U.S. Fish and Wildlife Service

Assessment of Bull Trout Distributions Upstream of Entiat Falls using Environmental DNA Analysis

Jose Vazquez and R.D. Nelle
U.S. Fish and Wildlife Service
Mid-Columbia Fish and Wildlife Conservation Office
Leavenworth, WA 98826

***On the cover**: Photograph of Entiat Falls. USFWS photograph by Mark Nelson.*

The correct citation for this report is:

Vazquez, J.A. and R.D. Nelle. 2020. Assessment of Bull Trout Distributions Upstream of Entiat Falls using Environmental DNA Analysis. U.S. Fish and Wildlife Service, Leavenworth, WA.

ASSESSMENT OF BULL TROUT DISTRIBUTIONS UPSTREAM OF ENTIAT FALLS USING ENVIRONMENTAL DNA ANALYSIS

Authored by

Jose A. Vazquez
R.D. Nelle

U.S. Fish and Wildlife Service
Mid-Columbia Fish and Wildlife Conservation Office
7501 Icicle Road
Leavenworth, WA 98826

Final Report
April 7, 2020

Disclaimers

Any findings and conclusions presented in this report are those of the authors and may not necessarily represent the views of the U.S. Fish and Wildlife Service.

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the federal government.

ASSESSMENT OF BULL TROUT DISTRIBUTIONS UPSTREAM OF ENTIAT FALLS USING ENVIRONMENTAL DNA ANALYSIS

Jose A. Vazquez and R.D. Nelle

U.S. Fish and Wildlife Service
Mid-Columbia Fish and Wildlife Conservation Office
7501 Icicle Rd.
Leavenworth WA, 98826

Abstract- The sections of the Entiat River and its tributaries upstream of Entiat Falls contain large quantities of potential Bull Trout habitat. Despite the presence of a fluvial Bull Trout population directly downstream of Entiat Falls, no Bull Trout have been documented in upstream reaches, suggesting the falls may be a barrier to Bull Trout movement. Past fisheries surveys performed upstream of Entiat Falls were limited to small portions of the total available habitat, and it is possible that resident or fluvial Bull Trout present upstream of the putative barrier escaped detection. 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 Upper Entiat watershed upstream of Entiat Falls, during summer and fall of 2017 we collected 52 eDNA samples from this area at one kilometer intervals within all potential Bull Trout 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 upstream of Entiat Falls. It is possible a few migratory Bull Trout avoided detection; however, our results imply a permanent rearing population of resident or fluvial Bull Trout is not present upstream of Entiat Falls. These results provide further evidence that Entiat Falls is likely a partial or permanent migration barrier to the fluvial Entiat River Bull Trout population. The results of this study will help influence potential management actions upstream of Entiat Falls, including nonnative species removal, translocation efforts, and passage improvement.

Table of Contents

List of Figures

Introduction

Bull Trout are listed as a threatened species under the Endangered Species Act due to declining populations caused by threats including habitat degradation, reductions in stream connectivity, and invasive species introductions (USFWS 2015a). Implementation of effective Bull Trout recovery actions requires detailed information about current Bull Trout distributions (USFWS 2010). Unfortunately, due to limited funding levels and difficultly accessing remote Bull Trout habitat, current distributions for many at-risk Bull Trout populations are poorly understood.

The Entiat River Core Area in the Mid-Columbia Basin contains two at-risk populations of fluvial Bull Trout, the Mad River and Entiat River populations (DeHaan and Neibauer 2012). The Entiat River population uses spawning and rearing habitat in the upper portions of the mainstem Entiat River between the Box Canyon area (rkm 46.5) and Entiat Falls (rkm 54.6). Models predict high quality Bull Trout habitat above Entiat Falls (Isaak et al. 2015, Hockman-Wert et al. 2016); however, Entiat Falls is considered a barrier to anadromy and is a presumed barrier to upstream Bull Trout migrations (Archibald and Johnson 2005, Nelson 2014).

Several studies have attempted to assess if Bull Trout are present upstream of Entiat Falls; however, neither resident nor migratory Bull Trout have been documented upstream of the putative barrier (Brown 1992, Proebstel et al. 1998, Archibald and Johnson 2005, USFWS 2015b). These studies utilized common fisheries techniques including electrofishing, angling, redd surveys, and day night and snorkeling. Past surveys were limited to a small subset of the available Bull Trout habitat upstream of Entiat Falls, often reaches accessible from nearby roads. Due to low Bull Trout detection probabilities associated with most fisheries techniques (Thurow et al. 2006), combined with the fact that a large portion of potential upper Entiat River Bull Trout habitat was never surveyed, it is possible that these studies failed to detect an isolated population of resident Bull Trout or low densities of migratory Bull Trout. A larger census of habitat above the falls is needed to determine if Bull Trout are present in unsurveyed habitat or if low densities of Bull Trout are present in previously surveyed areas.

Recent developments in environmental DNA (eDNA) analysis allow 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, Roghair et al. 2017). 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 upstream of Entiat Falls, we collected eDNA samples during the summer and fall of 2017 from locations in the Upper Entiat River Watershed. The sites encompassed the predicted range of potential Bull Trout habitat in this area. The objectives of this study were to determine if Bull Trout are present upstream of Entiat Falls and to assess the distributions of any Bull Trout populations detected in the Upper Entiat River Watershed during the study. The presence or probable absence of Bull Trout in the Upper Entiat River Watershed could affect future management and ESA recovery actions within the Entiat Basin.

Study Site

The Entiat River is a tributary to the Columbia River located in Central Washington. The river is approximately 68 km long and drains an area of 671 km² on the eastern side of the Cascade Mountains. Peak discharge in the Entiat River occurs during spring-runoff between March and June. The river's low discharge period occurs between August and March; although, rain events can elevate flows in October and November. Documented fish barriers within the Entiat River include Box Canyon at rkm 47.0, a seasonal fish barrier, and Entiat Falls at rkm 54.6 (Figure 1). The portion of the Entiat River Basin upstream of Box Canyon is generally referred to as the Upper Entiat River. Notable tributaries to the Upper Entiat River above Entiat Falls include Ice Creek, Snowy Brushy Creek, and the North Fork Entiat River and its primary tributary, Pyramid Creek.

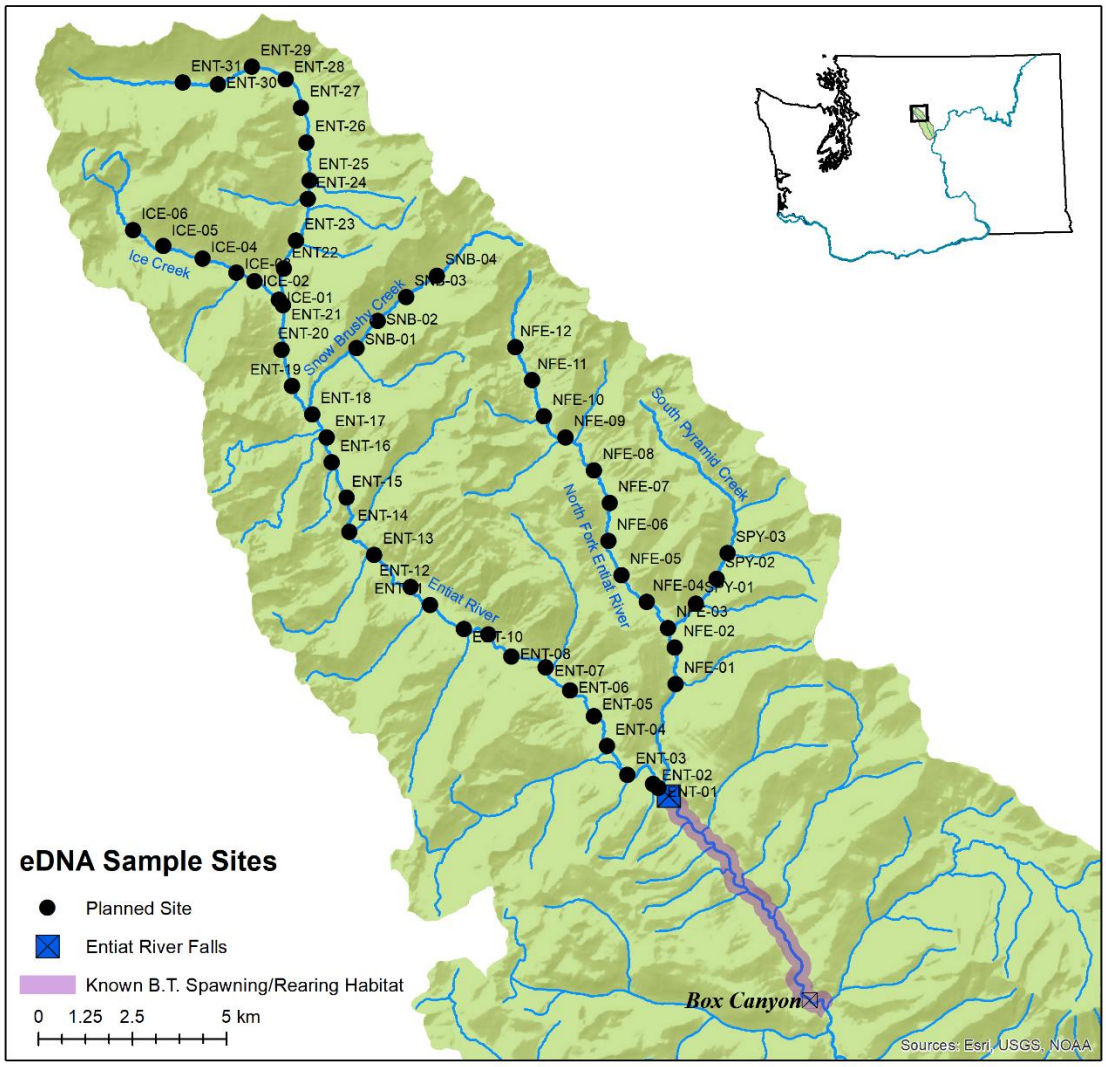

Figure 1. Documented Entiat River Bull Trout Spawning and Rearing Habitat and Planned eDNA Sites in the Upper Entiat Watershed. Displayed Bull Trout spawning and rearing habitat represents all known spawning and rearing habitat used by the Entiat River Bull Trout population. All eDNA collection sites were located upstream of Entiat Falls in Bull Trout habitat predicted by the Climate Shield Occurrence Model (n=56).

Methods

Field Collection

All planned eDNA sample sites were located upstream of Entiat Falls (Figure 1). Sampling occurred in areas containing potential juvenile Bull Trout rearing habitat as identified by the Bull Trout Climate Shield Occurrence Model (Isaak et al. 2015). Sample sites 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). In order to maximize detection probabilities, samples were collected during low flows in the summer and fall of 2017 when stream temperatures were moderate and turbidity levels were low. Fall sampling also coincided with the local Bull Trout spawning season, when migratory adult Bull Trout would be present at spawning grounds and spawning gametes would increase ambient Bull Trout eDNA concentrations. Sampling occurred in a downstream to upstream direction to avoid upstream contamination effects. 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. (2016). Sample collection involved filtering 5 L of stream water through a 1.5 µm glass filter 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. 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 Franklin et al. (2019). 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˚C/10 minutes (95˚C/15 s, 60˚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 52 samples were collected from the Upper Entiat River and its tributaries upstream of Entiat Falls between September 5, 2017 and October 12, 2017 (Appendix). Four additional sites were determined to be inaccessible by surveyors and were not sampled. All samples were

collected when stream temperatures were 4.5-11.5˚C except for four sites in the North Fork Entiat River where temperatures were 0-1.0˚C.

Quantitative PCR analysis found Bull Trout DNA did not amplify in any PCR replicates from the 52 tested samples, implying Bull Trout DNA was not detected in samples collected upstream of Entiat Falls in 2017 (Figure 2). There was no amplification of negative controls and the presence of PCR inhibitors was not detected in any sample, indicating laboratory contamination and sample inhibition did not influence PCR results.

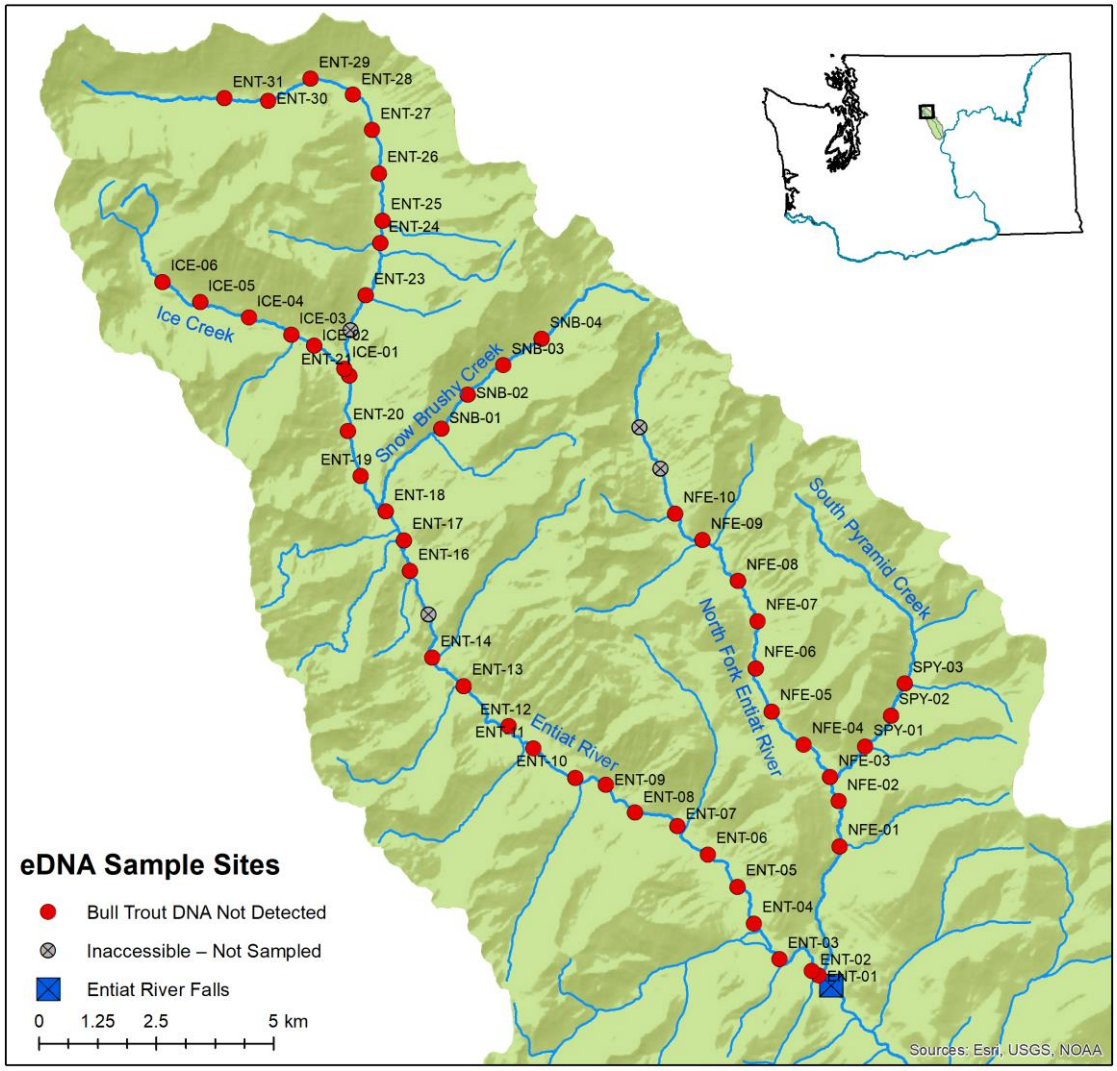

Figure 2. Bull Trout eDNA detection results from 2017 collection sites (n=52) in the Upper Entiat watershed upstream of Entiat Falls.

Discussion

The absence of detectable levels of Bull Trout DNA at sites sampled upstream of Entiat Falls in 2017 implies a rearing or spawning population of Bull Trout was likely not present upstream of the falls 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 when adults would be spawning under low stream discharge conditions resulted in high relative detection efficiencies within the study area that likely 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 population of resident Bull Trout or a sizeable population of rearing migratory Bull Trout were present. Based on our results, we advise that future management actions in the Entiat River Basin should assume a permanent Bull Trout population is not present upstream of Entiat Falls.

While the absence of detectable levels of Bull Trout DNA indicates a resident or rearing population upstream of Entiat Falls is unlikely; similar to other fishery techniques, the negative results seen in this study cannot prove the absence of extremely low densities of migratory or resident Bull Trout. Detection efficiencies of eDNA are influenced by distance and temporal related effects, including degradation and substrate interaction (Jerde et al. 2016, Shogren et al. 2017). At a one kilometer sampling interval, it is possible for DNA concentrations to fall below detectable levels if only a few individuals are present and they are located a significant distance from sample sites (Wilcox et al. 2016, Schumer et al. 2019). This implies a few migratory Bull Trout or the remnants of a resident population may have been present in sampled reaches and remained undetected during our study.

This study corroborates previous research and strongly suggests that Entiat Falls is currently a barrier to most or all Bull Trout upstream movement. Past research on migratory Bull Trout populations indicates that, when a barrier is easily passable, most migratory Bull Trout will use available spawning habitat above the barrier (McPhail and Baxter 1996, Schmetterling 2003). This behavior has been documented in the Upper Entiat River Bull Trout population, which successfully navigate Box Canyon, a substantial obstacle to most other migratory salmonids, to access upstream spawning habitat (Nelson and Nelle 2008). Large numbers of spawning adult Bull Trout upstream of Entiat Falls would likely have been detected by the small-interval eDNA analysis implemented during this study (Tilloston et al. 2018), implying that most Bull Trout were unable to pass the falls.

Given the likely absence of Bull Trout upstream of Entiat Falls, management actions that provide access to these areas could significantly increase the amount of habitat available to the Entiat River Bull Trout population. Indeed, introduction efforts including translocation and increasing passage at barriers have been shown to benefit Bull Trout populations in other systems (Hayes and Banish 2017). There are several factors that must be considered before undertaking similar efforts in the Upper Entiat River. Brook Trout are present upstream of Entiat Falls, and their presence may impede the establishment of Bull Trout populations (Rieman et al. 2006). The

current distributions of Brook Trout and their implications on potential Bull Trout introduction efforts needs to be assessed before commencing introduction or passage improvement efforts. Further, it is unknown if a Bull Trout population was historically present upstream of Entiat Falls, and the consequences of the introduction of Bull Trout to an area outside of their historic range warrants further consideration.

Conclusion

Environmental DNA analysis indicates that Bull Trout DNA was not detected at sample sites upstream of Entiat Falls, implying that, while low densities of Bull Trout may be present, rearing Bull Trout are likely not present above the falls. These results agree with findings from past studies and provide further evidence that Entiat Falls is a migratory barrier to Bull Trout.

Acknowledgments

Mark Inc (USFWS-MCFWCO) helped oversee daily sample collection and assisted project planning and implementation. Jakub Bednarek, Katy Pfannenstein, Gershom Bingham, Frederick Glassen, and Dustin Wagner (USFWS-MCFWCO) assisted sample collection. Tommy Franklin (National Genomics Center) oversaw laboratory analysis and sample equipment coordination. Michael Young, Kevin McKelvey, Michael Schwartz (Rocky Mountain Research Station) assisted project development and funding acquisition.

Literature Cited

Archibald, P. and E. Johnson. 2005. Snorkel survey of upper Entiat River, August-September 2004-2005. U.S. Forest Service, Entiat Ranger District, Wenatchee National Forest, Entiat, WA.

Baldigo, B.P., L.A. Sporn, S.D. George, and J.A. Ball. 2017. Efficacy of environmental DNA to detect and quantify Brook Trout populations in headwater streams of the Adirondack Mountains, New York. Transactions of the American Fisheries Society, 146(1), pp.99-111.

Brown, L. G. 1992. Draft management guide for the Bull Trout (*Salvelinus confluentus*) on the Wenatchee National Forest. Washington Department of Wildlife, Wenatchee, WA.

Carim, K.J., K.S. McKelvey, M.K. Young, T.M. Wilcox, and M.K. Schwartz. 2016. A protocol for collecting environmental DNA samples from streams. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula, MT.

DeHaan, P. and J. Neibauer. 2012. Analysis of genetic variation within and among upper Columbia River Bull Trout populations. U.S. Fish and Wildlife Service, Longview, WA.

Dysthe, J.C., T.W. Franklin, K.S. McKelvey, M.K. Young, and M.K. Schwartz. 2019. An improved environmental DNA assay for Bull Trout (*Salvelinus confluentus*) based on the ribosomal internal transcribed spacer I. PLoS ONE, 13(11), e020685.

Evans, N.T., P.D. Shirey, J.G. Wieringa, A.R. Mahon, and G.A. Lamberti. 2017. Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. Fisheries, 42(2), pp.90-99.

Franklin, T.W., K.S. McKelvey, J.D. Golding, D.H. Mason, J.C. Dysthe, K.L. Pilgrim, J.R. Squires, K.B. Aubry, R.A. Long, S.E. Greaves, C.M. Raley, S. Jackson, P. MacKay, J. Lisbon, J.D. Sauder, M.T. Pruss, D. Heffington, and M.K. Schwartz. 2019. Using environmental DNA methods to improve winter surveys for rare carnivores: DNA from snow and improved noninvasive techniques. Biological Conservation, 229, pp.50-58.

Fremier, A.K., K.M Strickler, J. Parzych, S, Powers, and C.S. Goldberg. 2019. Stream transport and retention of environmental DNA pulse releases in relation to hydrogeomorphic scaling factors. Environmental science & technology, *53*(12), pp.6640-6649.

Hayes, M.F. and N.P Banish. 2017. Translocation and reintroduction of native fishes: a review of Bull Trout (*Salvelinus confluentus*) with applications for future reintroductions. Endangered Species Research, 34, pp.191-209.

Hockman-Wert, D., J.B. Dunham, N. Chelgren. 2016. Rangewide climate vulnerability assessment for threatened Bull Trout. U.S. Geological Survey.

Isaak, D.J., M.K. Young, D.E., Nagel, D.L. Horan, and M.C. Groce. 2015. The cold-water climate shield: delineating refugia for preserving salmonid fishes through the 21st century. Global Change Biology, 21(7), pp.2540-2553.

Jane, S.F., T.M. Wilcox, K.S. McKelvey, M.K. Young, M.K. Schwartz, W.H. Lowe, B.H. Letcher, and A.R. Whiteley. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Molecular Ecology Resources, 15, pp.216–227.

Jerde, C.L., B.P. Olds, A.J. Shogren, E.A. Andruszkiewicz, A.R. Mahon, D.Bolster, and J.L. Tank. 2016. Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. Environmental Science & Technology, 50(16), pp.8770-8779.

McKelvey, K.S., M.K. Young, W.L. Knotek, K.J. Carim, K.J., T.M. Wilcox, T.M. Padgett-Stewart, and M.K. Schwartz. 2016. Sampling large geographic areas for rare species using environmental DNA: a study of Bull Trout (*Salvelinus confluentus*) occupancy in western Montana. Journal of Fish Biology, 88(3), pp.1215-1222.

McPhail, J.D. and J.S. Baxter. 1996. A review of Bull Trout (*Salvelinus confluentus*) life-history and habitat use in relation to compensation and improvement opportunities. Ministry of Environment, Lands and Parks, British Columbia.

Nelson, M.C and R.D. Nelle. 2008. Seasonal movements of adult fluvial Bull Trout in the Entiat River, WA 2003-2006. U.S. Fish and Wildlife Service, Leavenworth, WA.

Nelson, M.C. 2014. Spawning migrations of adult fluvial Bull Trout in the Entiat River, WA 2007 - 2013. U.S. Fish and Wildlife Service, Leavenworth, WA.

Proebstel, D.S., R.J. Behnke, and S.M. Noble. 1998. Identification of salmonid fishes from tributary streams and lakes of the Mid-Columbia Basin. U.S. Fish and Wildlife Service, Leavenworth, WA.

Rieman, B.E., J.T. Peterson, and D.L. Myers. 2006. Have Brook Trout (*Salvelinus fontinalis*) displaced Bull Trout (*Salvelinus confluentus*) along longitudinal gradients in central Idaho streams? Canadian Journal of Fisheries and Aquatic Sciences, 63(1), pp.63-78.

Roghair, C., C. Krause, T.W. Franklin, J. Farmer, and C.A. Dolloff. 2017. Inventory and monitoring of Brook Trout in the Santeetlah Creek Watershed, Nantahala National Forest, 2016-2017. U.S. Department of Agriculture, Forest Service, Southern Research Station, Blacksburg, VA

Shogren, A.J., J.L. Tank, E. Andruszkiewicz, B. Olds, A.R. Mahon, C.L. Jerde, and D. Bolster. 2017. Controls on eDNA movement in streams: Transport, retention, and resuspension. Scientific Reports, 7(1), p.5065.

Schmetterling, D.A. 2003. Reconnecting a fragmented river: movements of Westslope Cutthroat Trout and Bull Trout after transport upstream of Milltown Dam, Montana. North American Journal of Fisheries Management, 23(3), pp.721-731.

Schumer, G., K. Crowley, E. Maltz, M. Johnston, P. Anders, and S. Blankenship. 2019. Utilizing environmental DNA for fish eradication effectiveness monitoring in streams. Biological Invasions, 21(11), pp.3415-3426.

Thurow, R.F., J.T. Peterson, and J.W. Guzevich. 2006. Utility and validation of day and night snorkel counts for estimating Bull Trout abundance in first-to third-order streams. North American Journal of Fisheries Management, 26(1), pp.217-232.

Tillotson, M.D., R.P. Kelly, J.J. Duda, M. Hoy, J. Kralj, and T.P. Quinn. 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biological Conservation, 220, pp.1-11.

U.S. Fish and Wildlife Service. 2010. Bull Trout final Critical Habitat justification: Rationale for why habitat is essential, and documentation of occupancy. Boise, ID.

U.S. Fish and Wildlife Service. 2015a. Mid-Columbia Recovery Unit implementation plan for Bull Trout (*Salvelinus confluentus*). Portland, OR.

U.S. Fish and Wildlife Service. 2015b. Recovery plan for the coterminous United States population of Bull Trout (*Salvelinus confluentus*). Portland, OR.

Wilcox, T.M., K.S. McKelvey, M.K. Young, S.F. Jane, W.H. Lowe, A.R. Whiteley, and M.K. Schwartz. 2013. Robust detection of rare species using environmental DNA: The Importance of Primer Specificity. PLoS ONE, 8, e59520.

Wilcox, T.M., K.S. McKelvey, M.K. Young, A.J. Sepulveda, B.B. Shepard, S.F. Jane, A.R. Whiteley, W.H. Lowe, and M.K. Schwartz. 2016. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char (*Salvelinus fontinalis*). Biological Conservation, 194, pp.209-216.

Wilcox, T.M., M.K. Young, K.S. McKelvey, D.J. Isaak, D.L. Horan, and M.K. Schwartz. 2018. Fine-scale environmental DNA sampling reveals climate-mediated interactions between native and invasive trout species. Ecosphere, 9(11), e02500

Appendix

Table A1. Site Location Information and PCR Results from eDNA Samples Collected Upstream of Entiat Falls in 2017.

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	UTM Zone	UTM Northing	UTM Easting	Temperature (˚C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
Entiat River	1149-1	ENT-01	09/05/2017	10	5317709	0680571	11.0	5	0
Entiat River	1153-1	ENT-02	09/05/2017	10	5317794	0680416	11.0	5	0
Entiat River	1156-1	ENT-03	09/05/2017	10	5312992	0679710	11.0	5	0
Entiat River	1163-1	ENT-04	09/05/2017	10	5318715	0679111	11.5	5	0
Entiat River	1163-2	ENT-05	09/05/2017	10	5319477	0678705	11.5	5	0
Entiat River	1163-3	ENT-06	09/06/2017	10	5320113	0678018	10.0	5	0
Entiat River	1168-1	ENT-07	09/06/2017	10	5320674	0677328	10.0	5	0
Entiat River	1168-2	ENT-08	09/06/2017	10	5320893	0676396	10.5	5	0
Entiat River	1168-3	ENT-09	09/06/2017	10	5321438	0675734	11.0	5	0
Entiat River	1171-1	ENT-10	09/06/2017	10	5321536	0675071	11.5	5	0
Entiat River	1176-1	ENT-11	09/21/2017	10	5322094	0674128	6.0	5	0
Entiat River	1176-2	ENT-12	09/21/2017	10	5322535	0673572	7.0	5	0
Entiat River	1177-1	ENT-13	09/21/2017	10	5323316	0672544	7.0	5	0
Entiat River	1186-1	ENT-14	09/21/2017	10	5323316	0671833	7.5	5	0
Entiat River	1186-2	ENT-15	NA	NA	NA	NA	NA	NA	NA
Entiat River	1186-3	ENT-16	09/21/2017	10	5325689	0671220	6.5	5	0
Entiat River	1191-1	ENT-17	09/11/2017	10	5326300	0671017	8.5	5	0
Entiat River	1191-1	ENT-17	09/21/2017	10	5326335	0671041	6.5	5	0
Entiat River	1197-1	ENT-18	09/11/2017	10	5326920	0670607	9.0	5	0
Entiat River	1197-2	ENT-19	09/11/2017	10	5327632	0670011	10.0	5	0
Entiat River	1197-3	ENT-20	09/11/2017	10	5328579	0669669	10.5	5	0
Entiat River	1201-1	ENT-21	09/11/2017	10	5329759	0669610	10.0	5	0
Entiat River	1201-2	ENT-22	NA	NA	NA	NA	NA	NA	NA
Entiat River	1204-1	ENT-23	09/11/2017	10	5331501	0669825	9.0	5	0
Entiat River	1206-1	ENT-24	09/12/2017	10	5332638	0670051	4.5	5	0

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	UTM Zone	UTM Northing	UTM Easting	Temperature (˚C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
Entiat River	1211-1	ENT-25	09/12/2017	10	5333125	0670068	5.0	5	0
Entiat River	1211-2	ENT-26	09/12/2017	10	5334129	0669912	5.5	5	0
Entiat River	1211-3	ENT-27	09/12/2017	10	5335043	0669697	6.0	5	0
Entiat River	1211-4	ENT-28	09/12/2017	10	5335768	0669225	6.5	5	0
Entiat River	1211-5	ENT-29	09/12/2017	10	5336039	0668303	7.0	5	0
Entiat River	1211-6	ENT-30	09/12/2017	10	5335495	0667437	6.5	5	0
Entiat River	1211-7	ENT-31	09/12/2017	10	5335481	0666497	6.0	5	0
Ice Creek	1200-1	ICE-01	09/11/2017	10	5329899	0669487	9.0	5	0
Ice Creek	1200-2	ICE-02	09/13/2017	10	5330345	0668810	6.0	5	0
Ice Creek	1208-1	ICE-03	09/13/2017	10	5330540	0668308	6.0	5	0
Ice Creek	1208-2	ICE-04	09/13/2017	10	5330836	0667378	6.0	5	0
Ice Creek	1208-3	ICE-05	09/13/2017	10	5331090	0666314	7.5	5	0
Ice Creek	1208-4	ICE-06	09/13/2017	10	5331457	0665482	8.0	5	0
N.F. Entiat River	1164-1	NFE-01	09/22/2017	10	5320502	0680806	5.5	5	0
N.F. Entiat River	1164-2	NFE-02	09/22/2017	10	5321467	0680716	5.0	5	0
N.F. Entiat River	1180-1	NFE-03	09/22/2017	10	5321967	0680495	6.0	5	0
N.F. Entiat River	1180-2	NFE-04	09/23/2017	10	5322618	0679873	6.0	5	0
N.F. Entiat River	1180-3	NFE-05	09/23/2017	10	5323273	0679149	6.0	5	0
N.F. Entiat River	1180-4	NFE-06	09/23/2017	10	5324169	0678731	6.0	5	0
N.F. Entiat River	1180-5	NFE-07	10/12/2017	10	5325179	0678694	1.0	5	0
N.F. Entiat River	1180-6	NFE-08	10/12/2017	10	5326005	0678214	1.0	5	0
N.F. Entiat River	1183-1	NFE-09	10/12/2017	10	5326818	0677388	0.0	5	0
N.F. Entiat River	1195-1	NFE-10	10/12/2017	10	5327338	0676771	0.5	5	0
N.F. Entiat River	1195-2	NFE-11	NA	NA	NA	NA	NA	NA	NA
N.F. Entiat River	1195-3	NFE-12	NA	NA	NA	NA	NA	NA	NA
Snowy Brushy Creek	1198-1	SNB-01	09/14/2017	10	5328779	0671650	6.5	5	0
Snowy Brushy Creek	1198-2	SNB-02	09/14/2017	10	5329543	0672163	6.0	5	0
Snowy Brushy Creek	1198-3	SNB-03	09/14/2017	10	5330232	0672869	6.5	5	0

Stream Name	RMRS Site Name	MCFWCO Site Name	Collection Date	UTM Zone	UTM Northing	UTM Easting	Temperature (˚C)	Sample Volume (L)	PCR Wells with Bull Trout DNA (out of 3)
Snowy Brushy Creek	1198-4	SNB-04	09/14/2017	10	5330858	0673641	6.0	5	0
Snowy Brushy Creek	1170-1	SPY-01	09/22/2017	10	5322670	0681192	5.0	5	0
Snowy Brushy Creek	1170-2	SPY-02	09/22/2017	10	5323379	0681692	5.0	5	0
Snowy Brushy Creek	1173-1	SPY-03	09/22/2017	10	5324083	0681929	5.0	5	0

U. S. Fish and Wildlife Service
Mid-Columbia Fish and Wildlife Conservation Office
7501 Icicle Road
Leavenworth, WA

April 2020