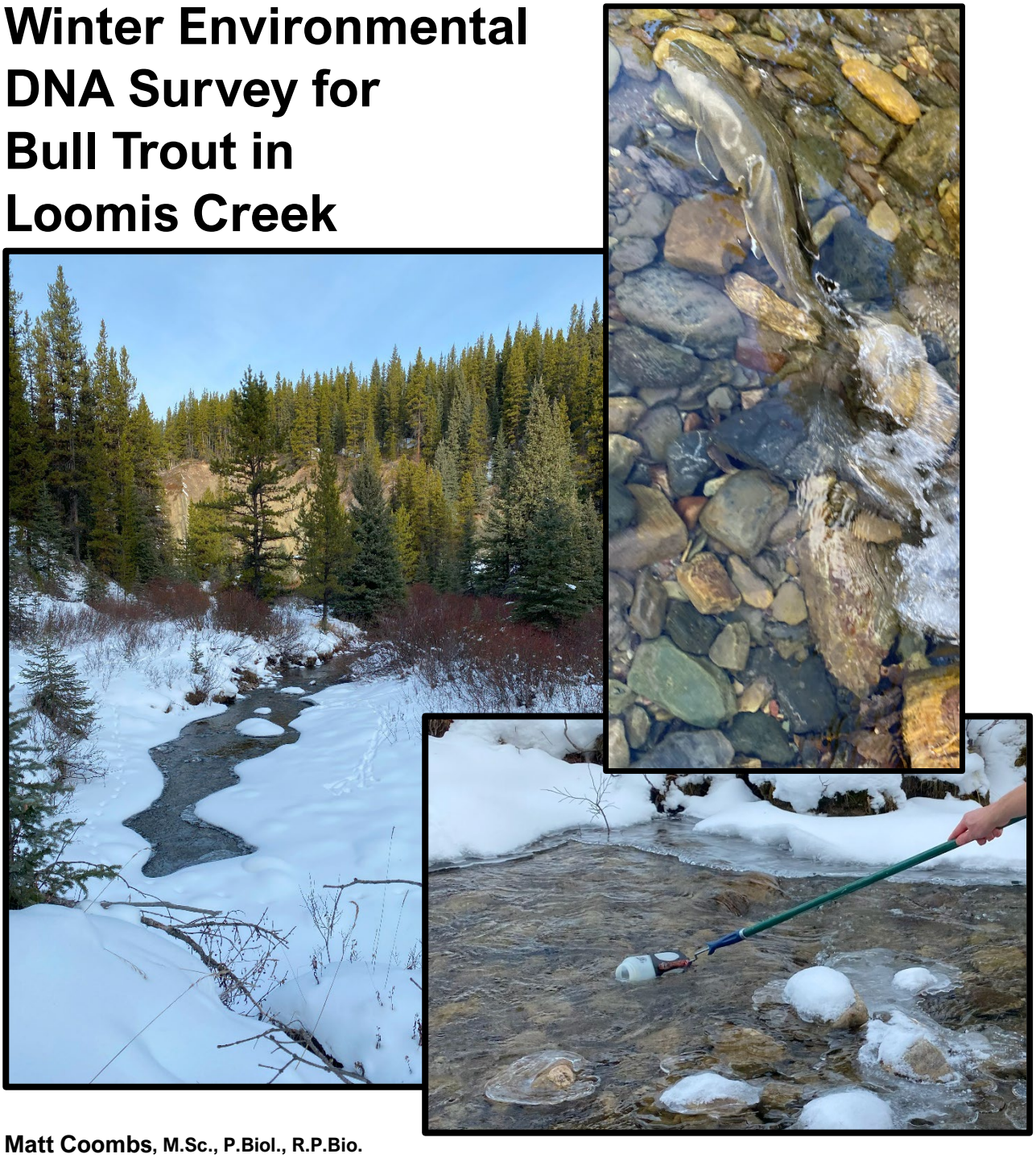
Winter Environmental DNA Survey for Bull Trout in Loomis Creek



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Introduction

The Canadian Parks and Wilderness Society (CPAWS) is dedicated to the protection of public land and freshwater. CPAWS southern Alberta chapter focuses on safeguarding, connecting, and expanding Alberta's parks and wilderness in the area.

In 2023 CPAWS learned of upcoming timber harvest by Spray Lake Sawmills Ltd. (SLS), now owned by West Fraser Mills Ltd. (West Fraser), within the Highwood River and Loomis Creek watersheds near Highwood Junction, Alberta. The area is part of the multi-use Kananaskis Country, adjacent to Don Getty Wildland Provincial Park. Referred to here as the Loomis Creek harvest plan, it involves constructing roads over watercourses that are legally designated as Critical Habitat for Bull Trout under the Species At Risk Act (SARA). Riparian areas designated as Critical Habitat will also be impacted, as well as groundwater recharge and seepage sites and other features, functions, and attributes that Bull Trout rely on for survival and reproduction.

In late November 2023 CPAWS retained Fintegrate Fisheries & Watershed Consulting Ltd. (Fintegrate) to conduct an environmental DNA (eDNA) survey to provide current and credible evidence assessing the likelihood that Bull Trout overwinter in Loomis Creek.

Environmental DNA Background

DNA (deoxyribonucleic acid) is the molecule that carries genetic information for the development and functioning of organisms. DNA is the code for the building blocks of all life, with unique, species-specific nucleotide chains held in a double stranded helix structure. DNA collected from the environment is referred to as eDNA.

All living things produce eDNA through cells, tissue, waste, or gametes being released into the environment. Animals are made up of trillions of cells, each with large amounts of DNA, resulting in eDNA being ubiquitous in the environment. This means the likelihood of detecting an organism like a fish in the environment by collecting a fragment of its DNA is higher than catching or seeing the fish itself.

Species-specific eDNA tests (assays) provide a non-invasive, efficient, and sensitive tool to identify habitat that certain species are using. In streams where the density of a target species is low, eDNA is transported downstream from an upstream source, allowing for more enhanced detection of the organism, compared to methods requiring capture or observation. Methods to sample and analyse eDNA are widely accepted to infer the occurrence of aquatic organisms near or upstream from a sampled site in lotic environments (Hobbs et al. 2019).

Cost savings using eDNA to identify trout habitat occupancy can be realized when it involves less sampling time and when equipment costs are lower than methods such as snorkelling or electrofishing surveys. Multiple and extensive sampling events with these observational or capture methods may be required to obtain as high a detection rate as can be reached in a single sampling event using eDNA methods. Harming sensitive species is also avoided using eDNA (i.e., no capture or habitat disturbance involved), and there is no need to obtain a Fisheries Research Licence from Alberta Environment and Protected Areas (AEPA) or a SARA permit from Fisheries and Oceans Canada (DFO), because there is no capture or handling of a listed species at risk (SAR).

Methods for collecting, filtering, preserving, and analysing eDNA are standardized in protocols such as the BC Ministry of Environment Environmental DNA protocol for freshwater aquatic ecosystems (Hobbs et al. 2017). The Canadian Standards Association has also published an eDNA reporting requirements and terminology standard (CSA 2021), and DFO has produced a guidance document on the use of eDNA (Abbott et al. 2021).

Project Background

CPAWS has been educating, engaging, and collaborating with Albertans and the provincial government and federal governments on the protection of East Slopes watersheds and native trout for many years. When it learned about the upcoming Loomis Creek harvest plan it started to raise public awareness regarding the Plan and engaged with Freshwater Research Ltd. (FWR) to identify numerous apparently unmitigable risks associated with direct and indirect impacts to Bull Trout and its Critical Habitat, both of which are protected in the area under SARA.

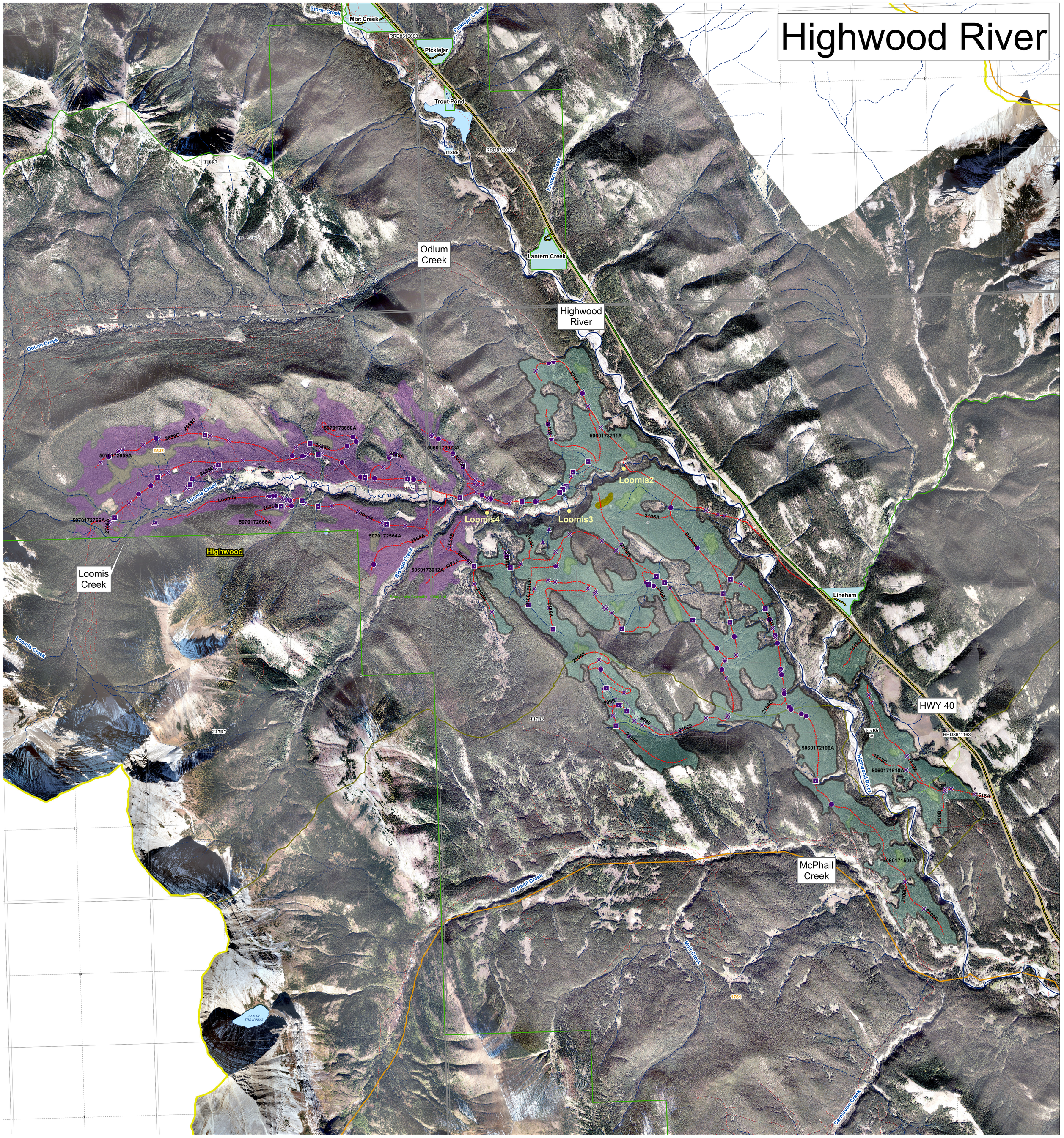
Relative to the larger Highwood River watershed, impacts of upcoming timber harvest are likely to be greatest in the Loomis Creek watershed due to the extensive clearcut logging and road footprint concentrated within this smaller watershed. Therefore, Fintegrate was asked to complete an eDNA assessment of Loomis Creek in November 2023 to provide current information on the presence of Bull Trout in the creek, suggesting a resident population that overwinters in the creek is present.

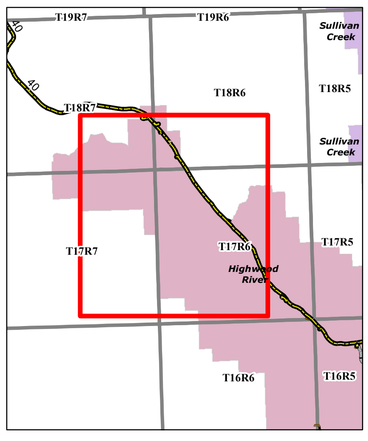
During fall 2023, Bull Trout spawning activity was observed by both Fintegrate and FWR in the Highwood River immediately downstream of the confluence of Loomis Creek and in the vicinity of a new road and bridge built over the Highwood River by SLS to access the harvest plan area. Bull Trout spawning activity in Loomis Creek itself was also previously documented in 2009 by Alberta Fish & Wildlife biologists (Eisler and Popowich 2010, Greg Eisler, *pers. comm.*), although the location of the redds has not been confirmed by Fintegrate. The gradient of Loomis Creek is steep near the Highwood River, where it descends through a narrow canyon, but the upper reaches are more suitable for spawning, rearing, and overwintering. However, whether spawning occurs in the upper reaches may not have been confirmed yet, and the redds observed in 2009 may have been directly at the confluence with the Highwood River within a short section of the stream in the floodplain that was lost after the 2013 flood (Dave Mayhood, *pers. comm.*).

An Annual Operating Plan (AOP) of the Loomis Creek harvest plan was provided to Fintegrate by CPAWS and shows timber harvest scheduled over two winters (2023-2024 and 2024-2025). Harvest in the first year is to occur along the Highwood River and in the lower portion of the Loomis Creek watershed, while in the second year it will occur in the headwaters of the Loomis Creek watershed (**Figure 1**).

With the above background information in hand, Fintegrate sampled eDNA at three sites along the creek on November 21, 2023. This brief report provides the location of these sites, reviews the sampling strategy, and interprets the results in the context of the existing understanding of eDNA production and transport from stream-dwelling salmonids.

Highwood River





FMA
- FMA

Transportation
- Transportation
- Road - Paved
- Road - Gravel
- Truck Trail
- Cutline/Trail
- Railway
- Other Trails
- Access Control

Hydrography
- Hydrography
- Ephemeral
- Intermittent
- Transitional
- Small Permanent
- Large Permanent

Points of Interest
- Cultural
- Grazing; Grazing
- Recreation
- Vegetation
- Water
- Wildlife

Cutblocks
- 2023
- 2024
- Retention

Roads
- Planned
- Laid Out
- Constructed

Planned Crossings
- Bridge
- Box Crib
- Half Pipe

Planned In Stream
- Cordoury
- Logfill
- Culvert
- X Drain

Active Crossings
- Bridge
- Box Crib
- Half Pipe

Active In Stream
- Culvert
- Cordoury
- Logfill
- X Drain

Stakeholder Landuse
- License of Occupation
- Mineral Surface Lease
- Pipeline Agreement
- Easement
- Forestry Road
- Misc. Permit
- Pipeline Installation Lease
- Parks Mineral Surface Lease
- Roadway
- Surface Material Lease
- REA Easement
- Right-of-Entry Agreement
- Country Road
- Surface Mineral Lease
- Vegetation Control Easement
- TP&R Easement
- TP&R License of Occupation
- Provincial Rec Area
- Road Corridor
- Ecological Reserve
- Heritage Rangeland
- Public Land Recreation Area
- Provincial Park
- PNT
- Wildland Provincial Park
- Provincial Sanctuary Corridor
- Natural Area
- Public Land Recreation Area
- National Parks
- IR 2020
- Grazing Allotments
- Grazing Leases
- Traplines
- Fence Lines - Internal
- SLS PSPs - Buffers
- Gov PSPs - Buffers

Designated Trails
- Shared Use Road
- Truck Trail (4x4)
- Quad
- Single Track
- Snow Vehicle Trails
- KC Trails

N W E S

1:15,000

0 0.275 0.55 1.1 1.65 2.2
Kilometers

Map Title: Highwood River 2023, 2024
Created By: Spray Lake Sawmills (1980) Ltd
Date Created: April 10, 2023
Author: Allen Mottet, RPF

Figure 1. Annual Operating Plan Map of the Loomis Creek harvest area showing eDNA sampling sites (Loomis, 4, Loomis3, Loomis2)

Local Bull Trout Population

Bull Trout require cold, clear flowing water to survive and both resident and fluvial life histories exist in the Highwood River watershed. Bull Trout spawning occurs during low flow periods in the fall, starting in late August or early September and continuing until mid-October. Egg incubation occurs over the winter and fry emerge early the following spring. Clean gravel that is free of sediment is also important for Bull Trout spawning. Stream-resident forms require smaller substrate size, while larger fluvial and adfluvial Bull Trout can spawn in larger substrate.

In the Highwood River watershed, risk factors facing Bull Trout include hybridization with, and displacement by, non-native Brook Trout (*Salvelinus fontinalis*) and changes in water quantity and quality and habitat degradation related to forestry, agriculture, irrigation, and climate change. Fluvial Bull Trout range over larger areas than Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), and the Highwood River supports a migratory fluvial Bull Trout population. Bull Trout can use different spawning tributaries from one year to the next, and individuals may not spawn consecutively year after year. As a result, trends in adult spawner abundance (often assessed by conducting redd counts), as well as juvenile abundance (often assessed by snorkel or electrofishing surveys) can be variable from one year to the next.

Little is known about the status of the Highwood River and Loomis Creek Bull Trout population. The most recent survey of the Highwood River was a fall snorkel survey in 2009, where most Bull Trout were observed in the headwaters of the river (Eisler and Popowich 2010). Migratory movements along the Highwood River have been confirmed through radio telemetry (Popowich and Paul 2006), but efforts to conduct mark-recapture population estimates have had limited success (Buchwald and Willis 2004). A 2018 subwatershed assessment in the Pekisko Creek drainage found very low Bull Trout abundance (Hurkett et al. 2018). The Fisheries and Wildlife Management Information System (FWMIS), accessed through the Fish and Wildlife Internet Mapping Tool (FWIMT), shows two electrofishing records of Bull Trout capture in Loomis Creek. One record from 1989 is upstream of Bishop Creek (three individuals captured) and one record from 2008 is further downstream (two individuals captured, FWIMT 2023).

Sample Site Selection

Given that eDNA is transported downstream, a series of three sites were sampled to evaluate how far up Loomis Creek Bull Trout are distributed from the Highwood River (**Figure 1**). Site selection in the field optimized detection probability by selecting microsites most appropriate for Bull Trout rearing and overwintering by considering gradient, flow, substrate type, and depth.

The site closest to the river (Loomis2, UTM 11U 656337E 5592961N) was approximately 1.5 km west of the new bridge and road recently constructed over the Highwood River and approximately 150 m upstream from where bridge construction over Loomis Creek is planned to occur. It was selected due to ease of access and an opening in the ice cover, proximity to the Highwood River (approximately 1 km upstream from the creek mouth), and a trout (unidentified species) and small pool created by a bedrock outcrop with suitable overwintering habitat observed nearby during an earlier survey on September 13, 2013. See site photos in **Appendix A**.

The site furthest upstream on Loomis Creek (Loomis4, 11U 654887E 5592565N) was approximately 3.2 km upstream from the Highwood River and 520 m downstream from the confluence of Bishop Creek. The site was selected due to ease of access from the hiking trail, lower gradient habitat with scour pools deep enough to provide suitable overwintering habitat, and the presence of flowing and ice-free reaches of stream. Numerous juvenile trout (unidentified species) were also observed in the area during an earlier survey on September 13, 2013. See site photos in **Appendix A**.

Further upstream near the confluence of Bishop Creek at 11U 654627E 5592591N, Loomis Creek was completely ice covered, and due to Project constraints and safety concerns, sampling at this location was not conducted. See **Appendix A** for a photo of Loomis Creek at this location.

The third site on Loomis Creek (Loomis3, 11U 655745E 5592594N) where eDNA was sampled was midway between the upper and lower sites and approximately 2 km upstream from the mouth of the creek at the Highwood River. The site was selected due to ease of access from the hiking trail, being located near the transition from the steeper gradient reach of the creek within a canyon and the shallower gradient upper reach, and the presence of deeper, flowing, and ice-free habitat with a large, deep pool suitable for overwintering approximately 500 m upstream. See site photos in **Appendix A**.

Sample Collection

Nalgene™ Wide-Mouth Lab Quality high density polyethylene one litre sample bottles were pre-cleaned with a 1:1 ratio of 6% bleach (weight by volume) and tap water. Bottles were then thoroughly rinsed with tap water and allowed to air dry.

Sampling eDNA followed the “grab and go” method outlined in Hobbs et al. (2017) where sample bottles are filled with site water standing on shore using an extendable pole, immediately placed in a cooler on ice, and filtered hours later. Avoiding streamside filtering reduces the necessary sampling gear required when collecting samples and allows sites to be accessed more quickly and easily.

At each sample site, sample bottles were labeled as field replicates A, B, and C together with the site code, date and time of collection, and geographic coordinates of the site. Each sample bottle was then rinsed again a minimum of three times with site water before being filled with site water. There was stream flow at all sites sampled, and rinsing occurred downstream of where each sample was collected. Samples were collected sequentially in an upstream direction. All three samples collected at each site were taken from within an area of approximately 10 m^2. The target volume of water for each field replicate was 2000 mL, which was collected in two 1 L bottles for each (i.e., bottle A1 & A2, B1 & B2, C1 & C2).

Samples were collected first at Loomis4, then Loomis3, and finally Loomis2. Sampling occurred over approximately 1 hour between 1000h and 1100h on November 21, 2023, and filtration was completed by 1400h the same day.

Sample Filtration

Samples were filtered in the order in which they were collected to minimize the time for eDNA to degrade before preservation.

Filtering occurred on a sterile workbench surface with a source of power using a vacuum pump and 2L vacuum flasks. Filters were 47 mm diameter cellulose nitrate (0.45 µm pore size; Thermo Fisher Scientific Inc., Ottawa, ON, Canada; Cat# N1452045).

Filters with eDNA were preserved by desiccation in coin envelopes placed in sealed Ziploc® bags with self-indicating silica desiccant (Dry & Dry Premium Orange Indicating Silica Gel Desiccant Beads, Brea, California, USA). Filters were vacuumed dry, folded in quarters, placed in coin envelopes, and then placed in Ziploc® bags with the silica desiccant, and shipped to Bureau Veritas for analysis on November 21, 2023.

One sample bottle was filled with distilled water as a negative control field blank that went through all the steps of filtration, preservation, and laboratory extraction and analysis.

Environmental DNA assay validation and use

The targeted, species-specific eDNA assay applied to test for the presence of genetic material from Bull Trout is referred to as eSACO3 by Bureau Veritas and Dr. Caren Helbing's Lab at the University of Victoria. It was originally developed by the US Forest Service for a range-wide assessment of Bull Trout distribution in the pacific northwest of the US (Dysthe et al. 2018). Assay reaction conditions for eSACO3 were modified by Dr. Caren Helbing's Lab to improve assay efficiency and selectivity, and these adjustments were adopted by Bureau Veritas.

The Bull Trout assay targets a 172 base pair region of the ITS1 ribosomal RNA gene within the nuclear genome. The assay has been previously validated *in vitro* with 15 tissue samples (fin clips) from Hidden Creek, in the upper Oldman River watershed, and *in situ* with eDNA samples collected at sites known to be occupied by Bull Trout throughout the Bow and Oldman river watersheds (Fintegrate 2022), including on the Highwood River at the point of diversion for the Women's Coulee Diversion Canal and in the headwaters of a nearby tributary, Pekisko Creek.

Quality Assurance and Control

Before analysis for Bull Trout eDNA occurred, all samples were tested with the IntegritE-DNA™ assay to confirm the integrity of DNA and rule out DNA degradation or PCR inhibition, which can produce false negative results indicating the target species is not present when in fact it is (Hobbs et al. 2019). False negatives can result from improper sampling handling or PCR inhibitors. The IntegritE-DNA™ assay is a primer/probe combination that amplifies chloroplast DNA from all algae species. Algal DNA is ubiquitous in all surface water, providing a means to evaluate the integrity of eDNA in any sample of water. The IntegritE-DNA™ assay used by Bureau Veritas was developed by Dr. Caren Helbing at the University of Victoria. If positive detection of algal DNA does not occur, this indicates that either DNA degradation has occurred during sample collection, processing, or storage or that PCR inhibition is occurring. The IntegirtE-DNA™ test was run as thirty cycles of qPCR performed on each of four laboratory technical replicates of the isolated DNA from the field blank and all environmental samples.

If samples did not pass the initial IntegirtE-DNA™ test, the isolated DNA was cleaned with a Zymo OneStep™ PCR Inhibitor Removal Kit (Cedarlane, Burlington, ON, Canada; Cat# D6030S) and retested. If algal DNA detection still did not occur, it was concluded that either DNA in the sample was degraded or that the concentration of inhibitors was too great to allow for effective removal. No further analysis occurred.

Environmental DNA analysis

Both the eSACO3 and IntegritE-DNA™ assay use a TaqMan polymerase probe and the IMMOLASE™ DNA polymerase enzyme. During development, multiple primer candidates were assessed (Helbing and Veldhoen 2017), and assay components were designed and selected to satisfy good targeted qPCR eDNA assay design criteria, including appropriate melting temperatures, free energies, lack of sequence runs or self/cross priming potential, etc. (Langlois et al. 2021). The most recent versions of the Helbing Laboratory technical bulletins available for the assays applied are attached (**Appendix B**), providing sensitivity and specificity test results.

Samples were analyzed in the order in which they were collected starting with the furthest upstream site (Loomis4) to minimize analysis costs. The lab was instructed to discontinue analysis of all other samples from additional sites further downstream once Bull Trout eDNA was detected at a site. Given that adult fish and spawning activity was observed near the mouth of Loomis Creek, eDNA detection at a point on Loomis Creek was assumed to infer a continuous distribution of Bull Trout upstream from the river to that point.

Samples were analyzed at the Bureau Veritas DNA Services laboratory (Bureau Veritas) in Guelph, Ontario. DNA extraction used one quarter of each filter, with the remaining samples kept in -20°C storage for 90 days before disposal. DNA extraction occurred using the DNeasy Blood and Tissue kit (Qiagen Inc. Mississauga, ON, Canada; Cat# 69506) following the methods outlined in Matthias et al. (2021) and Hobbs et al. (2019). DNA sample eluate from the spin column (150 µL in AE elution buffer) were stored at -20°C prior to analysis.

All DNA isolations and eDNA assay set-up was conducted in a laminar flow hood with a high efficiency particulate air filter. To eliminate the chances of a contamination event occurring, the workspace was cleaned with a 10% bleach solution prior to use and dedicated pipettes with filter tips were used.

DNA was isolated from each field replicate sample (3 per site; A, B, C). All sample locations were randomized on each analysis plate after being assigned sample processing numbers to reduce processing bias. Eight no-template controls (NTCs) and two positive controls were run per plate, with the NTCs positioned on the plate to spatially separate the positive controls from the samples (Hobbs et al. 2019). This was to ensure that if any cross contamination occurred it would be detected in the blank NTC wells. Positive controls consisted of synthetic double stranded DNA fragments corresponding to the eSACO3 amplicon at a concentration of 20 copies per reaction.

For all analyses, quantitative polymerase chain reaction (qPCR) was applied as the analysis technique. After all field samples were confirmed to contain viable DNA using the IntegirtE-DNA™ test, the test for Bull Trout eDNA was applied to eight technical replicates of the isolated DNA from each sample. The qPCR process followed is outlined in Veldhoen et al. (2016). For each replicate analysed, if amplification was detected within 50 cycles, the reaction was scored as a positive detection of the target sequence.

All primers and the TaqMan probe containing a 5'FAM reporter dye and 3'ZEN/Iowa Black FQ quencher were ordered from Integrated DNA Technologies (IDT; Coralville, IA, USA).

Interpreting environmental DNA results

Before eDNA analysis began, it was assumed that Bull Trout DNA was detected at a sample site if the target sequence was detected with the eSACO3 assay in at least one of the three field replicates (A, B, C) in at least one of eight laboratory replicates. It was also assumed that there was no contamination of the samples with Bull Trout eDNA from another site, and that no Bull Trout eDNA would be detected in the NTCs. Analysis of the NTCs supported this assumption.

Results

Bull Trout eDNA was detected in all eight laboratory replicates for each of the three field replicates at the first site sampled and analysed (Loomis4), which was the furthest upstream site (**Table 1**). Therefore, samples collected from the other two sites further downstream (Loomis3 and Loomis2) were not analysed, because it was assumed that Bull Trout were distributed at least as far upstream on Loomis Creek as Loomis4, near the confluence of Bishop Creek.

Results from applying the IntegritE-DNA™ assay before the Bull Trout assay was applied showed good DNA integrity, with amplifiable DNA detected in all four laboratory replicates in each of the three field replicates from Loomis4.

The time required to filter the samples and the sample volume filtered showed consistency across the three field replicates (**Table 1**). The estimated mean number of the target Bull Trout DNA sequence also showed consistency between the field replicates (**Table 1**).

Table 1. Results of eDNA analysis from Loomis4 near Bishop Creek, showing target sequence detection frequencies for the IntegritE-DNA™ and Bull Trout assays

Field replicate	Filtering time required (min:sec)	Sample volume filtered (ml)	Amplifiable DNA frequency (4 laboratory replicates)	Sample Clean-Up Required	eSACO3 Frequency (8 lab replicates)	Estimated mean target gene copy no. / L	Estimated mean target gene copy no. / L (SE)
A	9:58	1940	4/4	No	8/8	20148.3	624.2
B	9:42	1920	4/4	No	8/8	22647.1	938.6
C	7:46	1980	4/4	No	8/8	25665.2	730.9

Discussion and Interpretation of Results

Based on the above results, Bull Trout eDNA was strongly detected in all three field replicates in Loomis Creek near Bishop Creek in late November when much of the creek was covered in ice. Considering two previous electrofishing captures as well as records of spawning activity, Loomis Creek is likely used by Bull Trout throughout the year, supporting all life stages of the species.

As reviewed by Ostberg and Chase (2022), it is uncertain whether fertilized fish eggs shed eDNA. While some studies have been unable to detect target eDNA shed from eggs, there may be inter- and intraspecific differences as well as environmental factors that could influence this. The chorion membrane surrounding eggs is only permeable to low molecular weight molecules, which may also limit eDNA being shed from eggs into the aquatic environment.

The persistence of eDNA in lotic environments is short lived due to rapid downstream transport and degradation. This is the typical pattern observed in studies that track salmonid eDNA concentrations in lotic environments over time (e.g., Tillotson et al., 2018, Wood et al. 2021). Therefore, the strong detection of Bull Trout eDNA in Loomis Creek on November 21, 2023, was not the result of previous occupancy in the spring, summer, or fall.

Based on comparisons to traditional sampling methods (Piggott et al. 2021, Sard et al. 2019, Evans et al. 2017, Wilcox et al. 2016, Baldigo et al. 2016), it is assumed that eDNA sampling resulted in a higher detection probability than electrofishing. Until this Project, only electrofishing had been used to successfully detect the presence of Bull Trout in Loomis Creek.

The maximum distance over which eDNA is transported and can be detected from an upstream source depends on eDNA dilution, degradation, and deposition rates (Spence et al. 2021). Maximum or median detection distances for fish in lotic environments are typically reported to be on the order of hundreds of meters (Jane et al. 2015, Wilcox et al. 2016, Fremier et al. 2019, Robinson et al. 2019), although there are examples of detections 1-5 km downstream from a source (Schumer et al. 2019, Wood et al. 2021, Laporte et al. 2020).

Wilcox et al. (2016) estimated that the probabilities of detecting Brook Trout at low densities (1 fish per 100 m) using eDNA and electrofishing were 93% versus 45%, respectively. Strickland and Roberts (2019) estimated the detection probability for Roanoke Logperch (*Percina rex*) to be at least 89% and approaching 100% at many sites using eDNA. Piggott et al. (2021) produced a 100% detection probability for Rainbow Trout (*Oncorhynchus mykiss*) at occupied sites using eDNA compared to 80% with electrofishing.

These examples of high detection probabilities in stream environments support CPAWS using eDNA for this Project. The upstream limit of the species distribution is likely considerably further upstream in both Bishop and Loomis creeks. However, as the density of Bull Trout decreases, false negative results are possible, and the eDNA assays may not detect Bull Trout at low densities or at sites where individuals are large distances upstream from the sampling point.

Considering the target density of Bull Trout that would be required for detection under specific stream flows would be necessary to determine the optimal density of uniformly distributed sampling sites along Loomis Creek that would maximize detection rates and more accurately determine the upstream limits of the species distribution (Wood et al. 2020, Spence et al. 2021). However, the current Project was only an initial assessment of the utility of eDNA to assess the distribution of the species. Given low Bull Trout densities, additional resources and time would be necessary to design a study to evaluate the upstream limits with greater precision.

Cost savings using eDNA can be realized when it involves less sampling time and when equipment costs are lower than other conventional sampling methods. This is often the case for species that occur in low densities like Bull Trout in Loomis Creek. In small streams like this, more electrofishing effort may be required to obtain as high a detection rate as can be reached with less effort using eDNA methods.

Literature Cited

Abbott, C., Coulson, M., Gagné, N., Lacoursière-Roussel, A., Parent, G.J., Bajno, R., Dietrich, C., and May-McNally, S. 2021. Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/019. iv + 42 p. Available at: https://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2021/2021_019-eng.html

Baldigo, B.P., Sporn, L.A., George, S.D., and Ball, J.A. (2016). 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), 99-111.

Buchwald, V.G. and J.M. Willis. (2004). Sport Fish Population Studies in the Highwood River Watershed 1995-1996. Prepared by Sustainable Resource Development, Fish and Wildlife Division, Red Deer, Alberta.

Canadian Standards Association (CSA) (2021). National Standard of Canada CSA W214:21 Environmental DNA (eDNA) reporting requirements and terminology. ISBN 978-1-4883-3794-9.

Dysthe, J. C., Franklin, T. W., McKelvey, K. S., Young, M. K., & Schwartz, M. K. (2018). An improved environmental DNA assay for bull trout (*Salvelinus confluentus*) based on the ribosomal internal transcribed spacer I. PloS one, 13(11).

Eisler, G.R. and R. Popowich. (2010). Mountain Whitefish spawning assessment and fluvial Bull Trout redd survey in the Highwood River, 2009. Prepared for Fish and Wildlife Division, Alberta Sustainable Resource Development: 36 p. + 3 app.

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

Fintegrate Fisheries & Watershed Consulting Ltd. (Fintegrate). (2022). 2021-2022 Blackfoot Confederacy Tribal Council Native Trout Recovery Project. Blackfoot shared leadership and capacity support in native trout recovery addressing climate change, non-native species, and habitat loss. Aboriginal Fund for Species At Risk (AFSAR) Annual Report. Contribution Agreement Number: 2021AFSAR5014.

Fish and Wildlife Internet Mapping Tool (FWIMT). (2023). Fisheries and Wildlife Management Information System (FWMIS) accessed November 2023

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

Helbing, C.C. and Veldhoen, N.C. (2017). Canadian Patent Application CA# 2694374: Quantitative PCR-based Environmental DNA Assays. Available at: https://patents.justia.com/assignee/uvic-industry-partnerships-inc

Hobbs J., Round J.M., Allison M.J., and Helbing, C.C. (2019). Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLoS ONE 14(3): e0213849.

Hobbs, J., Goldberg, C., Helbing, C.C., Veldhoen, N. and E. Vincer. (2017). BC Ministry of Environment: Environmental DNA protocol for freshwater aquatic ecosystems Version 2.2 (File: 489-027.01; revised November 2017), 48 pp.

Hurkett, B., J. Blackburn, and L. Redman. (2018). Abundance, distribution, spawning, and thermal habitat of Westslope cutthroat trout and bull trout in the Stimson Creek watershed. Data Report, produced by Alberta Conservation Association, Sherwood Park, Alberta, Canada. 19 pp + App. https://www.ab-conservation.com/downloads/report_series/Stimson_Creek_Watershed.pdf

Jane, S.F., Wilcox, T.M., McKelvey, K.S., Young, M.K., Schwartz, M.K., Lowe, W.H., Letcher, B.H. and Whiteley, A.R. (2015). Distance, flow and PCR inhibition: e DNA dynamics in two headwater streams. Molecular ecology resources, 15(1), 216-227.

Langlois, V.S., Allison, M.J., Bergman, L.C., To, T.A., and Helbing, C.C. (2021). The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. Environmental DNA, 3(3), 519-527.

Laporte, M., Bougas, B., Côté, G., Champoux, O., Paradis, Y., Morin, J., and Bernatchez, L. (2020). Caged fish experiment and hydrodynamic bidimensional modeling highlight the importance to consider 2D dispersion in fluvial environmental DNA studies. Environmental DNA, 2(3), 362-372.

Matthias, L., Allison, M. J., Maslovat, C. Y., Hobbs, J., & Helbing, C. C. (2021). Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. Ecological Indicators, 131, 108187.

Ostberg, C.O., and Chase, D.M. (2022). Ontogeny of eDNA shedding during early development in Chinook Salmon (*Oncorhynchus tshawytscha*). Environmental DNA, 4(2), 339-348.

Piggott, M.P., Banks, S.C., Broadhurst, B.T., Fulton, C.J., and Lintermans, M. (2021). Comparison of traditional and environmental DNA survey methods for detecting rare and abundant freshwater fish. Aquatic Conservation: Marine and Freshwater Ecosystems, 31(1), 173-184.

Popowich, R. and A.J. Paul. (2006). Seasonal Movement Patterns and Habitat Selection of Bull Trout (*Salvelinus confluentus*) in Fluvial Environments. University of Alberta, Edmonton, Alberta. Prepared for Fisheries and Oceans Canada, Calgary, Alberta.

Robinson, A.T., Paroz, Y.M., Clement, M.J., Franklin, T.W., Dysthe, J.C., Young, M.K., McKelvey, K.S., and Carim, K.J. (2019). Environmental DNA sampling of small-bodied minnows: Performance relative to location, species, and traditional sampling. North American Journal of Fisheries Management, 39(5), 1073-1085.

Sard, N.M., Herbst, S.J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J.D., and Scribner, K.T. (2019). Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. Environmental DNA, 1(4), 368-384.

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

Spence, B.C., Rundio, D.E., Demetras, N.J., and Sedoryk, M. (2021). Efficacy of environmental DNA sampling to detect the occurrence of endangered coho salmon (*Oncorhynchus kisutch*) in Mediterranean-climate streams of California's central coast. Environmental DNA, 3(4), 727-744.

Strickland, G.J., and Roberts, J.H. (2019). Utility of eDNA and occupancy models for monitoring an endangered fish across diverse riverine habitats. Hydrobiologia, 826(1), 129-144.

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

Veldhoen, N., Hobbs, J., Ikonomou, G., Hii, M., Lesperance, M., & Helbing, C. C. (2016). Implementation of novel design features for qPCR-based eDNA assessment. PLoS One, 11(11), e0164907.

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

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M.T., Garry McBrine, C., Kinnison, M.T., McBrine, C.G., Pavey, S.A., and Gagné, N. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened salmon presence, abundance, and location. Frontiers in Ecology and Evolution, 9, 650717.

Appendix A – Project Photos

Plate A1: Loomis4, upstream view, November 21, 2023

Plate A2: Loomis4, downstream view, November 21, 2023

Plate A3: Loomis Creek near Bishop Creek, November 21, 2023

Plate A4: Loomis3, upstream view, November 21, 2023

Plate A5: Loomis3, downstream view, November 21, 2023

Plate A6: Deep pool 500 m upstream of Looms3, November 21, 2023

Plate A7: Loomis2, upstream view, November 21, 2023

Plate A8: Loomis2, downstream view, November 21, 2023

Plate A9: eDNA filtering, November 21, 2023

Appendix B – eDNA Assay Technical Bulletins

Helbing Lab
eDNA Inventory

Helbing Laboratory eDNA Technical Bulletin

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity[1-3].

General eDNA Assay Information

Target Species: Chloroplasts (Plant/Algae) | eDNA qPCR Tool: ePlant5 | Gene Target: 23S
Species Code: IntE-DNA | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD N/A 95% CI N/A Copies | LOQ N/A 95% CI N/A Copies | LOB N/A hits/8
Binomial-Poisson model for 8 technical replicates
Determined using eLowQuant R code[4]. | When the LOQ < LOD, use the LOD for the LOQ. | Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 3: 970-981. doi: 10.1002/edn3.220

Helbing Lab
eDNA Inventory

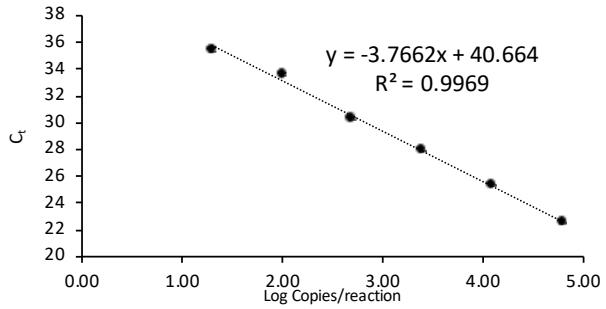
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

From N/A Technical Replicates

# Detects	# Copies	SE
0	N/A	N/A
1	N/A	N/A
2	N/A	N/A
3	N/A	N/A
4	N/A	N/A
5	N/A	N/A
6	N/A	N/A
7	N/A	N/A

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits



Binomial-Poisson model: N/A

Determined using eLowQuant R code[4].

Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency 84%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	23S	23S ribosomal RNA
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

Helbing Lab
eDNA Inventory

Helbing Laboratory eDNA Technical Bulletin

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity [1-3].

General eDNA Assay Information

Target Species: Bull Trout (*Salvelinus confluentus*) | eDNA qPCR Tool: eSACO3 | Gene Target: NC-ITS1
Species Code: te-SACO | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 | 95% CI 0.3-1.1 Copies/Rxn | LOQ 2 | 95% CI 1.3-4.2 Copies/Rxn | LOB 0 hits/8
Binomial-Poisson model for 8 technical replicates
Determined using eLowQuant R code [4].
Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-COCO	Slimy Sculpin (*Cottus cognatus)*	No	1	Yukon
te-ESLU	Northern Pike (*Esox lucius*)	No*	1	British Columbia
te-MIDO	Smallmouth Bass *(Micropterus dolomieu)*	No	1	British Columbia
te-MISA	Largemouth Bass *(Micropterus salmoides)*	No	1	British Columbia
te-ONCL	Cutthroat Trout *(Oncorhynchus clarkii)*	No	1	British Columbia
te-ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*	No	1	British Columbia
te-ONKE	Chum Salmon *(Oncorhynchus keta)*	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch)*	No	1	British Columbia
te-ONMY	Rainbow Trout *(Oncorhynchus mykiss)*	No	1	Alberta and British Columbia
te-ONNE	Sockeye Salmon *(Oncorhynchus nerka)*	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PRCY	Round Whitefish (P*rosopium cylindraceum)*	No	1	Yukon
te-SACO	Bull Trout (*Salvelinus confluentus*)	Yes	1	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma)*	No	1	British Columbia
te-SASA	Atlantic Salmon (*Salmo Salar)*	No	1	Nova Scotia
te-THAR	Arctic Grayling *(Thymallus arcticus)*	No	1	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus)*	No	1	British Columbia

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. https://doi.org/10.1002/edn3.82
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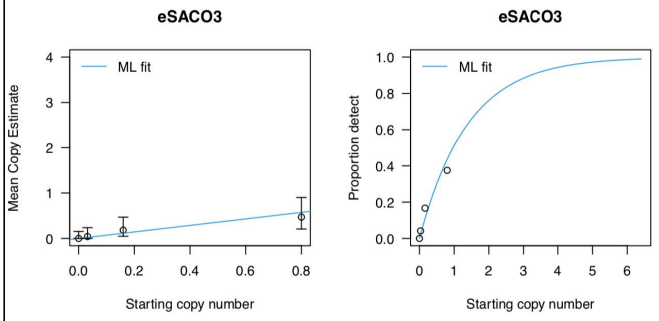
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 3: 970-981. doi: 10.1002/edn3.220

Helbing Lab
eDNA Inventory

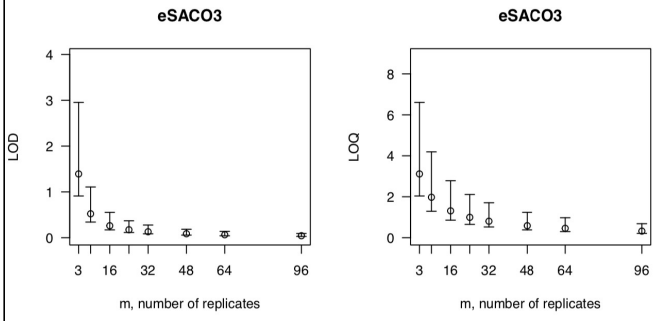
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



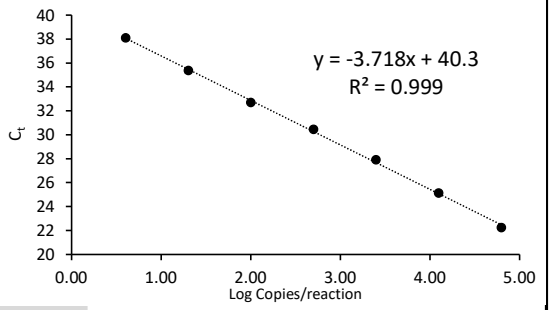
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.19	0.19
2	0.4	0.3
3	0.66	0.42
4	0.97	0.56
5	1.37	0.74
6	1.93	1
7	2.9	1.52

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



Efficiency 86%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	NC-ITS1	Nuclear internal transcribed spacer 1 gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error