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Assessment of Bull Trout Distributions in the Sinlahekin Creek Watershed using Environmental DNA Analysis

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On the cover: *Photograph of Upper Sinlahekin Creek Canyon. USFWS photograph by Jose Vazquez.*

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Abstract- The Sinlahekin Creek watershed located in the Similkameen River Basin upstream of Enloe Dam contains large quantities of potential Bull Trout habitat. Historic Bull Trout occupancy within the Sinlahekin Creek watershed is 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 watershed's total available habitat. It is possible that Bull Trout were present in the Sinlahekin Creek watershed and either 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 Sinlahekin Creek watershed, during the summers of 2018 and 2019 we collected 91 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 the Sinlahekin Creek watershed. Our results imply that Bull Trout are likely not extant within the watershed. The results of this study will help influence potential management actions within the Sinlahekin Creek watershed and larger Similkameen River Basin such as habitat restoration and native species reintroduction 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 (USDOI 1999). At the time of listing, Bull Trout distributions were poorly understood within large portions of their range, including many large watersheds in Central and Northeast Washington (USFWS 2002). While knowledge of Bull Trout distributions has increased since their listing, current distributions are still unknown in many watersheds (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 of large, understudied watershed where Bull Trout distributions are unknown is the Sinlahekin Creek watershed in Okanogan County, Washington. The Sinlahekin River is a major tributary to the Similkameen River in the Columbia River Basin. Upstream passage from other areas in the Columbia River Basin into the majority of the Similkameen River and its tributaries, including the Sinlahekin Creek watershed, was blocked by the construction of Enloe Dam in the 1920's (Inter-Fluve 2016). Bull Trout are presumed to be absent from the Similkameen River Basin upstream of Enloe Dam, and it is possible that they have been absent from the basin since the Wisconsin glaciation (McPhail and Baxter 1996, Haas and McPhail 2001). However, there are anecdotal reports of captured Bull Trout from within the Similkameen River Basin (BCMECCS 2021), and thorough fish distribution assessments have not been performed in many Similkameen River tributary systems, including the Sinlahekin Creek watershed.

Habitat prediction models indicate potential Bull Trout habitat is present in many headwater tributaries within the Sinlahekin Creek watershed (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 complete fish passage barriers, including Enloe Dam in the mainstem Similkameen river and several diversion and transportation related structures within the Sinlahekin Creek watershed, would likely have led to the extirpation of any migratory Bull Trout life histories that were previously present in the system. It is possible, however, that one or more resident Bull Trout populations are present in potential spawning and rearing habitat within the headwaters of the watershed. Past surveys performed within the Sinlahekin Creek watershed did not find Bull Trout but often identified Brook Trout (Cole et al. 2003, Ashbrook et al. 2010). 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 Sinlahekin Creek watershed, less likely (McHahon et al. 2007, Warnock and Rasmussen 2014, Howell 2018). Past surveys were limited in geographic scope; however, and a thorough assessment of all potential Bull Trout habitat is needed to accurately assess Bull Trout distributions in the watershed and evaluate the likelihood of their absence.

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, 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 in the Sinlahekin Creek watershed, we collected eDNA samples during the summers of 2018 and 2019 from sites encompassing the predicted range of potential Bull Trout spawning and rearing habitat within the watershed. The objectives of this study were to determine if Bull Trout are present in the Sinlahekin Creek watershed and to assess the distributions of any Bull Trout populations detected during the study. The presence or probable absence of Bull Trout in the Sinlahekin Creek watershed could affect future management actions within the Sinlahekin Creek watershed and the larger Similkameen River Basin, including post-dam removal fish management actions if Enloe Dam is removed.

Study Site Description

The Sinlahekin Creek watershed is a 724 km² watershed that flows into Palmer Lake, an 854 hectare warmwater lake that drains into the Similkameen River (Osborne et al. 2003). Palmer Lake and the Sinlahekin Creek watershed are located upstream of Enloe Dam, which blocks all upstream fish movement from the Okanogan and Columbia Rivers (Inter-Fluve 2016). Major tributaries in the Sinlahekin Creek watershed include Cecil Creek; Chopaka Creek; and Toats Coulee Creek and its tributaries, the North Fork, Middle Fork, and South Fork Toats Coulee creeks. Complete or significant fish passage barriers resulting from irrigation and transportation infrastructure are located in the lower or middle sections of Cecil Creek, Toats Coulee Creek, and Sinlahekin Creek (WDFW 2021). Due to these barriers, fish movement is likely not possible between most major Sinlahekin Creek tributaries. Sinlahekin Creek's low discharge period occurs between August and March; although, fall and winter rain events can temporarily elevate flows (NPCC 2001).

Within the Sinlahekin Creek watershed 23.1% of the land is federally owned, 70.9% is owned by Washington State, and 6.1% is privately owned (WARCO 2014). The majority of the federal property in the Sinlahekin Creek watershed is part of the USFS Okanogan-Wenatchee National Forest (138 km²). Property owned or managed by Washington State within the Sinlahekin Creek watershed includes the Sinlahekin Wildlife Area overseen by WDFW (44 km²) and the Loomis National Resource Conservation Area (83 km²) and Loomis State Forest (397 km²) managed by WA DNR. Cattle Grazing is currently allowed on WA DNR managed lands in the Sinlahekin Creek watershed, and timber harvesting and logging road development are permitted in Loomis State Forest as part of the Washington State Common School Trust Land Revenue Generation Program (Derr et al. 2005). These management practices have resulted in degraded riparian and stream habitat within impacted areas throughout the watershed (NPPC 2001).

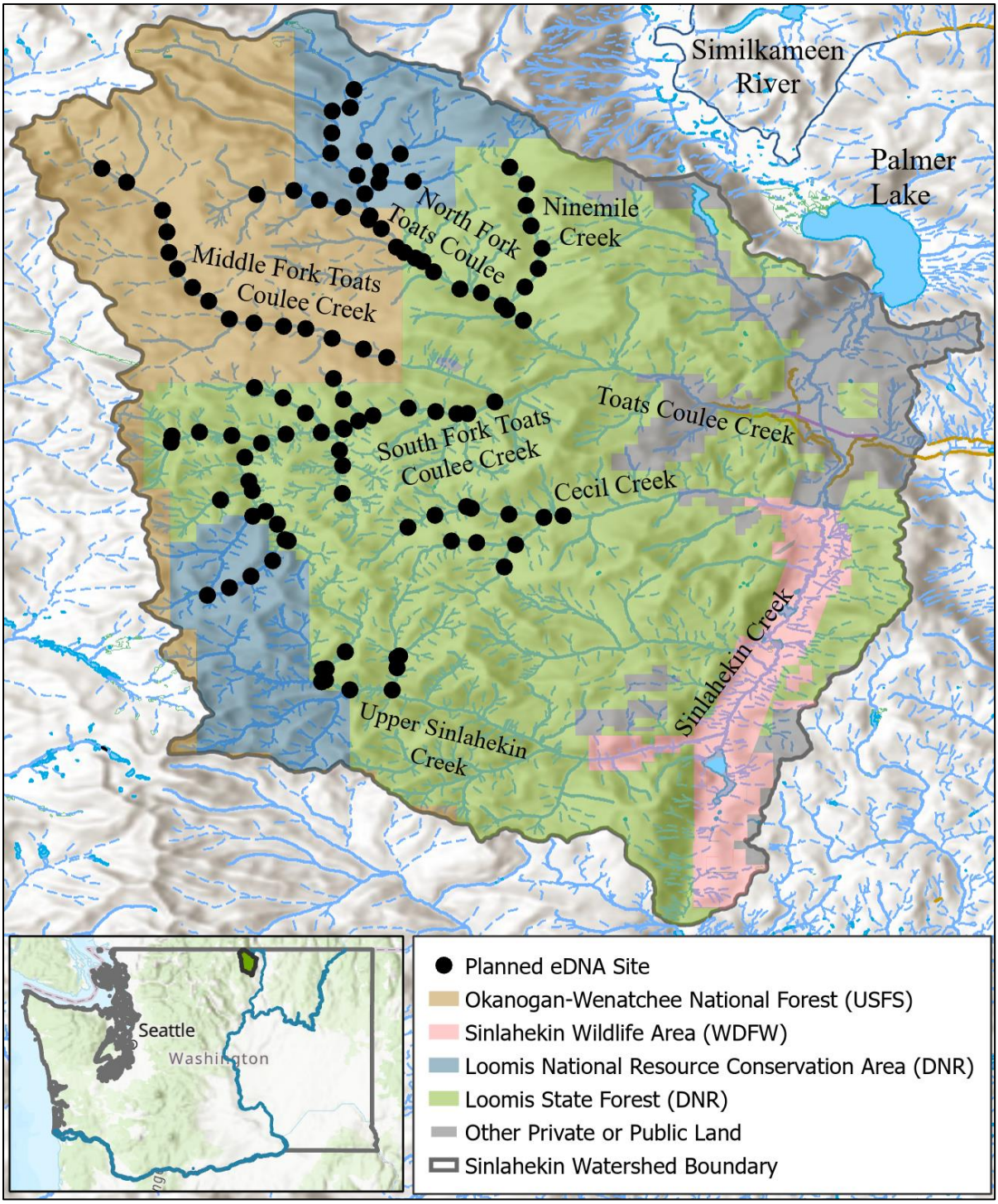

Figure 1. Planned eDNA Sites in the Sinlahekin Creek Watershed. All eDNA collection sites were located in potential Bull Trout habitat predicted by the Climate Shield Occurrence Model (n=111). Colored terrain represents land management areas within the Sinlahekin Creek watershed.

Methods

Field Collection

All planned eDNA sample sites were located within the Sinlahekin Creek watershed (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 >10%, 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 in the summers of 2018 and 2019 when stream temperatures were moderate and turbidity levels were low. Sampling occurred in a downstream to upstream direction to avoid contaminating downstream sample sites. 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 approximately 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 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˚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 91 samples were collected from the Sinlahekin Creek watershed during the first sampling period between September 4 and September 14, 2018 and the second period between July 2 and August 1, 2019 (Table A1). Eight planned sample sites were inaccessible to surveyors due to hazardous terrain and remote locations and were therefore not sampled (Table A2). Collections were not made from an additional 12 sites that were dry or had wetted widths <0.5m during site visits. All samples were collected when stream temperatures were 4.4-13.9˚C.

Quantitative PCR analysis found Bull Trout DNA did not amplify in any PCR replicates from the 91 tested samples, implying Bull Trout DNA was not detected in samples collected in the Sinlahekin Creek watershed in 2018 and 2019 (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.

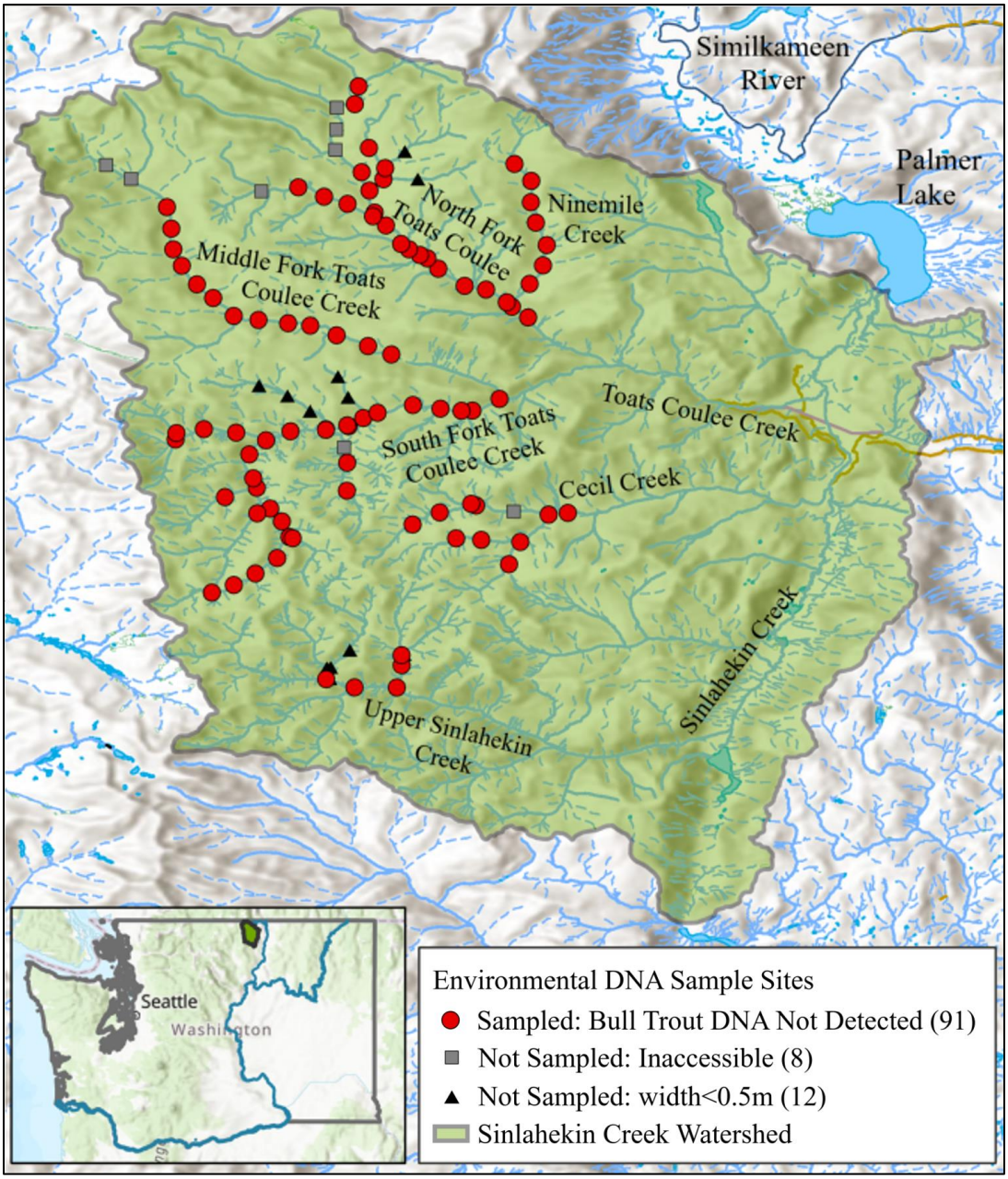

Figure 2. Bull Trout eDNA Detection Results From 2018-2019 Collection Sites in the Sinlahekin Creek Watershed.

Discussion

The absence of detectable levels of Bull Trout DNA in 2018 and 2019 at sample sites encompassing the majority of potential Bull Trout spawning and rearing habitat in the Sinlahekin Creek watershed implies Bull Trout were likely not present within the watershed 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 Sinlahekin 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 Sinlahekin 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 eight 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 Sinlahekin Creek watershed.

Due to the likely absence of Bull Trout within the Sinlahekin Creek watershed and the remainder of the Similkameen River Basin upstream of Enloe Dam, establishment of a Bull Trout population within the Sinlahekin Creek watershed will currently require introduction or reintroduction of the species. Even if Enloe Dam is removed, Bull Trout translocation will likely still be necessary due to the presumed absence of extant reproducing Bull Trout populations within the Okanogan Basin (USFWS 2015a). If Bull Trout introduction or reintroduction actions in the Sinlahekin Creek watershed are considered, several potential barriers to successful Bull Trout population establishment will need to be addressed for efforts to be effective. 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 local land management practices, including grazing and logging. Additionally, the introductions of novel species into previously unoccupied habitat is a controversial topic area (Seddon et al. 2014, Galloway et al. 2016, Hayes et al. 2017). If Bull Trout are found to have not historically occupied the Sinlahekin Creek watershed, 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 samples collected in 2018 and 2019 from the majority of potential Bull Trout spawning and rearing habitat within the Sinlahekin 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 Sinlahekin Creek system.

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Appendix

Table A1. Site Location Information and PCR Results From eDNA Samples Collected in the Sinlahekin Creek Watershed 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)
South Fork Toats Coulee Creek	101-1	SFTC-02	09/04/2018	48.83498	-119.78335	7.8	5	0
South Fork Toats Coulee Creek	103-1	SFTC-03	09/04/2018	48.83017	-119.79449	7.7	5	0
South Fork Toats Coulee Creek	107-1	SFTC-04	09/05/2018	48.83011	-119.79897	7.3	5	0
South Fork Toats Coulee Creek	114-1	SFTC-05	09/05/2018	48.83097	-119.80761	5.5	5	0
South Fork Toats Coulee Creek	114-2	SFTC-06	09/05/2018	48.83246	-119.81893	5.5	5	0
South Fork Toats Coulee Creek	114-3	SFTC-07	09/05/2018	48.82929	-119.83343	5.5	5	0
South Fork Toats Coulee Creek	111-1	SFTC-08	09/05/2018	48.82712	-119.83931	6.2	5	0
South Fork Toats Coulee Creek	108-1	SFTC-09	09/05/2018	48.82413	-119.84588	7.8	5	0
South Fork Toats Coulee Creek	110-1	SFTC-10	09/05/2018	48.82231	-119.85466	8.0	5	0
South Fork Toats Coulee Creek	110-2	SFTC-11	09/05/2018	48.82168	-119.86919	8.9	5	0
South Fork Toats Coulee Creek	94-1	SFTC-12	09/06/2018	48.81800	-119.87922	5.2	5	0
South Fork Toats Coulee Creek	94-2	SFTC-13	09/06/2018	48.81221	-119.88608	5.3	5	0
South Fork Toats Coulee Creek	88-1	SFTC-15	09/11/2018	48.79850	-119.88305	6.3	5	0
South Fork Toats Coulee Creek	94-3	SFTC-14	09/06/2018	48.80238	-119.88446	6.2	5	0
South Fork Toats Coulee Creek	78-1	SFTC-16	09/06/2018	48.78997	-119.87753	6.4	5	0
South Fork Toats Coulee Creek	78-2	SFTC-17	09/06/2018	48.78469	-119.87271	7.8	5	0
South Fork Toats Coulee Creek	64-1	SFTC-18	09/06/2018	48.77832	-119.86964	8.4	5	0
South Fork Toats Coulee Creek	64-2	SFTC-19	09/06/2018	48.76968	-119.87466	8.4	5	0
South Fork Toats Coulee Creek	64-3	SFTC-20	09/07/2018	48.76334	-119.88358	7.7	5	0
South Fork Toats Coulee Creek	64-4	SFTC-21	09/12/2018	48.75863	-119.89245	4.5	5	0
South Fork Toats Coulee Creek	64-5	SFTC-22	09/12/2018	48.75551	-119.90147	4.8	5	0
Cold Creek	124-1	COLD-01	09/05/2018	48.82252	-119.85463	6.0	5	0
South Fork Toats Coulee Trib 1	76-1	SFT1-01	09/06/2018	48.78801	-119.88283	5.0	5	0
South Fork Toats Coulee Trib 2	61-1	SFT2-01	09/06/2018	48.77781	-119.86830	7.2	5	0
Cougar Creek	81-1	COUG-01	09/05/2018	48.82375	-119.84531	6.0	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)
Cougar Creek	81-3	COUG-03	09/07/2018	48.80879	-119.84591	6.4	5	0
Cougar Creek	81-4	COUG-04	09/11/2018	48.79731	-119.84603	6.1	5	0
Crosby Creek	89-2	CROS-02	09/11/2018	48.79469	-119.89608	6.2	5	0
Cecil Creek	75-1	CECL-00	09/12/2018	48.78820	-119.75531	7.3	5	0
Cecil Creek	77-1	CECL-01	09/12/2018	48.78753	-119.76321	7.1	5	0
Cecil Creek	67-1	CECL-04	09/13/2018	48.79123	-119.79300	4.4	5	0
Cecil Creek	67-2	CECL-05	09/13/2018	48.78839	-119.80792	5.1	5	0
Cecil Creek	67-3	CECL-06	09/13/2018	48.78346	-119.81905	5.2	5	0
Chickadee Creek	66-1	CHIC-02	09/12/2018	48.78747	-119.76319	7.5	5	0
Chickadee Creek	50-2	CHIC-02	09/14/2018	48.76715	-119.77947	7.2	5	0
Hilltop Creek	117-2	HILT-01	09/12/2018	48.82113	-119.89147	5.6	5	0
Hilltop Creek	117-3	HILT-02	09/12/2018	48.82255	-119.90473	5.4	5	0
Hilltop Creek	97-1	HILT-03	09/12/2018	48.81834	-119.91637	4.9	5	0
Hilltop Tributary	122-1	HLTR-01	09/12/2018	48.82095	-119.91603	5.0	5	0
Cecil Tributary	85-1	CECT-01	09/13/2018	48.79194	-119.79499	5.3	5	0
North Fork Chickadee Creek	57-1	NFCH-01	09/13/2018	48.77628	-119.77482	6.2	5	0
North Fork Chickadee Creek	57-2	NFCH-02	09/13/2018	48.77713	-119.79086	5.8	5	0
North Fork Chickadee Creek	57-3	NFCH-03	09/13/2018	48.77776	-119.80117	5.4	5	0
Sinlahekin Creek	36-1	SINL-01	09/14/2018	48.71661	-119.84290	6.4	5	0
Sinlahekin Creek	43-1	SINL-02	09/14/2018	48.71996	-119.85454	6.4	5	0
Wood Pile Creek	39-1	WDPL-01	09/14/2018	48.71656	-119.82552	6.5	5	0
Wood Pile Creek	39-2	WDPL-02	09/14/2018	48.72571	-119.82353	6.7	5	0
Wood Pile Creek	45-1	WDPL-03	09/14/2018	48.72984	-119.82359	6.8	5	0
NF Toats Coulee	131-4	NFTC-02	07/22/2019	48.86841	-119.77161	12.6	5	0
NF Toats Coulee	135-1	NFTC-03	07/22/2019	48.87277	-119.77827	12.7	5	0
NF Toats Coulee	142-1	NFTC-04	07/22/2019	48.87462	-119.78036	12.7	5	0
NF Toats Coulee	142-2	NFTC-05	07/22/2019	48.87984	-119.78889	12.6	5	0
NF Toats Coulee	152-1	NFTC-06	07/23/2019	48.88137	-119.79767	10.8	5	0
NF Toats Coulee	159-1	NFTC-07	07/23/2019	48.88840	-119.80850	10.5	5	0
NF Toats Coulee	162-1	NFTC-08	07/23/2019	48.89267	-119.81295	10.8	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)
NF Toats Coulee	163-1	NFTC-09	07/23/2019	48.89405	-119.81606	11.2	5	0
NF Toats Coulee	169-2	NFTC-10	07/23/2019	48.89635	-119.82052	12.1	5	0
NF Toats Coulee	188-1	NFTC-11	07/23/2019	48.89857	-119.82377	12.1	5	0
NF Toats Coulee	188-2	NFTC-12	07/23/2019	48.90614	-119.83001	12.2	5	0
NF Toats Coulee	204-1	NFTC-13	07/23/2019	48.91142	-119.83463	12.0	5	0
NF Toats Coulee	236-1	NFTC-14	07/24/2019	48.92046	-119.83676	9.0	5	0
NF Toats Coulee	236-2	NFTC-15	07/24/2019	48.92800	-119.83996	9.7	5	0
Disappointment Creek	216-1	DISP-01	07/24/2019	48.92510	-119.83101	12.0	5	0
Disappointment Creek	235-1	DISP-02	07/24/2019	48.92968	-119.83029	11.3	5	0
Swamp Creek	245-2	SWMP-02	07/25/2019	48.93794	-119.83696	9.5	4.5	0
Olallie Creek	261-1	OLAL-02	07/25/2019	48.95591	-119.84278	7.1	5	0
Olallie Creek	261-2	OLAL-04	07/25/2019	48.96325	-119.84123	8.2	5	0
Deer Park	206-1	DRPR-01	07/23/2019	48.90991	-119.83531	11.5	5	0
Deer Park	206-2	DRPR-02	07/26/2019	48.91500	-119.84575	8.7	5	0
Deer Park	214-1	DRPR-03	07/26/2019	48.91795	-119.85540	8.7	5	0
Deer Park	214-2	DRPR-04	07/26/2019	48.92179	-119.86598	9.2	5	0
Nine Mile Creek	218-2	NINE-01	07/30/2019	48.88218	-119.77100	9.5	5	0
Nine Mile Creek	218-3	NINE-02	07/30/2019	48.88976	-119.76551	9.5	5	0
Nine Mile Creek	218-4	NINE-03	07/30/2019	48.89807	-119.76397	9.9	5	0
Nine Mile Creek	218-5	NINE-04	07/30/2019	48.90730	-119.76846	9.5	5	0
Nine Mile Creek	218-6	NINE-05	07/30/2019	48.91568	-119.77049	9.3	5	0
Nine Mile Creek	218-7	NINE-06	07/30/2019	48.92442	-119.77032	10.5	5	0
Nine Mile Creek	218-8	NINE-07	07/30/2019	48.93141	-119.77722	8.2	5	0
Middle Fork Toats Coulee	140-5	MFTC-01	07/31/2019	48.85323	-119.82777	11.2	5	0
Middle Fork Toats Coulee	140-6	MFTC-02	07/31/2019	48.85668	-119.83736	12.0	5	0
Middle Fork Toats Coulee	140-7	MFTC-03	07/31/2019	48.86108	-119.85024	13.9	5	0
Middle Fork Toats Coulee	140-8	MFTC-04	07/31/2019	48.86504	-119.86105	13.2	5	0
Middle Fork Toats Coulee	145-1	MFTC-05	07/31/2019	48.86603	-119.87016	13.6	5	0
Middle Fork Toats Coulee	145-2	MFTC-06	07/31/2019	48.86737	-119.88232	13.2	5	0
Middle Fork Toats Coulee	183-1	MFTC-07	07/31/2019	48.86903	-119.89247	12.9	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)
Middle Fork Toats Coulee	183-2	MFTC-08	07/31/2019	48.87638	-119.90094	12.7	5	0
Middle Fork Toats Coulee	183-3	MFTC-09	08/01/2019	48.88199	-119.90759	10.0	5	0
Middle Fork Toats Coulee	183-4	MFTC-10	08/01/2019	48.88963	-119.91379	10.1	5	0
Middle Fork Toats Coulee	223-1	MFTC-11	08/01/2019	48.89633	-119.91729	10.9	4.5	0
Middle Fork Toats Coulee	223-2	MFTC-12	08/01/2019	48.90483	-119.91811	11.6	5	0
Middle Fork Toats Coulee	223-3	MFTC-13	08/01/2019	48.91366	-119.91993	13.3	5	0

Table A2. Planned Sinlahekin Creek Watershed eDNA Sampling Locations Where eDNA samples Were not Collected During 2018-2019 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
Cecil Creek	77-2	CECL-02	09/12/2018	48.78877	-119.77753	Inaccessible
Chute Creek	60-1	CHUT-01	09/14/2018	48.72520	-119.85266	Width <0.5m
Chute Creek	60-2	CHUT-02	09/14/2018	48.73226	-119.84480	Width <0.5m
Cold Creek	124-2	COLD-02	09/05/2018	48.83035	-119.86114	Width <0.5m
Cold Creek	124-3	COLD-03	09/05/2018	48.83661	-119.87041	Width <0.5m
Cold Creek	124-4	COLD-04	09/05/2018	48.84082	-119.88213	Width <0.5m
Corduroy Creek	208-2	CORD-01	07/24/2019	48.92553	-119.81690	Width <0.5m
Cougar Creek	81-2	COUG-02	09/07/2018	48.81505	-119.84717	Inaccessible
Deer Park Creek	211-1	DRPR-05	07/26/2019	48.92017	-119.88112	Inaccessible
Disappointment Creek	235-2	DISP-03	07/24/2019	48.93689	-119.82224	Width <0.5m
Middle Fork Toats Coulee	263-1	MFTC-14	08/01/2019	48.92523	-119.93464	Inaccessible
Middle Fork Toats Coulee	263-2	MFTC-15	08/01/2019	48.93092	-119.94487	Inaccessible
North Fork Toats Coulee	247-1	NFTC-16	07/24/2019	48.93717	-119.85072	Inaccessible
North Fork Toats Coulee	247-2	NFTC-17	07/24/2019	48.94548	-119.85033	Inaccessible
Ollalie Creek	249-1	OLAL-01	07/24/2019	48.95448	-119.85016	Inaccessible
Parks Creek	125-2	PARK-01	09/05/2018	48.83603	-119.84555	Width <0.5m
Parks Creek	125-3	PARK-02	09/05/2018	48.84453	-119.84968	Width <0.5m
Timothy Creek	38-1	TIMO-01	09/14/2018	48.72058	-119.85294	Width <0.5m
Timothy Creek	53-1	TIMO-02	09/14/2018	48.72504	-119.85424	Width <0.5m
Woodpile Creek	45-2	WDPL-04	09/12/2018	48.73051	-119.82248	Width <0.5m

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