# Williston Arctic Grayling Distribution: Parsnip, Peace, and Dinosaur.

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3333 - 22ND AVENUE | PRINCE GEORGE, BC | V2N 1B4

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Prepared By:

Mike Stamford, M.Sc.
Stamford Environmental
877 West Bay Road | Gambier Island, BC | V0N 1V0
michaelstamford430@gmail.com

Ted Euchner, B.Sc., RP Bio.
Diversified Environmental Services
Box 6263|Fort St. John|BC V1J 4H7
teuchner@shaw.ca

Jeff Strohm, M.Sc.
1659 Pequod Cresc | Gabriola Island |BC | V0R 1X5
strohmjeffrey@gmail.com

Caitlin Pitt, M.Sc.
University of Northern BC, Molecular Biology & Genetics Lab
3333 University Way | Prince George, BC | V2N 4Z9
Caitlin.Pitt@unbc.ca

Brent Murray, PhD., Associate Professor
University of Northern BC, Molecular Biology & Genetics Lab
3333 University Way | Prince George, BC | V2N 4Z9
brent.murray@unbc.ca

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# Table of Contents

## List of Figures

## List of Tables

## Executive Summary

This project addresses priority action 1b3: “Undertake Arctic Grayling monitoring as per recommendations of the monitoring program and develop specific, prioritized recommendations for habitat-based actions, which correspond to the monitoring results.” The objective was to address the hypothesis that Arctic Grayling continue to use all critical habitats identified in Williston and Upper Peace core areas and potentially recovering some of their historical range, as suggested by 2019 eDNA results in Finlay Reach.

Four years of sampling with environmental DNA (eDNA) has successfully expanded the known range of summer habitat use for Arctic Grayling in the upper Peace Basin. Arctic Grayling eDNA was detected in five tributaries draining the eastern slopes of Williston Reservoir, and their proximity to surrounding core areas supports the hypothesis that Arctic Grayling might continue to migrate through the reservoir to complete their life history. Possibly, adults or juveniles originate from natal areas in surrounding core areas (e.g. Parsnip, Nation, Finlay, Ingenika) and migrate to summer rearing habitats in these tributaries. Possibly, populations with life history traits able to adapt to the flooded conditions are expanding their range in Finlay Reach and persist in Parsnip and Peace reaches. Alternatively, detection of eDNA at six consecutive sites in the larger sixth order Ospika River might suggest habitat for multiple life history stages sustain a previously unknown and potentially independent metapopulation that survived flooding in Finlay Reach.

Failure to detect Arctic Grayling eDNA at 60 sites distributed among 26 streams rejected the hypothesis that critical habitats continue to provide recruitment for Arctic Grayling in Parsnip and Peace reaches and Dinosaur Reservoir. Nonetheless, Arctic Grayling eDNA was detected in four small tributaries of Parsnip and Peace reaches and supports the hypothesis that Arctic Grayling might continue to migrate through the reservoir to complete their life history. Such populations potentially carry life history traits that facilitate survival and recovery in flooded areas.

The detection probability and false negative rate for the Arctic Grayling eDNA assay was evaluated by pairing eDNA samples with snorkel survey observations in five streams over a three year period: Ingenika River (2019), Wichcika Creek (2020), Hominka River (2020), Colbourne Creek (2021), and Reynolds Creek (2020 and 2021). The eDNA assay detected Arctic Grayling eDNA in 100% of sites where a minimum of three liters of river water was sampled and adults were observed within 1.5km upstream in snorkel surveys. The detection rate among one-liter triplicates decreased at the margins of habitat areas where adult densities were low and suggests filter volumes influence eDNA detection. Comparing between two eDNA sampling methods (i.e. Direct Filtering five liters, versus Triplicate one-liter Bottle Filling) suggest that filtering larger water volume increases detection probability and five liters can detect a single adult present at least 4.5 kilometers upstream.

## 1.0 Introduction

Arctic Grayling (*Thymallus arcticus*) are distributed throughout mainland Arctic drainages from the west coast of Hudson Bay to northern British Columbia, Alaska, and eastern and central Russia (Stamford and Taylor 2004). This project focuses on Peace River populations around Williston Reservoir, which was created in 1967 by the construction of the 183-m high W.A.C. Bennett Dam (Hirst 1991). The reservoir reached full pool in 1972, and flooded roughly 1,760 $km^2$ of the Peace, Finlay, and Parsnip valleys, resulting in the loss of 600 $km^2$ of large river habitat (Sebastian et al. 2003). This dramatic loss had substantial impacts on the surrounding First Nations communities, and seriously diminished habitats and ecological communities sustained within their traditional territories (Baker et al. 2000). Formation of Williston and Dinosaur reservoirs flooded over 100-kilometers of large river habitats in each of Finlay River, Parsnip River, and Peace River that lie within the traditional territories of McLeod Lake, Saulteau, West Moberly, and Tsay Keh Dene First Nations.

Within the Williston Reservoir watershed, the Arctic Grayling is of conservation concern and important to recreational fishers and local First Nations, so is recognized a priority fish species for management by both the Fish and Wildlife Compensation Program (FWCP 2014) and the British Columbia Ministry of Forests, Lands, Natural Resource Operations, and Rural Development (MFLNRORD). Arctic Grayling populations in the Williston Basin were listed Critically Imperiled by the British Columbia Conservation Data Centre after once abundant populations vanished from the Williston Reservoir and its small tributaries by the mid 1980s (Cannings and Ptolemy 1998). The status has since been downgraded to yellow (Apparently Secure) stemming from their ancestry, and inclusion in the South Beringian lineage, which is widely distributed in British Columbia (B.C. Conservation Data Centre 2011). Most recently, the FWCP Arctic Grayling Synthesis Report identified and evaluated eight Williston metapopulations (i.e. Core Areas, Figure 1), and assigned those in Upper Peace and Williston core areas at High Risk of extirpation (Stamford et al. 2017). The accuracy of the metapopulation structure in Upper Peace and Williston core areas is uncertain, however, as is their ability to sustain Arctic Grayling populations because the distribution of critical habitats remains incompletely defined for all life history stages. Filling this high immediacy data gap with increased sampling effort will improve understanding of threats, begin to identify factors that limit population productivity, and provide direction for conservation and enhancement actions aimed at these high-risk populations (Stamford et al. 2017; Hagen and Stamford 2017).

Watershed connectivity is crucial for Arctic Grayling because all life history stages undergo lengthy seasonal migrations between different habitat types (Blackman 2002; Stewart et al. 2007). These migrations occur between three main habitats: 1) summer feeding habitat, 2) overwinter refuge, and at reproductive maturity, 3) spawning habitat (Northcote 1993). Prior to the inundation of the upper Peace Basin, the Finlay, Parsnip, and Peace rivers provided core low gradient, and large river habitat for populations of Arctic Grayling (Withler 1959; Bruce and Starr 1985). Inventory sampling throughout Williston watershed suggest, however, that upper Peace Basin populations rarely enter lakes. Records indicate presence in inlet streams for only two lakes (Aiken Lake in Omineca Core Area, and Toodoggone Lake in Upper Finlay Core Area; Figure 1). Possibly, most metapopulations that survived the flooding lack the necessary

adaptations for adfluvial life history strategies and the presence of the reservoir might restrict access to habitats, as suggested by previous studies (Clarke et al. 2007). This scenario is especially dire for critical habitats in those small streams draining into Upper Peace and Williston Core Areas (e.g. smaller than 5$^{th}$ order; Hawkshaw et al. 2014), where vast habitat areas were lost, and post-flood Arctic Grayling records are rare (Figure 2; Williamson and Zimmerman 2004; Stamford et al. 2017).

Migratory behaviours are often adapted to local conditions among salmonids (e.g. Dodson et al. 2013), and phenotypic expression appears to have a strong genetic basis in grayling (e.g. Kaya and Jeannes 1995; Haugen and Vøllestad 2001; Froufe et al. 2005; Weis et al. 2006). Consequently, selection differentials probably promote divergent migratory behaviours in Arctic Grayling similar to other species (e.g. Jonsson and Jonsson 2001; Moore et al. 2013; Carim et al. 2017; Dodson et al. 2017; Wollebaek et al. 2018). Genetic and microchemistry studies support the hypothesis that Arctic Grayling survived flooding among demographically independent metapopulations and although individuals appear to move extensively within their home streams, they rarely migrate to habitats outside their core areas (Stamford and Taylor 2005; Clarke et al. 2015; Shrimpton et al. 2012; Shrimpton and Clarke 2012). Consequently, it appears unlikely that recruitment from surrounding core areas will rescue Arctic Grayling abundance in Upper Peace and Williston core areas and future populations will persist only in the larger streams that provide all the necessary habitat elements that sustain viable resident populations (Hawkshaw et al. 2014). The implications for species and habitat conservation (strategic objectives of the FWCP) are that continued habitat use in Williston and Upper Peace core areas might constitute ancestral migratory behaviours that continue to move through the reservoir to complete their life history.

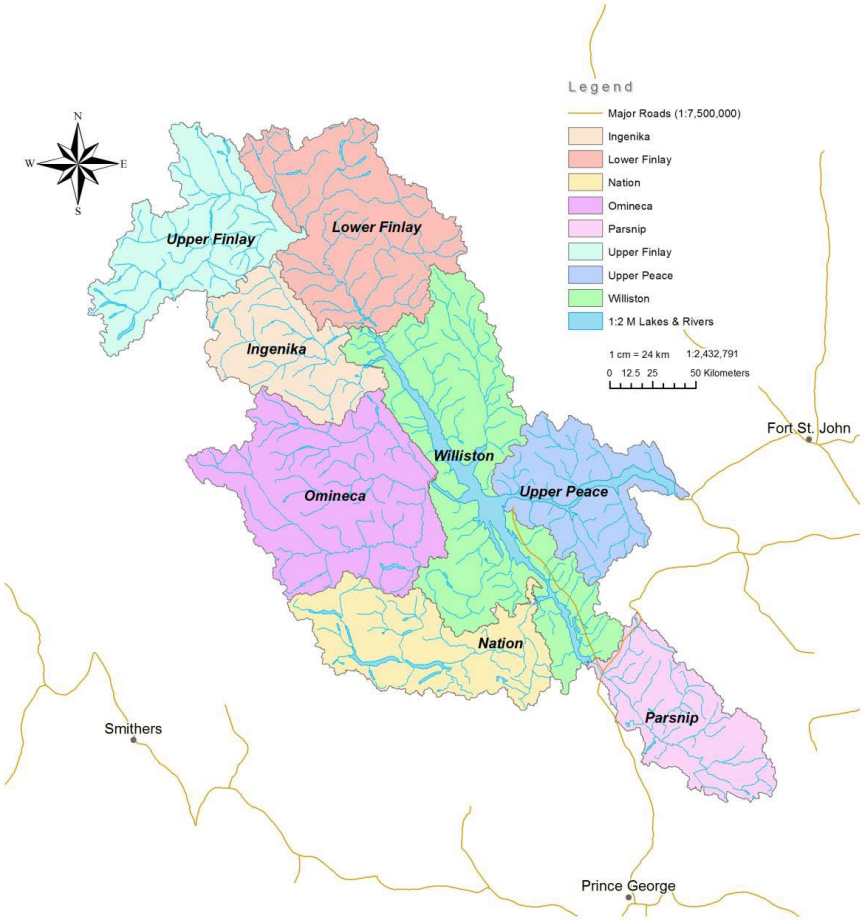



Figure 1: Arctic Grayling core areas of the Williston watershed which are thought to exist as demographically independent metapopulations (From Stamford et al. 2017).

Drawing from recent records and observations in Williston and Upper Peace core areas, and the different movement patterns observed among Arctic Grayling populations throughout the species' range, Stamford et al. (2015, 2017) hypothesized that Arctic Grayling adapted to large river habitats might continue to move through the reservoir to complete their life history. Possibly, once abundant large river adapted metapopulations survived flooding and continue to carry out their ancestral migrations that include small tributaries entering Williston Reservoir, and possibly Dinosaur Reservoir (Figure 3). Such metapopulations might be uniquely adapted and most appropriate for conservation and enhancement actions aimed at recovering the historical biocomplexity and abundance of Williston Arctic Grayling (Stamford et al. 2017). Recent post flood records of Arctic Grayling in the downstream reaches of tributaries entering

Williston and Dinosaur reservoirs were hypothesized to indicate presence of critical habitats but further sampling effort is needed to validate continued habitat use (Figure 2; Stamford et al. 2017).

Species distribution is a key measure of conservation status as it determines the geographic scope of threats and other factors that limit population size and trends and helps define enhancement actions. More broadly distributed species and populations, for instance, are often more resilient than those distributed more locally (e.g. recovery from disturbances might include rescue from surrounding less impacted habitats; McElhany et al. 2000). However, the prospect of conducting traditional inventory methods (snorkeling, electrofishing, angling, netting) within all core areas in a timely manner would require a massive investment and place a certain amount of handling stress on populations. In aquatic habitats, environmental DNA (eDNA) methods detect individuals via the cellular DNA-containing material that is constantly shed into the environment (Evans and Lamberti 2018). Without observing the organism, water samples are filtered in the field, concentrating cellular material on filter paper. Filters are then preserved for laboratory analysis where DNA is extracted, purified, and then taxonomically informative PCR primers are used to amplify specific sequences for species detection (Carim et al. 2016).

This multi-year project is using eDNA to begin addressing hypotheses of critical habitat use in high priority streams distributed in Williston and Upper Peace core areas, and Dinosaur Reservoir (Stamford et al. 2018). After two years of sampling (2018, 2019), the Arctic Grayling eDNA assay was validated with direct observations in snorkel surveys, and positive results support the findings from other studies that indicate a higher capture probability for eDNA relative to other sampling methods (e.g. Laramie et al. 2015; McKelvey et al. 2016; Wilcox et al. 2016; Stamford et al. 2020). Key results so far from this multi-year study are as follows:

- Arctic Grayling eDNA was detected in streams entering Finlay Reach, which has expanded the known range of critical habitats from a single stream (Figure 2) to five streams, including four which were previously thought extirpated by inundation (Williamson and Zimmerman 2004, 2005; Stamford et al. 2020).
- The pattern of positive results in tributaries of lower Finlay and Ingenika rivers supports the hypothesis that a large river adapted ecotype might continue to migrate among tributaries to complete their life history, which includes small streams entering Finlay Reach (Stamford et al. 2020).
- Arctic Grayling eDNA detection in Ospika River covered a contiguous 30 kilometers of complex habitat in the middle watershed, which suggests that multiple life history stages might constitute an independent metapopulation (i.e. potentially a separate core area; Stamford et al. 2020).
- In contrast, failure to detect eDNA in critical habitats draining the western slopes of Parsnip Reach suggests that Arctic Grayling are rare in that area. Possibly, the post flood records that identified critical habitats in Manson River and Fries Creek (Figure 2) might indicate a highly mobile population that avoided capture by our extensive eDNA sampling. Alternatively, the flooded western slopes of Parsnip Reach can no longer

support Arctic Grayling populations and the species is functionally extinct as previous assessments suggest (Williamson and Zimmerman 2005).

This report presents the findings from the third and final year (2020) of eDNA sampling, which has evaluated the distribution in critical habitats identified in Parsnip and Peace reaches in Williston Reservoir, and Dinosaur Reservoir (Figure 2). Sampling was also paired with snorkel surveys in six Parsnip River tributaries during 2020 and 2021 to improve the rigor of analyses estimating eDNA efficacy with larger sample sizes.

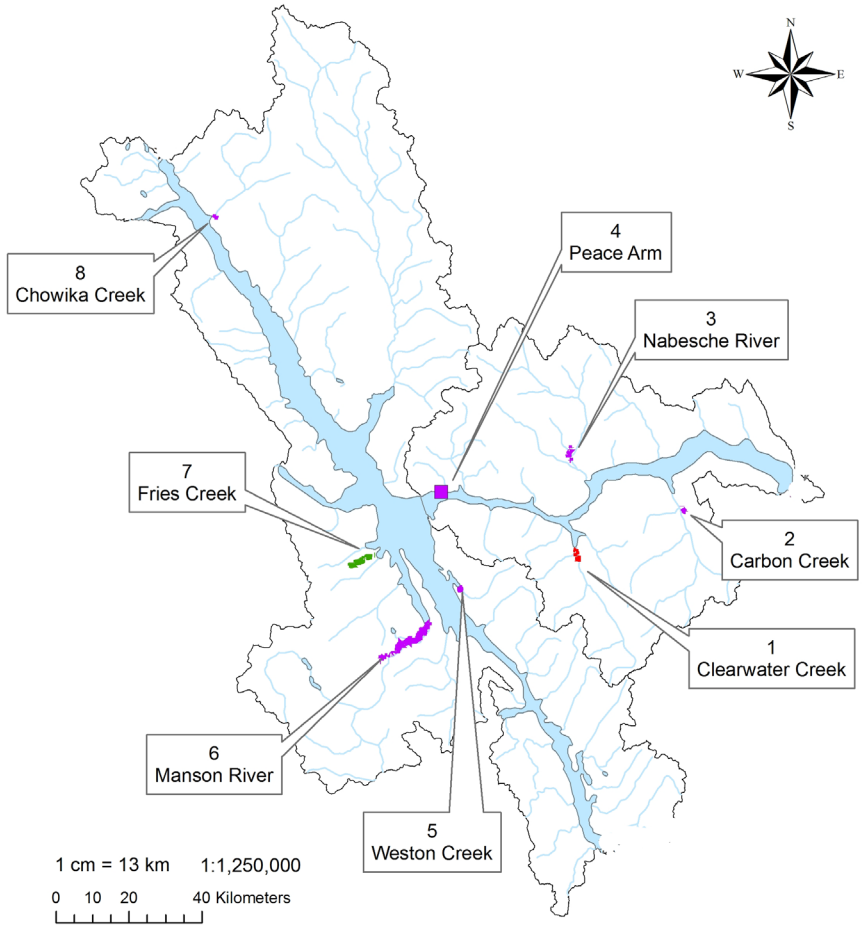



Figure 2: Arctic Grayling Critical habitats based on post 1988 population crash fish collection records and observations in Williston and Upper Peace core areas. Purple=Adult (>200mm fork length); Green=Yearling (100-200mm fork length); Red=Young of the year (fry <100mm fork length). Figure from Stamford et al. (2017).

## 2.0 Goals and Objectives

This project directly addresses sub-objective 1b: Conserve and enhance Arctic Grayling and improve understanding of limiting factors. Distribution information for Williston and Upper Peace Core Areas, and Dinosaur Reservoir is top priority for completing Step 1 in the sequence of monitoring needs and addresses recurring data gap #7: Unknown present-day distribution of

Arctic Grayling (Hagen and Stamford 2017). The results from this project is a first step toward enabling: 1) further actions to protect remnant populations; 2) evaluation of key habitats and life history characteristics associated with factors that limit population productivity; 3) improved evaluation of conservation status; 4) evaluation of potential enhancement options.

A prioritized list of 77 eDNA sample sites were distributed among 22 streams draining into Parsnip Reach, Peace Reach, and Dinosaur Reservoir, and four snorkel streams draining the Parsnip River watershed. The objectives were successfully addressed and within budget after sampling 93% of sites that were planned (Table 1).

Table 1: Number of eDNA sample sites used to complete the evaluation of Arctic Grayling distribution in Williston and Dinosaur reservoirs, and the detection probability relative to snorkel observations in Parsnip River tributaries. Critical habitat streams have recent (post 1988 crash) records indicating Arctic Grayling presence; presumed extirpated streams have records of rearing Arctic Grayling dating before 1988 (e.g. Bruce and Star 1985). Sampling for eDNA was carried out during August of 2020 and 2021.

| Objective | Total Sample Sites | | Number of streams sampled | |
|---|---|---|---|---|
| | Sites Planned | Sites Sampled | Critical habitat streams | Presumed extirpated streams |
| 1. Evaluate Reservoir Tributaries | 48 | 43 | 6 | 16 |
| 2. Evaluate Detection Probability | 29 | 29 | 2 | 2 |

## 2.1 Objective 1: Evaluating distribution in reservoir tributaries.

Assuming that historical presence of Arctic Grayling upstream of flooded areas indicates presence of suitable rearing habitats, all Arctic Grayling records and observations (i.e. including those before 1988 when populations were abundant; Bruce and Starr 1985; Cannings and Ptolemy 1998) were used to select sample sites throughout Parsnip and Peace reaches of the Williston, and Dinosaur reservoirs (Figure 4; see Stamford et al. 2018). The objective was to address the hypothesis that Arctic Grayling might be recovering their historical range in Williston Reservoir. A range expansion from their current 'known distribution' (i.e. critical habitats; Figure 2, 3) could be occurring in two forms based on life history stages observed:

1. **Isolation with critical habitat present**: Records of fry in Clearwater River (Langston and Blackman 1993), descriptions of abundant spawning populations in Carbon Creek (BC Research 1976), and above barrier adults in Nabesche River (Langston and Blackman 1993) suggest that critical habitats of Upper Peace Core Area include natal areas (i.e. spawning and fry rearing, Figure 2, 3), that might have escaped flooding and perhaps provides recruitment to a locally moving metapopulation (Stamford et al. 2018).
2. **Migration and adaptation**: Observations of rearing adult (>200mm fork length) Arctic Grayling in Weston and Scott creeks suggest that Arctic Grayling might move from surrounding natal areas (e.g. Parsnip, Nation core areas) to rear in the flooded river mouths (Stamford et al. 2018).

With regard to sampling in Dinosaur Reservoir, a single record from 1984 in Gething Creek was available, but more recent habitat descriptions and absence after significant sampling effort suggest Arctic Grayling may no longer be present (Diversified Environmental Services 2011). Therefore, eDNA will be used to gather an alternative line of evidence of this extirpation event.

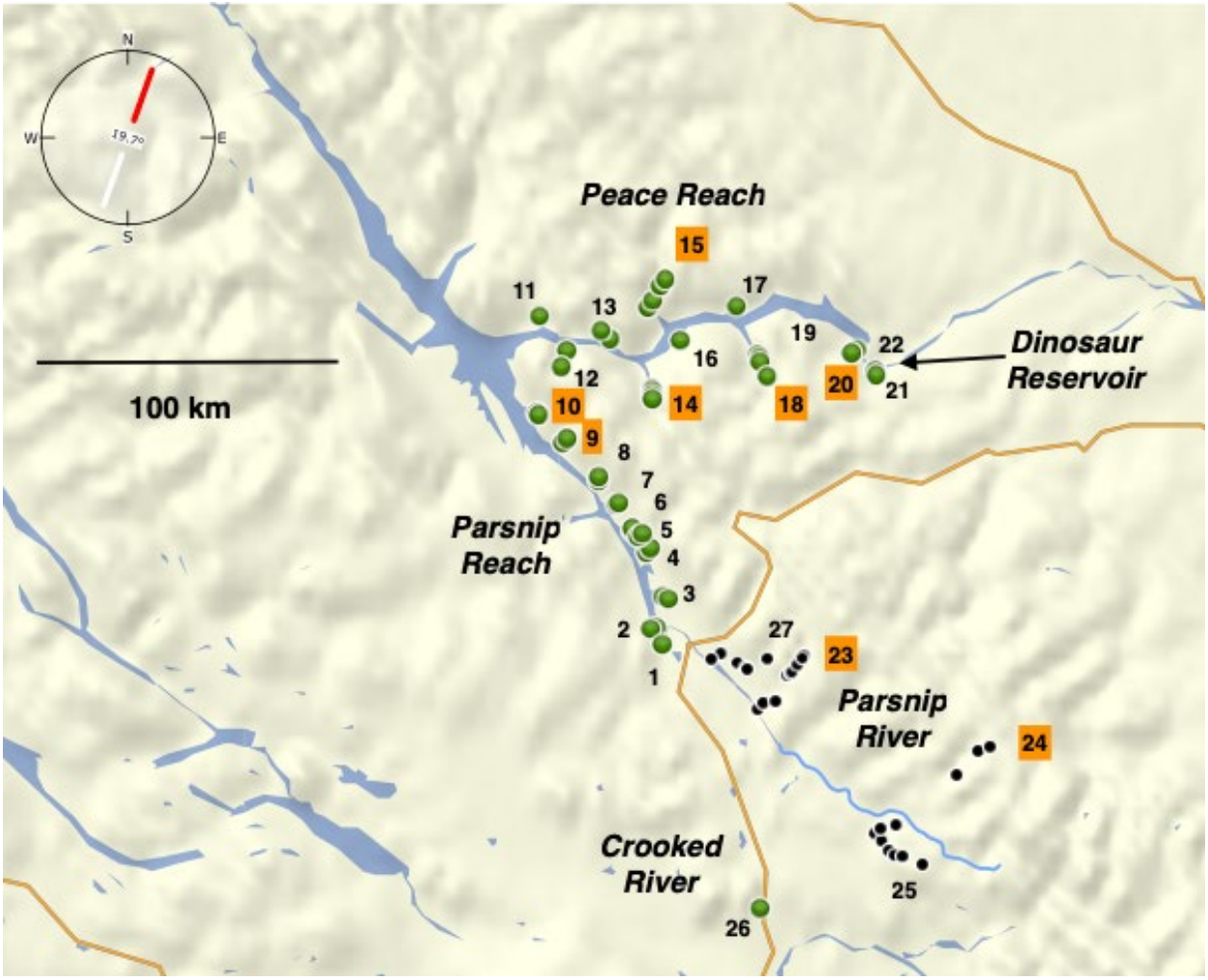



Figure 3: Spatial distribution of Arctic Grayling eDNA samples collected during summer 2020 and 2021. Green dots are sites in Parsnip and Peace reaches, and Dinosaur Reservoir sampled to evaluated putative critical habitat use; black dots are sites in Parsnip River tributaries that were paired with snorkel surveys; a single site in Crooked River was used for field methods training both in 2018 and 2020. Numbers next to sample groups correspond to: 1 Pack River, 2 Lignite Creek, 3 Gagnon Creek, 4 Mugaha Creek, 5 Tutu Creek, 6 Tony Creek, 7 Cutthumb Creek, 8 Patsuk Creek, 9 Scott Creek, 10 Weston Creek, 11 Wicked River, 12 Selwyn Creek, 13 Bernard Creek, 14 Clearwater River, 15 Nabesche River, 16 Pardonet Creek, 17 Schooler Creek, 18 Carbon Creek, 19 Stott Creek, 20 Gething Creek, 21 Johnson Creek, 22 Dinosaur Reservoir, 23 Reynolds Creek, 24 Hominka River, 25 Wichcika Creek, 26 Crooked River; 27 Colbourne Creek. Orange highlight show streams with critical habitats updated in the seed report (Stamford et al. 2018)

## 2.2 Objective 2: Evaluate eDNA detection probability with paired snorkel observations.

Without knowledge of the limits of our eDNA assay (e.g. how far upstream and what fish densities does eDNA detection rate reach 0%), it is difficult to explain unexpected failures to detect presence (e.g. Anzac River in 2018). The results from 2018, for instance, found detection and non-detection in adjacent sites in two streams (Ospika River, Davis River) and seven reservoir sites failed to detect Arctic grayling eDNA even though they were adjacent to streams where eDNA was detected. This suggests that eDNA settled below detectable concentration between sample sites both in streams (0.6 to 2 km apart) and the reservoir (between 1 and 11 km apart). Alternatively, other variables influenced eDNA detection and there is poor concordance between eDNA detection and Arctic grayling presence. To distinguish between these alternatives relating eDNA detection and the presence of Arctic Grayling upstream, eDNA sampling was paired with snorkel observations.

The distributions of presence/absence data aligned perfectly between eDNA detections and presence/absence in snorkel observations in Ingenika River during 2019 and supports the findings in other studies that suggest eDNA persists up to 1.5 kilometers in streams (e.g. Jane et al. 2014; Laramie et al. 2015; McKelvey et al. 2016; Wilcox et al. 2016). The influences from environmental variables (e.g. water chemistry, discharge, turbulence, temperature) on the persistence of eDNA in streams remain incompletely resolved (Carim et al. 2016), however, and the distance eDNA persists in the water column might vary among streams. Furthermore, only seven sites were successfully paired with direct snorkel observations in Ingenika River, which limited evaluation of eDNA detection of our assay, relative to fish density and distance upstream in snorkel observations. Therefore, the objectives for increasing eDNA sampling in snorkel streams were: 1. to increase the sample size of eDNA sites paired with snorkel observations; 2. evaluate the detection probability in more than one stream.

Table 2: Number of eDNA sites collected in snorkel survey streams, including Ingenika River (2019) and Parsnip tributaries (2020/2021). Sampling included sites not paired with snorkel surveys to evaluate the distribution of critical habitats outside of snorkel surveys. Distances between eDNA sites included both paired and not paired with snorkel observations.

| Sample Year | Number of snorkel streams | eDNA sites sampled | No. eDNA sites at the dowsntream end of index sections | | | Mean distance between sites (km) |
|---|---|---|---|---|---|---|
| | | | Total | GR Observed | GR Not Observed | |
| Ingenika 2019 | 1 | 12 | 7 | 4 | 3 | 1.7 |
| Parsnip Tributairies 2020/21 | 4 | 29 | 15 | 11 | 4 | 5.6 |
| Combined | 5 | 41 | 22 | 15 | 7 | 4.1 |

FWCP funded snorkel survey studies (PEA-F20-F-2963, PEA-F21-F-3203, respectively) provided helicopter support, field personnel to collect eDNA, and direct observations of Arctic Grayling upstream of eDNA sites. The eDNA results also informed the snorkel surveys and the distribution of critical habitats by evaluating presence/absence beyond the range of snorkel surveys (Strohm et al. 2020; Hagen and Stamford 2021A).

# 3.0 Methods

## 3.1 Sample collection

Sample collection training for both the instream filtration (Section 3.1.2) and bottle filling (Section 3.1.3) methods were carried out in the Crooked River (stream 26; Figure 3) and served as field negatives during 2018 and 2020 (Strohm et al. 2019). Consistent with previous sampling, the instream filtration method was used to evaluate the distribution of Arctic Grayling eDNA among streams entering Williston (Parsnip and Peace reaches) and Dinosaur reservoirs between July 31 and August 12, 2020 (Objective 1; Figure 3). A minimum of two sites per stream spaced roughly 1.5 km apart around previous Arctic Grayling records guided field site selections. Four sites selected in Nabesche River, Carbon Creek, and Clearwater Creek were intended to evaluate potential natal areas, including at least one site in each stream situated upstream of observed barriers to assess potential presence of resident populations (Stamford et al. 2017). Habitats were visually assessed, and final site selections determined from the helicopter. Sample sites were situated as near as possible and downstream of observed low gradient rearing areas (e.g. deep pools, glides) or at the planned site coordinates.

The bottle filling method and associated short sample collection time was key for integrating eDNA sampling with snorkel surveys (Stamford et al. 2020). To evaluate possible differences in the detection probabilities of eDNA the two methods were paired at 10 sites situated among low densities of Arctic Grayling, and presumably low concentrations of eDNA.

## 3.2 Control sampling

Two control samples were collected each sampling day to detect equipment cross contamination: one prior to collection of the first eDNA sample, and one after the final eDNA sample was collected. Controls were collected some distance away from the sampling streams and sample bottles to avoid direct contamination. Each control sample consisted of 1 L of distilled water filtered through the assembly, the filter was folded into quarters (filtrate side in) and stored cool and dark in a 95% ethanol-filled cryovial, which upon arrival in the laboratory was stored at -20 $^{o}$C until analyses.

## 3.3 Instream Filtering Method

Sample collection methods in 2021, 2020, 2019, and 2018 are described by Carim et al. (2016) and used by Jane (2015), McKelvey et al. (2016), and Wilcox et al. (2016) to estimate eDNA detection probability in streams. The method, which captures eDNA on 1.5μm pore size glass fiber filters from five liters of water pumped directly from the environment, can detect presence when fish are sparsely distributed in small headwater streams (e.g. one fish per kilometer and about 750m away; Jane et al. 2015; McKelvey et al. 2016). In this study the instream water filtering and sample collection used a portable peristaltic pump to draw water through a filter assembly fitted with 1.5μm pore sized glass fiber filters. The filter assembly was placed directly into the stream in locations with flow to ensure captured eDNA originated from upstream. At sites where stream margins had minimal flow and sites in the reservoirs, sterile bottles were used to collect from flowing water or some distance offshore to bring water to the filter apparatus.

All equipment was carried in a 50 L backpack in the field (Table 3). Site kits were assembled in the laboratory in 4 L resealable bags prior to deployment and each contained enough supplies to collect one sample. Items 1, 2, 3 and 4 (Table 3) constituted a site kit. Aside from the forceps, all site kit contents were single use. Forceps were decontaminated after use by soaking in 50% bleach solution for 20 minutes and rinsing three times with distilled water. The filtering apparatus consisted of items 2, 5, 6, 7, 8, and 9 (Table 3, Figure 4). A Geopump Peristaltic Pump (Geotech, CO) pumped the water through the filter assembly on the inflow end of the tubing and into the outflow bucket, which tracked the volume filtered. The eDNA sample was collected on the filter as water passed through it. Sampling apparatus was set up on shore in a dry (if possible) and flat area where all sampling equipment was easily assembled. Sampling personnel avoided entering the water and remained downstream of the sampling location when setting up the equipment and during sampling to prevent contamination. Samples were not collected from eddies or splash pools where DNA could wash off contaminated materials, flow upstream, and contaminate the sample (Carim et al. 2016).

Prior to sample collection, the pump and tubing were rinsed with stream water for 5 min downstream from where sampling was to occur. Once complete, the pump was moved to the desired sampling location upstream, the filter assembly was attached to the tubing adaptor, and placed in the stream. A single replicate was collected by pumping 3-5 L of water directly from the stream through the filters. Filter that became clogged before 3 L of water was drawn through were replaced with a new filter to complete the total 3-5 L filtered water volume and both filters labelled the same (e.g. 1 of 2, 2 of 2, etc). At the end of each run the filter apparatus was removed from the stream and the pump was run for one minute to dry the filter. The filter was folded into quarters (filtrate side in) and inserted into an ethanol-filled cryovial and stored cool and dark on ice, until delivery to the laboratory where cryovials were stored at -20$^{o}$C until analyses. When multiple filters (maximum 3) were required to complete a single site or replicate, each was stored in separate cryovials and then combined in the laboratory and analyzed as a single replicate.

Table 3: Equipment required for in-field collection of eDNA samples.

| Site Kit | Filtering Apparatus | Other Equipment |
|---|---|---|
| 1. nitril gloves | 5. peristaltic pump | 10. 1L bottles |
| 2. filter assembly | 6. pump battery with backup | 11. garbage bags for used supplies |
| 3. glass fiber filters | 7. power cord | 12. battery charger |
| 4. forceps | 8. tubing with funnel adaptor | 13. 95% ethanol-filled cryovialsfor sample preservation and storage |
| | 9. graduated outflow bucket | 14. clean sample bags |
| | | 15. datasheets |
| | | 16. permanent markers, pencils |
| | | 17. GPS unit |
| | | 18. camera |
| | | 19. cooler with ice packs |
| | | 20. Distilled water for control samples |
| | | 21. bleach to sterilize bottles and equipment. |
| | | 22. Thermometer |


Figure 4: Instream filtering apparatus for eDNA sample collection consisted of the filter assembly (2), peristaltic pump (5), pump battery (6), power cord (7), tubing with funnel adaptor (8), graduated outflow bucket (9), clean sample bag (14), and cooler (19).

## 3.3 Bottle filling method

Samples were collected from flowing water in triplicate 1L HDPE bottles and filtered at the end of the day (Helbing and Hobbs 2019). Prior to use, and between sampling events, all bottles and lids were cleaned with a 50% bleach solution on the inside and outside. Bottles were then rinsed with tap water. Upon arrival at the sampling site bottles and lids were thoroughly rinsed in flowing river water to ensure all traces of bleach were removed. A few steps upstream were taken, and then bottles were dunked and filled while being held in a position upstream from the sampling biologist. Samples were kept in a cooler with ice packs until being filtered at the end of the day, and a maximum of eight hours after collection.

Small pore sized filters (0.45 μm Mix Cellulose Ester (MCE) as recommended by Helbing and Hobbs (2019) were used to capture eDNA from the one-liter triplicate bottle samples from clear flowing snorkel streams. When sampling turbid water, the coarse pore sized glass fiber (1.5μm)

filters were used to minimize clogging. Filters were placed in the funnel assembly on top of a vacuum flask, and a GAST pump was used to draw the full 1 L sample from each triplicate through the filter, which was then folded and inserted in a cryovial containing 95% ethanol and stored in a freezer until delivery to the laboratory. Forceps were cleaned with 50% bleach and rinsed thoroughly with distilled water between samples to avoid cross contamination. Control blanks were collected before and after each filtering event and handled in the same manner as eDNA samples (see Section 3.1.1).

## 3.4 Paired eDNA sampling with snorkel observations

To examine the ability of our eDNA assay to detect Arctic Grayling relative to direct observations upstream (Objective 2, Section 2.2), eDNA sample sites were collected among snorkel observations in five streams, including Ingenika River, and four Parsnip River tributaries (Table 2). Sampling for eDNA occurred in Ingenika watershed between August 5 and 7, 2019, Wichcika, Hominka, and Reynolds watersheds between August 18 to 30, 2020, and Reynolds and Colbourne creeks between August 10 and 20, 2021. Environmental DNA was always sampled from a downstream to upstream direction and most (70%) sites received triplicate one-liter bottle sampling in the morning before snorkelers entered the water. The instream filtering method was used in 2021 to sample 11 sites in Reynolds and Colbourne creeks and took place 24 hours after snorkel surveys were completed: assuming sufficient time to clear stream reaches of eDNA contamination from the snorkelers but also assume Arctic Grayling had not moved from previous snorkel observations.

A total of 41 eDNA sites were distributed in snorkel streams, including 22 sites paired directly at the downstream ends of index sections and 19 sites distributed between and upstream from snorkel observations (Table 2). Spacing between eDNA sites was relatively consistent (roughly 1.5km apart) in Ingenika River, and previous snorkel data was used to ensure sites were paired with both expected presence and absence observations (i.e. Cowie and Blackman 2012; Hagen et al. 2019). Site selection in reconnaissance Parsnip tributaries had no prior snorkel observations to inform the sampling strategy and 15 sites were paired at the downstream ends of index sections. Spacing between eDNA sites was variable but coordinates were collected where Arctic Grayling were observed to inform the distance upstream from the eDNA sites.

The same snorkeling methods were used in all streams to rigorously enumerate Arctic Grayling and other species, using three (in larger streams) or two (in smaller streams) tandem snorkelers (Figure 5). For a detailed description of the snorkel methods, see Strohm et al. (2020) and Hagen and Stamford (2021).

## 3.5 eDNA detection between sampling methods

Pumping five liters directly from the stream for about 15 minutes using the instream filtering method (Section 3.1.2) might capture larger quantities of eDNA from the water column relative to dunking only three liters (triplicates) using the bottle filling method (Section 3.1.2). Filtering larger water volumes might also increase the chance of collecting rare large clumps of eDNA originating from further upstream (e.g. Wilcox et al. 2016). Consequently, the direct filtering method is hypothesized to have a higher detection probability and detection distance than

bottle filling method (e.g. Wilcox et al. 2016; Carim et al. 2016). Alternatively, the detection probability is similar between the two methods when a minimum of three liters is filtered (see filter comparisons, Stamford et al. 2020).

To distinguish between these alternative hypotheses about the influence of sampling methods on the detection of eDNA, both the instream filtering and bottle filling methods were paired at 10 sites distributed among four streams. Each site was associated with low densities of Arctic Grayling as determined from snorkel observations and other sampling methods, which suggest eDNA concentrations might also be low and possibly close to the detection limits between the methods. These included three sites in Reynolds Creek, which were spaced sequentially 1.5, 3.0, and 4.5 kilometers downstream from a single adult snorkel observation.

Figure 5: Snorkel survey crew demonstrating the organization needed for effective fish counts during the 2019 Ingenika swims, and eDNA sampling.

## 3.6 Molecular methods

For each site, filters were processed with an extraction control for each extraction batch. Environmental DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen, Toronto, ON) following a modified protocol for water filters. For each filter, a first (i) and a second (ii) eDNA elution (50 µl each) was collected. After the initial sample "i" eDNA was eluted from its purification column, a second volume (ii) of elution buffer was run through it to collect any DNA that remained bound to it. For each filter, the first elution (i) was analyzed in duplicate for the presence of Arctic grayling DNA, with the second sample analyzed only when the "i" sample showed signs of PCR inhibition or low signal.

Two published TaqMan assays for Arctic Grayling were used (Rodgers et al. 2018; Carim et al. 2016), which were designed for populations on the North Slope of Alaska, and Montana, respectively, and both were previously tested against other salmonids and non-salmonids. The specificity and sensitivity against Arctic Grayling DNA isolates from Northern BC, as well as

other co-occurring fish species (Mountain Whitefish *Prosopium williamsoni*, Rainbow Trout *Oncorhynchus mykiss*, Northern Pikeminnow *Ptychocheilus oregonensus*, Sockeye Salmon *O. nerka*, Dolly Varden *Salvelinus malma*, Brook Trout *S. fontinalus*) were checked before use in this study (Stamford et al. 2018). Both assays target the same gene (cytochrome c oxidase 1), with the additional assay, hereby referred to as "GRAY2," being specific to a region downstream of the previously used "GRAY1". GRAY1 utilizes a FAM reporter, whereas GRAY2 uses HEX and both assays were assessed in a single duplex reaction. The same reporter dyes are used for the inhibitor test duplex reaction which uses the LAMBDA and ePLANT assays (Stamford et al. 2020). Digital droplet PCR (ddPCR) was used to assess the detection of the target species. Briefly, samples were run in 2, 20 μL reactions (this samples 10 μl, 1/5 of the eDNA recovered in the elution tested) using mastermix for probes (no UTP) and droplets were generated using the AutoDG and run on a QX200 Droplet Reader (Bio-Rad Laboratories, Mississauga, ON). A signal of 4 or more positive droplets was used to determine positive detection. GRAY1 and GRAY2 were tested across a temperature gradient using synthetic DNA which contained both target regions to determine the optimal annealing temperature and to confirm the sensitivity of the duplex reaction down to single copy number for both assays.

### 3.7 Data analyses

A Chi square test was used to compare the distributions of eDNA detection/not detection with the known distributions of Arctic Grayling. The known distribution was defined by post 1988 sampling records, which were also used to identify critical habitat streams in Williston and Upper Peace core areas (Figure 3). To compare the distributions among sites collected from snorkel index streams the known distribution was defined by snorkel observations: Arctic Grayling present in upstream snorkel index sites. Arctic Grayling were assumed absent in eDNA sites located upstream of snorkel surveys. Among triplicate samples the detection probability was calculated as the average site detection rate ± SE to compare between direct snorkel observations (Arctic Grayling observed/not observed upstream) among streams. Individual site detections were the number of 1 L bottles that detected eDNA (between 1 and 3) divided by the total number of replicates collected (n=3). The false negative rate was the number of 1 L replicates that failed to detect eDNA divided by the total number of replicates collected among sites where eDNA was detected.

## 4.0 Results and Discussion

### 4.1 Laboratory Results

Among the 156 samples and field controls (Blanks) submitted for analyses during Fall 2020, and 2021, Arctic Grayling eDNA was not detected in any field distilled water controls (n=83). Five samples showed a weak signal that was too low to be confirmed after retesting, including two bottle replicates collected from two sites in snorkeled streams (Wichcika and Reynolds creeks), two direct filtering sites in Parsnip Reach streams (Mugaha Creek 01, Lignite Creek 01), and one of two samples collected during training in Crooked River where Arctic Grayling have never been observed. The weak signal detected in these samples were assumed to have failed to detect Arctic Grayling eDNA in the following analyses.

## 4.2 Distribution of eDNA and critical habitat use by Arctic Grayling in streams draining into Parsnip and Peace reaches and Dinosaur Reservoir.

Difficult access (no helicopter landing sites) prevented sampling in four sites distributed among three Peace Reach streams and a total of 45 eDNA samples were collected from Parsnip and Peace reaches and Dinosaur Reservoir (Table 4, 5; Figure 6). The relationship between the distributions of Arctic Grayling eDNA detections and their assumed presence in critical habitats and absence in other streams was not significance (Fisher's Exact p (*no association*) = 0.07). Both eDNA and historical sampling records agreed among 22 sites and suggest the species no longer rear in 12 streams draining into Parsnip and Peace reaches and Dinosaur Reservoir (Table 4). Environmental DNA also failed to detect Arctic Grayling at 18 sites distributed among the six critical habitat streams ('*known distribution*', Table 4; Figure 6), however, which does not support the hypothesis that Arctic Grayling are adapting to the flooded conditions and expanding their range in Parsnip and Peace Reaches and Dinosaur Reservoir. Absence in critical spawning and rearing habitats does not support the hypothesis that Peace Reach and Dinosaur Reservoir continue to sustain Arctic Grayling metapopulations. Arctic Grayling appear to be exceedingly rare in Parsnip and Peace reaches and absent in Dinosaur Reservoir (Table 4; Figure 6).

Table 4: Environmental DNA results from sample sites in critical habitat streams (Arctic Grayling present) and other streams with no recent records (Arctic Grayling absent) among streams draining into Parsnip, and Peace reaches and Dinosaur Reservoir.

| Known Distribution (Critical Habitats) | eDNA Detected | eDNA not detected | Total |
|---|---|---|---|
| Present | 0 | 18 | 18 |
| Absent | 5 | 22 | 27 |
| Total | 5 | 40 | 45 |

Remarkably, a recent angler report of adult Arctic Grayling captured in Carbon Creek one year after eDNA sampling (summer 2021, Ted Euchner, pers. com.) might suggest that adults move among streams to find summer rearing habitats. Similarly, detection of Arctic Grayling eDNA at five sites outside their known distribution in Parsnip and Peace reaches (Cutthumb, Tony, Tutu and Pardonet creek, 20% of samples) supports the hypothesis that Arctic Grayling continue to migrate through the reservoir to complete their life history (Table 4; Figure 6). Potentially these third and fourth order streams (Table 5) provide rearing habitat for vagile life history stages (e.g. juvenile, subadult, adult) originating from natal areas in other streams (e.g. Nation, Parsnip core areas). This scenario supports the hypothesis that a divergent large river adapted metapopulation might have survived flooding and continue their ancestral migratory behaviour to the flooded habitats. Possibly, individuals move among rearing habitats and their presence in east Parsnip and Peace Reach streams, including the critical habitats, is temporally variable. Follow-up fish sampling to identify life history stages present, identify natal origins (e.g. genetics, microchemistry) will begin distinguishing between these alternative hypotheses about

migratory behaviours associated with Parsnip Reach streams (Stamford et al. 2017; Hagen and Stamford 2017).

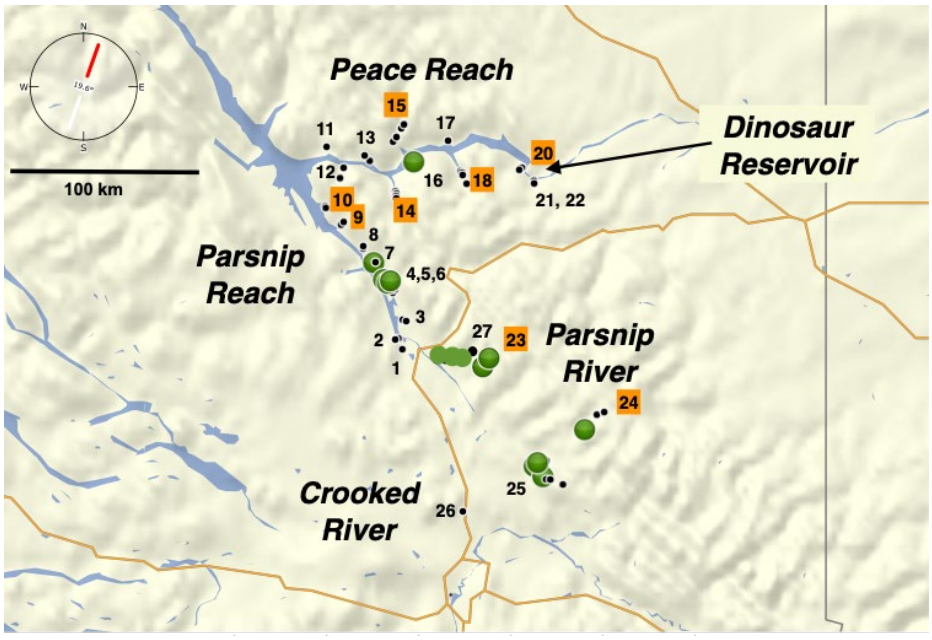



Figure 6: Distribution of Arctic Grayling eDNA results in Parsnip River, Parsnip and Peace reaches, and Dinosaur Reservoir. Small black dots = Arctic Grayling eDNA not Detected; Large Green Dots= Arctic Grayling eDNA detected. Numbers next to sample groups correspond to: 1 Pack River, 2 Lignite Creek, 3 Gagnon Creek, 4 Mugaha Creek, 5 Tutu Creek, 6 Tony Creek, 7 Cutthumb Creek, 8 Patsuk Creek, 9 Scott Creek, 10 Weston Creek, 11 Wicked River, 12 Selwyn Creek, 13 Bernard Creek, 14 Clearwater River, 15 Nabesch River, 16 Pardonet Creek, 17 Schooler Creek, 18 Carbon Creek, 19 Stott Creek, 20 Gething Creek, 21 Johnson Creek, 22 Dinosaur Reservoir, 23 Reynolds Creek, 24 Hominka River, 25 Wichcika Creek, 26 Crooked River, 27 Colbourne Creek. Orange highlighted stream labels indicate critical habitats.

Table 5: Summary of eDNA samples collected during August 2020 to evaluate the distribution of Arctic Grayling eDNA in streams draining into Parsnip and Peace reaches and Dinosaur Reservoir.

| Stream Number* | Reach | Stream | Stream Order | No. eDNA Sites | Sample Sequence** | eDNA Results (1= GR detected; 0= GR NOT detected) | Historic Distribution (1=GR present; 0=GR Absent)*** | Temperature oC | Turbidity**** |
|---|---|---|---|---|---|---|---|---|---|
| 26 | West Parsnip | Crooked River | 6 | 1 | 00 | 0 | 0 | 20 | C |
| 1 | West Parsnip | Pack River | 6 | 1 | 0 | 0 | 0 | 18 | L |
| 2 | West Parsnip | Lignite Creek | 4 | 2 | 00 | 0 | 0 | 11 | C |
| 3 | East Parsnip | Gagnon Creek | 4 | 2 | 00 | 0 | 0 | 10 | C |
| 4 | East Parsnip | Mugaha Creek | 5 | 2 | 00 | 0 | 0 | 9.5 | C |
| 5 | East Parsnip | Tutu Creek | 3 | 2 | 11 | 1 | 0 | 9 | C |
| 6 | East Parsnip | Tony Creek | 3 | 2 | 10 | 1 | 0 | 9 | C |
| 7 | East Parsnip | Cutthumb Creek | 4 | 2 | 10 | 1 | 0 | 10 | C |
| 8 | East Parsnip | Patsuk Creek | 3 | 2 | 00 | 0 | 0 | 9 | C |
| 9 | East Parsnip | Scott Creek | 4 | 2 | 00 | 0 | 1 | 11 | L |
| 10 | East Parsnip | Weston Creek | 3 | 2 | 00 | 0 | 1 | 12 | C |
| 11 | North Peace | Wicked River | 5 | 1 | 0 | 0 | 0 | 8 | C |
| 12 | South Peace | Selwyn Creek | 4 | 2 | 00 | 0 | 0 | 7 | C |
| 13 | North Peace | Bernard Creek | 3 | 2 | 00 | 0 | 0 | 6 | C |
| 14 | South Peace | Clearwater Creek | 5 | 4 | 0000 | 0 | 1 | 10 | C |
| 15 | North Peace | Nabesche River | 5 | 4 | 0000 | 0 | 1 | 11 | T |
| 16 | South Peace | Pardonet Creek | 4 | 1 | 1 | 1 | 0 | 9 | L |
| 17 | North Peace | Schooler Creek | 4 | 1 | 0 | 0 | 0 | 8 | C |
| 18 | South Peace | Carbon Creek | 6 | 4 | 0000 | 0 | 1 | 9 | L |
| 19 | South Peace | Stott Creek | 3 | 0 | na | na | 0 | | |
| 20 | Dinosaur Reservoir | Gething Creek | 5 | 2 | 00 | 0 | 1 | 11 | C |
| 21 | Dinosaur Reservoir | Johnson Creek | 5 | 2 | 00 | 0 | 0 | 17 | M |
| 22 | Dinosaur Reservoir | Dinosaur Reservoir | na | 1 | 0 | 0 | 0 | 12 | M |

* Numbers correspond to Figure 3

** Listed from left to right samples located downstream to upstream

*** 1=critical habitat identified in Stamford et al. 2018

**** C=Clear; L=Slightly Turbid; M=Moderately Turbid; T=Turbid

## 4.3 Detection of eDNA in snorkel survey streams.

A total of 41 eDNA sites were distributed among 20 snorkel index sections in five streams and 26 (63%) sites received triplicate filters from 1 L bottle sampling, 14 (30%) sites received single filter sampling, and three sites (7%) compared eDNA detections between the bottle filling and direct filtering methods downstream of a grayling observation (Table 6; Figure 7). Among the single filter sites, the bottle sampling failed to detect Arctic Grayling at all four sites distributed in Ingenika River and Wichcika Creek situated upstream or at the margins of snorkel sites (Table 6). The 5 L direct filtering method detected eDNA at five out of eight sites distributed in Colbourne and Reynolds creeks both within the ranges and upstream of snorkel observations (Figure 7E, F; Table 6). Among the 28 eDNA sites situated downstream of snorkel observations (including paired sites and three sites between snorkel sections), Arctic Grayling were observed present between zero and 17.5 kilometers upstream from the nearest eDNA sites (Table 6).

Table 6: Distribution of eDNA triplicate samples among snorkel survey sections in five Upper Peace Basin watersheds, collected during August 2019, 2020, and 2021. Note, single replicates in Reynolds and Colbourne creeks received direct filtering method (5 Liters).

| Watershed | Sample Year | Snorkel Survey section* | eDNA Site Name | eDNA Site Location (stream km) | UTM | Upstream snorkel GR count | eDNA Outcome ** | Closest Upstream GR Observation (km) | eDNA method*** | Total Water Volume filtered (L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingenika River (Upper ) | 2019 | na | HW2 | 122.5 | 9 V 667553 6304912 | na | 000 | | BF Triplicates | 3.00 |
| Ingenika River (Upper ) | 2019 | na | HW1 | 120.5 | 9 V 669035 6305290 | na | 000 | | BF Triplicates | 3.00 |
| Ingenika River (Upper ) | 2019 | Site 1 | U5 | 118.5 | 9 V 670285 6305599 | 0 | 000 | | BF Triplicates | 3.00 |
| Ingenika River (Upper ) | 2019 | Site 2 | U4 | 117 | 9 V 671647 6305889 | 0 | 000 | | BF Triplicates | 3.00 |
| Ingenika River (Upper ) | 2019 | Site 2 | U3 | 115 | 9 V 673250 6306274 | 0 | 0 | | BF Single | 1.00 |
| Ingenika River (Upper ) | 2019 | Site 2, 3 | U2 | 113 | 9 V 675010 6306253 | 3 | 010 | <1.5km | BF Triplicates | 3.00 |
| Ingenika River (Upper ) | 2019 | Site 3 | U1 | 112 | 9 V 675652 6305611 | 1 | 011 | <1.5km | BF Triplicates | 3.00 |
| Ingenika River (Middle) | 2019 | Site 9 | M3 | 89 | 10 V 327497 6299405 | 8 | 111 | <1.5km | BF Triplicates | 3.00 |
| Ingenika River (Middle) | 2019 | Site 9 | M2 | 87.5 | 10 V 328594 6298982 | 1 | 111 | <1.5km | BF Triplicates | 3.00 |
| Ingenika River (Middle) | 2019 | Site 9 | M1 | 86 | 10 V 329493 6298627 | na | 111 | <1.5km | BF Triplicates | 3.00 |
| Ingenika River (Wrede Creek) | 2019 | na | Trib4B | 79.7 | 10 V 332889 6294219 | na | 111 | na | BF Triplicates | 3.00 |
| Ingenika River (Wrede Creek) | 2019 | na | Trib4A | 78 | 10 V 334061 6295318 | na | 100 | na | BF Triplicates | 3.00 |
| Hominka River | 2020 | na | 1U | 52 | 10 U 588098 6074755 | na | 000 | | BF Triplicates | 3.00 |
| Hominka River | 2020 | 1U-1L | 1L | 48 | 10 U 584647 6073246 | 0 | 000 | | BF Triplicates | 3.00 |
| Hominka River | 2020 | 4U-5L | 5L | 35 | 10 U 578705 6065100 | 64 | 111 | 0km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | na | Wich_HW | 27 and 37 | 10 U 569085 6035073 | na | 0 | | BF Single | 6.00 |
| Wichcika Creek | 2020 | na | Wich_5U | 27 | 10 U 563176 6037770 | na | 0 | | BF Single | 3.00 |
| Wichcika Creek | 2020 | 27km-23km | Wich_5L | 23 | 10 U 560931 6037916 | 1 | 0 | 2.8km | BF Single | 3.00 |
| Wichcika Creek | 2020 | 22km-20km | Wich_4L | 20 | 10 U 559270 6039434 | 2 | 001 | 1.5km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | 22km-20km | Wich_3U | 15 | 10 U 557071 6042787 | na | 000 | 6.5km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | 15km-11km | Wich_3L | 11 | 10 U 555038 6045112 | 5 | 011 | 1.3km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | 15km-11km | Wich_upper | 10 | 10 U 555329 6045631 | na | 100 | 1km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | 10km-7km | Wich_middle | 7 | 10 U 556658 6046918 | 0 | 111 | 4km | BF Triplicates | 3.00 |
| Wichcika Creek | 2020 | 5km-1km | Wich_lower | 1 | 10 U 561139 6048056 | 1 | 000 | 2km | BF Triplicates | 3.00 |
| Reynolds Creek | 2021 | 36km-34km | Ray7 | 34 | 10 U 533870 6104485 | 0 | 0 | na | DF Single | 5.00 |
| Reynold Creek | 2020 | na | Reyn_33U | 33 | 10 U 533339 6103547 | na | 000 | | BF Triplicates | 3.00 |
| Reynold Creek | 2020 | na | Reyn_30U | 30 | 10 U 532069 6101402 | na | 000 | | BF Triplicates | 3.00 |
| Reynold Creek | 2020 | 30km- 26km | Reyn_26D | 26 | 10 U 530405 6098824 | 6 | 111 | 1.3km | BF Triplicates | 3.00 |
| Reynolds Creek | 2021 | 30km-26km | Ray6 | 26 | 10 U 530689 6099225 | 2 | 11 | 1.5km | DF & BF | 8.00 |
| Reynolds Creek | 2021 | 30km-26km | Ray5 | 24.5 | 10 U 530059 6098567 | 2 | 10 | 3.0km | DF & BF | 8.00 |
| Reynolds Creek | 2021 | 30km-26km | Ray4 | 23 | 10 U 529154 6097785 | 2 | 10 | 4.5km | DF & BF | 8.00 |
| Reynolds Creek (Chuyuzega Creek) | 2021 | na | CHO1 | 10 | 10 U 525815 6089006 | na | 1 | na | DF Single | 5.00 |
| Reynolds Creek | 2021 | 12km-10km, 20km-16km | Ray3 | 10.2 | 10 U 525820 6089088 | 0 | 1 | 17.5km | DF Single | 5.00 |
| Reynolds Creek | 2021 | 6km-2km | Ray2 | 5 | 10 U 522124 6088588 | 10 | 1 | 0km | DF Single | 5.00 |
| Reynolds Creek | 2021 | 6km-2km | Ray1 | 1 | 10 U 520620 6086391 | 10 | 1 | 0km | DF Single | 5.00 |
| Colbourne Creek | 2021 | na | COL5 | 27 | 10 U 523268 6103326 | na | 0 | na | DF Single | 5.00 |
| Colbourne Creek (Trib17) | 2021 | na | COL_Trib17 | 17.5 | 10 U 517451 6099800 | na | 0 | na | DF Single | 5.00 |
| Colbourne Creek | 2021 | na | COL3 | 17 | 10 U 517142 6100196 | na | 001 | na | BF Triplicates | 3.00 |
| Colbourne Creek | 2021 | 17km-13km | COL2 | 13 | 10 U 514655 6101948 | 0 | 011 | na | BF Triplicates | 3.00 |
| Colbourne Creek | 2021 | 9km-5km | COL1 | 5 | 10 U 509775 6104891 | 0 | 010 | na | BF Triplicates | 3.00 |
| Colbourne Creek | 2021 | 3km-1km | COL4 | 0.5 | 10 U 507029 6103183 | 0 | 1 | na | DF Single | 5.00 |

* Sites listed from upstream to downstream within watersheds; na = no snorkel survey upstream

** 1= GR detected; 0= GR Not detected

*** BF= Bottle Filling Method; DF= Direct Filtering Method; Single= eDNA captured onto a single filter; Triplicate= eDNA captured onto three filters each from 1 L water samples and processed separately in the l

Arctic Grayling eDNA was detected in 59% of sites collected from snorkel streams, including seven sites where they were expected to be absent (Table 7). These include three sites in tributaries where post 1988 records suggest absence (Wrede Creek and Chuyuzega Creek in

Ingenika and Reynolds watersheds, respectively), and four sites in Colbourne Creek where both snorkel observations and sampling records failed to detect Arctic Grayling (Table 6, 7, Figure 7). The eDNA sampling failed to detect Arctic Grayling at three sites in Wichcika Creek situated downstream of snorkel index sites where Arctic Grayling were observed between 2.0 and 6.5 kilometers upstream (Table 6, 7; Figure 7C). In total, 76% of eDNA results matched the 'known distribution' of Arctic Grayling in snorkel streams ($\chi^2 = 9.6$, df = 1, *P* (no association) = 0.002; Table 7).

Among the 25 sites paired at the downstream ends of snorkel index sites, 18 (72%) eDNA detections matched the presence/absence in snorkel observations, while eDNA was detected at five sites where snorkeling failed, and eDNA failed to detect at two sites where snorkeling observed Arctic Grayling 2.0 and 2.8 km upstream, respectively ($\chi^2 = 4.00$, df = 1, *P* (no association) = 0.045; Table 8). Close associations between eDNA detection and other observations make the data appropriate for evaluating the detection rate for eDNA.

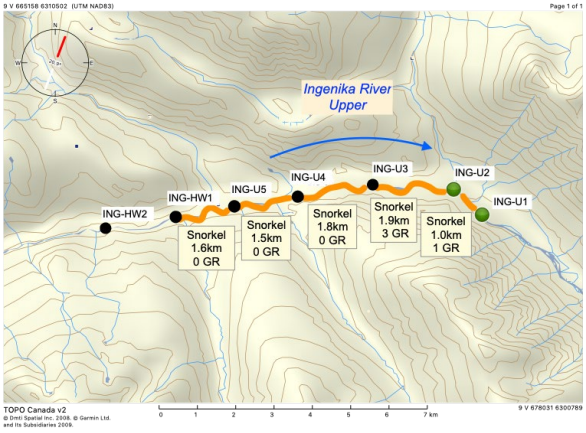
Table 7: Association between eDNA detection and the "known" distribution of Arctic Grayling in five snorkel index streams collected during August 2020 and 2021. The known distribution was determined from all sampling records, including snorkel observations and other post 1988 sampling efforts.

| Known Distribution | eDNA | | Total |
|---|---|---|---|
| | Detected | Not detected | |
| Present | 17 | 3 | 20 |
| Absent | 7 | 14 | 21 |
| Total | 24 | 17 | 41 |

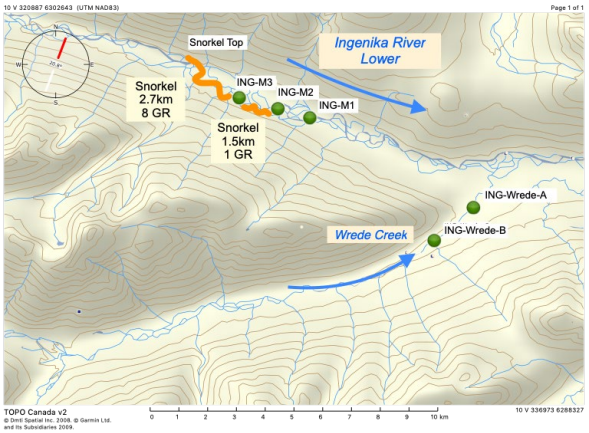
Table 8: Association between eDNA detection and observations of Arctic Grayling in upstream snorkel sites collected during August 2020 and 2021.

| Snorkel Observation | eDNA | | Total |
|---|---|---|---|
| | Detected | Not detected | |
| Observed | 13 | 2 | 15 |
| Not Observed | 5 | 5 | 10 |
| Total | 18 | 7 | 25 |

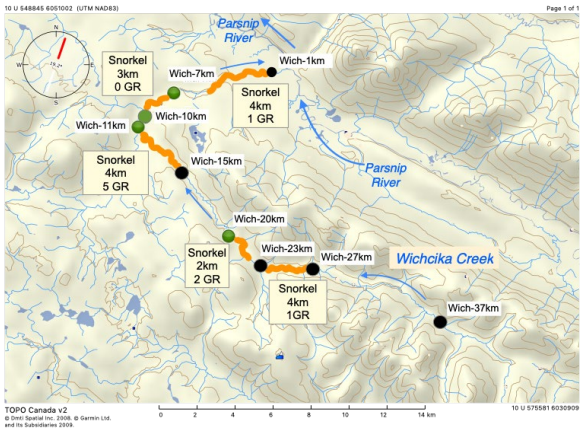
A: Ingenika Upper 2019



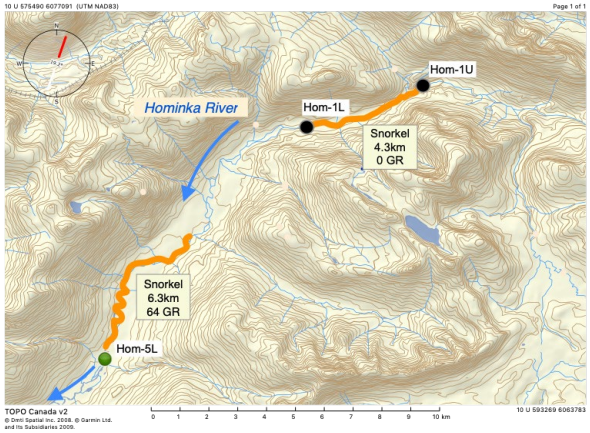

B: Ingenika Lower 2019



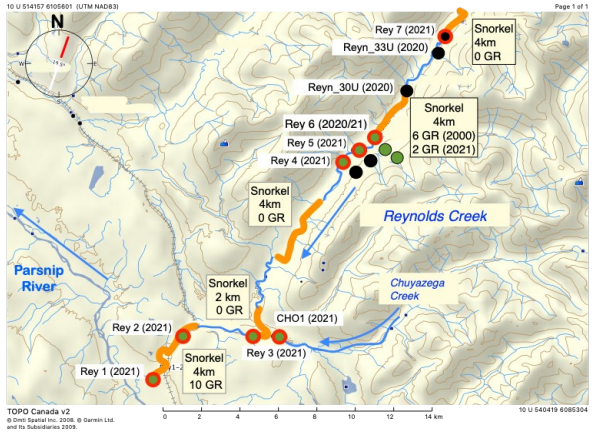

C: Wichcika Creek 2020




D: Hominka River 2020




E: Reynolds Creek-2020 and 2021.




F: Colbourne Creek-2021

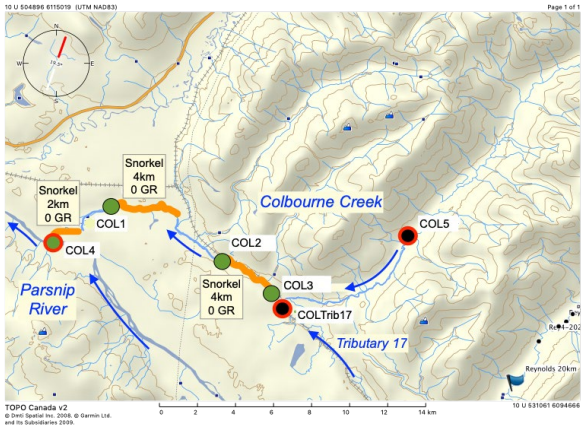



Figure 7: Distribution of eDNA sites (green and black symbols) relative to snorkel index sections (orange shaded sections) in A: Ingenika River Upper; B: Ingenika River Lower; C: Wichcika Creek; D: Hominka River; E: Reynolds Creek; F Colbourne Creek. Green = Arctic Grayling eDNA *Detected*; Black = Arctic Grayling eDNA *Not Detected*. Labels next to snorkel sections indicate the distance snorkeled (km), and the counts of Arctic Grayling (GR) observed. Red borders around eDNA sites highlight the Instream filtering method used in Colbourne and Reynolds creeks. Blue arrows indicate direction of stream flow. See Figure 3 for locations of Parsnip tributaries.

Arctic Grayling eDNA detection among one-liter triplicates appears to be influenced by distance and densities of Arctic Grayling observed upstream (Figure 7). Highest detection rates (all three triplicates positive) illustrate the hub of Arctic Grayling snorkel distributions in Ingenika River, Hominka River, and Reynolds Creek and lower detection rates (<3 positive triplicates) appear to be associated with lower densities of Arctic Grayling. For example, lower detection rates among triplicates in Ingenika at the upstream margins of snorkel observations might suggest there was lower eDNA concentration in the water column (Table 6; Figure 7A). Similarly, generally lower detection rates among triplicates from Wichcika Creek and Colbourne Creek and associated low densities or absence (in Colbourne Creek) in snorkel observations, relative to other streams (Hagen and Stamford 2021), might suggest these streams also contained relatively low eDNA concentrations (Table 6). Fish abundance appears to be a good predictor of downstream eDNA concentration in other studies (e.g. Wilcox et al. 2016), and lower detection rates among our triplicates might illustrate small populations or marginal habitats.

The single highest detection rate (all triplicates positive) in Wichcika Creek was associated with a snorkel negative site, which suggests adults avoided observation by snorkelers. Alternatively, suspended eDNA concentration was high relative to upstream due to presence of other life history stages less visible to snorkeler (e.g. fry, juveniles). Although the eDNA detections cannot distinguish between these alternatives, a general tendency for Arctic Grayling life histories to sort within streams with adults further upstream and younger life histories downstream (e.g. Hughes 1999) supports the second alternative. Similarly, presence of fry or juveniles might be the source of eDNA detected in Colbourne Creek where 10 kilometers of snorkeling failed to observe a single adult (Table 6; Figure 7F). Arctic Grayling fry often accumulate downstream in natal streams, and higher detection rates downstream might suggest Colbourne Creek and Wichcika Creek provide critical natal habitats, potentially for adults that migrate to other streams to rear in Parsnip Core Area. Possibly, these fifth order streams promote homing behaviour and the eDNA results signify components of biodiversity critical for the resilience in Parsnip Core Area.

Among the 96 1 L bottles collected in triplicate from snorkeled streams there were four non-ordered outcomes and the assay failed to detect Arctic Grayling eDNA in 62 replicates (Table 9). Among 39 replicates collected from snorkel positive sites, 19 failed to detect Arctic Grayling eDNA and suggests a 49% false negative rate among 1 L samples. Including all 51 replicates collected from eDNA positive sites (i.e. eDNA detected in at least one triplicate), 17 were negative and suggests a 33% false negative rate overall. Among all triplicate negative results (i.e. ‘000’, Table 9), four were associated with snorkel positives and in each instance adult Arctic Grayling were observed further than 1.5km upstream (Table 6). Triplicate positives (‘111’) were consistently located within the hub of Arctic Grayling distributions in snorkel streams, and false negative rates increased near the margins of habitat use where snorkel counts were lower. False negatives at three eDNA sites together with absence in snorkel surveys in Colbourne Creek (Table 6), for instance, suggests the watershed sustains low abundance of Arctic Grayling. The false negative rate of 33% among one-liter samples suggest that a minimum of three liters of water filtered per site is sufficient to detect a single Arctic Grayling adult present within 1.5

kilometers upstream, including near the margins of their habitat areas, and in sites where they are rare in streams.

Table 9: Percentage of sites with one of four possible eDNA detection outcomes with triplicate 1 L water sampling among 32 sites (96 1 L bottles) distributed in Ingenika River, Wichcika Creek, Hominka River, Reynolds Creek, and Colbourne Creek.

| Possible outcomes with triplicate sampling | Number of sites | Percent of sites |
|---|---|---|
| 000 | 15 | 47% |
| 100, 010, 001 | 7 | 22% |
| 110, 101, 011 | 3 | 9% |
| 111 | 7 | 22% |
| Total | 32 | 100% |

0= GR eDNA not detected; 1= GR eDNA detected

## 4.4 Detection of Arctic Grayling eDNA between bottle filling and direct filtering methods.

Remarkably, failure to detect Arctic Grayling eDNA in bottles collected downstream of two snorkel positive sites in Wichcika Creek and two sites in Reynolds Creek (Figure 7C, E) suggests eDNA settled below detection concentration within 2 kilometers from snorkel observations (Table 6). However, Arctic Grayling eDNA was detected at 100% of sites where Arctic Grayling were observed 1.5km or closer upstream (11 sites, Table 6). In Reynolds Creek, both 3 L bottle filling and 5 L direct filtering methods detected eDNA 1.5km downstream from a single snorkel observed adult while only the direct filtering detected eDNA further downstream 3 and 4.5 kilometers (Figure 7E, Table 9). Generally, among trials comparing the two methods, direct filtering detected eDNA more frequently than bottle filling, which supports the hypothesis that larger filter volumes have higher detection probability. Possibly filtering directly larger water volume over an extended period increases the chance of collecting rare large eDNA particles that might have longer transport distances than smaller particles (Wilcox et al. 2016).

Table 10: Results from paired sampling trials comparing detection of Arctic Grayling eDNA between direct filtering and bottle filling methods. Water volumes were pumped through single filters for all except three sites where bottle fill triplicates were filtered separately. Paired sampling trials in 2019 took place during flood conditions and potentially influenced eDNA detections by direct filtering water from newly flooded habitats. Trials in Reynolds Creek were located downstream from an adult observed 27.5 kilometers upstream from the mouth. Only the direct filtering method was used during 2018.

| Trial | Stream | Site (year sampled) | Sampling method* | | Comment |
|---|---|---|---|---|---|
| | | | Direct Filtering (5L) | Bottle Fill (3L) | |
| 1 | Reynolds Creek | Rey4-23 km (2021) | 1 | 0 | GR observed 4.5 km upstream |
| 2 | Reynolds Creek | Rey5-24.5 km (2021) | 1 | 0 | GR observed 3.0 km upstream |
| 3 | Reynolds Creek | Rey6-26 km (2021) | 1 | 1 | GR observed 1.5 km upstream |
| 4 | Ospika River | Main1 (2021) | 1 | 0 | GR eDNA detected in 2018 |
| 5 | Ospika River | Main1 (2019) | 0 | 001 | GR eDNA detected in 2018 |
| 6 | Ospika River | Main2 (2021) | 1 | 1 | GR eDNA detected in 2018 |
| 7 | Ospika River | Main2 (2019) | 0 | 110 | GR eDNA detected in 2018 |
| 8 | Ospika River (Gavreau Creek) | Trib1-Site1 (2021) | 0 | 0 | GR eDNA NOT detected in 2019 |
| 9 | Chowika Creek | Chow1 (2019) | 1 | 0 | GR eDNA detected in 2018 |
| 10 | Davis River | Dav1 (2019) | 0 | 010 | GR eDNA detected in 2018 |

* 1= GR eDNA detected; 0= GR eDNA NOT detected

## 4.5 Summary of eDNA results after four years of sampling in Upper Peace Basin

In stark contrast to the 2020 results in the south and eastern parts of Williston and Dinosaur Reservoir, the 2018 and 2019 sampling of eDNA significantly expanded the known range of Arctic Grayling summer habitat use in Finlay Reach (Figure 8). The repeatable detection (in 2018, 2019, and 2021) of Arctic Grayling eDNA in five streams draining the eastern slopes of Finlay Reach and one Reservoir site near the mouth of Finlay River supports the hypothesis that Arctic Grayling migrate through the reservoir to rear in small streams (Stamford et al. 2017). Furthermore, eDNA detected in tributaries of Finlay and Ingenika rivers supports the hypothesis that a potentially distinct large river adapted life history type might continue to migrate to the ancestral rearing habitats in small streams. Alternatively, the eDNA detected in Finlay Reach streams has identified local populations that complete their life history without migrating through the reservoir as suggested by microchemistry data that found no evidence that Arctic grayling use reservoir (Clarke et al. 2007). A contiguous distribution of eDNA in the sixth order

Ospika River spanning over 30 kilometers including habitats in the mainstem and tributaries supports this second alternative hypothesis. Possibly, Ospika River and adjacent Finlay Reach streams support multiple life history stages and the eDNA sampling has discovered new core areas. Potentially, or hypothetically, the numerous positive eDNA sites in Finlay Reach streams reflect the rearing areas for multiple Arctic Grayling metapopulations that might originate from up to three natal areas in the Finlay, Ingenika, and Ospika rivers. Follow-up fish sampling and analyses are needed to: 1. Determine the life history stages present; 2. Determine the demographic relationships among Finlay Reach core areas.

The eDNA results from 2020 sampling suggest that Arctic Grayling are rare in Parsnip and Peace reaches, and Dinosaur Reservoir and supports the hypothesis proposed by Williamson and Zimmerman (2004, 2005) that the species has become functionally extinct due to flooding. Remarkably, repeat sampling in both 2018 and 2019 among 19 sample sites in streams draining the western slopes of Parsnip Reach failed to support the hypothesis proposed by Stamford et al. (2017) that multiple life history stages of Arctic Grayling might continue to support a metapopulation in Manson River and surrounding small tributaries (Figure 9). Similarly, sampling in 2020 from 45 sites and 22 streams toward the east in Williston and Dinosaur reservoirs failed to support the hypothesis that Arctic Grayling are expanding their range into small streams entering the reservoir environment, as previously suggested by the eDNA results in Finlay Reach (Figure 8). The hypothesis that critical habitats continue to support rearing Arctic Grayling was rejected, having not a single positive eDNA result from 18 sites in six putative critical habitat streams entering Parsnip Reach, Peace Reach, and Dinosaur Reservoir (see Table 4; Figure 6). Intriguingly, and unexpectedly, Arctic Grayling eDNA was detected at five sites and supports the hypothesis that Arctic Grayling continue to rear in small streams, and possibly migrate through the reservoir from surrounding natal areas. Potentially, a large river adapted life history migrates through the reservoir to rear among small tributaries, possibly originating from natal areas in surrounding core areas (e.g. Nation, Parsnip core areas).

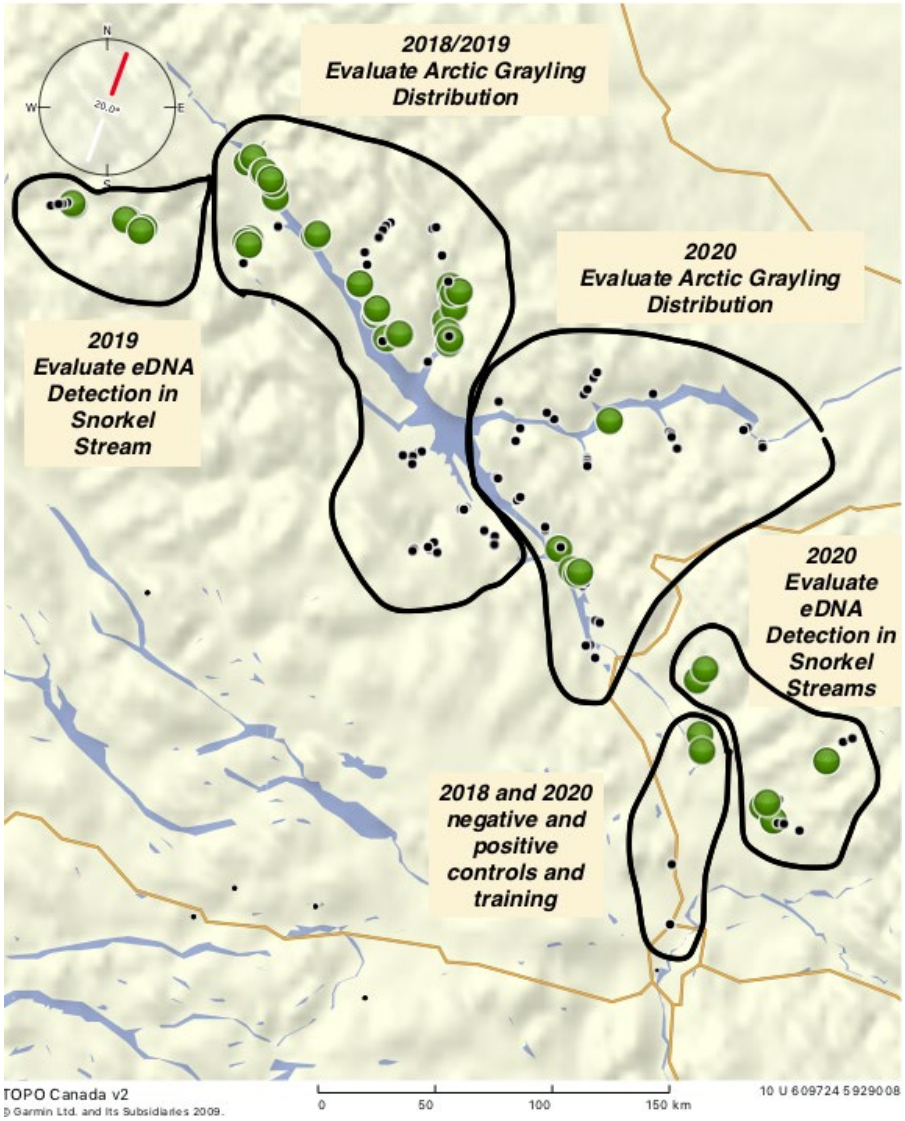



Figure 8: Distribution of Arctic Grayling eDNA sampling after three field seasons, including 2018, 2019 and 2020. Small black dots = Arctic Grayling eDNA not Detected; Large Green Dots= Arctic Grayling eDNA detected.

## 5.0 Conclusions and recommendations

1. Although Arctic Grayling eDNA was rare in the south and eastern portions of flooded Upper Peace Basin and absent in Dinosaur Reservoir, the eDNA results also suggest the species continues to rear in sparsely distributed small streams entering Parsnip and Peace reaches. Sampling for fish aimed at identifying life history stages present and use techniques (e.g. microchemistry, genetics) to determine migratory patterns and demographic connections with surrounding natal areas. Given the rare occurrence and potentially dynamic nature of their distribution and movement patterns among small tributaries, continued monitoring using eDNA might inform a sampling strategy. Populations that migrate through the reservoir potentially carry life history traits best tuned for recovery in flooded conditions (Hagen and Stamford 2017).

2. The detection probability appears to be consistent among streams, and both bottle filling and direct filtering methods detected Arctic Grayling eDNA when adults were observed within 1.5 kilometers upstream in snorkel index sections. Detection rates decreased among one-liter triplicates, however, when adult densities were low and suggests filter volume influences detection probability. Three liters of filtered river water detected a single adult Arctic Grayling present within 1.5 kilometers upstream, while five liters detected eDNA 4.5 kilometers away. Sample spacing and minimum volumes used in the direct filtering method used throughout this study has rigorously evaluated the distribution of Arctic Grayling eDNA among tributaries entering Williston and Dinosaur reservoirs.

3. Arctic Grayling eDNA detected in Parsnip, Peace, and Finlay Reach streams provide direction for fish sampling focused on identifying the different life history stages present and begin investigating the demographic connections. Sampling should address the hypothesis that surrounding natal areas (e.g. Parsnip, Nation, Ingenika, Finlay, Ospika rivers) might provide recruitment to metapopulations that migrate through the reservoir to complete their life history. Such populations potentially carry ancestral life history traits that are best adapted for recovery to the flooded environment of Williston Reservoir (Hagen and Stamford 2017).

4. Given the success using eDNA to identify the summer rearing distribution in this study, there is great potential for using eDNA to identify other seasonal critical habitat use such as spawning, and overwintering areas.

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