2020-2021 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

Contribution Agreement Number: 2020AFSAR6010-01 (Year 1 of 3)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

List of Acronyms and Abbreviations

Acronym / Abbreviation	Definition
AFSAR	Alberta Environment and Parks
BFC	Blackfoot Confederacy Tribal Council
DFO	Fisheries and Oceans Canada
SARA	*Species at Risk Act*
SAR	Species at risk
WLNP	Waterton Lakes National Park

1 Project Overview

The Blackfoot Confederacy Tribal Council (BFC) is a not-for-profit regional managing organization that officially opened in July 2018 to serve the following Nations: Amskapi Piikani, Kainai, Piikani and Siksika. The Blackfoot Confederacy Tribal Council and member Nations developed a Blackfoot Confederacy Environment and Land Framework which facilitates several action-based working groups to assist in the coordination, communication and integration of land and natural resource issues both on and off-reserve. Aquatic species at risk (SAR) has been identified as a priority under the Blackfoot Confederacy Aohkii (Water) working group. Native trout are significant, aquatic biocultural artifacts that the Blackfoot seek to conserve and restore. The BFC native trout restoration project (the Project), which is titled "Blackfoot shared leadership and capacity support in native trout recovery addressing climate change, non-native species, and habitat loss", is an outcome of this working group.

1.1 Purpose of Project

The purposes of the Project are to:

1. Build Blackfoot capacity and expertise as guardians of East Slope watershed integrity through training and mentorship in assessing, managing, and restoring instream and riparian native trout habitat.
2. Gather Blackfoot values and holistic ways of knowing about the study area, water, and native trout and bring this together with scientifically derived watershed values in a respectful manner that increases Blackfoot engagement in native trout conservation and restoration activities.

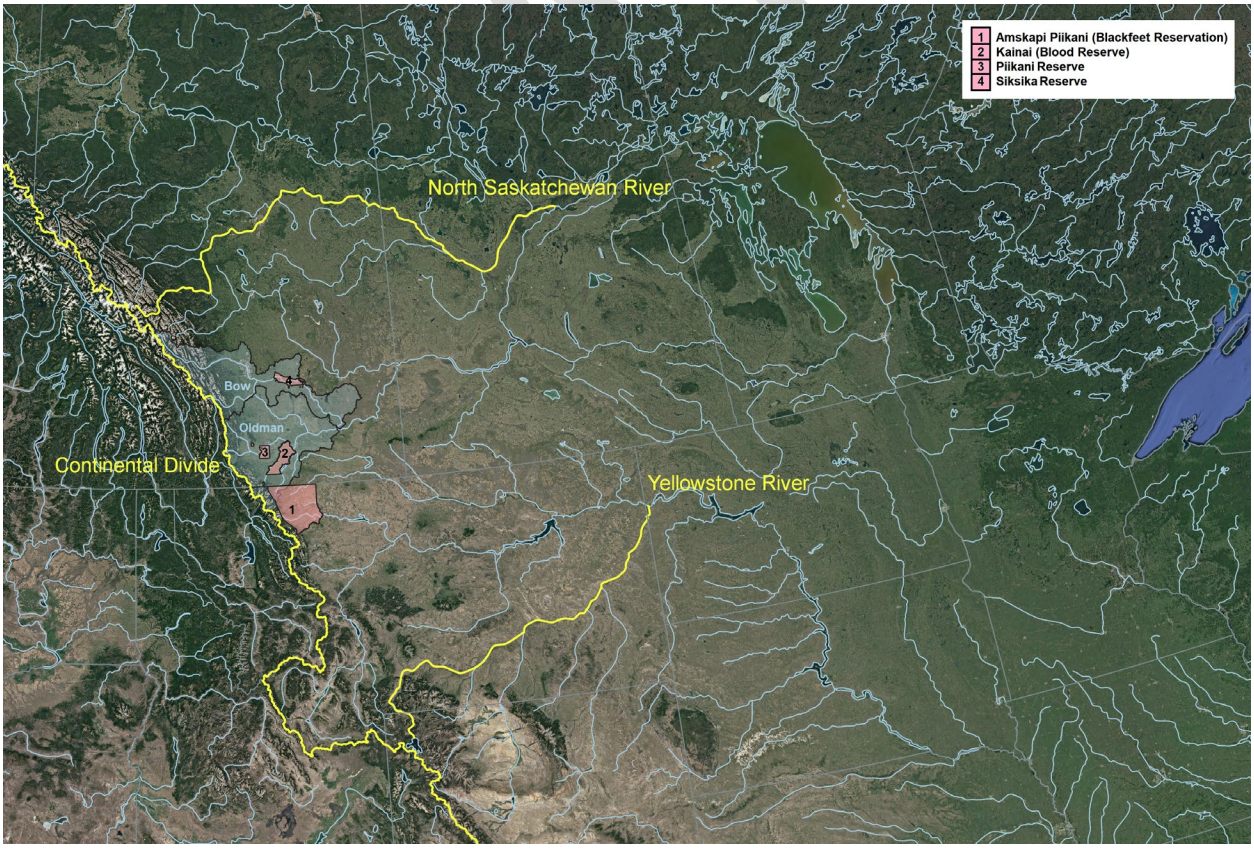

Figure 1.1 Project study area within the Blackfoot Territory

1.2 Focal Species

In the headwaters of the South Saskatchewan River only three native fish species originally occupied the uppermost drainages: Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), Bull Trout (*Salvelinus fontinalis*), and Mountain Whitefish (*Prosopium williamsoni*). The two trout species are the focus of the Project.

Bull Trout are one of the most widely distributed native salmonids in western North America, with the species' native range extending from northern Nevada and California to southern Yukon and anadromous populations extending along the Pacific coast from Oregon to the southern eastern coast of Alaska. In contrast, while Westslope Cutthroat Trout have the broadest range of any of the 14 cutthroat trout species, the species' range is much more restricted than that of Bull Trout, extending from Idaho and northern parts of Yellowstone National Park just as far north as southeast British Columbia and southwestern Alberta.

1.3 Study Area

The South Saskatchewan River watershed (specifically the Bow and Oldman subwatersheds) is the study area of the Project. The Oldman watershed extends into Montana and includes part of the Amskapi Piikani (Blackfeet) Reservation. The study area is unique in that it represents the southern-most extent of the native range of Bull Trout on the East Slopes of the Rockies and also supports the only native Westslope Cutthroat Trout populations east of the continental divide. The overlapping distributions of Bull Trout and Westslope Cutthroat Trout in the study area near the outward extent of their ranges highlights the functional conservation value of the study area in terms of the fish habitat and water quality it provides.

In the South Saskatchewan River watershed, Bull Trout and Westslope Cutthroat Trout are both provincially and federally listed as Threatened species. In 2007 Westslope Cutthroat Trout were designated as a Threatened species under Alberta's *Wildlife Act* following a recommendation by Alberta's Endangered Species Conservation Committee (ESCC) and its Scientific Subcommittee (SSC). Similarly, in 2014 Bull Trout were designated as a Threatened species under Alberta's Wildlife Act following recommendation by Alberta's ESCC. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) designated Westslope Cutthroat Trout in Alberta as Threatened in 2005, with the species listed as such under the *Species at Risk Act* (SARA) in 2013. Similarly, COSEWIC designated Bull Trout as Threatened in 2012, with the species listed as such under SARA in 2019. Under SARA, the Threatened designation is a "…species that is likely to become an endangered species if nothing is done to reverse the factors leading to its extirpation or extinction."

As required under SARA, critical habitat for Bull Trout and Westslope Cutthroat Trout has been identified for these species. Instream habitat for both species is protected legally by critical habitat orders and is broadly designated throughout the study area. Critical habitat for Bull Trout covers large portions of the study area, while critical habitat for Westslope Cutthroat Trout is less broadly distributed than that for Bull Trout and is restricted to stream segments where Westslope Cutthroat Trout were determined to be genetically pure and reaches upstream from these reaches as well as some areas with near genetic purity. Riparian critical habitat has also been designated for both species at a width of 30 m on all designated reaches of stream.

1.4 Significance of Study Area to Blackfoot People

The South Saskatchewan River flows through the middle of Blackfoot Territory. The East Slopes of the Rocky Mountains are traditionally, and to this day, Blackfoot Territory. Water is an integral part of Blackfoot culture - memorialized with ceremony, songs, and artifacts passed down through the generations. Blackfoot People believe everything is connected and has a spirit, and that the Rocky Mountains were created from water as the "Backbone of the World." The health and well-being of the East Slopes is the health and well-being of the people, their culture, and all other living entities in the region.

1.5 Project Team

The Project is managed by Kimmy Houle, BFC Land and Environment Director. Kimmy is a member of the Kainai Nation (Blood Tribe) and resides in Piikani Nation. Kimmy coordinates with the individual Blackfoot Nations to identify staff and Elders that are able to participate in the Project. She also acts as a liaison between the Project, the CEO of the BFC, and individual Blackfoot Nations. Kimmy administers the funding received from AFSAR together with the BFC finance department, including managing the project budget, paying invoices for professional services, purchasing equipment, reimbursing Nation staff for travel costs, providing honorariums to Elders, and covering the translation costs for Traditional Ecological Knowledge (TEK) interviews. Kimmy has over 10 years of experience working in roles as Aboriginal Relations Advisor and Engagement Advisor for Alberta Environment and Parks and Alberta Indigenous Relations. This work focused on a variety of land and natural resource management issues. She has worked in her role with the Blackfoot Confederacy Tribal Council for over two years on a variety of environmental issues. She has extensive experience working with the Blackfoot Nations, with strong relationships with land and consultation managers as well as environment and fish and wildlife staff in each Nation. Kimmy holds a Bachelor of Management degree.

Elliot Fox is the Assistant Project Manager. Elliot is a member of the Kainai Nation (Blood Tribe) and is currently working as an independent consultant. Elliot's role is liaising with the Nations and their staff, acting as an ambassador for the project in meetings and presentations with Project partners, and coordinating Nation staff in field activities. Elliot also conducted TEK interviews with Blackfoot Elders, transcribed the knowledge gathered, and prepared a report for the BFC and its Nations. Elliot has 27 years of training and experience working as a technician for federal and provincial natural resource management agencies that are Blackfoot partners (e.g., Parks Canada, Alberta Fish & Wildlife), non-government agencies (e.g., the Earthwatch Institute & the Resilience Institute), as well as serving as a Land Manager for two of the three Blackfoot Nations in Alberta (Kainai, Piikani) for 16 of those years.

Lands departments from three of the Blackfoot Nations supported the Project in Year 1. Due to covid-19 restrictions and the Canada-US border closure, the fourth Blackfoot Nation, Amskapi Piikani (the Blackfeet Nation), was unable to participate as originally planned. Irene Solway, Environmental Coordinator with Siksika Land Management supported the Project by providing Lance Breaker as a field technician. Noreen Plain Eagle, Piikani Lands Manager, supported the Project by providing Brent and Blair Little Mustache as field technicians. Kansie Fox, Environmental Protection Manager, Blood Tribe Land Management, supported the Project by providing Alvin First Rider as a field technician. Kansie also helped mentor staff through the habitat restoration activities of the Project. All four of the field technicians attended two days of training at the outset of the project and then participated in field activities throughout Year 1 of the Project. Due to Lands staff having fulltime responsibilities in their roles managing reserve lands already, not all staff were able to participate every that day activities occurred, and field activities were generally only schedule once per week. The BFC envisions the Project growing and as capacity within the Nations increases, one or more field technicians may be able to be dedicated to the native trout project on a part-time or even full-time basis, depending on funding availability.

Matt Coombs works as the Project Biologist. He is the director of Fintegrate Fisheries & Watershed Consulting Ltd, a private consulting firm he operates in the Crowsnest Pass near two of the three Nations where Kimmy Houle and Elliot Fox work (Piikani and Kainai Nations). Matt has 12 years of experience working for Alberta Environment and Parks as a fisheries biologist in southern Alberta and has since worked in the consulting industry for the last three years. Matt is well connected with the BFC's partner agencies and NGOs and has broad local knowledge of biocultural values along the East Slopes. As a professional Biologist, Matt provides technical science support to the Project. Matt organized the two-day training course that kicked off the Project in Year 1, providing outdoor classroom instruction together with the BFC's government and NGO partners. Matt also helps plan the habitat assessment and restoration activities and coordinates environmental DNA (eDNA) sampling.

1.6 Project Partners

The BFC appreciates the support of its 2020-2021 Project partners from a range of federal and provincial government agencies, academia, and NGOs.

Table 1.1 2020-2021 BFC Project partners

Partner	2020-2021 contribution
Alberta Environment and Parks	• Stream temperature loggers and installation equipment • Whirling disease decontamination equipment and supplies • Outdoor classroom training • Outdoor classroom facilities
Oldman Watershed Council	• Outdoor classroom training • Organized habitat restoration activities
Cows & Fish (Alberta Riparian Management Society)	• Outdoor classroom training • Organized habitat restoration activities
University of Victoria, Helbing Lab	• Environmental DNA technical support analysis
Trout Unlimited Canada	• Outdoor classroom training • Organized habitat restoration activities
AJM Environmental Inc.	• Discounted rate for project biologist
Parks Canada	• Loan of equipment

1.7 Project Objectives

The Project objectives were to:

1. Provide outdoor classroom training covering East Slope watershed processes, native trout conservation, impacts of non-native trout, fish identification, importance of riparian and instream fish habitat, spawning behaviour, habitat assessment using eDNA, stream temperature data loggers, redd counts, and riparian habitat restoration.
2. Engage Blackfoot Nation staff in hands on training and mentorship in community-based habitat assessment techniques that can be used to identify and prioritize riparian and fish habitat restoration opportunities and priorities. Focus on designated critical habitat and the use of non-invasive methods (eDNA, temperature monitoring, and redd counts).
3. Mentor Blackfoot Nation staff in implementing riparian restoration activities on reaches of streams designated as Westslope Cutthroat Trout and Bull Trout critical habitat.
4. Interview Elders from the four Blackfoot Nations to collect TEK about water, native trout streams and rivers within Blackfoot Territory, and the significance of fish and fish habitat. Bring this knowledge together with the scientific understanding of Westslope Cutthroat Trout and Bull Trout within Blackfoot Territory.

2 Methods

All Project methods followed the AFSAR 2020-2021 Program Guidelines, and the activities outlined in the BFC's 2020-2021 proposal.

2.1 Project Timing

Project funding from AFSAR was announced to BFC by DFO on April 23, 2020. Due to concerns and uncertainty related to covid-19 and the reduced funding that was initially provided, the project start was delayed. This meant Westslope Cutthroat Trout spawning surveys only occurred for one day (July 1, 2020), and the Project did not officially start until August 12-13, 2020, when a two-day outdoor training course was organized by the Project Biologist and the BFC's NGO partners. Following this event, Project activities continued every week until mid-September, with some additional activities occurring in October, November, and January 2021. Due to the need to redesign the eDNA assay for Rainbow Trout (*Oncorhynchus mykiss*) and a high demand for laboratory reagents resulting from covid-19, receipt of eDNA results has been delayed, which has delayed reporting for Year 1 of the Project.

2.2 Alignment with Regional Priorities

As part of DFO's Central & Arctic Region, the South Saskatchewan River Basin was identified in the AFSAR Program Guidelines as a priority area. Westslope Cutthroat Trout were identified as a priority species, and although Bull Trout were not, this was simply because the Saskatchewan-Nelson species was not listed as Threatened under SARA until August 2019 at which time the 2020-2021 Program Guidelines would have already been written. Bull Trout are listed as a priority species in the 2021-2022 Program Guidelines. Four regional priority threats identified in the 2020-2021 Program Guidelines were addressed in Year 1 of the Project:

- Habitat loss or degradation
- Impacts from introduced non-native trout species
- Restrictions to fish passage
- Loss of Indigenous Knowledge related to presence and utilization of important habitats

2.3 Project Activities

Six of the eight eligible activity categories outlined in the 2021-2022 Program Guidelines were addressed as part of the Project:

- Surveys, Inventories and Monitoring: identifying sites for habitat restoration; assessing the presence of SAR and SAR habitat to target, design, and carry out a current (or future) stewardship project;
- Habitat Improvement: Enhance or restore SAR habitat; changing land/water management or land/water use practices to benefit SAR and improve habitat quality;
- Human Impact Mitigation: direct intervention to protect, mitigate, or prevent human activities that immediately threaten SAR;
- Outreach & Education: sharing knowledge on specific actions to be taken to protect SAR; raising awareness about SAR conservation needs; promoting community level stewardship to improve attitudes and change behaviour.

- Indigenous Knowledge Documentation, Management, and Use: community-led documentation following appropriate protocols, management and use of indigenous knowledge; documentation through interviews focused on SAR and their habitats.

Developing SAR land use guidelines/best practices as part of the "Program Planning and Development" eligible activity may be an action the BFC could take in the future but has not yet been taken. Similarly, assessing the social and biological results and effectiveness of stewardship activities as part of the "Project and Program Evaluation" is another eligible activity that may be taken at some point in the future.

2.3.1 Surveys, Inventories and Monitoring

As part of the Surveys, Inventories and Monitoring activity category, the Project assessed designated critical habitat by conducting spawning surveys (redd counts), monitoring temperature with data loggers, and testing if the habitat was occupied using eDNA. This updated and refined the current known distribution and habitat use of Westslope Cutthroat Trout and Bull Trout to prioritize future conservation and recovery efforts, including habitat improvement and human impact mitigation.

Spawning Surveys

Spawning surveys were conducted on foot while wading in the streams in the upstream direction with polarized sunglasses and a hat with a brim to improve visibility into the water and the ability to identify fish. Crews consisted of two or more members scanning back and forth from bank to bank and looking for bull trout redds and/or spawning activity. At each redd crews recorded the redd position in the channel, redd length (cm), redd width (cm), water depth (cm), and dominant substrate type. For superimposed redds the total number of pits was counted and a representative water depth for the entire spawning area was taken. The presence of any fish observed was also noted. GPS locations were also recorded for each redd as well as any riparian or stream bank restoration opportunities in the vicinity of the site.

Redd surveys were only conducted in the Oldman watershed in Year 1. Streams in the Bow watershed could not be surveyed due to limited staff resources. With the Blackfoot Nation Lands staff having other fulltime responsibilities, surveying streams for redds with two successive passes separated by one week was also not possible. Instead, each reach of stream was only surveyed once, and efforts were made to time surveys when spawning was most likely to be observed. Due to the delay in the Project officially starting, only one day of Westslope Cutthroat Trout spawning survey was conducted by Elliot Fox and Matt Coombs. The survey was conducted on July 1, 2020 on Beaver Creek, a tributary to the Oldman River that flows through Piikani Nation (Piikani Indian Reserve No. 147). In the fall, eight days of Bull Trout spawning surveys were conducted with Kimmy Houle, Elliot Fox, Alvin First Rider, Blair Little Mustache, Brent Little Mustache, and Lance Braker all participating together with Matt Coombs. The surveys were conducted between September 9-17, 2020 on Hidden Creek, the upper Oldman River and Livingstone River above their confluence and below the waterfalls of each of these streams as well as on Dutch Creek, Racehorse Creek, and Daisy Creek. The Oldman watershed was selected by BFC as a manageable area within the broader study area within which they could conduct the Bull Trout spawning surveys over a one-week period. The streams the Project focused on had been previously identified as the primary spawning tributaries by the Alberta Conservation Association (ACA, Hurkett et al. 2011).

One additional day of Bull Trout spawning surveys was conducted on October 14, 2020 in Waterton Lakes National Park (WLNP) on the North Belly River. This survey was led by Paul Harper, who was the Ecologist Team Leader for the Aquatic Conservation and Restoration at the time. Elliot Fox and Alvin First Rider participated in the survey. The site was accessed by helicopter and commenced in Canada, although the section of stream surveyed does cross the boarder with the United States and the survey ended in Montana.

Stream temperature monitoring

Like spawning surveys, stream temperature monitoring was only conducted in the Oldman watershed in Year 1; streams in the Bow watershed could not be monitored due to limited staff resources. Given that Alberta Environment and Parks (AEP) was already running an established a provincial stream temperature monitoring program along the East Slopes in 2020, the Project supported AEP by adopting some of the monitoring sites that were previously established. The Project also followed AEP's stream temperature data logger installation and monitoring protocol (AEP 2019). Blackfoot field technicians checked for stream temperature loggers at eight sites previously established by AEP and were able to find the loggers and successfully download the data from four of these sites (Table 2.1). A new logger was installed at one site where the AEP logger could not be found in the headwaters of Beaver Creek, a tributary to the Oldman River that flows through Piikani Nation. An additional six loggers were installed at sites the Blackfoot considered high priority monitoring sites.

Table 2.1 2020-2021 BFC Project steam temperature monitoring activities

Waterbody Name	Site ID	Installer	BFC Project's 2020 action
Pincher Creek	W20-003	BFC	Initial logger installation
Pincher Creek	W20-001	BFC	Initial logger installation
Castle River	W18-107	AEP	Found logger data downloaded
Belly River	W18-103	AEP	Found logger; data downloaded
Belly River	W18-152	AEP	Found logger; data downloaded
North Belly River	W18-620a	AEP	Found logger; data downloaded
Payne Outlet Canal	W18-153	AEP	Logger not found; no replacement installed
Payne Inlet creek	W18-154	AEP	Logger not found; no replacement installed
Beaver Creek	W20-006	BFC	Initial logger installation
Beaver Creek	W18-802	AEP	Logger not found; replacement logger installed
Oldman River	W20-002	BFC	Initial logger installation
Oldman River	W18-082	AEP	Found logger; data downloaded
St. Mary River	W20-004	BFC	Initial logger installation
Lee Creek	W20-005	BFC	Initial logger installation
Waterton River	W18-105	AEP	Logger not found; no replacement installed

eDNA assay development and validation, sampling, and analysis

As a leading expert in the use of targeted, species-specific eDNA testing in Canada, the Project partnered with Dr. Caren Helbing at the University of Victoria for eDNA assay development and validation and eDNA analysis. Dr. Helbing met virtually with Kimmy Houle and Matt Coombs multiple times during Year 1 to discuss eDNA analysis.

At the onset of the Project, it was decided that lab (*in vitro*) and field (*in situ*) verification of the eDNA assays would be incorporated into Year 1 given that eDNA sampling was an approved activity under the DFO AFSAR Contribution Agreement and BFC was eager to have the Nation field technicians start using the

sampling method as a tool for native trout habitat assessment. The Helbing lab had assays available for Westslope Cutthroat Trout, Bull Trout, and Rainbow Trout at the outset of the project, and anticipated developing assays for Brook Trout (*Salvelinus fontinalis*) and Lake Trout (*Salvelinus namaycush*). A Bull Trout assay that does not result in false positives when eDNA from these other two char species is present in the sample and that can distinguish between all three charr species was deemed to be a priority for the Project, given Brook Trout and Lake Trout are invasive in parts of the study area (e.g., Castle River watersheds).

To initiate the process of further assay development and validation, Elliot Fox requested voucher tissue samples from AEP for four key target species: Westslope Cutthroat Trout, Bull Trout, Rainbow Trout, and Brook Trout. Elliot met with Andreas Luek, Senior Fisheries Biologist with AEP, and obtained fin clips from these species. These samples were submitted to the Helbing Lab for an initial *in vitro* verification that existing assays that were already developed and used by the lab would work with Alberta populations in the Project study area (Table 2.2). Additional wild-source Brook Trout tissue samples were collected by Project field technicians by electrofishing on Beaver Mines Creek under the supervision of the Project Biologist on November 18 (Table 2.2), and additional Lake Trout (*Salvelinus namaycush*) tissue samples were also provided by Brian Meagher, Fisheries Biologist with AEP (Table 2.2).

Results from this initial lab verification process were received on September 10, 2020. While the assays for Westslope Cutthroat Trout and Bull Trout were successfully validated, the results suggested the Rainbow Trout assay was not distinguishing Westslope Cutthroat Trout from Rainbow Trout. This was confirmed in a later meeting, at which time the Helbing Lab indicated that redesigning the Rainbow Trout assay would be necessary. The BFC subsequently applied to the ACA Research Grant fund in November 2020 and were successful in receiving funding in March 2021 to develop and further validate environmental DNA assays for native Westslope Cutthroat Trout and Bull Trout and non-native Rainbow Trout and Brook Trout within the Blackfoot territory for citizen science and community-based monitoring. This is now being done, with the Rainbow Trout assay redesigned, Book Trout and Lake Trout assays under development, and plans to eventually develop a new Bull Trout assay as well.

A total of 13 sites were sampled for eDNA as part of the Project, including 12 sites in the Oldman watershed and 1 site in the Bow watershed (Table 2.3). Sites known to be occupied were sampled to confirm the assays worked, and while the number of known occupied sites sampled was low (site OR2 for Westslope Cutthroat Trout, site LC1 for Rainbow Trout), additional sites for these and other species like Bull Trout and Brook Trout will be sampled in future years. One additional site (CC1) where the populations of Westslope Cutthroat Trout and Bull Trout are believed to have been extirpated was sampled as a negative field control on the same day sampling other sites where fish were present was also done to confirm cross contamination between sites was not occurring.

Sampling eDNA followed the “grab and go” method (Hobbs 2021). Nalgene™ Wide-Mouth Lab Quality HDPE Bottles (1L) were filled with site water while standing on shore using an extendable pole without entering the water. Filled sample bottles were stored in a cooler on ice and each full 1L sample was filtered within 12 hours following sample collection. Filtering occurred on a sterile workbench surface with a source of power using a vacuum pump and 2L vacuum flasks. All samples were filtered through a cellulose nitrate 0.45µm pore size 100mL filter (STERLITECH™). Filters were vacuumed dry, folded in quarters, placed in coin envelopes, and then placed in Ziploc™ bags with a silica desiccant before being shipped to the Helbing research lab for analysis. One sample bottle was filled with distilled water during each day of sampling as a field blank (negative control). Blank samples went through all the steps of eDNA collection, filtration, preservation, and testing. Collection bottles and all collection equipment were sterilized with 50% bleach solution and rinsed with distilled water between re-use from one site to the next and one sampling day to the next.

Year 1 of the project was preliminary, and limited funding and staff resources were available. eDNA sampling sites were broadly distributed across the Oldman watershed with one additional sampling site in the Bow watershed. Negative eDNA results must be interpreted keeping in mind that more sample sites and sampling multiple times throughout the year would be required on each stream that was sampled to achieve

higher detection probabilities necessary to determine with greater confidence whether the target species are absent.

As is recommended in DFO's eDNA guidance document (Abbott et al. 2021), the following information is summarized for the eDNA methods applied and a copy of DFO's eDNA reporting template is attached (**Appendix A**). Targeted-specific assays were applied individually to test for the presence of genetic material from each species in each sample. Technical bulletins for these assays are attached (**Appendix B**), providing sensitivity and specificity test results. All assays use quantitative polymerase chain reaction (qPCR) and the TaqMan enzyme (Helbing and Hobbs 2019). Three field replicate samples were collected at each eDNA sampling location, and 8 laboratory technical replicates were generated for each of the eDNA assays that were applied. Separate sets of 4 laboratory technical replicates from each field replicate were used to confirm viable DNA could be amplified from each sample and test for qPCR inhibition and DNA degradation (i.e., IntegritE-DNA™ method, Hobbs et al. 2019). A total of 50 cycles of qPCR were then performed for all assays.

Table 2.2 Tissue samples submitted for *in vitro* verification of eDNA assays.

Species	Source	No. of samples	Collection Date	Source
Westslope Cutthroat Trout	Girardi Creek	7	Jul 2017	AEP, Andreas Luek (July 24, 2020)
Westslope Cutthroat Trout	Lost Creek	8	Sept 2015	AEP, Andreas Luek (July 24, 2020)
Westslope Cutthroat Trout	Allison Creek Brood Trout Station – Job Lake origin	15	Aug 4, 2020	AEP, Laine Ripley (Aug 4, 2020)
Bull Trout	Hidden Creek	15	Oct 2011	AEP, Andreas Luek (July 24, 2020)
Rainbow Trout	Raven Hatchery	12	Unknown	AEP, Andreas Luek (July 24, 2020)
Rainbow Trout	Lyons Creek	5	Sept 2015	AEP, Andreas Luek (July 24, 2020)
Brook Trout	Allison Creek Brood Trout Station	15	Aug 4, 2020	AEP, Laine Ripley
Brook Trout	Beaver Mines Creek	19	Nov 18, 2020	Project electrofishing under FRL 20-1617
Lake Trout	Crowsnest River	6	Jun 13, 2013	AEP, Brian Meagher (Dec 9, 2020)

2.3.2 Habitat Improvement

As part of the Habitat Improvement activity category, the Project restored riparian habitat on stream reaches designated as critical habitat for Westslope Cutthroat Trout and Bull Trout. Habitat improvement was conducted at four sites in the Bow and Oldman watersheds (Table 2.4). Restoration activities were organized by some of the Project partners (i.e., Oldman Watershed Council, Cows and Fish, Trout Unlimited Canada), as well as other partner organizations (e.g., Ghost River Watershed Alliance, Elbow River Partnership). Willow stakes, harvested in advance during the spring and kept dormant over the summer in cold storage, were supplied by the Oldman Watershed Council and Cows and Fish. These partners also provided all the equipment needed for the restoration work (sledgehammers, planting bars, pruning loppers). The three techniques used to promote revegetation of bare ground and reduce erosion and

sediment inputs to the streams at the riparian restoration sites were live willow staking (~100 stakes per site), bioengineering with wattle fences and willow fascines installed perpendicular to the eroding slope, and "top-dressing" with coarse woody debris.

Table 2.3 eDNA sampling site locations and dates in Year 1 (2020).

Site Code	Watercourse Location	Watershed Basin	Sample Date	Lat/Long Location
OR2	Oldman River at Cache Creek	Oldman	Aug 20	50.05101 -114.58805
SC1	Shale Creek upstream of road	Oldman	Aug 20	49.98713 -114.49076
PC1	Pincher Creek at Brocket	Oldman	Aug 26	49.549611 -113.771981
PC2	Pincher Creek at Alberta Ranch	Oldman	Aug 26	49.405486 -114.016135
CCT1	Chipman Creek Tributary	Oldman	Aug 26	49.4035 -114.057889
PO1	Payne Lake outlet canal	Oldman	Sept 3	49.105181 -113.631196
TC1	Tough Creek at Pole Haven entrance	Oldman	Sept 3	49.064523 -113.553405
LC1	Lee Creek at Beazer	Oldman	Sept 3	49.112159 -113.487611
ETC1	Evan-Thomas Creek above Hwy 40	Bow	Sept 23	50.88137 -115.12107
LYC1	Lyons Creek at Sartoris Road	Oldman	Sept 23	49.570372 -114.453231
CC1	Crowsnest Creek above falls	Oldman	Sept 23	49.603558 -114.68235
SM1	St. Mary River at Kimball bridge	Oldman	Nov 3	49.091033 -113.221869
SM2	St. Mary River at Hwy 5	Oldman	Nov 3	49.224606 -113.265874

Table 2.4 Habitat restoration site locations and dates in Year 1 (2020).

Watercourse Location	Watershed Basin	Restoration Date	Lat/Long Location
Dutch Creek at closed random camping area	Oldman	Sept 16	49.911158 -114.487731
Dutch Creek at closed ford adjacent to bridge	Oldman	Sept 16	49.910278 -114.511894
Silvester Creek at pipeline crossing right-of-way closed to OHVs	Bow	Sept 24	50.847844 -114.717458
Waiparous Creek closed OHV crossing	Bow	Sept 26	51.382010 -114.995891
Beaver Creek at closed OHV ford crossing	Oldman	Oct 5	49.8209 -113.959717

2.3.3 Human Impact Mitigation

Human impact mitigation is a broad activity category that encompasses some of the eligible activities under "Surveys, Inventories and Monitoring" and "Habitat Improvement" categories:

- Habitat restoration work was a method the Project used to mitigate human-caused riparian damage that immediately threatens Westslope Cutthroat Trout and Bull Trout.

- During the spawning surveys, the Project also identified and documented additional riparian sites where habitat restoration is necessary.
- Stream temperature monitoring and eDNA sampling is a method to identify areas where Bull Trout habitat is being impacted by water diversion for irrigation. Some of the temperature monitoring sites downstream of diversions will monitor whether temperatures are suitable for Bull Trout. Some of these areas were also sampled for eDNA to search for any evidence they were occupied by Bull Trout.

While riparian habitat restoration was the only direct intervention method that occurred in Year 1, other activities in Year 1 contributed to future actions that will be included in the human impact mitigation category. Specifically, Blackfoot Nation staff and community members have previously participated in fish rescues funded by AEP and conducted by Trout Unlimited. Bull Trout entrainment downstream of diversion structures or in water diverted for irrigation is an ongoing occurrence in the Oldman watershed and fish rescues are meant to mitigate this. Bull Trout can move downstream of irrigation dams and diversion structures to areas where they may not be able to spawn, and in future years the Project would like to translocate Bull Trout rescued from the canal systems to areas where these fish can access spawning habitat. There may also be options to adjust water management regimes to ensure adequate flows are maintained so water temperatures remain cool enough for Bull Trout in areas where they cannot access cooler habitat further upstream.

2.3.4 Outreach & Education

The primary method the Project implemented for sharing knowledge on specific actions to be taken to protect Westslope Cutthroat Trout and Bull Trout was hands on field-based mentorship of Blackfoot Nation staff by technical experts. The Project officially started August 12-13, 2020, when a two-day outdoor training course was organized by the Project Biologist and the BFC's NGO partners.

Day 1

The course opened with William Big Bull, an elder with the Piikani Nation, giving a blessing and talking about the knowledge that is inherent in everything; the hills and mountains, water, fish and wildlife. The training occurred in the upper Oldman watershed, an area William knows well, and Blackfoot Nation staff learned from him about the significance of the area to their people and some of the seasonal cycles that occur in the area (e.g., spawning).

Training throughout the day took place at an outdoor picnic shelter classroom and focused on watershed processes and the headwater environments of the East Slopes were Westslope Cutthroat Trout and Bull Trout persist. Topics included native and non-native trout identification, range contraction, loss of biodiversity and populations, fishing regulations, and provincial and federal legislation protecting these species. The causes of declines in native trout populations, including the effects of overfishing, hybridization, and habitat degradation, were discussed as well as efforts to reduce these threats. The features, functions, and attributes of instream and riparian fish habitat were discussed, as well as the designation of critical habitat under SARA. Differences in the temperature requirements and spawning behaviour of the two species were discussed. Cows and Fish presented on the importance of riparian areas for creating and sustaining fish habitat, buffering the effects of floods, maintaining groundwater inputs to support base flows, keeping temperatures cool, and filtering runoff. The Oldman Watershed Council reviewed ongoing factors damaging fish habitat and highlighted work being done in the watershed to restore fish habitat. Trout Unlimited followed this up with a review of non-native and native trout species identification and helped the Nation Staff work through a fish ID test for training purposes.

In the afternoon of Day 1 a tour of Dutch Creek occurred. Examples of riparian habitat damage and restoration work that had been done by the Oldman Watershed Council were visited and stream side discussions about restoration techniques occurred.

Day 2

The second day of the course started with a presentation in the outdoor picnic shelter classroom by Alberta Environment and Parks on the lifecycle of the whirling disease parasite (*Myxobolus cerebralis*) and the impacts it can have on many salmonid species, including Westslope Cutthroat Trout. The history of the origin of the parasite in North America, past testing for whirling disease within Alberta, and the first positive detection in 2016 were covered. There was also a demonstration of decontamination procedures, and decontamination supplies were provided to BFC by AEP as in-kind support.

Whirling disease knowledge sharing was followed by a demonstration of how to set up stream temperature data loggers, including installation and downloading data. Methods for constructing a protective housing for the data loggers, installing them underwater using rebar stakes or epoxy glue on large boulders, recording logger locations in a provincial database using AEP datasheets, and downloading data and input into AEP's loadforms for data submission were reviewed. The Blackfoot Nation staff were able to practice installing and locating data loggers in the Oldman River as well as downloading data from the loggers.

Riparian restoration training

Classroom-based riparian restoration training was provided to the Blackfoot Nation staff by a leading expert in the field, Dave Polster, through funding received by the Ghost River Watershed Alliance from the Alberta Conservation Association. Classroom training occurred on September 25, 2020 in the Wiaparous Village, with Dave presenting and sharing a restoration manual. Dave also provided hands-on training to the Blackfoot Nation staff on riparian restoration activities for two days (September 24 and 26) on Silvester Creek and Waiparous Creek.

Two additional days of riparian restoration training, as outlined above, occurred on Dutch Creek on September 16, 2020 and Beaver Creek on October 5, 2020. On both days, Blackfoot received riparian restoration training from riparian specialists from Cows and Fish.

Mentored learning

Beyond the outreach and training covered above, Blackfoot Nation staff spent 14 additional days working alongside the Project Biologist. This included eDNA sampling, stream temperature logger installation, and Bull Trout redd surveys (Table 2.5). Considerable practical experience was gained during this time in the various methods of assessing and restoring habitat, including a deeper understanding of Westslope Cutthroat Trout and Bull Trout conservation requirements.

2.3.5 Indigenous Knowledge Documentation, Management, and Use

Blackfoot knowledge and values related to the study area, water, and native trout was documented by Elliot Fox through interviews he conducted of elders from each of the four Nations. The objective was to acquire information on the traditional and current significance of fish and water as well as place names within Blackfoot Territory. Six (6) respected elders from each of the four nations of the Blackfoot Confederacy, Kainai (Blood Tribe), Piikani (Peigan Nation), Siksika (Siksika Nation) and Aamskappi Piikunni (Blackfeet Nation, Montana, USA) were interviewed. Some interviews were in person while physically distancing due to covid-19 restrictions, while others were conducted by phone or video call. Elliot transcribed most of the interviews, although two were conducted in fluent Blackfoot and two translators assisted Elliot transcribe these interviews. The three questions asked were:

1. How are fish significant to the Blackfoot people?

2. How is water significant to the Blackfoot people?

3. What are some traditional Blackfoot place names in Blackfoot Territory?

The Blackfoot Nations hold the knowledge from the interviews, and a separate report summarizing the knowledge has been written and will be shared where appropriate.

Table 2.4 Year 1 Habitat assessment and restoration activities and dates (2020).

Date	Activities				Participating BFC Nations		
	eDNA	Temperature	Redds	Habitat restoration	Kainai	Siksika	Piikani
Jul 1			✓		✓		
Aug 20	✓	✓			✓		✓
Aug 26	✓	✓			✓	✓	✓
Sept 3	✓	✓			✓	✓	✓
Sept 9			✓				✓
Sept 10			✓		✓		✓
Sept 11			✓		✓		
Sept 12			✓		✓		
Sept 14			✓		✓		
Sept 15			✓		✓		
Sept 16				✓	✓	✓	✓
Sept 17			✓		✓	✓	✓
Sept 23	✓				✓		
Sept 24				✓	✓		
Sept 25				✓	✓		
Sept 26				✓	✓		
Oct 5				✓	✓		
Oct 14			✓		✓		
Nov 3	✓	✓			✓		
Nov 17	✓				✓		

3 Results

Results from Year 1 of the Project are focused primarily on the "Surveys, Inventories and Monitoring" eligible activity. Participation by Nation staff, topics covered, and methods used in the "Habitat Improvement" and "Outreach & Education" activities are already presented above in the Methods section. Opportunities for Human Impact Mitigation that were identified during the project are presented as results below in Section 3.4. A summary of TEK collected as part of the project by Elliot Fox is presented in a separate report.

3.1 Spawning surveys

The single Westslope Cutthroat Trout spawning survey conducted on Beaver Creek was only conducted over the upper most reach of the stream upstream of the highest culvert crossing on the Beaver Creek Road (Figure 3.1). The reach of stream surveyed was approximately 1300 m long and started at the road. This was the only reach of stream that could be surveyed on July 1, 2020, because heavy rain the day before resulted in the rest of the creek downstream from this location being too turbid to be surveyed. A single spawning pair of fish was observed immediately upstream from the culvert crossing at the road. Spawning downstream of the crossing, though not observed, is suspected to also occur.

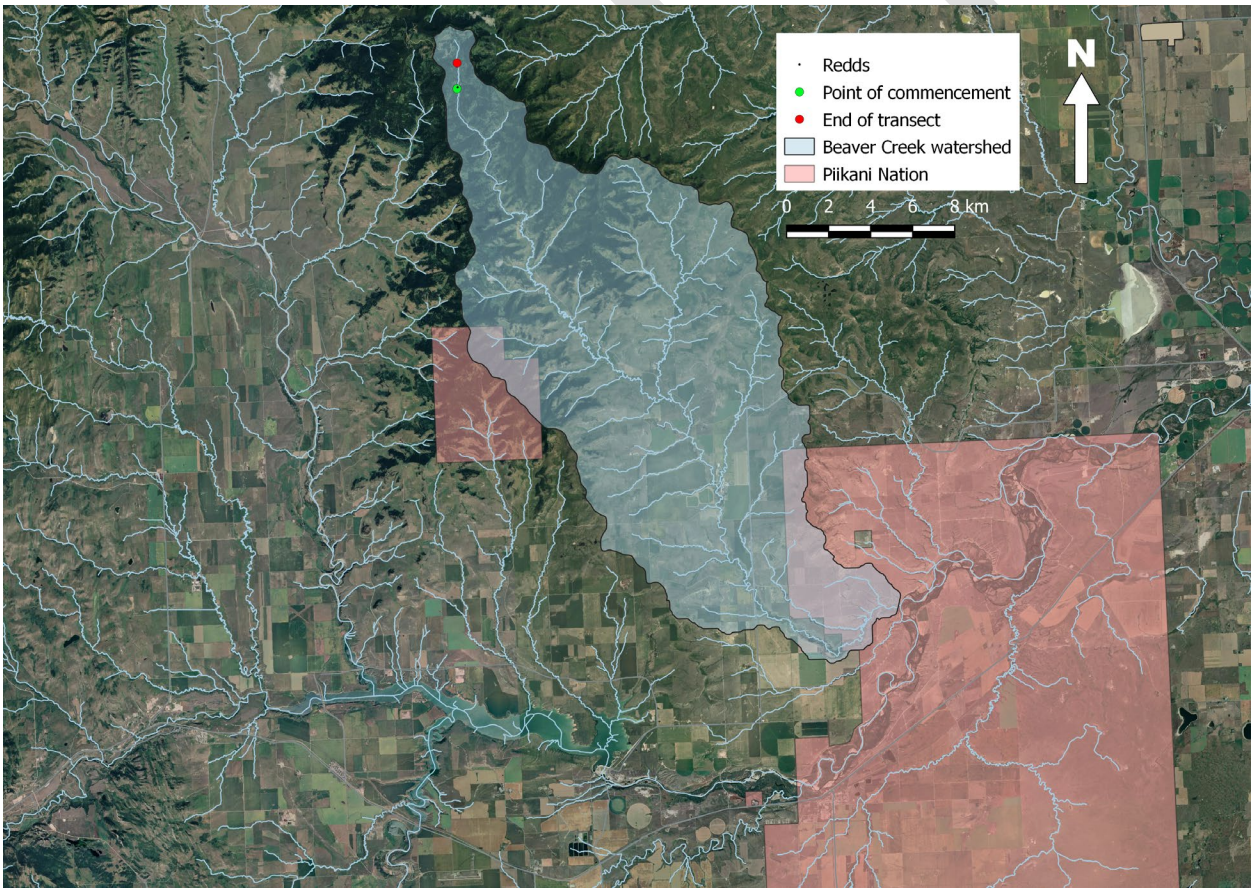

Figure 3.1 Beaver Creek Westslope Cutthroat Trout redd survey (July 1, 2020)

Bull Trout spawning surveys in the upper Oldman watershed occurred over 7 days. Of the stream reaches surveyed, Hidden Creek had the most redds (34), followed closely by the upper Oldman River (31, Figure 3.2). The Livingstone River below Livingstone Falls, Dutch Creek, and Racehorse Creek had one half to one third as many redds (Figure 3.2). Only 3 redds were observed on Daisy Creek (Figure 3.2).

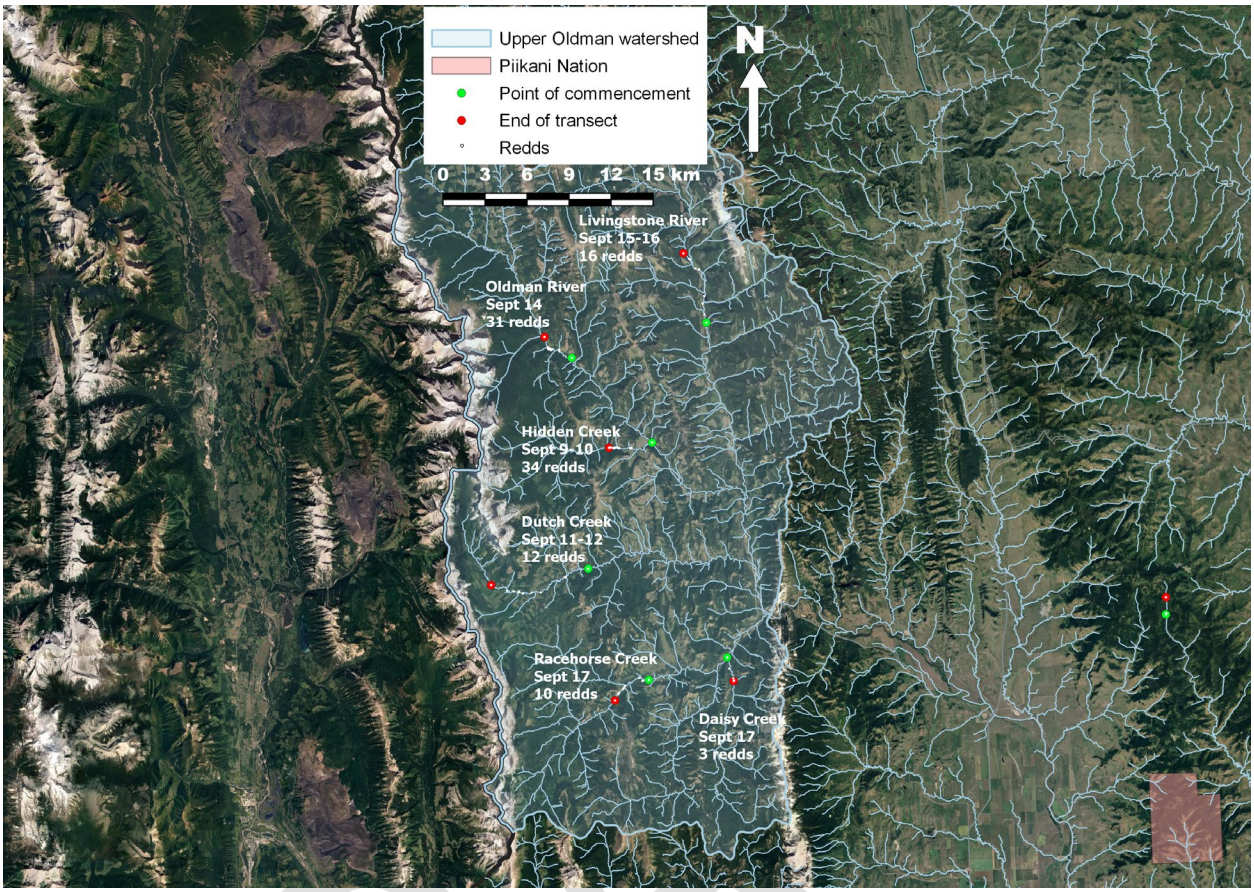

Figure 3.2 Oldman watershed Bull Trout redd surveys (September 9-17, 2020)

The North Belly River redd survey that was conducted on October 14, 2020 resulted in 23 definite redds and 4 possible redds being observed (Figure 3.3). The survey commenced in Canada upstream from the Blood Timber Limit and ended in Glacier Nation Park (Montana, USA) at Miche Wabun Falls. According to Paul Harper with WLNP, this was the highest number of redds counted in the North Belly in over the last decade.

All redd survey raw data has been submitted to AEP for entry into the FWMIS database using AEP's electronic loadform. This loadform is also being submitted to DFO with this report.

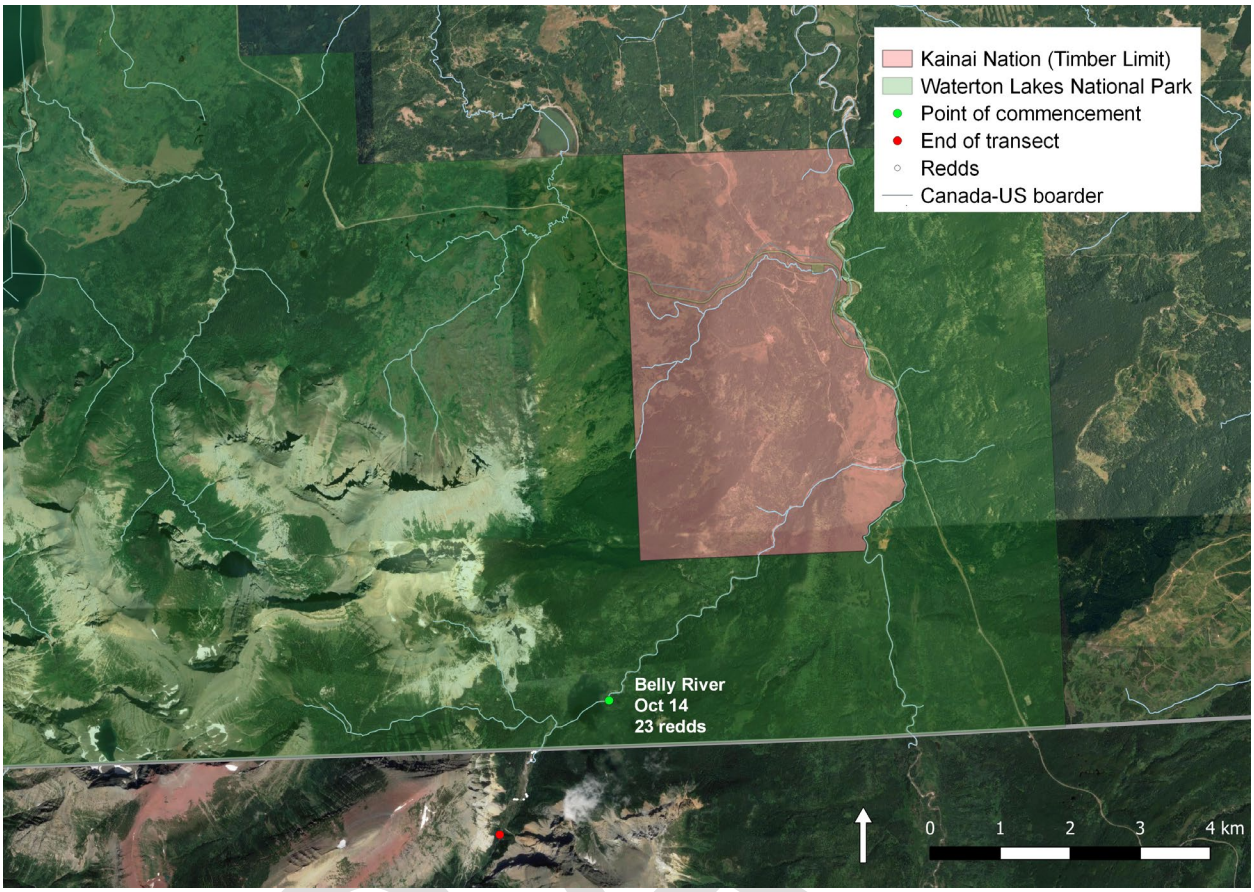

Figure 3.3 Belly River Trout redd surveys (October 14, 2020)

3.2 Stream temperature monitoring

Locations of stream temperature data loggers installed by the Project for the first time are summarized below (Table 3.1). As no data are yet available from these sites, site locations are all that can be reported in Year 1. AEP's datasheets were used to record descriptions of the sites, and these are included with this report along with site photos (**Appendix C**). These loggers have also been added to AEP's online database:

https://www.google.com/maps/d/edit?mid=1F4jsAVrf3l9aVvZkX7kJXRh0H4XdhQ2F&usp

Temperature loggers were installed on Pincher Creek at the same two locations were eDNA was sampled. One location was near the mouth, and the other was roughly halfway between the mouth and known spawning habitat in the headwaters. This was done to assess whether elevated temperatures on the lower reaches of the creek may be limiting migration of Bull Trout from the Oldman River in the headwaters of Pincher Creek for spawning. It is not known whether Bull Trout that currently spawning in the headwaters of Pincher Creek include fish migrating from the Oldman River. A temperature logger was also installed in the Oldman River downstream from the mouth of Pincher Creek to compare temperatures in the river and creek. The post-spawning migration period for Bull Trout can range from May to December (DFO 2020). Habitat that Bull Trout use for foraging, migration, and overwintering typically has mean August temperatures no greater than 14°C (Jones et al. 2014), and upper incipient lethal temperatures of 20.9°C and 23.5°C have been reported for Bull Trout for 60- and 7-day exposures, respectively (Selong et al. 2001).

Table 3.1 New steam temperature loggers installed in 2020

Waterbody	Site ID	Date	Lat/Long	Location
Pincher Creek	W20-003	Aug 26	49.405914 -114.016053	Left upstream bank, ~100m upstream of Township Road 5-4 near the Alberta Ranch
Pincher Creek	W20-001	Aug 26	49.549599 -113.772003	Left upstream bank, ~500m upstream of Oldman River on Piikani Reserve
Beaver Creek	W20-006	Oct 5	49.629667 -113.712936	Left upstream bank, ~20 m downstream from road & ~1200 m from Oldman River
Oldman River	W20-002	Aug 26	49.552728 -113.763017	Left upstream bank, ~100 m upstream from High Bush parking area
St. Mary River	W20-004	Nov 3	49.224167 -113.26611	Right upstream bank, ~40 m upstream of Hwy 5 bridge on Blood Tribe Reserve
Lee Creek	W20-005	Nov 3	49.222778 -113.266944	Left upstream bank, ~100 m upstream from St Mark River

Temperature loggers were also installed on the St. Mary River immediately below Lee Creek and in Lee Creek immediately upstream from the river for similar reasons as described above for Pincher Creek and Oldman River. While Fitch (1997) concluded that migratory bull trout no longer occur in the St. Mary River in Alberta, these temperature loggers are intended to assess the thermal suitability of the lower reaches of Lee Creek together with eDNA sampling to assess habitat occupancy. Bull Trout critical habitat has been designated in Alberta on both Lee Creek and the St. Mary River, but only downstream as far as Beazer and Kimball, respectively. Tagging data from U.S. Fish and Wildlife Service has confirmed migratory Bull Trout do occur in the St. Mary River downstream of the diversion dam in Montana that directs water from the St. Mary River to the Milk River and that at least some of these fish can move upstream over the dam to access spawning tributaries flowing into the St. Mary lakes (Mogen and Keading 2005). Both the Canadian Bull Trout Recovery Strategy (DFO 2020) and the U.S. Bull Trout Recovery Plan (USFW 2015) include goals to reconnect isolated bull trout populations, improve fish passage, and address fish entrainment. Rehabilitation of the St. Mary River Diversion in Montana is a step towards doing this and is likely to occur soon (St. Mary's Reinvestment Act). The project is designed to improve fish passage over the structure and exclude fish from the diversion (Heiner and Wagner 2016). Given that the St. Mary River downstream to at least Lee Creek and all of Lee Creek were historically occupied Bull Trout habitat, it is important to make reciprocal efforts to identify migration routes and assess habitat suitability on the Canadian portions of these streams.

A temperature logger was also installed at the mouth of Beaver Creek on the Piikani Reserve to compare with the stream temperature in the headwaters of Beaver Creek that was previously installed by AEP. This will help assess whether the full length of the creek is suitable habitat for cold water trout species and whether Rainbow Trout may be moving into the creek from the Oldman River and could hybridize with the pure Westslope Cutthroat Trout in the headwaters.

As presented in Table 2.1, four of the loggers previously installed by AEP could not be found. There loggers were in the Payne Outlet Canal, Payne Inlet creek, the headwaters of Beaver Creek, and the Waterton River near Standoff. Only the logger in the headwaters of Beaver Creek was replaced by the Project with a new logger. Data from one other logger (Belly River at Standoff) was downloaded but has been misplaced and cannot be reported at this time. When the data is located it will be reported separately.

Daily average stream temperature data downloaded from the remaining four loggers that were previously installed by AEP show a typical season pattern of warming (Figure 3.4). All raw temperature data have been submitted to AEP for entry into the provincial stream temperature database using AEP's electronic loadform. This loadform is also being submitted to DFO with this report. The Project was unaware that the logger on

the Oldman River upstream of the Oldman Reservoir (W18-082) had already had the data downloaded by the University of Alberta less than 2 weeks before the Blackfoot Nations downloaded data again. This is why the temperature record presented for this logger is short compared to the records for the other three data loggers. The North Belly River shows stream temperatures typical of a Bull Trout spawning tributary, with temperatures during the winter being greater than zero suggesting the influence of groundwater.

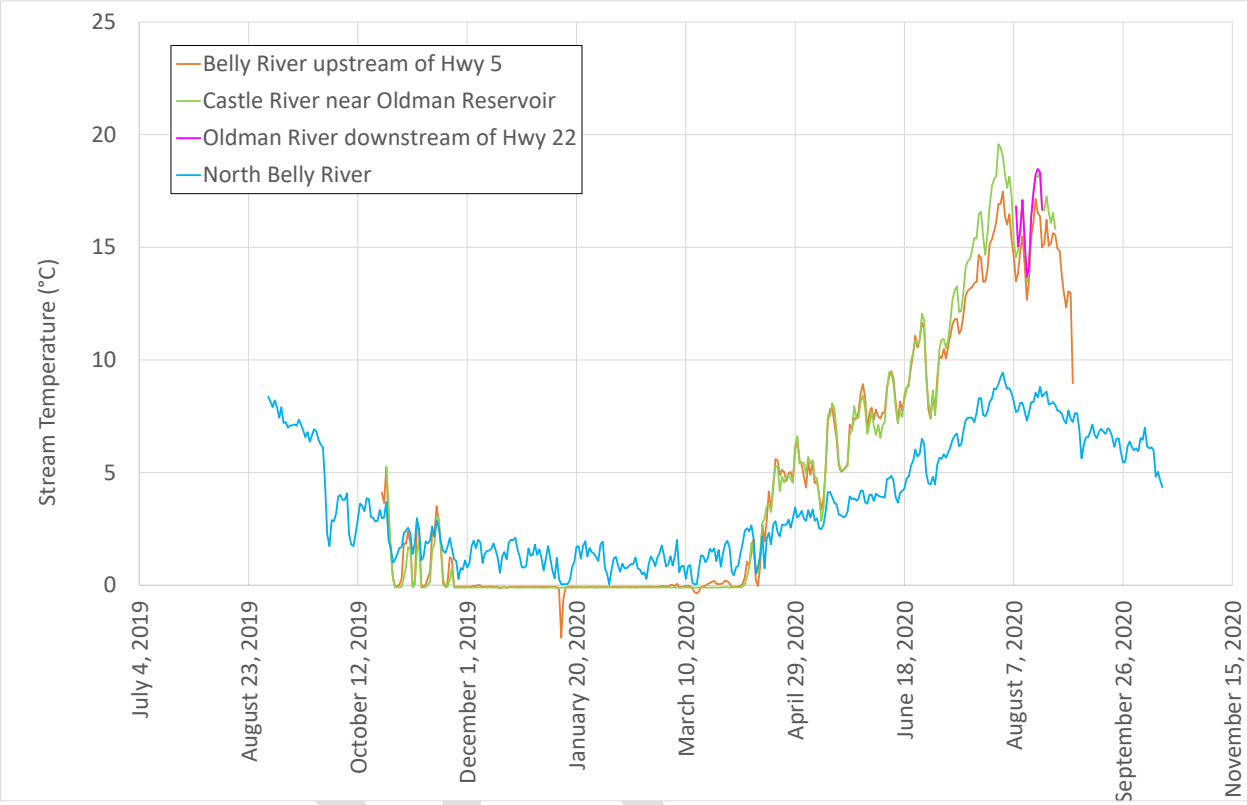

Figure 3.4 Daily 24-hour temperature averages for datasets downloaded by the Project.

3.3 eDNA sampling

One taxon-specific eDNA assay (i.e., detects any fish) and three species-specific eDNA assays (i.e., Bull Trout, Westslope Cutthroat Trout, Rainbow Trout) were applied to all Project samples (Table 3.2). Results confirmed the presence of Westslope Cutthroat Trout in the Oldman River at Cache Creek, which was already known. This site was sampled as a known occupied site to confirm the species-specific assay worked. Results from Shale Creek were the first to document Westslope Cutthroat Trout in this creek upstream of the Oldman River Road. Young-of-year (YOY) were observed while eDNA water samples were collected, and although they were suspected to be Westslope Cutthroat Trout, the eDNA assay confirmed this. The results from two other samples suggest populations of Westslope Cutthroat Trout persist in an area where there is a limited understanding of the distribution of the species. A sample from Tough Creek near the mouth with Lee Creek and a sample further downstream on Lee Creek at the hamlet of Beazer both had Westslope Cutthroat Trout genetic material detected. The eDNA sample collected from Evan-Thomas Creek, a tributary to the Kananaskis River with documented Westslope Cutthroat Trout present as recently as 2015 (Mayhood 2017) did not have genetic material from this species detected. Genetic material from Bull Trout was not detected in Pincher Creek on the Piikani Reserve near Brocket, however it was detected further upstream of the town of Pincher Creek near the Alberta Ranch. Similarly, genetic material from Bull Trout was not detected in the St. Mary River at Hwy 5 on the Kainai Reserve but was very weakly detected (1 detection out of 24 technical replicates) further upstream at the Kimball bridge (Hwy 501).

No genetic material from any fish was detected in Crowsnest Creek above the falls. This watershed is suspected to have been originally occupied by Bull Trout that used the creek for spawning (Fitch 1997), and Westslope Cutthroat Trout were still present in the creek in the 1970's (Fitch 1978). However, both species have not been observed for decades and the site was sampled as a field-based negative control as a QA/QC measure to check for cross contamination between samples from different sites.

No AEP loadform currently exists for submission of eDNA results. Therefore, in addition to Appendix A and B (DFO eDNA Reporting Template and eDNA Assay Technical Bulletins) all raw eDNA results are submitted to DFO with this report in a Microsoft Excel spreadsheet.

3.4 Human Impact Mitigation

Riparian habitat restoration activities all focused on revegetating bare ground that is part of the 30 m buffer designated as Westslope Cutthroat Trout and Bull Trout critical habitat (DFO 2019, DFO 2020). The bare ground was the result of off-highway vehicle use, recreational vehicle camping, and livestock grazing within this area. The Project restored riparian areas at specific sites that AEP was discouraging or legally prohibiting motorized access. All sites were selected by the Project partners (e.g., Cows and Fish, Oldman Watershed Council, Trout Unlimited) with approval by AEP. While the ability to exclude grazing on the sites does not exist currently, planting woody vegetation (i.e., willow stacking) and covering sites with woody debris is also intended to discourage heavy use of the sites by livestock. During the spawning surveys, additional sites where riparian habitat restoration is needed were also identified. Some examples of these sites are provided (Table 3.3).

Table 3.2 Year 1 eDNA detections from 3 field and 24 lab replicates.

Watercourse Location	Assay results[1]											
	eFish			Bull Trout			Westslope Cutthroat			Rainbow Trout		
Field replicate	A	B	C	A	B	C	A	B	C	A	B	C
Oldman River at Cache Creek	8/8	8/8	8/8	0/8	0/8	0/8	8/8	8/8	8/8	0/8	0/8	0/8
Shale Creek upstream of road	7/8	8/8	8/8	0/8	0/8	0/8	**8/8**	**8/8**	**8/8**	0/8	0/8	0/8
Pincher Creek near Brocket	8/8	8/8	8/8	**0/8**	**0/8**	**0/8**	1/8	2/8	0/8	8/8	7/8	5/8
Pincher Creek at Alberta Ranch	8/8	8/8	8/8	**1/8**	**2/8**	**8/8**	4/8	8/8	8/8	8/8	8/8	8/8
Chipman Creek Tributary	8/8	8/8	8/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Payne Lake outlet canal	8/8	8/8	8/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Tough Creek at Pole Haven entrance	8/8	8/8	8/8	0/8	0/8	0/8	**8/8**	**8/8**	**8/8**	4/8	3/8	3/8
Lee Creek at Beazer	8/8	8/8	8/8	0/8	0/8	0/8	0/8	2/8	3/8	**8/8**	**8/8**	**8/8**
Evan-Thomas Creek above Hwy 40	2/8	5/8	6/8	0/8	0/8	0/8	**0/8**	**0/8**	**0/8**	0/8	0/8	0/8
Lyons Creek at Sartoris Road	8/8	8/8	8/8	0/8	0/8	0/8	0/8	0/8	0/8	8/8	8/8	8/8
Crowsnest Creek above falls	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
St. Mary River at Kimball bridge	8/8	8/8	8/8	**0/8**	**0/8**	**1/8**	0/8	0/8	0/8	0/8	0/8	0/8
St. Mary River at Hwy 5	8/8	8/8	8/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8

[1] Green = confirmed detection of DNA from target. Orange = suspected detection. See Appendix A and Hobbs (2021) for how field and lab replicate results were interpreted.

Table 3.3 Sites with riparian restoration needs identified during spawning surveys

Watercourse	Lat/Long	Location	Site Description
Beaver Creek	49.858277, -113.978085	Furthest upstream crossing on the Beaver Creek Road	Random camping area within 30 m of habitat where Westslope Cutthroat Trout were observed spawning
Hidden Creek	49.978518 -114.516911	North bank, half way between old bridge crossing and the falls	Groundwater intercepted and creating sedimentation on an undesignated off-highway vehicle trail within 30 m of where Bull Trout observed spawning
Dutch Creek	49.891183 -114.634862, 49.891201 -114.633830, 49.887707 -114.626638	The creek is crossed three times by the North Fork Pass trail	Off-highway vehicles fording creek on a designated trail open during spawning season (AEP 2021), sedimentation of Bull Trout spawning habitat observed
Racehorse Creek	49.816150, -114.527322	The pipeline crosses the creek upstream of North Racehorse Creek confluence	Abandoned pipeline right-of-way used as an undesignated off-highway vehicle trail, sedimentation of Bull Trout spawning habitat likely occurring

3.5 Indigenous Knowledge Documentation, Management, and Use

Responses to the interview questions Elliot Fox asked the six Blackfoot elders are summarized in a separate report. All six elders did have knowledge of Blackfoot traditional use of fish for food, including some references to native trout, methods used to catch fish, and locations they were caught. Water is very significant to Blackfoot people, both spiritually and physically as a source of life. The Blackfoot place names for fish, rivers, and mountains throughout the East Slopes show the significance of the area to their culture.

4 Summary and Recommendations

4.1 Summary

The Blackfoot Confederacy's Native Trout Recovery Project developed after aquatic SAR were prioritized by the Aohkii (Water) working group. Year 1 established the purpose of the Project as both building Blackfoot capacity and expertise as guardians of East Slope watershed integrity and gathering Blackfoot values and holistic ways of knowing about the study area, water, and native trout. Westslope Cutthroat Trout and Bull Trout were the focus of the Project for two reasons. Firstly, the legal status of these species under SARA makes them eligible for AFSAR funding. Secondly, these species are two examples of numerous native fish species in the Blackfoot territory that are significant aquatic biocultural artifacts that the Blackfoot seek to conserve and restore. The Bow and Oldman watersheds were selected as the study area because they are central to Blackfoot territory and are the only place where both Westslope Cutthroat Trout and Bull Trout occur together as native trout in Alberta.

A strong team was established in Year 1 of the Project, with Kimmy Houle as Project Manager, and Elliot Fox as Assistant Project Manager. The three Alberta Blackfoot Nations provided field technicians to participate and training and mentorship through the Project. This was a significant step forward for the Blackfoot Confederacy in terms of building capacity to work on native trout recovery and engaging the Blackfoot Nation staff in the process.

All the objectives for the Project that were set in Year 1 were met. Outdoor classroom training covered watershed processes, native trout conservation, impacts of non-native trout, fish identification, importance of riparian and instream fish habitat, spawning behaviour, habitat assessment using eDNA, stream temperature data loggers, spawning surveys, and riparian habitat restoration. An elder from Piikani Nation provided context for the training by reviewing integrated Blackfoot connections and knowledge of the East Slopes environment. A tour of local watershed restoration efforts took place, and there was hands on training in temperature monitoring and eDNA sampling. Through a total of 20 days conducting community-based habitat assessment and restoration work with technical experts, the field technicians then received considerable hands-on mentorship following up on the initial training topics. Elders from all four Blackfoot Nations were also interviewed. Considerable TEK that shows a strong Blackfoot connection to the East Slopes, native trout, and water was documented.

Spawning surveys (redd counts) identified Westslope Cutthroat Trout spawning habitat that is at risk of riparian damage in Beaver Creek, which flows into the Piikani Nation. Similarly threatened Bull Trout spawning habitat was identified upstream of Piikani Nation in the upper Oldman (Napi's Playground) on six streams where cumulative effects of many different land uses are altering watershed processes and encroaching on riparian areas (See Ecohydrological Assessment of the Upper Oldman River – 2020/2021 Habitat Stewardship Program Report, DFO CA No. 2020-HSP-C&A-004). An isolated resident population of Bull Trout threatened by invasive non-native Brook Trout and habitat fragmentation from irrigation dams was also monitored with redd counts upstream of the Blood Tribe Timber Limit on the North Belly River. Overall, all the redd surveys highlighted were spawning habitat occurs so future riparian restoration and human impact mitigation activities can focus on these areas. There was no analysis of redd numbers in 2020 relative to data from previous years. Some spawning habitat was apparently surveyed for the first time, confirming spawning in reaches of stream where it was previously only suspected and establishing a point of reference for future spawning surveys.

Stream temperature monitoring focused on thermal suitability of Bull Trout habitat on streams affected by irrigation dams and diversions. Temperature monitoring also occurred on one Westslope Cutthroat Trout stream where riparian habitat degradation and hybridization with Rainbow Trout are threats. In total, six new temperature loggers were installed, and data was downloaded from four additional loggers previously installed by AEP. Overall, the data collected was limited in Year 1. Additional years of temperature

monitoring will be necessary to understand how stream temperature is affecting native trout in each specific stream.

Sampling eDNA identified one previously undocumented Westslope Cutthroat Trout population in Shale Creek in the upper Oldman watershed, isolated above a culvert that is at least a partial barrier to fish movement. It also suggested that no Westslope Cutthroat Trout remain in Evan-Thomas Creek, a population recently suspected to have been extirpated. Bull Trout eDNA was detected upstream of the town of Pincher Creek, but not in Pincher Creek near the confluence with the Oldman River on the Piikani Reserve. Additional eDNA sampling is needed throughout the migration period to determine if Bull Trout from the Oldman River migrate upstream to spawn in Pincher Creek. Bull Trout eDNA was suspected to have been detected upstream of the town of Cardston on the St. Mary River, but was not detected in Lee Creek or downstream of Cardston on the St. Mary River. As for Pincher Creek, additional sampling is needed throughout the migration period to determine if Bull Trout in the St. Mary River migrate as far downstream as Lee Creek and move up Lee Creek to spawn in its headwaters.

Riparian habitat restoration activities all focused on revegetating bare ground that is part of the 30 m buffer designated as Westslope Cutthroat Trout and Bull Trout critical habitat (DFO 2019, DFO 2020). Both classroom and field-based training was provided. Expert practitioners led the Blackfoot staff in willow staking and bioengineering with wattle fences and willow fascines.

4.2 Recommendations

The following recommendations are made for the Project as it continues:

1. Engage with more Blackfoot Nation staff to build more capacity through continued training and mentorship as in Year 1. Include staff from the Consultation offices who work as liaisons with government and industry for projects within Blackfoot territory off reserves.
2. Incorporate TEK from Year 1 into training and habitat assessment and restoration activities.
3. Expand habitat assessment and restoration areas to areas that are a priority for the BFC Nations.
4. Assess more spawning habitat with redd surveys in areas with limited or a lack of recent data.
5. Further assess habitat fragmentation at the downstream limits of occupied Bull Trout habitat. Use eDNA and stream temperature to evaluate the effects of dams and weirs and changes in water quantity and quality resulting from irrigation and riparian damage. Establish more monitoring sites and sample eDNA more frequently throughout the Bull Trout migration period.
6. Assess habitat fragmentation on more headwater streams by sampling eDNA upstream of culvert crossings to evaluate opportunities to restore fish passage.
7. Evaluate whether Rainbow Trout invasion of pure Westslope Cutthroat Trout populations is occurring using eDNA sampling.
8. Restore more riparian habitat using willow staking and bioengineering techniques.
9. Relocate Bull Trout rescued from irrigation systems to areas where these fish can spawn.

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Appendix A DFO eDNA Reporting Template

eDNA Reporting Template

I. eDNA Testing Sample Submission Information

Report Title:	2020-2021 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			
Project Number:	Contribution Agreement Number: 2020AFSAR6010-01 (Year 1 of 3)		**Date of Final Reporting:** 2021-06-28	
Service Provider Information	Type:	Academia	Requesting Organization Information	Organization Name:
	Contact Name: Dr. Caren Helbing			Contact Name: Dr. Caren Helbing
	Address: Department of Biochemistry & Microbiology, University of Victoria			Contact Phone: (250)721-6146
	Contact Phone: (250)721-6146			Contact Email: chelbing@uvic.ca
	Contact Email: chelbing@uvic.ca			
	LAB ACCREDITATION / CERTIFICATION:			

Executive Summary - Study Objectives, Rationale, and Main Finding(s) derived from both eDNA samples and controls

See attached report

Appendices (Required)	Check to confirm inclusion		**Appendices (Additional)**
Appendix 1: Maps of the study sites and sampling locations	☑		*Appendix 5:*
Appendix 2: Contamination prevention procedures	☑		*Appendix 6:*
Appendix 3: qPCR protocol	☑		*Appendix 7:*
Appendix 4: Metadata and qPCR data	☑		*Appendix 8:*

II. Study Design and eDNA Sampling

A. Study information	*A.1 Species targeted (common and Latin):*	Westslope Cutthroat Trout (Oncorhynchus clarkii lewisi), Bull Trout (Salvelinus fontinalis), Rainbow Trout (Oncorhynchus mykis	
	A.2 Study objectives:	Species Detection for Conservation	
	A.3 Geographic location and/or region:	See attached report	
	A.4 Sampling date (range):	Start: 2020-08-12	Finish: 2020-11-03
	A.5 Sample types:	Freshwater	
	A.6 Mapping databases (list all):		
B. Study design	*B.1 Type(s) of ecosystem:*	River	
	B.2 Sampling design (how does sampling optimize species detection for study goal?):	Sampling was conducted on streams where the target species are known to occur at sites where current occupancy was unknown. Timing of sampling was dictated by the Project schedule more than to optimize detection. Sampling occurred during low flows in late summer and fall.	
	B.3 Number of sites sampled:	11 streams	
	B.4 Number of stations sampled within sites (add explanation for variation among sites):	13 stations (two streams, Pincher Creek and St. Mary River had two stations)	
	B.5 Number of field sample replicates:	3 replicate samples of 1L from each station	
	B.6 Time series (number of times sites and stations were sampled):	Once	
	B.7 Environmental conditions, relevant observations, and additional field data:	Water quality was not recorded, samples were not collected following any significant precipitation events	
	B.8 Field blanks and field controls (describe and give numbers):	Field Blanks: Distilled water negative controls (5). Field positive controls: 3 replicate environmental samples from a station where the target organism is known to occur. 1 station per target species.	
C. eDNA sample collection	*C.1 Env. sample collection method:*	Select From Dropdown	
	C.2 Volume / weight sampled:	1L of water per field replicate	
	C.3 Sample depth(s):	~0-30 cm	
	C.4 Field sample storage/time to processing:	Samples put on ice in cooler immediately to avoid UV exposure. Samples filtered within 12 hours after collection.	
	C.5 Sample processing method (list disposable equipment; preservative used):	Vacuum Pump	
	C.6 Filter type and pore size:	Cellulose nitrate 0.45µm pore size 100mL filter (STERLITECH)	
	C.7 Sample preservation:	Desiccant	

For instructions on how to fill out the reporting template, refer to the following DFO research document: 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.

III. eDNA Sample Analysis - Laboratory Methods		
D. DNA extraction	*D.1 Name of commercial kit or protocol:*	QIAGEN, DNeasy Blood and Tissue Kit, CAT# 69504
	D.2 Reference protocol:	Hobbs et al., 2019, DOI:10.1371/journal.pone.0213849
	D.3 DNA extraction controls:	Negative control: Distilled water samples, one per day of filtration. Positive control: IntegritE-DNA assay for successful DNA extract
	D.4 Proportion of total sample:	DNA is extracted from 1/4 of the filter membrane.
	D.5 DNA elution volume:	150 µL Ultrapure DNase free water.
	D.6 Extracted eDNA storage conditions:	Silica desiccant and storage at -20 degrees C. Allison et al., 2021, DOI:10.1186/s13104-021-05530-x)
E. qPCR assay	*E.1 Assay Name:*	eFish1: Klymus et al., 2019, DOI:10.1002/edn3.29. eSACO3: Dysthe et al. 2018, DOI:10.1002/ece3.3898, run conditions modified by the Helbing lab. eONCL4/eONMY5: Helbing, unpublished
	E.2 Assay Type:	qPCR (Probe Based)
	E.3 Level of assay validation:	Level 5 (Operational)
	E.4 Specificity data:	Refer to technical bulletins for each assay
	E.5 Dilution and volume of DNA used:	2 µL undiluted DNA per reaction.
	E.6 qPCR positive and negative controls:	Positive controls: Synthetic DNA fragments at 20 copies/reaction, in duplicate per qPCR plate. Negative controls: No template controls (NTC) of PCR grade water, 8 replicates per qPCR plate.
	E.7 Technical replicates per sample:	8 technical replicates per target taxa assay per sample; 4 technical replicates per sample for IntegritE-DNA for QC.
	E.8 Inhibition tests:	The IntegritE-DNA assay is done on every sample. It uses endogenous chloroplast DNA to indicate sample inhibition or degradation. It also acts as an extraction control. We require 4/4 technical replicates to pass at a Cq <27. If a sample does not pass, we use an inhibitor removal kit (CedarLane CAT#D6030) and retest with the IntegritE-DNA assay. If sample continues to fail, inhibition or degradation are suspected. Typically most distilled water samples test negative. Hobbs et al. 2019. 2020 DOI:10.1371/journal.pone.0213849: DOI:10.1002/edn3.82
	E.9 Number of qPCR cycles:	50

IV. Summary of eDNA Results		
F. Reporting control results	*F.1 Criteria to determine if controls passed or failed:*	Field negative controls: A pass is 0/8 technical replicates. Field positive controls: 3 replicate environmental samples collected per station where the organism is known to occur. A pass is at least one of the replicates detecting ≥ 2/8 technical replicates. Extraction controls: see D.3. Inhibition, degradation, and extraction controls: see E.8. qPCR positive controls: Synthetic DNA at 20 copies/reaction, 2/2 technical replicates required to pass per plate. qPCR negative controls: Distilled water, 0/8 technical replicates required to pass per plate.
	F.2 Positive control results (report each type separately):	qPCR positive controls: IntegritE-DNA: 3/3 96-well plates passed, 6/6 technical replicates. eFish1: 1/1 plate passed, 2/2 technical replicates. eONCL4: 3/3 plates passed, 6/6 technical replicates. eSACO3: 3/3 plates passed, 6/6 technical replicates. Field positive controls: number of field positive stations per species assay.
	F.3 Negative control results (report each type separately):	qPCR negative controls: IntegritE-DNA: 3/3 96-well plates passed, 24/24 technical replicates. eFish1: 1/1 plate passed, 8/8 technical replicates. eONCL4: 3/3 plates passed, 24/24 technical replicates. eSACO3: 3/3 plates passed, 24/24 technical replicates. Field negative controls: 5/5 distilled water samples passed.
	F.4 Failed controls (report and explain):	None.
G. Reporting eDNA sample results	*G.1 Calculated LOD:*	eFish1: LOD 0.9 copies/reaction (CI95%: 0.65-1.48). Lesperance et al, 2021, DOI:10.1002/edn3.220.
	G.2 QA/QC qPCR results:	IntegritE-DNA: 156/156 positive detections/total technical replicates, eFish1: 0/24 , eONCL4: 78/192, eSACO3: 12/192.
	G.3 Other qPCR results:	IntegritE-DNA: 0/156 negative detections/total technical replicates, eFish1: 24/24 , eONCL4: 114/192, eSACO3: 180/192.
	G.4 Determination of sample-level results:	Criteria for sample-level results: 0/8 technical replicates is negative, 1/8 is suspected, ≥ 2/8 is positive. IntegritE-DNA: 39/39 samples were positive, eFish1: 24/24 samples were positive, eONCL4: 2/24 samples were positive, eSACO3: 2/24 samples were positive, 2/24 samples were suspected.
	G.5 Determination of station-level results:	Criteria for station-level results: Positive = at least 1/3 station sample replicates is 2/8 or higher. Suspected if only one sample at the station = 1/8. If 2+ samples from a station = 1/8, the station is considered positive. If all samples at a station are negative, the station is negative. eFish1: 0/1 station was positive, eONCL4: 4/8 stations were positive, eSACO3: 1/8 stations were positive, 1/8 stations were suspected.
	G.6 Determination of site-level results:	This data set only included station-level sampling.
H. Closing statements	*H.1 Disclaimer (any additional information to help explain results for any samples, stations, or sites):*	See attached report
	H.2 Summary of eDNA detection:	See attached report
	H.3 Future recommendations:	See attached report

For instructions on how to fill out the reporting template, refer to the following DFO research document: 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.

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.

General eDNA Assay Information

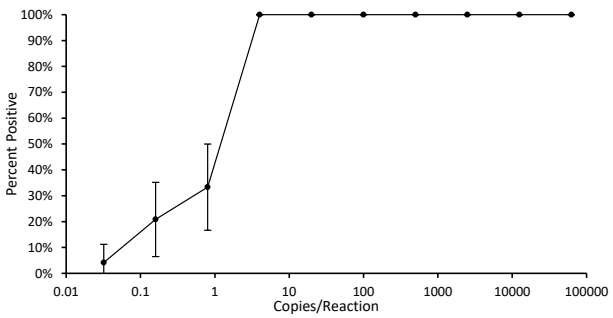
Target Species: Cutthroat Trout *(Oncorhynchus clarkii)*
Species Abbreviation: ONCL
eDNA qPCR Tool: eONCL4
eDNA qPCR Format: TaqMan

eDNA Assay Specificity Tests

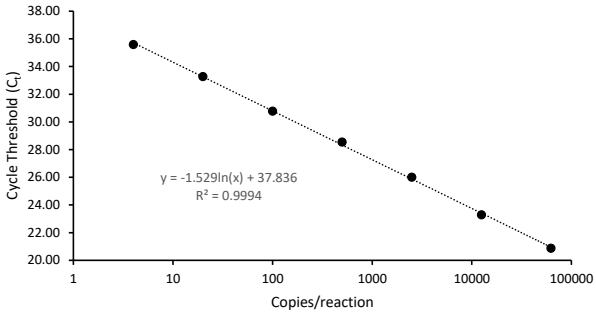
Species:	ONTS	ONKI	ONNE	ONGO	ONKE	ONMY	ONCL	SASA	HOSA	NTC
Detection:	No	No	No	No	No	No	Yes	No	No	No

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=25

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA



>100 copies/reaction were tested with n=8 technical replicates.
≤100 copies/reaction were tested with n=24 technical replicates.



The relationship between Cycle Threshold and Copy Number does not necessarily remain linear when fewer than 100% of technical replicates are positive.

Abbreviations

eDNA	environmental DNA
gDNA	Total Genomic DNA extracted from voucher specimen tissue or swabs
HOSA	Human (*Homo sapiens*)
NTC	qPCR no template control
ONCL	Cuttthroat Trout *(Oncorhynchus clarkii)*
ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*
ONKE	Chum Salmon *(Oncorhynchus keta)*
ONKI	Coho Salmon (*Oncorhynchus kisutch)*
ONMY	Rainbow Trout *(Oncorhynchus mykiss)*
ONNE	Sockeye Salmon *(Oncorhynchus nerka)*
ONTS	Chinook Salmon(*Oncorhynchus tschawytscha*)
qPCR	quantitative real-time polymerase chain reaction
SASA	Atlantic salmon (*Salmo salar*)

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. 2020; 00: 1– 12. 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. Klymus, KE, Merkes, CM, Allison, MJ, Goldberg, CS, Helbing, CC, Hunter, ME, Jackson, CA, Lance, RF, Mangan, AM, Monroe, EM, Piaggio, AJ, Stokdyk, JP, Wilson, CC, Richter, CA (2019) Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA. 2019; 00: 1– 12. https://doi.org/10.1002/edn3.29

4. Veldhoen N, Hobbs J, Ikonomou G, Hii M, Lesperance M, Helbing, CC (2016) Implementation of novel design features for qPCR-based eDNA assessment. PLOS ONE 11(11): e0164907. https://doi.org/10.1371/journal.pone.0164907

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.

General eDNA Assay Information

Target Species: Bull Trout (*Salvelinus confluentus*)
Species Abbreviation: SACO
eDNA qPCR Tool: eSACO3
eDNA qPCR Format: TaqMan

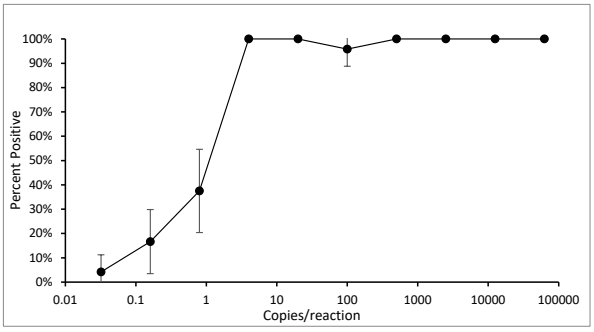
eDNA Assay Specificity Tests

Species:	SACO	COCO	ESLU	HOSA	MIDO	MISA	ONCL	ONGO	ONKE	ONKI
Detection:	Yes	No	No*	No	No	No	No	No	No	No

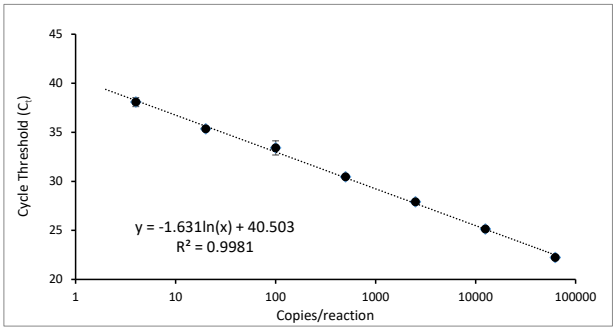
Species:	ONMY	ONNE	ONTS	PRCY	SAMA	SAFO	SASA	THAR	THPA	NTC
Detection:	No	No	No	No	No	No	No	No	No	No

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=25
*This tool weakly detects *Esox lucius* DNA (1/25 replicates). Unlikely to contribute to field-relevant positive results.

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA



>100 copies/reaction were tested with n=8 technical replicates.
≤100 copies/reaction were tested with n=24 technical replicates.



The relationship between Cycle Threshold and Copy Number does not necessarily remain linear when fewer than 100% of technical replicates are positive.

Abbreviations

COCO	Slimy Sculpin (*Cottus cognatus)*
eDNA	environmental DNA
ESLU	Northern Pike (*Esox lucius*)
gDNA	Total Genomic DNA extracted from voucher specimen tissue or swabs
HOSA	Human (*Homo sapiens*)
MIDO	Smallmouth Bass *(Micropterus dolomieu)*
MISA	Largemouth Bass *(Micropterus salmoides)*
NTC	qPCR no template control
ONCL	Cutthroat Trout - Westslope and Coastal (*Oncorhynchus clarki lewisi, O. clarkii clarkii)*
ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*
ONKE	Chum Salmon *(Oncorhynchus keta)*
ONKI	Coho Salmon (*Oncorhynchus kisutch)*
ONMY	Rainbow Trout *(Oncorhynchus mykiss)*
ONNE	Sockeye Salmon *(Oncorhynchus nerka)*
ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)
PRCY	Round Whitefish (P*rosopium cylindraceum)*
qPCR	quantitative real-time polymerase chain reaction
SACO	Bull Trout (*Salvelinus confluentus*)
SAFO	Brook Trout (*Salvelinus fontinalis*)
SAMA	Dolly Varden (*Salvelinus malma)*
SASA	Atlantic Salmon (*Salmo Salar)*
THAR	Arctic Grayling *(Thymallus arcticus)*
THPA	Eulachon (*Thaleichthys pacificus)*

References

1. 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. PLOS ONE. http://doi.org/10.1371/journal.pone.0206851

2. 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. 2020; 00: 1– 12. https://doi.org/10.1002/edn3.82

3. 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

4. Klymus, KE, Merkes, CM, Allison, MJ, Goldberg, CS, Helbing, CC, Hunter, ME, Jackson, CA, Lance, RF, Mangan, AM, Monroe, EM, Piaggio, AJ, Stokdyk, JP, Wilson, CC, Richter, CA (2019) Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA. 2019; 00: 1– 12. https://doi.org/10.1002/edn3.29

5. Veldhoen N, Hobbs J, Ikonomou G, Hii M, Lesperance M, Helbing, CC (2016) Implementation of novel design features for qPCR-based eDNA assessment. PLOS ONE 11(11): e0164907. https://doi.org/10.1371/journal.pone.0164907

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.

General eDNA Assay Information

Target Species:	Rainbow Trout (*Oncorhynchus mykiss*)
Species Abbreviation:	ONMY
eDNA qPCR Tool:	eONMY5
eDNA qPCR Format:	TaqMan

eDNA Assay Specificity Tests

Species:	ONMY	CAFA	COCO	HOSA	MIDO	MISA	ONCL	ONGO	ONKE
Detection:	Yes	No	No	No	No	No	No	No	No

Species:	ONKI	ONNE	ONTS	SACO	SAFO	SAMA	THAR	NTC
Detection:	No	No	No	No	No	No	No	No

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=25

Abbreviations

CAFA	Dog (*Canis familiaris*)
COCO	Slimy sculpin (*Cottus cognatus*)
eDNA	environmental DNA
gDNA	Total Genomic DNA extracted from voucher specimen tissue or swabs
HOSA	Human (*Homo sapiens*)
MIDO	Smallmouth Bass *(Micropterus dolomieu)*
MISA	Largemouth Bass *(Micropterus salmoides)*
NTC	qPCR no template control
ONCL	Cutthroat Trout - Westslope and Coastal (*Oncorhynchus clarki lewisi, O. clarkii clarkii)*
ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*
ONKE	Chum Salmon (*Oncorhynchus keta*)
ONKI	Coho Salmon (*Oncorhynchus kisutch*)
ONMY	Rainbow Trout (*Oncorhynchus mykiss*)
ONNE	Sockeye Salmon (*Oncorhynchus nerka*)
ONTS	Chinook Salmon (*Oncorhynchus tshawytscha*)
qPCR	quantitative real-time polymerase chain reaction
SACO	Bull Trout (*Salvelinus confluentus*)
SAFO	Brook Trout (*Salvelinus fontinalis*)
SAMA	Dolly Varden (*Salvelinus malma*)
THAR	Arctic Grayling (*Thymallus arcticus*)

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. 2020; 00: 1– 12.
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. Klymus, KE, Merkes, CM, Allison, MJ, Goldberg, CS, Helbing, CC, Hunter, ME, Jackson, CA, Lance, RF, Mangan, AM, Monroe, EM, Piaggio, AJ, Stokdyk, JP, Wilson, CC, Richter, CA (2019) Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA. 2019; 00: 1– 12.
4. Veldhoen N, Hobbs J, Ikonomou G, Hii M, Lesperance M, Helbing, CC (2016) Implementation of novel design features for qPCR-based eDNA assessment. PLOS ONE 11(11): e0164907. https://doi.org/10.1371/journal.pone.0164907

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.

General eDNA Assay Information

Target Species: Various Fish Species
Species Abbreviation: Fish
eDNA qPCR Tool: eFish1
eDNA qPCR Format: TaqMan

eDNA Assay Specificity Tests

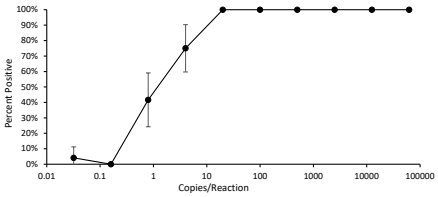
Species:	ANRO	COCO	ESLU	HOSA	LICA	MIDO	MISA	ONCL	ONGO	ONKE	ONKI	ONMY	ONNE	ONTS	PRCY	SACO	SAMA	SASA	THAR	THPA	NTC
Detection (n=25):	Yes	Yes	Yes	No	No	Yes*	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes*	No

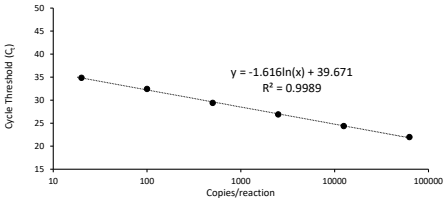
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=25

Species:	AMMA	AMTI	ANBO	ANVA	ASMO	ASTR	COFL	DRBU	DRPO	ENES	LICL	LIPI	LISY	PSMA	RAAU	RACA	RALU	RAPR	SOBE	SPIN	TAGR
Detection (n=2):	Yes**	No	No	No	Yes**	Yes **	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes**	No

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA. Technical replicates: n=2
*This tool weakly detects Smallmouth Bass and Eulachon DNA
**This tool may detect Long-toed Salamander, Rocky Mountain Tailed Frog, Coastal Tailed Frog, and Great Basin Spadefoot DNA if present

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA





>100 copies/reaction were tested with n=8 technical replicates.
≤100 copies/reaction were tested with n=24 technical replicates.
The eFish1 gBlocks sequence is based on Chinook Salmon (Oncorhynchus tschawytscha)

The relationship between Cycle Threshold and Copy Number does not necessarily remain linear when fewer than 100% of technical replicates are positive.

eDNA Assay Sensitivity Test using tissue-derived gDNA

COCO

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	96%	7%
0.2	32%	16%
0.04	8%	10%
0.008	0%	0%
0	0%	0%

ONCL

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	20%	14%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONGO

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	100%	0%
0.2	100%	0%
0.04	92%	10%
0.008	20%	14%
0	0%	0%

ONKE

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	100%	0%
0.2	40%	17%
0.04	16%	13%
0.008	0%	0%
0	0%	0%

ONKI

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	40%	17%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONMY

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	88%	11%
0.2	36%	17%
0.04	16%	13%
0.008	0%	0%
0	0%	0%

ONNE

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	4%	7%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

ONTS

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	64%	17%
0.2	20%	14%
0.04	0%	0%
0.008	4%	7%
0	0%	0%

PRCY

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	4%	7%
0.2	0%	0%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

SASA

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	96%	7%
1	28%	16%
0.2	12%	11%
0.04	0%	0%
0.008	0%	0%
0	0%	0%

THAR

DNA (µg/L)	Detection Frequency (n=25)	Binomial Standard error (n=8)
5	100%	0%
1	100%	0%
0.2	68%	16%
0.04	20%	14%
0.008	8%	10%
0	0%	0%

Abbreviations

AMMA	Long-toed Salamander (*Ambystoma macrodactylum*)
AMTI	Tiger salamander (*Ambystoma tigrinum*)
ANBO	Western Toad (*Anaxyrus (Bufo) boreas*)
ANRO	American Eel *(Anguilla rostrata)*
ANVA	Wandering Salamander (*Aneides vagran* s)
ASMO	Rocky Mountain Tailed Frog (*Ascaphus montanus*)
ASTR	Pacific Tailed frog (*Ascaphus truei*)
COCO	Slimy Sculpin (*cottus cognatus)*
COFL	Asian Clam (*Corbicula fluminea*)
DRBU	Quagga Mussel (*Dreissena bugensis*)
DRPO	Zebra Mussel (*Dreissena polymorpha*)
eDNA	environmental DNA
ENES	Ensatina (*Ensatina eschscholtzii*)
ESLU	Northern Pike *(Esox lucius)*
gDNA	Total Genomic DNA extracted from voucher specimen tissue or swabs
HOSA	Human (*Homo sapiens*)
LICA	American Bullfrog *(Lithobates(Rana) catesbeiana)*
LICL	Green Frog (*Lithobates (Rana) clamitans*)
LIPI	Northern Leopard Frog (*Lithobates (Rana) pipiens*)
LISY	Wood Frog (*Lithobates sylvaticus*)
MIDO	Smallmouth Bass *(Micropterus dolomieu)*
MISA	Largemouth Bass *(Micropterus salmoides)*
NTC	qPCR no template control
ONCL	Cuttthroat Trout *(Oncorhynchus clarkii)*
ONGO	Pink Salmon *(Oncorhynchus gorbuscha)*
ONKE	Chum Salmon *(Oncorhynchus keta)*
ONKI	Coho Salmon (*Oncorhynchus kisutch)*
ONMY	Rainbow Trout *(Oncorhynchus mykiss)*
ONNE	Sockeye Salmon *(Oncorhynchus nerka)*
ONTS	Chinook Salmon(*Oncorhynchus tschawytscha*)
PRCY	Round Whitefish (*prosopium cylindraceum)*
PSMA	Boreal chorus frog (*Pseudacris maculata*)
qPCR	quantitative real-time polymerase chain reaction
RAAU	Northern Red-legged Frog (*Rana aurora*)
RACA	Cascades Frog (*Rana cascadae*)
RALU	Columbia spotted frog (*Rana luteiventris*)
RAPR	Oregon Spotted Frog (*Rana pretiosa*)
SACO	Bull Trout *(Salvelinus confluentus)*
SAMA	Dolly Varden (*Salvelinus malma)*
SASA	Atlantic Salmon (*Salmo Salar)*
SOBE	Pacific Water Shrew (*Sorex bendirii*)
SPIN	Great Basin Spadefoot (*Spea intermontana*)
TAGR	Rough-skinned Newt (*Taricha granulosa*)
THAR	Arctic Grayling *(Thymallus arcticus)*
THPA	Eulachon *(Thaleichthys pacificus)*

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Appendix C Stream Temperature Datasheets

Alberta Government

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: 03/09/2020	**Waterbody:** BELLY RIVER		**Site ID: W18-** 152	**WBID:**	
Latitude:		**Longitude:**			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org:		**Crew Members:**			
Installation Method		**Data Logger Serial #**	20270766		
Status:	Install / (Field Check) / (Data Download) / Retrieval				
Field Check:	☑ Logger operational	☐ Logger with problems	**Logger Issue:** NA / (Out of water) / Vandalism / Buried / Lost / Other:		
24h Time:		**Memory Used (%):**	~22%	**Battery (%):**	100%

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- LOGGER OUT OF WATER (CHECKED @ 1130 hrs
- LAST CHECKED 10/22/2019 @ 1300 hrs
- Removed Logger/moved down stream in the middle of stream 3 meters down from old spot.
- Down stream from exposed boulder. Directly behind boulder.
- Left old rebar in old spot.

Site Photos: ☑ **Datasheet Photo:** ☑ **Data Logger Download:** ☑ **Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: 03/09/2020	**Waterbody:** Belly River		**Site ID: W18-** 103	**WBID:**	
Latitude: same		**Longitude:** same			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFC		**Crew Members:**	EF, BLM, AFR		
Installation Method	Rebar	**Data Logger Serial #**	20300013		
Status: Good	Install / (Field Check) / (Data Download) / Retrieval				
Field Check:	☑ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:		
24h Time:	1530h	**Memory Used (%):**	17%	**Battery (%):**	100%

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Found on RUB
- No trees or woody debris near logger
- ~250m DS of bridge
- D/S of aspen stand and fence line, 75m US on RUB

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Logger Download:** ☑ **Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

On Elliot Fox of phone or ipad Alvin's


W18-152

W18-103

Alberta Government

Date: 03/09/2020	**Waterbody:** Unnamed to Payne Lake		**Site ID: W18-** 418-154	**WBID:**	
Latitude:		**Longitude:**			
Site Access:	Truck / ATV / Heli / Other:				
Sampling Org:	BFC		**Crew Members:**	BFC (with Matt Coombs)	
Installation Method			**Data Logger Serial #**		
Status:	Install / (Field Check) / Data Download / Retrieval				
Field Check:	☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / (Lost) / Other:		
24h Time:		**Memory Used (%):**		**Battery (%):**	

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Unable to find, looked in small creek flowing into Payne Lake on the downstream side of the road
- Extensive cattle activity may have buried logger in mud

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: 03/09/2020	**Waterbody:** Payne Lake Outlet Canal		**Site ID: W18-** 153	**WBID:**	
Latitude:		**Longitude:**			
Site Access:	Truck / ATV / Heli / Other:				
Sampling Org:	BFC		**Crew Members:**	BFC (with Matt Coombs)	
Installation Method			**Data Logger Serial #**		
Status:	Install / (Field Check) / Data Download / Retrieval				
Field Check:	☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / (Lost) / Other:		
24h Time:		**Memory Used (%):**		**Battery (%):**	

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Unable to find, looked in canal upstream from road
- Steep banks prevented thorough search

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Alberta Government

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:	10/5/20	**Waterbody:**	BEAVER CREEK	**Site ID: W18-**	W20-006	**WBID:**	
Latitude:	49.62966 E	**Longitude:**	-113.7129 N				
Site Access:	(Truck) / ATV / Heli / Other:						
Sampling Org:	BFC-AFSAR	**Crew Members:**	MATT & ELLIOT				
Installation Method	REBAR	**Data Logger Serial #**	20300073				
Status:	(Install) / Field Check / Data Download / Retrieval						
Field Check:	☑ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:				
24h Time:	1530	**Memory Used (%):**	0	**Battery (%):**	100%		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- INSTALLED BETWEEN 2 CONCRETE SLABS (RIPRAP) ON LUB ~50cm DEEP
- INSTALLED ~20m D/S FROM CULVERT ON GRAVEL ROAD NEAR REGGIE ENGLISH RESIDENCE ON PIIKANI NATION
- ~1Km NORTH OF OLDMAN RIVER

Site Photos: ☑	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to acp.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:		**Waterbody:**		**Site ID: W18-**		**WBID:**	
Latitude:		**Longitude:**					
Site Access:	Truck / ATV / Heli / Other:						
Sampling Org:		**Crew Members:**					
Installation Method		**Data Logger Serial #**					
Status:	Install / Field Check / Data Download / Retrieval						
Field Check:	☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:				
24h Time:		**Memory Used (%):**		**Battery (%):**			

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to acp.wdsurveillance@gov.ab.ca

W20-006 LOOKING UPSTREAM

W20-006 LOOKING UPSTREAM


W20-006

Alberta Government

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:	Aug 26 '20	**Waterbody:**	Pincher Creek	**Site ID:** W20-	003
WBID:	1335				
Latitude:	49.405914	**Longitude:**	-114.016053		
Site Access:	(Truck) / ATV / Heli / Other:				
Sampling Org:	BFC	**Crew Members:**	Matt, Elliot, Kimmy, Blair, Alvin		
Installation Method	Rebar	**Data Logger Serial #**	20300119		
Status:	(Install) / Field Check / Data Download / Retrieval				
Field Check:	☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:		
24h Time:		**Memory Used (%):**		**Battery (%):**	

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- On left upstream bank ~100m upstream from Twp Rd 5-4, which was accessed from Hwy 6
- At base of ~10' tall cottonwood against the bank
- Logger is near the "Alberta Ranch"
- Twp Rd 5-4 is visible from logger location

Site Photos: ☑	**Datasheet Photo:** ☑	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:	Aug. 26-20	**Waterbody:**	Pincher Creek	**Site ID:** W18-	W20-001
WBID:	1335				
Latitude:	49.549599	**Longitude:**	-113.772003		
Site Access:	(Truck) / ATV / Heli / Other:				
Sampling Org:	BFC	**Crew Members:**	Kimmy, Blair, Matt, Elliot, Lance, Alvin		
Installation Method	Rebar	**Data Logger Serial #**	20300096		
Status:	(Install) / Field Check / Data Download / Retrieval				
Field Check:	☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:		
24h Time:		**Memory Used (%):**	0%	**Battery (%):**	100%

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- On left upstream bank ~500m upstream from Oldman River
- Accessed from High Bush area on road off Hwy 3
- Downstream of a large bolder underwater and larger bolders on shore
- 1-2m off left bank

Site Photos: ☐	**Datasheet Photo:** ☑	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

W20-003 LOOKING UPSTREAM

W20-001 LOOKING UPSTREAM

W20-001 LOOKING DOWNSTREAM

Alberta Government

Date: August 20, 2020	**Waterbody:** Oldman River		**Site ID: W18-** W18-082	**WBID:** 2166	
Latitude: 49.726243		**Longitude:** 114.082662			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFC		**Crew Members:** Matt, Elliot, Carleigh, Kimmy, Blair			
Installation Method Rebar		**Data Logger Serial #** 20300029			
Status: Install / Field Check / (Data Download) / Retrieval					
Field Check: ☑ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:			
24h Time: 15:10	**Memory Used (%):** 3%		**Battery (%):** Full		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

~10m D/S OF LOGGER IS AN OVERHANGING COTTONWOOD ON LEFT BANK;
COTTONWOOD IS ALIVE, BUT GROWING FROM OLD STUMP;
U/S FROM CREVICE ~150m ON RIGHT BANK
↳ large ravine

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: Aug 26/20	**Waterbody:** Oldman River		**Site ID: W18-** W18-002	**WBID:** 2166	
Latitude: N 49.552728		**Longitude:** W 113.763017			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFC		**Crew Members:** Blair, Alvin, Kimmy, Lance, Elliot, Matt			
Installation Method Epoxy		**Data Logger Serial #** 20300102			
Status: (Install) / Field Check / Data Download / Retrieval					
Field Check: ☐ Logger operational	☐ Logger with problems	**Logger Issue:** (NA) / Out of water / Vandalism / Buried / Lost / Other:			
24h Time: 10 AM	**Memory Used (%):** 0%		**Battery (%):** Full		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- 100 m upstream of parking lot in area known as High Bush
- windmills directly North
- 2 Big Boulders immediately upstream of logger
- on side channel → Left Upstream bank, ~830m downstream of Pincher Creek confluence
- loggers only 20cm below water surface at time of install

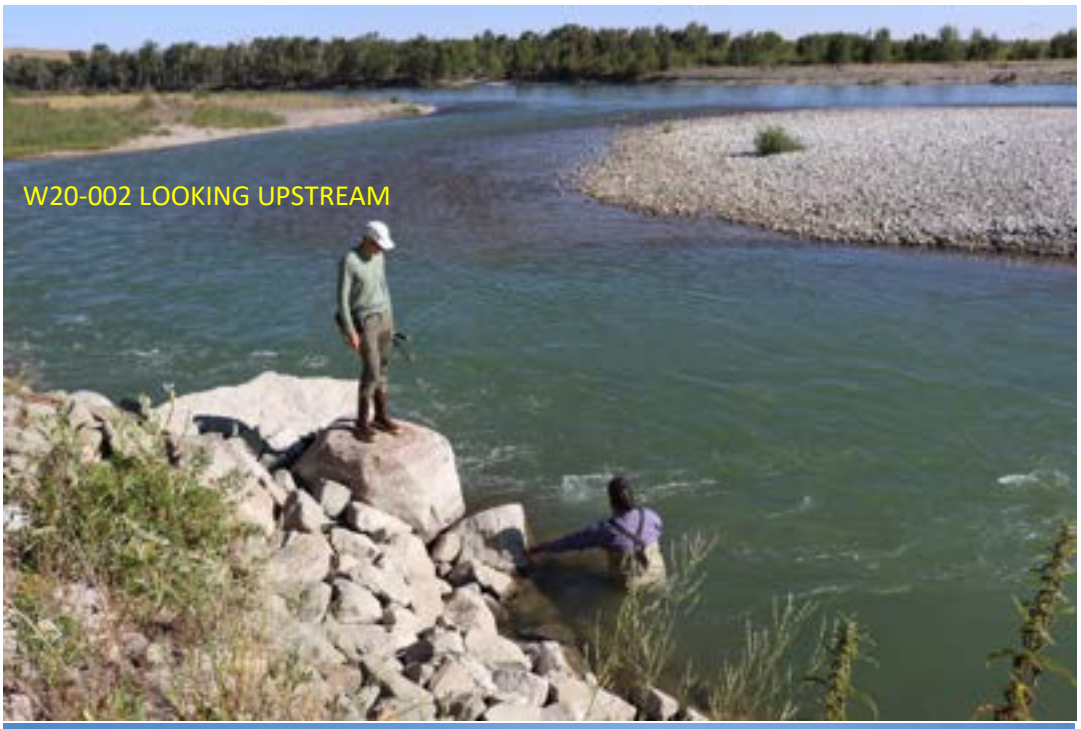
Site Photos: ☑	**Datasheet Photo:** ☑	**Data Logger Download:** ☐	**Data Submission:** ☐

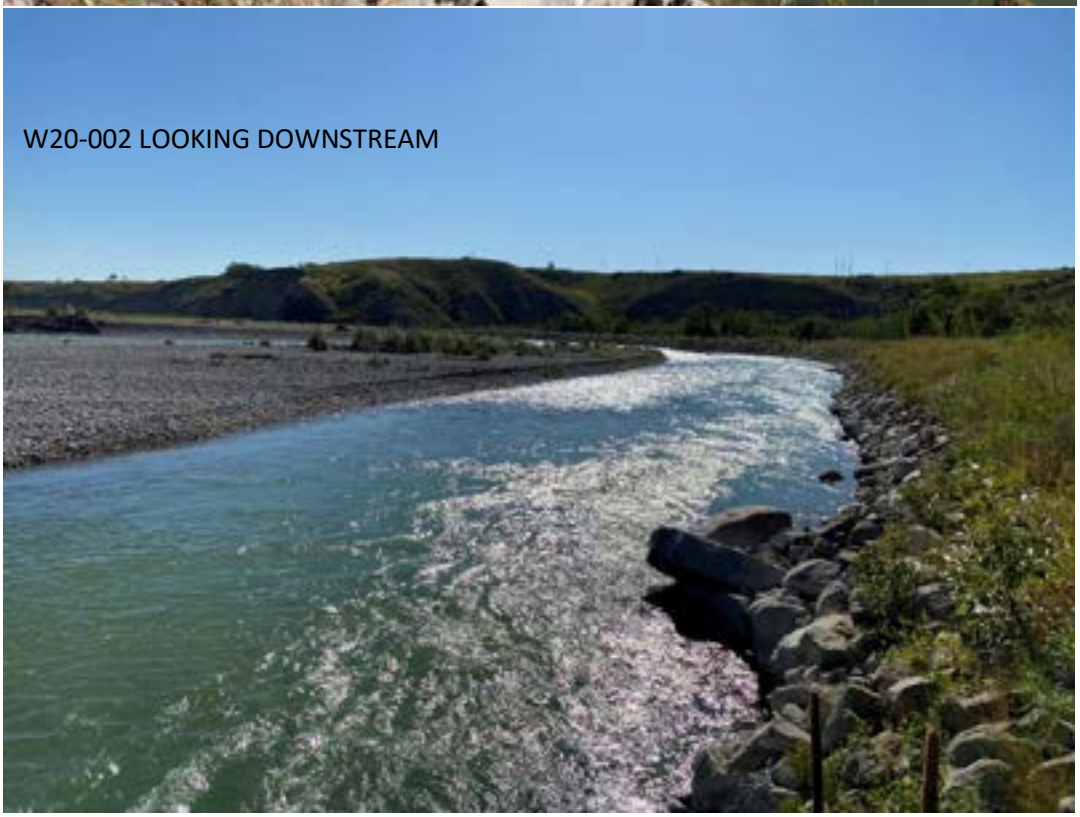
** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

↳ Logger had to be re-installed deeper in water on Jan 6, 2021

W18-082 LOOKING DOWNSTREAM

W18-082 LOOKING UPSTREAM


W20-002 LOOKING UPSTREAM


W20-002 LOOKING DOWNSTREAM

W20-002 LOOKING UPSTREAM

Alberta Government

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:	Nov 3, 2020	**Waterbody:**	St Mary River	**Site ID: W18-**	W20-004	**WBID:**	2232
Latitude:	49.224167°			**Longitude:**	-113.266111°		
Site Access:	(Truck) / ATV / Heli / Other:						
Sampling Org:	BFC			**Crew Members:**	MC, AFR, EF		
Installation Method	Epoxy			**Data Logger Serial #**	SN20300088		
Status:	(Install) / Field Check / Data Download / Retrieval						
Field Check:	□ Logger operational	□ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:				
24h Time:	1215h	**Memory Used (%):**	0%	**Battery (%):**	100%		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Epoxy glued to rip rap on right upstream bank where 3 cottonwood trunks a growing from the same stump
- on the upstream side of the Hwy 5 bridge on Blood Tribe lands
- logger is ~40m downstream from Lee Creek

Site Photos: ☐ **Datasheet Photo:** ☐ **Data Logger Download:** ☐ **Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:	Nov 3, 2020	**Waterbody:**	Lee Creek	**Site ID: W18-**	W20-005	**WBID:**	934
Latitude:	49.222778°			**Longitude:**	-113.266944°		
Site Access:	(Truck) / ATV / Heli / Other:						
Sampling Org:	BFC			**Crew Members:**	MC, AFR, EF		
Installation Method	Rebar			**Data Logger Serial #**	SN 20300085		
Status:	(Install) / Field Check / Data Download / Retrieval						
Field Check:	□ Logger operational	□ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:				
24h Time:	1230h	**Memory Used (%):**	0%	**Battery (%):**	100%		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Logger stated into bank horizontally below a large boulder on the left upstream bank
- Logger is downstream of a piece of slumping bank and woody debris
- logger is on Blood Tribe lands

Site Photos: ☐ **Datasheet Photo:** ☐ **Data Logger Download:** ☐ **Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca


W20-004 LOOKING DOWNSTREAM
ALVIN IN RED IS AT LOGGER


W20-004 LOOKING UPSTREAM


W20-005 LOOKING DOWNSTREAM


W20-005 LOOKING UPSTREAM

Alberta Government

Date: Aug 26, '20	**Waterbody:** Castle River		**Site ID: W18-** 107	**WBID:** 2004	
Latitude: 49.515880		**Longitude:** -114.04150			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFL		**Crew Members:** BFL			
Installation Method Epoxy		**Data Logger Serial #** 20300150			
Status: Install / (Field Check) / (Data Download) / Retrieval					
Field Check: ☑ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other: Data only available from Oct 24, 2019			
24h Time: 1604h	**Memory Used (%):** 17		**Battery (%):** 100		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

- Went through gate on Left upstream side of bridge
- Closed gate behind us, public access seems to be permitted if gate kept closed
- Parked at fence line and walked ~600m upstream to site
- Installation instructions correct, data downloaded and logger re-configured

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☑	**Data Submission:** ☐

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Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date:		**Waterbody:**		**Site ID: W18-**	**WBID:**
Latitude:		**Longitude:**			
Site Access: Truck / ATV / Heli / Other:					
Sampling Org:		**Crew Members:**			
Installation Method		**Data Logger Serial #**			
Status: Install / Field Check / Data Download / Retrieval					
Field Check: ☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / Lost / Other:			
24h Time:	**Memory Used (%):**		**Battery (%):**		

Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Alberta Government

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: 03/09/2020	**Waterbody:** Waterton River		**Site ID: W18-** 105	**WBID:**	
Latitude:		**Longitude:**			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFC		**Crew Members:** BFC (with Matt Coombs)			
Installation Method		**Data Logger Serial #**			
Status: Install / Field Check / Data Download / Retrieval					
Field Check: ☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / (Lost) / Other:			
24h Time:	**Memory Used (%):**		**Battery (%):**		
Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)					
- Looked extensively, but bank showed lots of erosion and logger was placed across from swimming beach and RV Park					
Site Photos: ☐	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐		

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring

Date: Oct 5, 2020	**Waterbody:** Beaver Creek		**Site ID: W18-** 802	**WBID:**	
Latitude: 49.832336		**Longitude:** -113.959725			
Site Access: (Truck) / ATV / Heli / Other:					
Sampling Org: BFC		**Crew Members:**			
Installation Method Rebar		**Data Logger Serial #** 20300074			
Status: (Install) / Field Check / Data Download / Retrieval					
Field Check: ☐ Logger operational	☐ Logger with problems	**Logger Issue:** NA / Out of water / Vandalism / Buried / (Lost) / Other: re-installed			
24h Time:	**Memory Used (%):** 0		**Battery (%):** 100%		
Site Notes: (Field Check: concerns and mitigations; Retrieval: notes on dewatered, buried, evidence of fouling/vandalism)					
- Original logger not found, new logger installed on right upstream bank at base of sawed off stumps					
Site Photos: ☑	**Datasheet Photo:** ☐	**Data Logger Download:** ☐	**Data Submission:** ☐		

** Reminder: Send all datasheets, photos and data to aep.wdsurveillance@gov.ab.ca

Whirling Disease Wild Surveillance Datasheet – Stream Temperature Monitoring


W18-802

Appendix D eDNA Collection Datasheets

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Aug 20, '20	**24h Time:** 1125h		**Waterbody:** Oldman River	**Site ID:** OldR2	
Latitude: 50.051			**Longitude:** -114.588		
Crew Members: ML, EF, KS, CGS, BLM					
Number of sample bottles filled: 3			**Sample condition:** (Clear) / Contains: substrate / existing turbidity		
Sample storage: (Cooler / On ice) / Covered / Uncovered		**Air temp:** 20	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl) / Gravel-sand / Silt-organics		**Gradient:** Steep / Moderate / None	**Flow:** High / Low / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected from Right upstream bank with collection pole
- Samples collected above major waterfall on Oldman River where only westslope cutthroat trout occur

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Aug 20, '20	**24h Time:** 1320h		**Waterbody:** Shale Creek	**Site ID:** SC1	
Latitude: 49.9871			**Longitude:** -114.491		
Crew Members: ML, EF, KS, CGS, BLM					
Number of sample bottles filled: 3			**Sample condition:** (Clear) / Contains: substrate / existing turbidity		
Sample storage: (Cooler) / On ice / Covered / Uncovered		**Air temp:** 20	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: Bould-cobl / Gravel-sand / Silt-organics		**Gradient:** Steep / Moderate / None	**Flow:** High / Low / None		
Fish observed: (Yes) / No / Possible sighting		**Species/Family:**	Young of year, suspected cutthroat trout		

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected from the creek ~100 m upstream from culvert on Oldman River road, culvert was nearly filled with gravel and creek was not flowing at the culvert at the time of sampling
- Culvert is a seasonal barrier to fish passage, possibly a permanent barrier

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Submission:** ☐


OLDR2 LOOKING UPSTREAM


SC1 LOOKING UPSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Aug 26, '21	**24h Time:** 1145h	**Waterbody:** Pincher Ck		**Site ID:** PC1	
Latitude: 49.5496		**Longitude:** -113.772			
Crew Members: KS, EF, MC, AFR, BLM, LB					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: (Cooler / On ice) / Covered / Uncovered		**Air temp:**	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl / Gravel-sand / Silt-organics)		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected upstream of temperature logger after logger was installed
- collected from left upstream bank
- small amount of substrate in one sample

Site Photos: ☑	**Datasheet Photo:** ☐	**Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Aug 26, '21	**24h Time:** 1415h	**Waterbody:** Pincher Ck		**Site ID:** PC2	
Latitude: 49.4055		**Longitude:** -114.016			
Crew Members: KS, EF, MC, AFR, BLM, LB					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: (Cooler / On ice) / Covered / Uncovered		**Air temp:**	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl / Gravel-sand) / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected from upstream of temperature logger after logger was installed
- collected from right upstream bank
- cattle grazing in area

Site Photos: ☑	**Datasheet Photo:** ☐	**Data Submission:** ☐


PC1


PC2 LOOKING UPSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Aug 26, '20	**24h Time:** 1500h	**Waterbody:** Chippman Cr Trib	**Site ID:** CCT1		
Latitude: 49.4035		**Longitude:** -114.058			
Crew Members: KS, EF, ML, AFR, BLM, LB					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: (Cooler / On ice) / Covered / Uncovered		**Air temp:**	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: Bould-cobl / (Gravel-sand) / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected from this small creek on the upstream side of the road to assess fish passage
- Creek is reported by local land owner to historically be occupied by Bull Trout

Site Photos: ☑	**Datasheet Photo:** ☐	**Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date:	**24h Time:**	**Waterbody:**	**Site ID:**		
Latitude:		**Longitude:**			
Crew Members:					
Number of sample bottles filled:		**Sample condition:**	Clean / Contains: substrate / existing turbidity		
Sample storage:	Cooler / On ice / Covered / Uncovered	**Air temp:**	**12h Precip:**	None / Drizzle / Light / Heavy	
Dominant Substrate:	Bould-cobl / Gravel-sand / Silt-organics	**Gradient:**	Steep / Moderate / None	**Flow:**	High / Low / None
Fish observed:	Yes / No / Possible sighting	**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Submission:** ☐


CCT1 LOOKING DOWNSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: 09/03/20	**24h Time:** 1000		**Waterbody:** Payne Lake Outlet Cam	**Site ID:** PayneOutlet1	
Latitude: 49.104861°N			**Longitude:** -113.630522°W		
Crew Members: BFC crew					
Number of sample bottles filled: 3			**Sample condition:** (Clean) / Contains: substrate / existing turbidity		
Sample storage: (Cooler) / On ice / Covered / Uncovered		**Air temp:** 17°C	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: Bould-cobl / Gravel-sand / Silt-organics		**Gradient:** Steep / Moderate (None)	**Flow:** High / (Low) / None		
Fish observed: (Yes) / No / Possible sighting		**Species/Family:** Mottled Sculpin			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

Water diversion is possibly inhibiting migration of fish spp. Road is close

Site Photos: ☐ **Datasheet Photo:** ☐ **Data Submission:** ☐

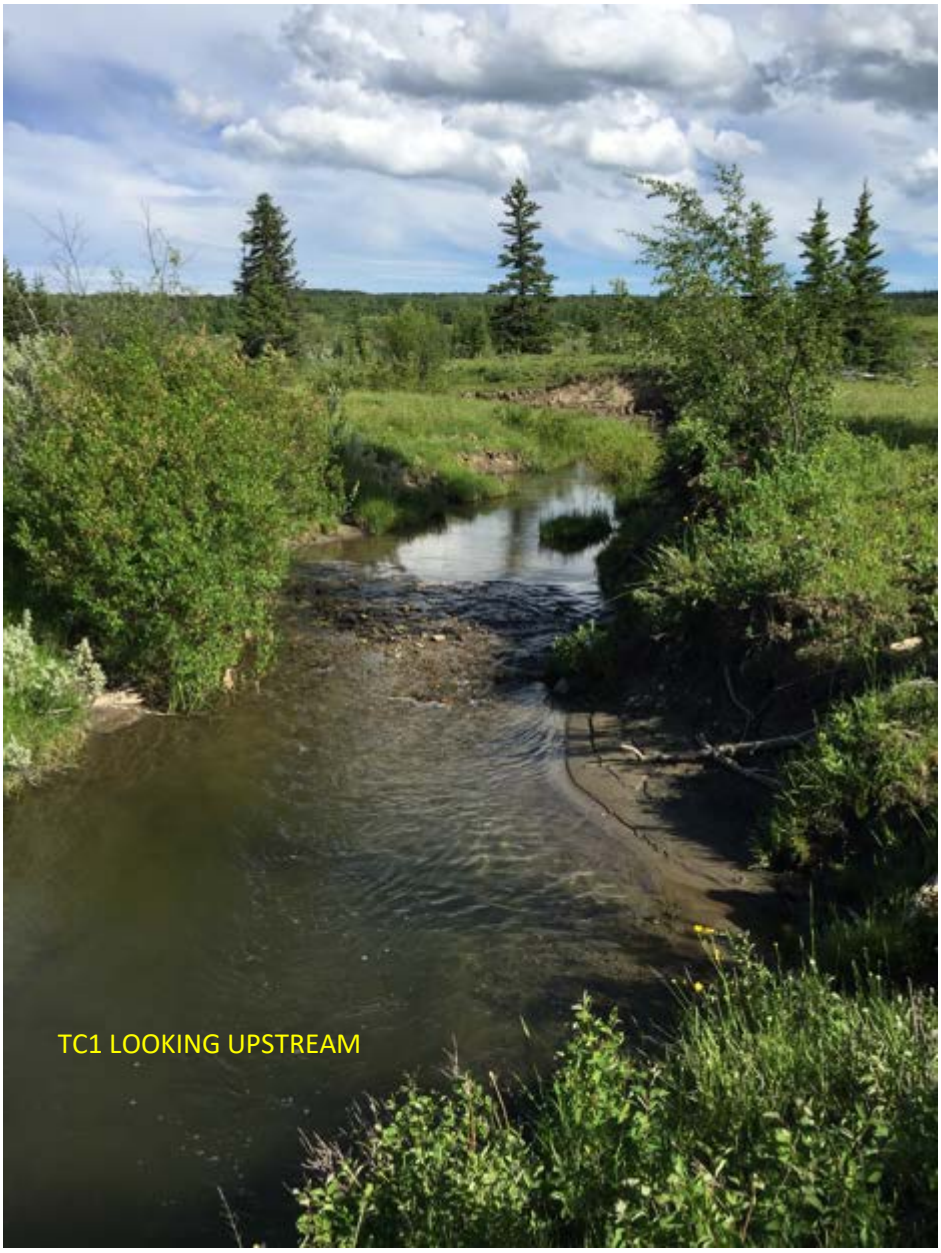
Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: 09/03/20	**24h Time:** 13:20		**Waterbody:** Tough Creek	**Site ID:** Tough Creek 1	
Latitude: 49.064523°N			**Longitude:** -113.553405°W		
Crew Members: BFC Crew					
Number of sample bottles filled: 3			**Sample condition:** (Clear) / Contains: substrate / existing turbidity		
Sample storage: (Cooler) / On ice / Covered / Uncovered		**Air temp:** 22°	**12h Precip:** None / Drizzle / Light / Heavy		
Dominant Substrate: Bould-cobl / Gravel-sand / (Silt-organics)		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: (Yes) / No / Possible sighting		**Species/Family:** Cutthroat (Rainbow?)			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples taken upstream of cattle crossing
- Riffle pool-run sequence
- Minimal overhanging foliage
- Samples taken from Tough Creek, east of access road into pasture (Pok Haven Grazing Lease)

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Submission:** ☐



TC1 LOOKING UPSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: 09/03/20	**24h Time:** 2:00	**Waterbody:** Lee Creek		**Site ID:** LeeC1	
Latitude: 49.112159		**Longitude:** -113.487611			
Crew Members: BFC Crew					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: (Cooler) / On ice / Covered / Uncovered		**Air temp:** 20°	**12h Precip:** None / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl) / (Gravel-sand) / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Site seemed to be used as swimming area.
- Samples collected from upstream side of the road

Site Photos: ☐	**Datasheet Photo:** ☐	**Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: 2024/09/23	**24h Time:** 1236h MDT	**Waterbody:** EVAN-THOMAS		**Site ID:** EVAN-THOMAS C1	
Latitude: 50.88237		**Longitude:** 115.12107 ±4m			
Crew Members: MATT COOMBS A. FIRSTRIDER D MAYHOOD / ELEV. 1547m ±4m					
Number of sample bottles filled: A, B, C. C1, C2, C3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: Cooler / (On ice) / Covered / Uncovered		**Air temp:**	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl) / Gravel-sand / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

SWIMMERS FROM ABOVE (5-30m) WASHED OUT FOOTBRIDGE

Site Photos: ☑	**Datasheet Photo:** ☐	**Data Submission:** ☐


ETC1 - LOOKINGDOWNSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Sept 23, '20	**24h Time:** 1800h	**Waterbody:** Lyons Ck		**Site ID:** LC1	
Latitude: 49.5704		**Longitude:** -114.45			
Crew Members: BFC crew					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: Cooler / On ice / Covered / Uncovered		**Air temp:** 20	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl / Gravel-sand) / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High (Low) None		
Fish observed: (Yes) No / Possible sighting		**Species/Family:** Rainbow Trout			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected downstream of Sattors Road adjacent to some recent bank stabilization work

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Sept 23, '20	**24h Time:** 1900h	**Waterbody:** Crowsnest Ck		**Site ID:** CC1	
Latitude: 49.6036		**Longitude:** -114.68			
Crew Members: BFC crew					
Number of sample bottles filled: 3		**Sample condition:** (Clean) / Contains: substrate / existing turbidity			
Sample storage: Cooler / On ice / Covered / Uncovered		**Air temp:** 20	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl) / Gravel-sand / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High (Low) / None		
Fish observed: Yes (No) Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Samples collected upstream of falls on Crowsnest Creek that are likely a fish passage barrier
- Sampling site is ~10m from Tent Mountain Mine road

Site Photos: ☑ **Datasheet Photo:** ☐ **Data Submission:** ☐


LC1 - LOOKINGDOWNSTREAM


CC1 LOOKING UPSTREAM

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Nov 3, '20	**24h Time:** 10:30am		**Waterbody:** St Mary River	**Site ID:**	
Latitude: 49.091			**Longitude:** -113.22		
Crew Members: MC, EF, AFR					
Number of sample bottles filled: 3			**Sample condition:** (Clean) / Contains: substrate / existing turbidity		
Sample storage: (Cooler) / (On ice) / Covered / Uncovered		**Air temp:** 18°C	**12h Precip:** (None) / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl) / Gravel-sand / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: Yes / (No) / Possible sighting		**Species/Family:**			

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Upstream of kimball Bridge (Hwy # 501)
- LUB — left upstream bank
- Silt on all substrate
- Water appeared clear

Site Photos: ☐ **Datasheet Photo:** ☐ **Data Submission:** ☐

Blackfoot Confederacy Native Trout Environmental DNA (eDNA) Collection Datasheet

Date: Nov 3, '20	**24h Time:**		**Waterbody:** St Mary River	**Site ID:**	
Latitude: 49.2246			**Longitude:** -113.27		
Crew Members: MC, EF, AFR					
Number of sample bottles filled: 3			**Sample condition:** (Clean) / Contains: substrate / existing turbidity		
Sample storage: (Cooler) / On ice / Covered / Uncovered		**Air temp:**	**12h Precip:** None / Drizzle / Light / Heavy		
Dominant Substrate: (Bould-cobl / Gravel-sand) / Silt-organics		**Gradient:** Steep / (Moderate) / None	**Flow:** High / (Low) / None		
Fish observed: (Yes) / No / Possible sighting		**Species/Family:**	just observed rising		

Site Notes: (Collection concerns and mitigations; Site notes on habitat condition, damage or threats, species to test for, surrounding land use)

- Collected from RUB standing on rip rap
- taken up from bridge
- Lee ck visible upstream of site
- Site immediately upstream of Hwy #5

Site Photos: ☐ **Datasheet Photo:** ☐ **Data Submission:** ☐


ST MARY RIVER AT KIMBALL


ST MARY RIVER AT HWY 5