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Assessment of Bull Trout Distributions in the Headwaters of the Naneum Creek Watershed Using Environmental DNA Analysis



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On the cover: Photograph of Naneum Creek. USFWS photograph by Jake Blakley.

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Abstract- The Naneum Creek watershed located in the upper Yakima River Basin contains large quantities of potential Bull Trout spawning and rearing habitat within its headwaters. Historic Bull Trout occupancy within Naneum Creek's headwaters are unknown, and no Bull Trout were found during past fisheries surveys in the area; however, due to limited resources, local surveys have been restricted to small portions of the upper watershed's total available habitat. It is possible that Bull Trout were present in Naneum Creek's headwaters and escaped detection during previous surveys or were present in unsurveyed areas. Recent advances in environmental DNA (eDNA) analysis allow for the rapid assessment of fish distributions in large sections of stream habitat. To assess Bull Trout distributions throughout the headwaters of Naneum Creek, during July 2020 we collected 54 eDNA samples from this area at one-kilometer intervals within all potential Bull Trout spawning and rearing habitat predicted by the Climate Shield Occurrence Model. All eDNA samples were tested for Bull Trout DNA by the National Genomics Center using quantitative PCR analysis. Bull Trout DNA was not detected in any samples collected within Naneum Creek's headwaters. Our results imply that Bull Trout are likely not extant within the potential spawning and rearing habitat in the Naneum Creek watershed. The results of this study will help influence potential management actions within the Naneum Creek watershed such as habitat restoration and native species conservation efforts.

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Introduction

Bull Trout were listed as a threatened species throughout the coterminous United States under the Endangered Species Act in 1999 (USDOJ 1999). At the time of listing, Bull Trout distributions were poorly understood within large portions of their range, including many large watersheds in Central Washington (USFWS 2002). While knowledge of Bull Trout distributions has increased since their listing, current distributions are still unknown in many areas (Reiss et al. 2012, USFWS 2015a). Implementation of effective Bull Trout recovery actions requires detailed information about current Bull Trout distributions, and the USFWS Bull Trout Recovery Plan prioritizes evaluating Bull Trout distributions within unsurveyed potential Bull Trout habitat (USFWS 2015b).

One large, understudied watershed where Bull Trout distributions are largely unknown is the Naneum Creek watershed located in Washington's Yakima Basin. Habitat prediction models indicate potential Bull Trout habitat is present in many headwater tributaries within the upper portions of the Naneum Creek watershed, hereafter referred to as the upper Naneum Creek subwatershed (Isaak et al. 2015, Hockman-Wert et al. 2016). The historic presence of Bull Trout within this predicted habitat is unknown; however, the construction of several downstream diversion and transportation related fish passage barriers would likely have led to the extirpation of any migratory Bull Trout life histories that were previously present in the Naneum Creek system (Haring 2001). It is possible, however, that one or more isolated resident Bull Trout populations are present in potential spawning and rearing habitat within the headwaters of the Naneum Creek watershed. Past surveys performed within this region did not find Bull Trout but often identified Brook Trout (KCCD 2013, Bowler et al. 2017, Randall personal comm. 2019). Brook Trout can outcompete resident Bull Trout, which makes the long-term survival of resident Bull Trout populations in areas where Brook Trout are present, such as the upper Naneum Creek subwatershed, less likely (McHahon et al. 2007, Warnock and Rasmussen 2014, Howell 2018). Past Naneum Creek surveys were limited in geographic scope; however, and a thorough assessment of all potential Bull Trout habitat in the upper portions of the watershed is needed to accurately assess Bull Trout distributions in the upper Naneum Creek subwatershed and evaluate the likelihood of their absence from potential spawning and rearing habitat.

Recent advancements in environmental DNA (eDNA) analysis allow for the rapid assessment of Bull Trout distributions in large watersheds. Several studies indicate eDNA surveys can be implemented more quickly than traditional fisheries surveys (Baldigo et al. 2017, Evans et al. 2017, Carim et al. 2020, Sanches and Schreier 2020). Research also indicates eDNA sampling is often more capable of detecting low densities of Bull Trout in headwater streams than traditional fisheries techniques (McKelvey et al. 2016, Wilcox et al. 2018).

To quickly and efficiently examine the distribution of Bull Trout in the headwaters of the Naneum Creek watershed, we collected eDNA samples during the summer of 2020 from sites encompassing the predicted range of potential Bull Trout spawning and rearing habitat within the upper Naneum Creek subwatershed. The objectives of this study were to determine if Bull Trout are present in the Upper Naneum Creek subwatershed and to assess the distributions of any Bull Trout populations detected during the study. The presence or probable absence of Bull Trout in the headwaters of the Naneum Creek watershed could affect future management actions within

the watershed including passage improvements, habitat restoration, and non-native species removal.

Study Site Description

Naneum Creek is a 55.0 rkm long, 5th order tributary to the Yakima River in the Upper Yakima River Basin that drains a 328 km² area before entering Wilson Creek and the Yakima River near Ellensburg, Washington (USGS 2022). Peak discharge in Naneum Creek occurs during spring-runoff between March and June (USGS 2023). Naneum Creek's low discharge period occurs between July and March; although, fall and winter rain events can temporarily elevate flows (Parrish 2017, USGS 2023). Major tributaries in the Naneum Creek watershed include Upper Wilson Creek, Boulder Creek, Swift Creek, and Pearson Creek.

The majority of coldwater fish habitat (mean August temperature $\leq 15^{\circ}\text{C}$) in Naneum Creek is located in the upper portions of the watershed upstream of the Upper Wilson Creek confluence (Isaak et al. 2017), referred to here as the upper Naneum Creek subwatershed. This 178.1 km² area is comprised primarily of federal and Washington State owned land (WARCO 2014). The upper Naneum Creek subwatershed has been heavily impacted by contemporary fires, which have burned 69.1% of the watershed since 2012 (WADNR 2021). These fires have severely impacted riparian habitat throughout the upper portions of the Naneum Creek watershed and have likely led to local stream temperature increases that have resulted in decreases in available coldwater habitat within the area (Roccanova 2018). Currently, the scale and magnitude of warming within stream habitat in the Upper Naneum Creek subwatershed has not been assessed.

The middle and lower portions of the Naneum Creek watershed that the upper Naneum Creek subwatershed flows into are comprised primarily of agricultural lands (KCCD 2013, JEG 2017) and contain stream habitat that exceed Bull Trout thermal maximums in the summer (Isaak et al. 2017). This area also contains several significant fish passage barriers resulting from irrigation and transportation infrastructure (JEG 2017, Parrish 2017, WDFW 2023). Due to these barriers, upstream fish movement into the upper portions of the Naneum Creek watershed is not possible, resulting in fish populations in the upper Naneum Creek subwatershed that are isolated from larger metapopulations in the remainder of the Yakima Basin (Conley et al. 2009, JEG 2017).

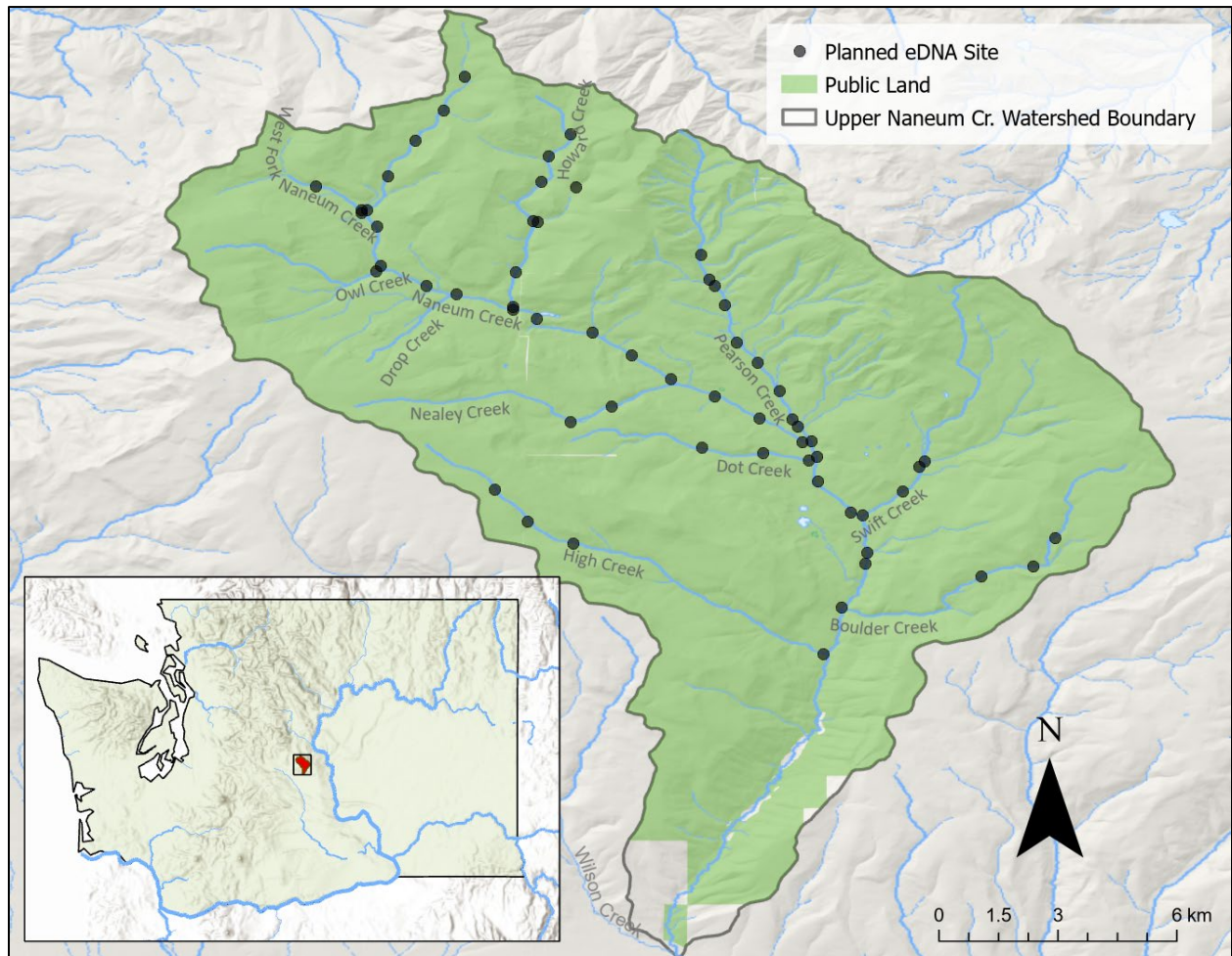


Figure 1. Planned eDNA Sites in the Naneum Creek Watershed. All eDNA collection sites were located in potential Bull Trout habitat predicted by the Climate Shield Occurrence Model (n=61).

Methods

Field Collection

All planned eDNA sample sites were located in the headwaters of Naneum Creek within the upper Naneum Creek subwatershed (Figure 1). Sampling occurred in areas containing potential Bull Trout spawning and juvenile rearing habitat as identified by the Bull Trout Climate Shield Occurrence Model (Isaak et al. 2015). Sample sites within predicted spawning and rearing habitat were separated by approximately one river kilometer, a spatial distribution that provides a high probability of detecting rearing Bull Trout populations in headwater streams (McKelvey et al. 2016). Samples were not collected from sites within reaches where field observations or available data indicated base flow wetted widths were <0.5m, average gradient was >15%, or flows were intermittent as these reaches are not expected to support perennial Bull Trout populations (Rich et al. 2004, Carim et al 2016b). In order to maximize detection probabilities,

samples were collected during low flows when stream temperatures were moderate and turbidity levels were low (Laramie et al. 2015, Curtis et al. 2021). Sampling occurred in a downstream to upstream direction to avoid upstream contamination effects (Jane et al. 2015, USFWS 2022). When possible, all samples from a continuous stream reach were collected in a single day to minimize temporal effects. When a reach could not be sampled in a single day, all samples were collected within a two-week period.

Samples were collected according to the protocol developed by Carim et al. (2016b). Sample collection involved filtering 5 L of stream water through a 1.5 μm glass filter (GE HealthCare) using a Global Water sp200 peristaltic pump. Surveyors used single-use filtration and sample processing supplies to minimize the risk of cross-site contamination. A single sample was collected at each visited site. A field control was also collected at the first site visited each day by filtering 500 mL of distilled water following the same collection procedures used to collect standard field samples. Following collection, filter samples were stored on silica desiccant until they could be transferred to a -20°C freezer for storage. Frozen samples were transferred to the National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT for laboratory analysis and archival storage.

Laboratory Analysis

At the NGC, filter paper samples were halved, and one side was archived at -20°C for future analysis. DNA from the remaining half of each filter was extracted using Qiagen DNEasy Blood and Tissue Kit following a modified protocol described in Carim et al. (2016a). Extracted samples were analyzed for the presence of Bull Trout mitochondrial DNA using DNA markers developed at the NGC (Wilcox et al. 2013, Dysthe et al. 2019). Each sample was analyzed in triplicate on a StepOne Plus qPCR Instrument or a QuantStudio 3 qPCR System. Thermocycling conditions were $95^{\circ}\text{C}/10$ minutes ($95^{\circ}\text{C}/15$ s, $60^{\circ}\text{C}/60$ s) and 45 cycles. We considered samples to contain Bull Trout DNA if linear amplification occurred in one or more of the three qPCR reactions.

During analysis, each PCR plate included at least one set of triplicate positive and negative controls to validate testing and ensure there was no contamination during DNA extraction or qPCR setup. All sample reactions included an internal positive control to test for the presence of PCR inhibitors. If the internal positive control appeared inhibited (i.e., amplification of the internal positive control was reduced), the sample was treated with a PCR inhibitor removal kit and re-analyzed in triplicate. To minimize potential DNA loss during inhibitor removal, laboratory staff extracted the second half of the sample filter from inhibited samples and combined all extracted DNA from a given sample to obtain ~ 200 μl of extracted DNA.

Results

A total of 54 samples were collected from the Upper Naneum Creek subwatershed between July 9 and July 30, 2020 (Appendix Table A1). Six planned sample sites were inaccessible to surveyors due to hazardous terrain and remote locations and were therefore not sampled (Appendix Table A2). Collections were not made from one additional site that had a wetted

width <0.5m in West Fork Naneum Creek. All samples were collected when stream temperatures were 7.5-18.6°C.

Quantitative PCR analysis found Bull Trout DNA did not amplify in any PCR replicates from the 54 tested samples, implying Bull Trout DNA was not detected in samples collected in the Upper Naneum Creek subwatershed in 2020 (Figure 2). There was no amplification of negative field or laboratory controls, and the presence of PCR inhibitors was not detected in any sample, indicating field equipment and laboratory contamination as well as sample inhibition did not influence PCR results.

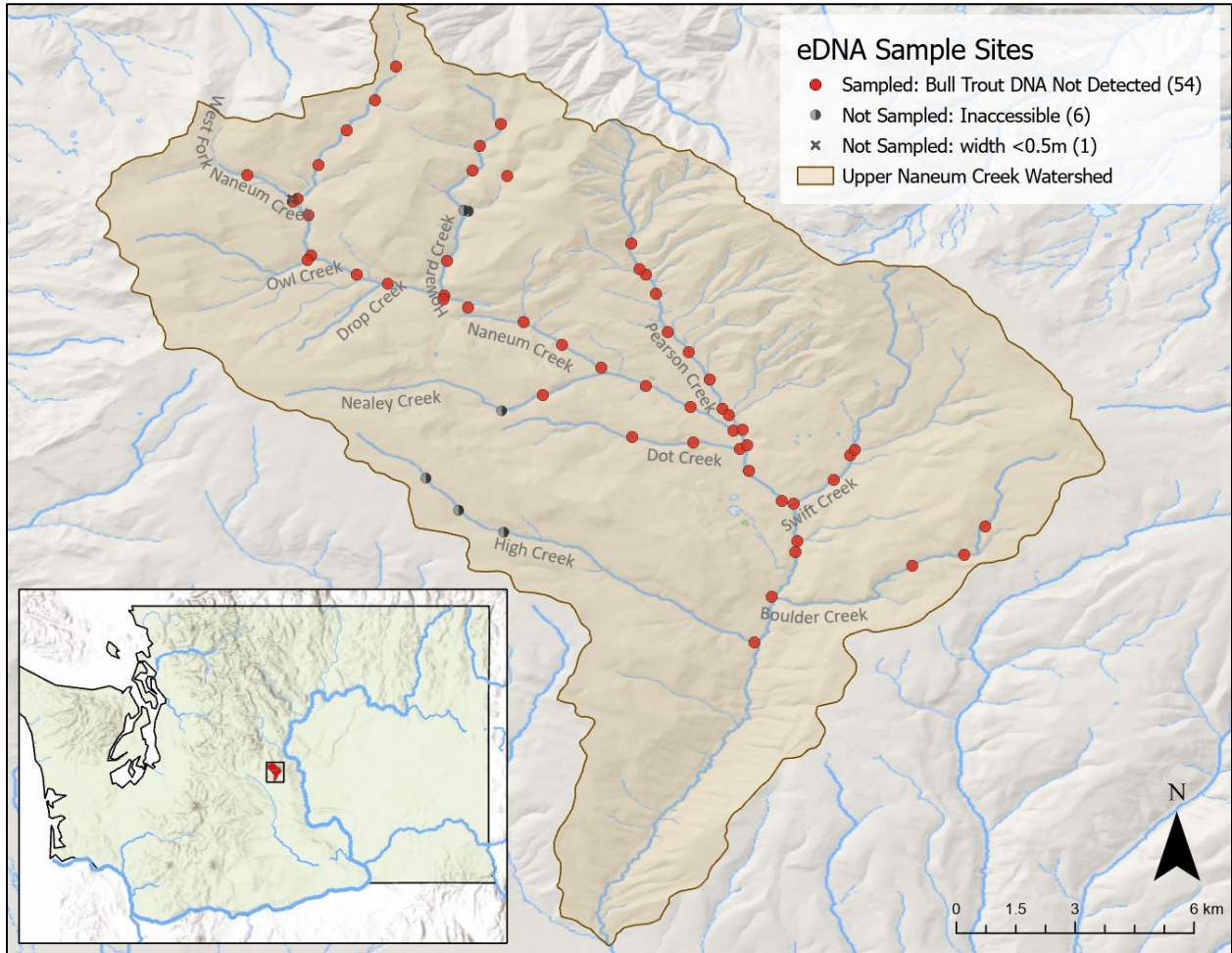


Figure 2. Bull Trout eDNA Detection Results From 2018-2019 Collection Sites in the Naneum Creek Watershed.

Discussion

The absence of detectable levels of Bull Trout DNA in 2020 at sample sites encompassing the majority of potential Bull Trout spawning and rearing habitat in the Upper Naneum Creek subwatershed implies a rearing Bull Trout population was likely not present within the headwaters of Naneum Creek during sample collection. Specific eDNA detection efficiencies within many lotic systems, including Mid-Columbia River tributaries, are variable and

undocumented due to the numerous physiological and environmental variables that influence eDNA detection rates (Jane et al. 2015, Wilcox et al. 2016, Fremier et al. 2019). Despite detection efficiency variation, our use of one-kilometer sampling intervals combined with our strategy of sampling during low stream discharge conditions likely resulted in high relative detection efficiencies within the study area that equaled or exceeded the detection efficiencies of other Bull Trout census methods (McKelvey et al. 2016). By using these methods, Bull Trout DNA would have likely been detected if a sustainable, rearing population of Bull Trout was present.

It is possible that a sufficiently small Bull Trout population present within sampled reaches at very low densities may have evaded detection using the employed eDNA methods (Wilcox et al. 2016, Schumer et al. 2019, Penaluna et al. 2021). It is also possible that Bull Trout were present in potential Bull Trout habitat that was inaccessible during our surveys. We believe that both of these scenarios are unlikely. The survival of small, isolated Bull Trout populations over long periods of time has been documented (Hudson et al. 2017, Howell 2018); however, these populations were successfully detected using the employed eDNA methods when eDNA surveys were performed in their native spawning and rearing areas (Young et al. 2020). Additionally, the disconnection of Naneum Creek and its tributaries from a larger Bull Trout meta-population, the degraded state of several spawning and rearing reaches, and the existence of Brook Trout in spawning and rearing areas makes the continued survival of a small, undetected Naneum Creek population less likely (Dunham and Rieman 1999, Rieman et al. 2006, USFWS 2015b). The existence of Bull Trout in unsurveyed reaches is also unlikely, since only six planned sites in identified potential Bull Trout habitat were not sampled. Many of these inaccessible sites were surrounded by surveyed areas where Bull Trout DNA was not detected, and several reaches containing inaccessible sites were expected to be too small to support Bull Trout populations based on observed conditions at nearby surveyed sites. While the eDNA methods employed during this study cannot guarantee the absolute absence of Bull Trout from the study area, based on our results, we advise that future management actions assume Bull Trout are not extant within the upper Naneum Creek subwatershed, and that any Bull Trout presence within the Naneum Creek watershed is limited to possible, unassessed foraging, winter, and migration (FMO) habitat usage by subadult and migrant adults in portions of the lower Naneum Creek accessible from adjacent Yakima River FMO habitat.

Establishment of a Bull Trout population within the upper Naneum Creek subwatershed will currently require the assisted introduction or reintroduction of the species or passage and habitat improvements in downstream FMO habitat that facilitate natural colonization. Several potential barriers to successful Bull Trout population establishment will need to be addressed before natural or assisted introductions are likely to succeed. These establishment barriers include the presence of Brook Trout in potential Bull Trout spawning and rearing habitat in the watershed; the current lack of connectivity within the system; and the degraded state of potential Bull Trout habitat caused by recent fires which may have resulted in increased local stream temperatures (JEG 2017, Rocanova 2018). We suggest that managers undertake habitat restoration and passage improvements throughout the Naneum Creek watershed and perform Brook Trout removal in the upper Naneum Creek subwatershed if Bull Trout population establishment is considered a priority. Given the slow rates of Bull Trout colonization in distant, disconnected habitat (Dunham et al. 2011) and the degraded state of potential FMO habitat within lower

Naneum Creek, we also suggest that any managers prioritizing Bull Trout establishment in the upper Naneum Creek subwatershed consider translocating Bull Trout from other healthy Bull Trout populations, especially those with a resident life history component capable of surviving in isolated headwater systems.

Conclusion

Environmental DNA analysis indicates that Bull Trout DNA was not detected at samples collected in 2020 from the majority of potential Bull Trout spawning and rearing habitat within the Naneum Creek watershed. The absence of detectable levels of Bull Trout DNA within the samples, combined with the lack of connectivity and the presence of population persistence threats within the watershed, such Brook Trout, implies Bull Trout are likely not extant within the headwaters of the Naneum Creek watershed.

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Appendix

Table A1. Site Location Information and qPCR Results From eDNA Samples Collected in the Upper Naneum Creek Subwatershed in 2018 and 2019.

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	Latitude	Longitude	Temperature (°C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
Boulder Creek	828-4	BOLD-01	7/30/2020	47.206081	-120.409234	11.6	5	0
Boulder Creek	828-5	BOLD-02	7/30/2020	47.208546	-120.397500	11	5	0
Boulder Creek	828-6	BOLD-03	7/30/2020	47.214887	-120.392620	12.2	5	0
Dot Creek	861-1	DOT-01	7/29/2020	47.232463	-120.448357	11	5	0
Dot Creek	861-2	DOT-02	7/29/2020	47.234067	-120.458805	11.8	5	0
Dot Creek	861-3	DOT-03	7/29/2020	47.235299	-120.472729	14	5	0
E.F. Howard Creek	1021-2	EFHO-02	7/27/2020	47.294432	-120.501132	9.2	5	0
High Creek	810-1	HIGH-01	7/28/2020	47.188690	-120.445110	16	5	0
Howard Creek	985-1	HOWA-01	7/16/2020	47.267212	-120.515401	13.1	5	0
Howard Creek	985-2	HOWA-02	7/20/2020	47.275166	-120.514855	15.3	5	0
Howard Creek	1044-2	HOWA-04	7/27/2020	47.295577	-120.509020	9	5	0
Howard Creek	1044-3	HOWA-05	7/27/2020	47.301304	-120.507328	11.3	5	0
Howard Creek	1044-4	HOWA-06	7/27/2020	47.306314	-120.502488	8.9	5	0
Naneum Creek	806-1	NANE-01	7/09/2020	47.199092	-120.441012	10	5	0
Naneum Creek	806-2	NANE-02	7/09/2020	47.209121	-120.435657	10	5	0
Naneum Creek	825-1	NANE-03	7/09/2020	47.211553	-120.435221	10.5	5	0
Naneum Creek	847-1	NANE-04	7/09/2020	47.220706	-120.438828	10.4	5	0
Naneum Creek	847-2	NANE-05	7/09/2020	47.227671	-120.446368	11.7	5	0
Naneum Creek	850-1	NANE-06	7/09/2020	47.233358	-120.446605	12	5	0
Naneum Creek	884-1	NANE-07	7/14/2020	47.236687	-120.449809	10	5	0
Naneum Creek	884-2	NANE-08	7/14/2020	47.241969	-120.459604	11	5	0
Naneum Creek	884-3	NANE-09	7/14/2020	47.246922	-120.469703	12.5	5	0
Naneum Creek	910-1	NANE-10	7/14/2020	47.250961	-120.479771	13.7	5	0
Naneum Creek	910-2	NANE-11	7/14/2020	47.256203	-120.488658	13.5	5	0

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	Latitude	Longitude	Temperature (°C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
Naneum Creek	922-1	NANE-12	7/14/2020	47.261362	-120.497404	13	5	0
Naneum Creek	932-1	NANE-13	7/16/2020	47.264560	-120.510108	12.1	5	0
Naneum Creek	950-1	NANE-14	7/16/2020	47.266553	-120.515527	13.3	5	0
Naneum Creek	953-1	NANE-15	7/16/2020	47.272017	-120.535181	14.1	5	0
Naneum Creek	977-1	NANE-15	7/16/2020	47.270094	-120.528275	14	5	0
Naneum Creek	1011-1	NANE-17	7/17/2020	47.276482	-120.545570	8	5	0
Naneum Creek	1011-2	NANE-18	7/17/2020	47.285539	-120.546295	10	5	0
Naneum Creek	1083-1	NANE-19	7/17/2020	47.289194	-120.548703	11.5	5	0
Naneum Creek	1083-2	NANE-20	7/17/2020	47.296868	-120.543886	13.1	5	0
Naneum Creek	1083-3	NANE-21	7/20/2020	47.304849	-120.537542	7.5	5	0
Naneum Creek	1083-4	NANE-22	7/20/2020	47.311653	-120.531179	11.3	5	0
Naneum Creek	1083-5	NANE-23	7/20/2020	47.319281	-120.526448	8.6	5	0
Nealy Creek	897-2	NEAL-01	7/29/2020	47.244752	-120.493083	10.9	5	0
Owl Creek	971-1	OWLC-01	7/17/2020	47.275297	-120.546448	8.4	5	0
Pearson Creek	858-1	PEAR-01	7/22/2020	47.236854	-120.447761	13.2	5	0
Pearson Creek	860-1	PEAR-02	7/22/2020	47.240119	-120.450898	13.2	5	0
Pearson Creek	885-1	PEAR-03	7/22/2020	47.241670	-120.452240	13.9	5	0
Pearson Creek	885-2	PEAR-04	7/22/2020	47.248240	-120.455106	18.5	5	0
Pearson Creek	896-1	PEAR-05	7/22/2020	47.254525	-120.459952	18.6	5	0
Pearson Creek	924-1	PEAR-06	7/22/2020	47.259129	-120.464766	17.2	5	0
Pearson Creek	924-2	PEAR-07	7/28/2020	47.267730	-120.467400	11	5	0
Pearson Creek	928-1	PEAR-08	7/28/2020	47.272080	-120.469670	10.5	5	0
Pearson Creek	954-1	PEAR-09	7/28/2020	47.273360	-120.471050	9.6	5	0
Pearson Creek	1017-1	PEAR-10	7/28/2020	47.279030	-120.472900	9.6	5	0
Swift Creek	832-1	SWIF-01	7/28/2020	47.220050	-120.436170	11	5	0
Swift Creek	832-2	SWIF-02	7/30/2020	47.225416	-120.427069	8.9	5	0
Swift Creek	835-1	SWIF-03	7/30/2020	47.230990	-120.423312	9.5	5	0
Swift Creek Tributary	845-1	SWTB-01	7/30/2020	47.232311	-120.422253	13.9	5	0
W.F. Naneum Creek	1013-1	WFNA-01	7/17/2020	47.288557	-120.549767	8	5	0

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	Latitude	Longitude	Temperature (°C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
W.F. Naneum Creek	1050-2	WFNA-03	7/17/2020	47.294573	-120.560125	12.4	5	0

Table A2. Planned Upper Naneum Creek Subwatershed eDNA Sampling Locations Where eDNA samples Were not Collected During 2020 Surveys. Samples were not collected from sites that were too small to support rearing Bull Trout populations (<0.5m wide) and sites that could not be safely accessed by survey teams.

Stream Name	RMRS Site Name	MCFWCO Site Name	Attempted Collection Date	Latitude	Longitude	Reason Skipped
E.F. Howard Creek	102-01	EFHO-01	7/27/2020	-120.509907	47.286429	Inaccessible
High Creek	867-3	HIGH-03	7/30/2020	-120.501893	47.213660	Inaccessible
High Creek	867-4	HIGH-04	7/30/2020	-120.512148	47.218625	Inaccessible
High Creek	867-5	HIGH-05	7/30/2020	-120.519547	47.225931	Inaccessible
Howard Creek	1044-1	HOWA-03	7/27/2020	-120.510956	47.286632	Inaccessible
Nealy Creek	897-3	NEAL-02	7/30/2020	-120.502369	47.241198	Inaccessible
W.F. Naneum Creek	1050-1	WFNA-02	7/17/2020	-120.549944	47.289210	Width <0.5m

**U. S. Fish and Wildlife Service
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Leavenworth, WA**



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