions could effectively be made to address a potential brook trout invasion largely due to the contradicting (electrofishing vs. eDNA) results.

The overall objective of the 2019 efforts in the Little Malheur was to determine the extent of invasive brook trout presence in the main stem Little Malheur River and its tributaries. BPT aimed to confirm and locate brook trout presence to provide information for a fast, interagency response. 2019 sampling involved another electrofishing effort paired with intensive eDNA sampling.

3.2 Methods

Study Area: The Little Malheur River and tributaries

The Little Malheur River is a tributary to the North Fork Malheur River in Eastern Oregon (Figure 3.1). The core sampling area for 2018 and 2019 sampling efforts originated at the intersection of the FS-16 Road and the Little Malheur River. This location was hypothesized to have the highest likelihood of illegal brook trout introductions. Electrofishing and eDNA sampling efforts were conducted the first two weeks of August 2019. Several tributaries flow into the Little Malheur near that location: Larch Creek, Canteen Creek, Camp Creek, Unnamed Creek, Anderson Creek, and Squaw Creek (Figure 3.2).

Field Methods

eDNA

BPT took 34 eDNA samples throughout the mainstem Little Malheur as well as on five tributaries. DNA samples were taken at tributary confluences in the main stem Little Malheur and at the U.S. Forest Service, (USFS) property boundary downstream of the 16-road crossing. A sample and a replicate (2 total samples) were taken at these specific sites. This was to heighten the ability of capturing DNA (Schumer et al. 2019). The goal for the selected tributaries was to take eDNA samples (a single sample) every 500-meters starting from the confluence with the Little Malheur and working upstream until half the tributary length was sampled or a fish barrier was reached. Private property, stream discontinuity, and unsuitable sampling sites caused for some discrepancy between sampling distance.

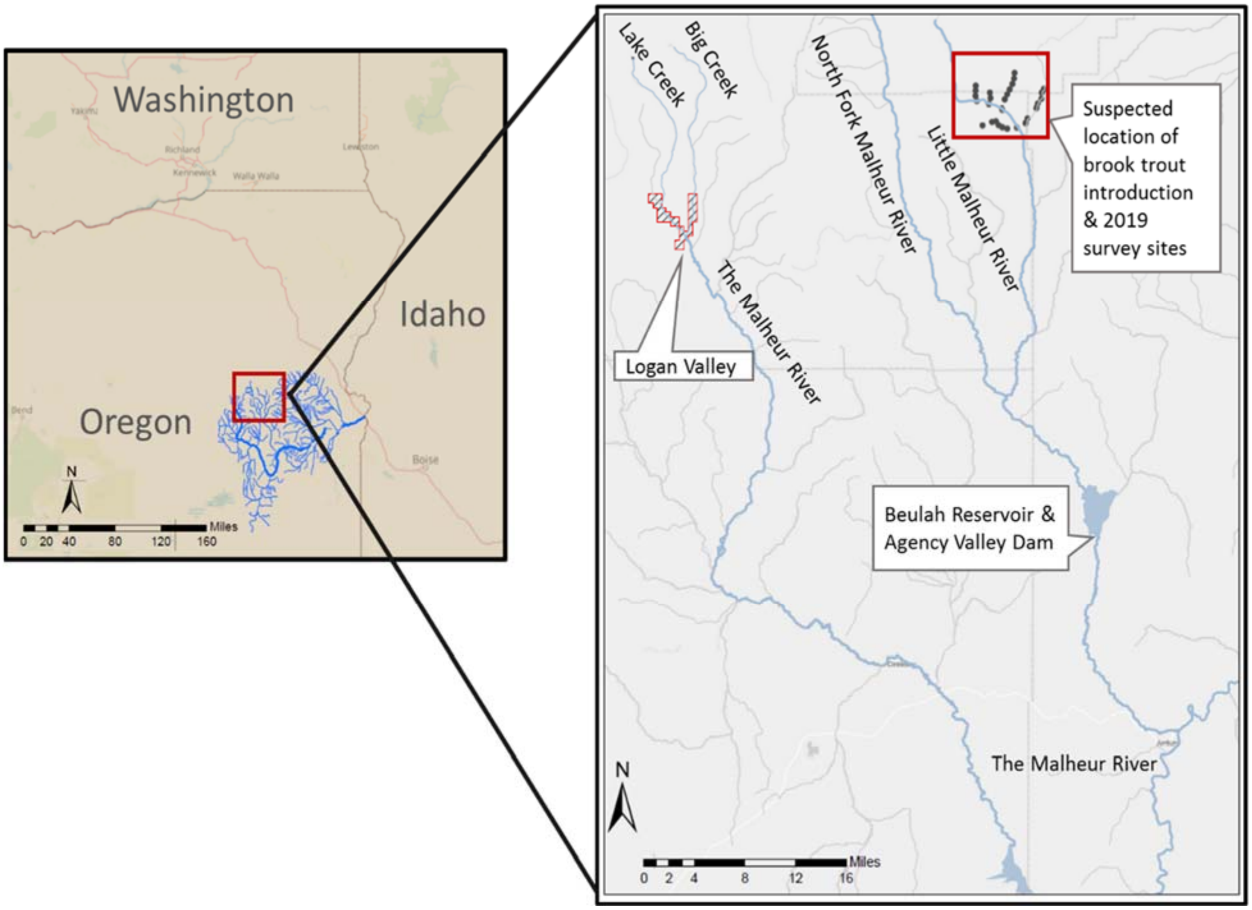

Figure 3.1 Map of Malheur Watershed with the Little Malheur River 2019 Sampling Location and eDNA sites (represented by grey circles)

BPT eDNA sampling occurred the day prior to electrofishing to avoid potential site contamination. Wading gear was decontaminated with a bleach dilution before eDNA sampling efforts began and between each different location. Field equipment, methods, and protocol for eDNA samples are as described in the 2017 BPT Annual Report (Crowley, 2017). Briefly, each water sample was a single liter in volume. Samplers remained downstream of the thalweg when taking the water sample(s) from the top ~3 inches of the water column. Samples were pumped through sterile silicone tubing using a handheld, battery powered drill into a Serivex filter (Millipore ®). All Sterivex filters were labeled, placed in new, individual ziplock bags, and immediately put on ice and hiked out after which eDNA samples were stored in a dry ice cooler at camp until they could be placed in a -20 °C freezer.

Electrofishing

BPT Fisheries conducted upstream, single-pass electroshocking without block nets using a LR24 Smith-Root backpack electrofisher. Electroshocking occurred in the tributaries (Larch Creek, Camp Creek, Anderson Creek, and Squaw Creek) and in the Little Malheur River. In the mainstem Little Malheur, at least ~300 meters of stream above and below the FS-16 road

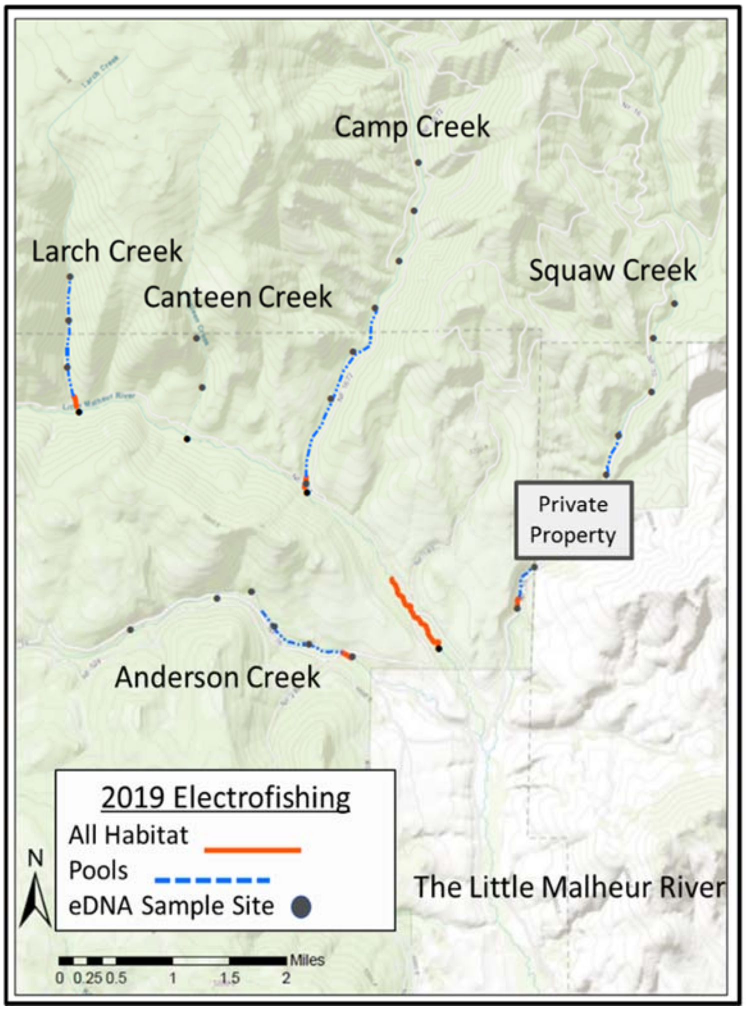

Figure 3.2 2019 electrofishing methods and eDNA sample locations

crossing was fully electroshocked. In the tributaries, all available habitat of the first 100 meters (starting at the confluence) was fully shocked. After which, shocking shifted to every third pool. If no brook trout were encountered after five consecutive, 'third pool' sections crews worked upstream targeting only pools which were deemed to be exceptional habitat for a total electroshocking reach of at least ~1,000 meters (Figure 3.2). All native captured fish were counted and released downstream after being fully revived. Any encountered brook trout was to be photographed, measured (fork length), georeferenced, and removed from the stream (euthanized).

Lab Methods

2019 BPT samples were mailed on ice to Smith Root, Inc. Smith Root partners with Precision Biomonitoring Inc. to analyze eDNA samples. Precision Biomonitoring extracted DNA from the Sterivex filters using the DNeasy Blood and Tissue Kit and following the protocol developed through the Rocky Mountain Genomics Center (with a few modifications due to the Sterivex filters) (Crookes 2019). After DNA was extracted from each sample, total DNA was quantified, and the samples were prepped for qPCR. Quantitative real-time PCR (qPCR assay) was run in triplicate for all samples to detect presence of brook trout DNA (Crookes 2019).

3.3 Results

The BPT electofished the Little Malheur and four of its tributaries. Larch and Canteen Creek both had shortened survey sites because of potential fish barriers which were believed to impede further upstream fish movement. One tributary, Canteen Creek was not electofished due to water temperatures. Another tributary, Unnamed Creek, was dry and therefore not surveyed.

eDNA

BPT had a total of 34 eDNA samples analyzed by Precision Biomonitoring Inc. These samples represented 29 different locations. The eDNA samples taken above the suspected fish barriers came back negative for brook trout DNA. This solidified the predictions that the sampled locations were likely effective fish barriers. In total, six locations tested positive for brook trout DNA. These were the lowermost sites in Camp Creek, Squaw Creek, and Anderson Creek (Figure 3.4). Three of the locations resulted in 100% detection as 3/3 qPCR replicates tested positive for brook trout DNA (Appendix Table 1), and the third site on Camp Creek had the greatest amount of DNA (an average of 7.88 gene copies / reaction) (Appendix Table 1, Figure 3.4).

Electroshocking

BPT electroshocked ~600 meters of the Little Malheur, upstream and downstream of a major road crossing (FS-16), and a total of four tributaries. Six fish species were encountered:

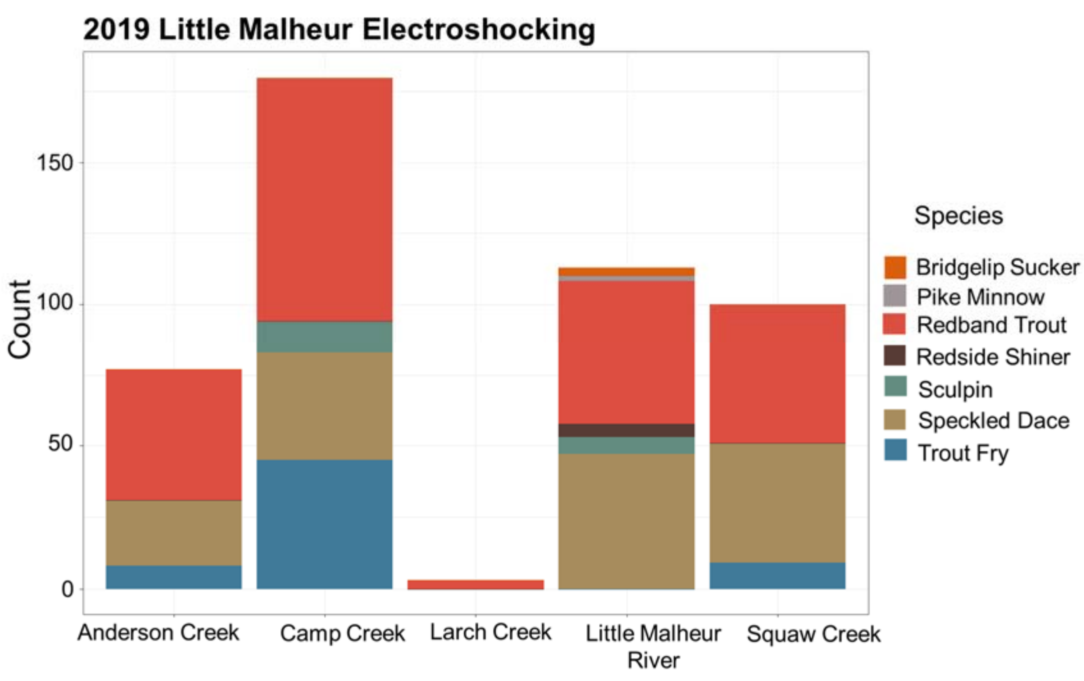

Figure 3.3 2019 electroshocking results (reach length varied among sites)

bridgelip sucker (*Catostomus columbianus*), northern pikeminnow *(Ptychocheilus oregonensis)*, redband trout (*Oncorhynchus mykiss gairdneri)*, redside shiner (*Richardsonius balteatus)*, sculpin (*Cottoidea spp.)*, and speckled dace *(Rhinichthys osculus)* (Figure 3.3). Three locations surveyed also had 'unidentified' trout fry (trout fry less than 50 mm). The 600-meter Little Malheur reach had the highest species composition as compared to the tributary sites. No brook trout were encountered during the electrofishing surveyed.

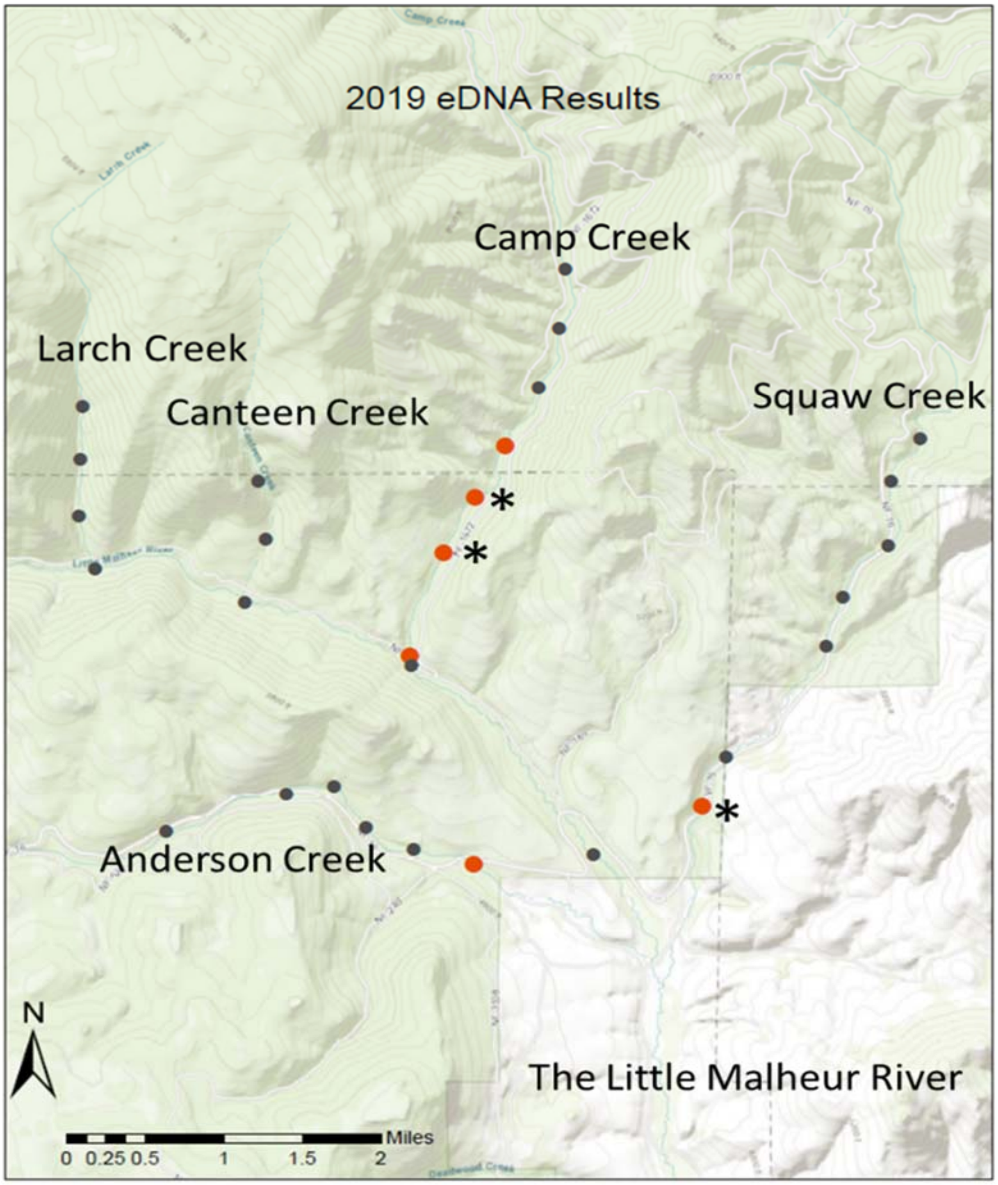

Figure 3.4 2019 eDNA results. Grey = locations which tested negative for brook trout DNA. Orange = locations which tested positive for brook trout DNA. Sites denoted with (*) have 100% detection among three qPCR replicates

3.4 Discussion

Invasive brook trout threaten a North Fork of the Malheur tributary

Although the 2019 study location in the Little Malheur River is not known to support bull trout, it drains into the North Fork of the Malheur (which is critical bull trout habitat). The results of the 2019 BPT eDNA sampling effort revealed brook trout presence in the Little Malheur which may have future, negative impacts on North Fork bull trout habitat. BPT eDNA samplings have successfully captured brook trout presence at a finer scale than conventional sampling methods. Two years of intensive electrofishing efforts in 2018 (BPT 2018) and 2019 have not resulted in conclusive brook trout presence, however eDNA sampling is demonstrating to have greater sensitivity in species detection and be more cost-effective method in fisheries management (Wicox et al. 2016).

The ability of electrofishing to effectively survey for brook trout in the Little Malheur was made difficult in several locations throughout the different tributaries for different reasons. Anderson Creek was heavily populated by redband trout throughout the survey reach, however multiple portions of the stream were discontinuous. It is worth noting that the tributaries which had positive eDNA results all contained 'unidentified trout fry.' Several of the fry were identified as redband trout. However, many salmonid fry smaller than 50 mm were counted and not speciated due to crew variation in fish ID experience. Multiple pools in Squaw Creek were unable to be thoroughly electofished due to size and depth. These pools were also heavily vegetated. Many fish were spotted but not captured in these pools (redband were captured around the perimeter). Squaw Creek also had private property in the middle of the electrofishing reach and could not be sampled. A crew sampled the lower 400-meters below the property and a crew sampled the first 500-meters upstream of the property boundary. Finally, electrofishing was temperature limited (Canteen Creek was too hot to survey) and the ~600-meter survey on the Little Malheur had to be done in two days due to temperature limitations.

Many of the limitations in electrofishing the Little Malheur and tributaries were alleviated by eDNA sampling. The eDNA sampling was not as limited by temperature. deep/vegetated pools, or habitat complexity. Benefits of using eDNA include: the ability to speciate brook trout from redband trout fry, less time/crew needed for sampling efforts, and the ability to cover a greater reach length. Regardless of these benefits, some of the problems experienced with electrofishing also impacted eDNA sampling. The discontinuity of Anderson Creek affected the ability to get 500-meters of continuous stream between each sample. Some sites sampled smaller distances. A section of Anderson was marsh/bog habitat with very slow flow and an eDNA sample was not taken in that section. Private property caused discontinuity in eDNA sampling for a lower portion of the sample reach. Despite the limitations in the eDNA sampling during the 2019 field season, eDNA confirmed what electrofishing was unable to capture: brook trout are present in the Little Malheur system.

The costs of managing invasive species are high

Brook trout are the primary threat to the neighboring bull trout population in the Upper Malheur River. A 10-year, interagency, large-scale eradication effort has been planned to tackle the issue using the piscicide rotenone (TAC 2017, BPT 2018-09). Though determined less costly and more effective that continuing to suppress brook trout using mechanical methods (Crowley, 2017), the planned eradication efforts detailed in the Upper Malheur River Bull Trout Conservation Strategy will require immense funding and resources. These efforts involve the time and collaboration of multiple agencies, pre and post treatment data collection, applying and adhering to multiple regulatory criteria, and treatment cost. The Upper Malheur has been deemed worth the effort due to its status as its current status critical bull trout habitat (USFWS 2002, 2015). The high cost associated with effectively managing invasive brook trout being experienced in the Upper Malheur, provides the need to quickly and accurately address the smallest chance of brook trout presence in the neighboring North Fork Subbasin.

Future Recommendations

Although the higher stream temperatures in the Little Malheur may be acting as a thermal barrier deterring downstream migration to the North Fork, continued monitoring will provide a greater understanding into the extent of the invasion. Since the Little Malheur tested positive for brook trout DNA, BPT will work with partner agencies to quickly form a management plan and aggressively address the invasion. BPT will meet with the TAC in spring 2020 to inform collaborators of the eDNA results. The issue of brook trout in the North Fork basing is of grave concern to the BPT and partner TAC agencies.

Acknowledgements

BPT Fisheries would like to acknowledge Prairie City Forest Service for their collaboration on this project. During the second week of Little Malheur eDNA and electrofishing sampling BPT fisheries was joined by the Prairie City Forest service hydrology crew. The crew consisted of a permanent technician and her two seasonal hydrologists. (Hydrologist: Hazel Wood, Lead: Jordan Bass, Technicians: Samuel Spengler and Isabella Moody). The crew also was hosting an exchange student from Spain. Together Forest Service and BPT electofished and eDNA sampled: Anderson Creek, Camp Creek, and Squaw Creek.

BPT is very grateful for the support and guidance of the TAC (Oregon Department of Fish and Wildlife, U.S. Bureau of Reclamation, U.S. Forest Service, and U.S. Fish and Wildlife Service). BPT has been collaborating closely with the TAC and values the insight and partnerships regarding brook trout presence in the Little Malheur as well as future issues challenging Malheur River Bull Trout Recovery.

BPT Fisheries also thanks Smith Root Inc. and Precision Biomonitoring Inc. for the fast turnover time on the eDNA samples as well and the results in a report prepared to BPT by Precision Biomonitoring.

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Appendices

Appendix Table 1: qPCR Results

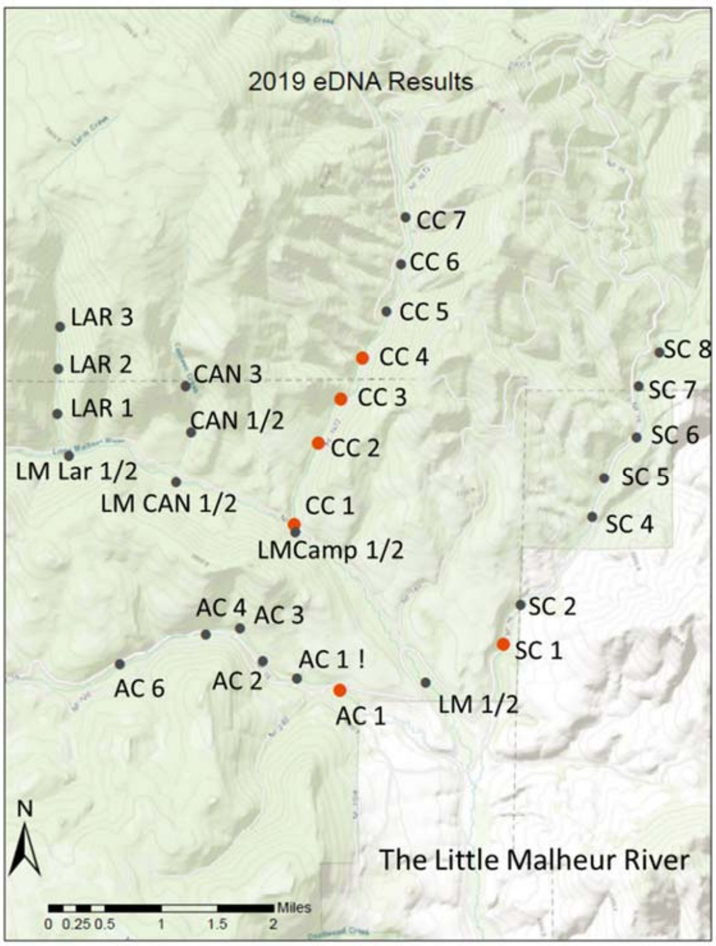
Table 1: Results of qPCR and DNA yield analysis. Samples are listed in order of the date in which the field water was extracted (see Sample Table). Results for qPCR are reported as Cq, and where calculable, copy number of sturgeon target DNA molecules per PCR reaction. Copy number is reported by reference to the standard curve used to validate the assay. A lower Cq value is indicative of higher levels of target DNA. Mean IPC Cq is reported across technical replicates for each sample. DNA yields are reported within the capable range of the Qubit 2.0™ Fluorometer, 10 – 12000 ng mL^{-1}.

Sample	qPCR Cq Value			qPCR Copy #			Mean IPC Cq	DNA Yield (ng mL^{-1})
LM 1	0	0	0	0	0	0	28.16	55
LM 2	0	0	0	0	0	0	28.17	426
AC 1	0	38.50	39.57	0	1.142	< 1*	27.91	1240
SC 1	35.82	35.74	37.30	9.84	10.42	3.37	27.91	602
CC 1	0	39.81	0	0	< 1*	0	27.67	112
LmLar 1	0	0	0	0	0	0	27.80	828
LmLar 2	0	0	0	0	0	0	28.25	2340
LAR 1	0	0	0	0	0	0	27.76	1170
LAR 2	0	0	0	0	0	0	27.88	1160
LAR 3	0	0	0	0	0	0	27.91	226
LMCAMP 1	0	0	0	0	0	0	27.86	308
LMCAMP 2	0	0	0	0	0	0	27.67	326
LM CAN 1	0	0	0	0	0	0	27.69	178
LM CAN 2	0	0	0	0	0	0	28.01	258
CAN 3	0	0	0	0	0	0	27.65	131
CAN 2	0	0	0	0	0	0	27.60	412
CAN 1	0	0	0	0	0	0	27.86	606
AC 6	0	0	0	0	0	0	27.67	586
AC 4	0	0	0	0	0	0	27.56	432
AC 3	0	0	0	0	0	0	27.72	940
AC 2	0	0	0	0	0	0	27.59	764
AC 1 !	0	0	0	0	0	0	28.07	770
CC 2	38.15	39.08	37.77	1.89	< 1*	2.40	27.80	920
CC 3	39.38	39.16	37.93	< 1*	< 1*	2.14	27.69	690
CC 4	0	39.45	0	0	< 1*	0	27.56	195
CC 5	0	0	0	0	0	0	27.39	1580

Appendix Table 1 (continued) This table was provided by Precision Biomonitoring Inc. to the BPT (Crookes, 2019) and Sample names correspond to the map below **(Figure 3.5 A)**

Sample	qPCR Cq Value			qPCR Copy #			Mean IPC Cq	DNA Yield (ng mL^{-1})
CC 6	0	0	0	0	0	0	27.62	1060
CC 7	0	0	0	0	0	0	28.22	133
SC 2	0	0	0	0	0	0	28.01	216
SC 8	0	0	0	0	0	0	28.01	598
SC 7	0	0	0	0	0	0	27.50	264
SC 6	0	0	0	0	0	0	27.11	568
SC 5	0	0	0	0	0	0	27.14	368
SC 4	0	0	0	0	0	0	27.73	276

0: No Cq observed. * = Cq above standard curve y-intercept



Appendix Figure 3.5: Sample ID and locations corresponding to Table 1**.** Grey= Negative for brook trout DNA; Orange= positive for brook trout DNA.

Supplement: Evaluate the Life History of Salmonids in the Malheur Subbasin 2019 Baseline Data Collection and Outreach

The Burns Paiute Tribe Fisheries Program has been actively collecting baseline data, as in the Upper Malheur Watershed Bull Trout Conservation Plan (TAC 2017). The bull trout recovery Technical Assessment Committee (TAC) has outlined the necessary data collection needed to prepare for a potential piscicide treatment. 2019 data collection included 1) surveying and mapping springs in upper Lake Creek and 2) increasing education using the "Help Native Fish" outreach developed for BPT and the TAC by Samara Group.

Upper Lake Creek Tributary Mapping

The 2019 field season concluded with an effort to map out the seeps and springs feeding into High Lake and upper Lake Creek. These surveys took place in the late summer. Flows are at their lowest during this time, allowing for the survey to focus on 1) the perennial tributaries with enough water flowing that they may serve as sanctuary to brook trout during rotenone treatment and 2) accurate representation of hydrologic features during the time the treatment would take place. The BPT 2015 field crew mapped out 'features of consideration' (seen as the red points on Figure A). These features included: locations where seeps and springs flow into upper Lake Creek, remnant pools, subterranean flow, and side channels (BPT 2018). 2019 surveys used the 2015 points as a starting point and walked up several springs until the source was

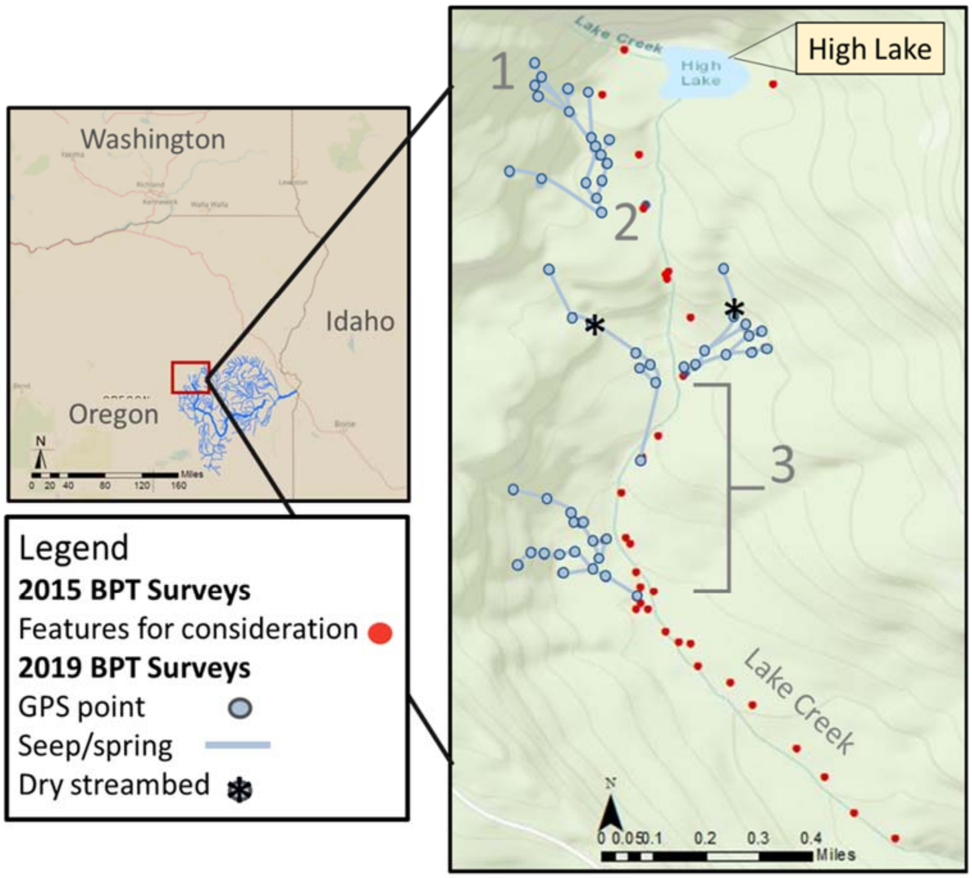


Figure A: Lake Creek Tributaries mapped in 2019 (**1**) High gradient, heavily vegetated seeps/springs **(2)** Low gradient meadow with multiple channels/spring inputs **(3)** Three springs downstream of meadow habitat noted as likely candidates for fish refugia during rotenone treatment (*) Indicates location where all upstream points are dry/intermittent located. Multiple springs were mapped to the source (Figure A) however; there are still springs/seeps which will still need to be mapped in the 2020 field season.

2019 Outreach

Figure B: Help Native Fish was developed for BPT by Samara Group resulting in this recognizable logo currently used for outreach.

The BPT Fisheries Program expanded public outreach efforts in 2019. In 2018, BPT hired the outside consulting group (Samara Group), to develop an outreach/education plan for this project (Figure B). Help Native Fish includes a website: www.helpnativefish.com (Figure B), posters, and informative brochures. Outreach has been extensive. Posters were placed on busy trailheads starting summer 2019. Staffing a Help Native Fish booth at the two local county fairs allowed for BPT Fisheries to engage and educate nearly 900 people. BPT also staffed a Held Native Fish Booth The website provides information on Upper Malheur River Bull Trout status, current management actions, and will be continually maintained and updated by BPT to provide information regarding bull trout recovery and fisheries management efforts/successes.

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