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2019 WILLISTON ARCTIC GRAYLING DISTRIBUTION: eDNA MONITORING

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2019 Williston Arctic Grayling Distribution: eDNA Monitoring

FWCP Project Number: PEA-F20-F-2965

Prepared For

FISH AND WILDLIFE COMPENSATION PROGRAM
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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.” Two years of sampling with environmental DNA (eDNA) has successfully expanded the known range of summer habitat use for Arctic Grayling in Williston Core Area, including tributaries entering Ingenika River, Finlay River, Ospika River, and four streams draining the eastern slopes of Finlay Reach. Previously, sampling records from these streams suggested Arctic Grayling had been extirpated. Presence upstream in two of these streams, Collins Creek and Ospika River, suggests they might sustain more than one life history stage and possibly independent metapopulations. In particular, the distribution of Arctic Grayling in Ospika River covers a habitat area of at least 20 kilometers in the middle river including three tributaries. Positive results in Finlay Arm near the mouth of Finlay River suggest Arctic Grayling use Williston Reservoir either to rear or to migrate to other streams to complete their life history. Movements through the reservoir might explain some of the positive results in smaller streams draining the eastern slopes of Finlay Reach (i.e. Chowika Creek, Davis River, Lafferty Creek, Collins Creek, Ospika River) that might not sustain all life history stages.

Failure to detect Arctic Grayling eDNA at 19 sites distributed in Manson River, Blackwater Creek, Fries Creek, and Strandberg Creek on the west side of Parsnip Reach suggests Arctic Grayling are too rare and possibly highly mobile and avoided capture in these slow meandering streams. Validating post-flood records of fry, yearlings, or adults in these streams using eDNA was unsuccessful.

We evaluated the detection probability and false negative rate for our Arctic Grayling assay by pairing eDNA samples with snorkel survey observations in Ingenika River. We also initiated laboratory components, including a digital droplet PCR platform, the ePLANT and Lambda DNA, which improved evaluation of the eDNA quality in samples and the potential for false negatives. We also streamlined our field sampling methods and apparatus using lab trials to determine that larger pore sized filters outperform the smaller pore sized filters when a minimum volume of water (1-liter) is filtered from sample sites. Larger pore size filters significantly improve sampling efficiency, especially in turbid streams.

We determined that our assay detected Arctic Grayling in 100% of sites where they were observed within 1.5km upstream in snorkel surveys, and not detected in 100% of sites where Arctic Grayling were not observed. The detection rate decreased at the margins of habitat areas as determined by snorkel observations. An overall false negative rate of 30% among these marginal sites suggests a minimum of three liters of water filtered from sites is required to avoid collecting false negatives at sample sites. Field trials comparing collection methods were confounded by high water conditions at the time of sampling and suggest that using bottles to collect from flowing water improves eDNA detection in larger rivers. Direct filtering from stream margins of larger streams collected false negatives, while wading out into the current at the same sites detected Grayling eDNA at four positive sites. We confirmed presence of Arctic Grayling eDNA in the lower Anzac River by sampling from riffle habitat in 2019, while at the same site in 2018 direct filtering on the edge of a pool resulted in a false negative.

Acknowledgements

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1 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 1760 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 in habitat has had substantial impacts on the surrounding ecological communities, including the Arctic Grayling (Baker et al. 2000).

Within the Williston Reservoir watershed, the Arctic Grayling is of conservation concern and important to recreational fishers and local First Nations, so it 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). They were since 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 Repor*t identified and evaluated eight Williston metapopulations (i.e. Core Areas, Figure 1.1), 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, as is their ability to sustain Arctic Grayling populations because the distribution of critical habitats for all life history stages remain poorly defined. 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).

Watershed connectivity is crucial for Arctic Grayling as all life history stages undergo lengthy seasonal migrations between different habitat types (Blackman 2002; Stewart et al. 2007a). 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 Williston Reservoir, 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). Grayling rarely enter lakes within the Williston watershed, which suggests most metapopulations might lack the necessary adaptations for adfluvial life history strategies. Consequently, the presence of the reservoir might restrict access to habitats, especially in those streams draining into Upper Peace and Williston Core Areas, where vast habitat areas were lost and post-flood Arctic Grayling records are rare (Williamson and Zimmerman 2004; Stamford et al. 2017). Alternatively, migratory behaviours are adapted to move locally, and current populations rarely migrated to habitats beyond their home stream. Genetic and microchemistry studies

support the second hypothesis (Stamford and Taylor 2005; Clarke et al. 2007; Shrimpton et al. 2012; Shrimpton and Clarke 2012), and it appears unlikely that Arctic Grayling abundance in Upper Peace and Williston core areas are being rescued by recruitment from surrounding core areas[1] (Figure 1.1). The implications for species and habitat conservation (strategic objectives of the FWCP) are that continued habitat use in Williston and Upper Peace core areas (Figure 1.1) might constitute ancestral migratory behaviours that currently include movements through the reservoir to complete their life history. Alternatively, remnant populations persist only in streams entering Williston and Upper Peace core areas that provide all the necessary habitat elements that sustain viable resident populations (Hawkshaw et al. 2014). Such metapopulations might be uniquely adapted and most appropriate for conservation and enhancement actions aimed at recovering the historical biocomplexity and abundance of Williston Grayling (Stamford et al. 2017). Post flood Arctic Grayling records in the downstream reaches of tributaries draining the eastern slopes of Finlay Arm and western slopes of Parsnip Arm were hypothesized to indicate presence of critical habitats for Arctic Grayling (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 (McElhany et al. 2000). However, the prospect of conducting traditional inventory studies (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 itself, 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 habitat use in high priority streams entering Williston and Dinosaur reservoirs (see Table 1 *in* Stamford et al. 2018). Results from the first field season (i.e. 2018) have identified Arctic Grayling presence in five Finlay Reach streams and one site in Williston Reservoir where other sampling methods (e.g. electrofishing, angling, seining) failed to detect their presence (Williamson and Zimmerman 2004, 2005; Stamford et al. 2017; Strohm et al. 2019). These eDNA results have effectively expanded the known range of habitat use for Arctic Grayling into Finlay Arm streams previously thought functionally extinct and suggest they might move through the reservoir to complete their life history in more than one stream. Previous work suggests that Arctic grayling do not spend enough time in Williston reservoir to develop an elemental signature in their otoliths (Clarke et al. 2007). However, the eDNA results support the findings from other studies that indicate higher capture probability for eDNA relative to other sampling methods (e.g. Laramie et al. 2015;

[1] Metapopulation structure in Williston Arctic Grayling appears to have evolved prior to reservoir formation (Stamford and Taylor 2005).

McKelvey et al. 2016). However, the results also failed to detect Arctic Grayling eDNA in an expected positive site (Anzac River), which was difficult to explain without having estimated a false negative rate for our Grayling assay. In 2019, as described in this report, the sampling rationale was largely directed by efforts to refine the methodology and expand sampling around positive results from 2018 and improve resolution of the known Arctic grayling distributions.

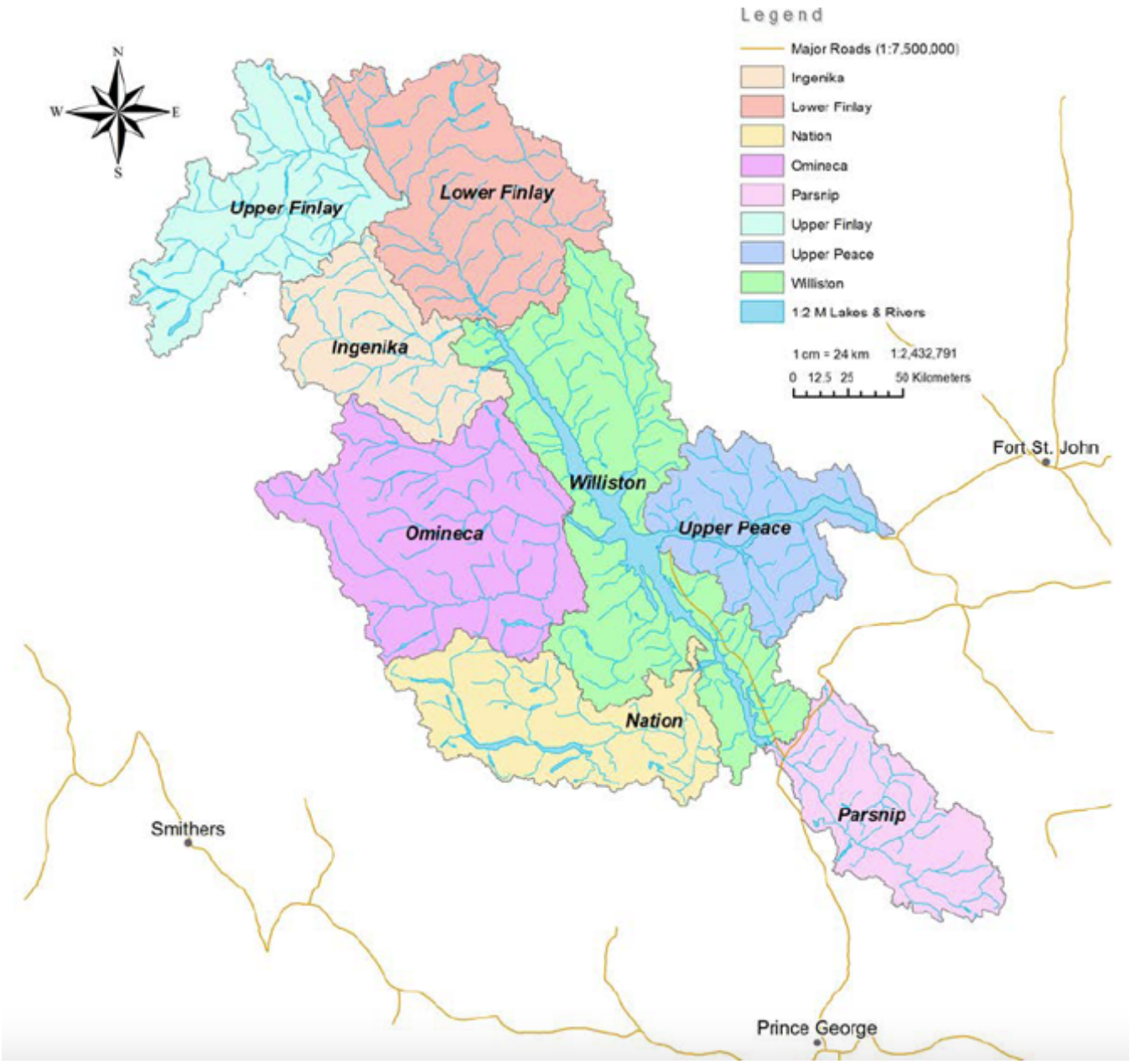

Figure 1.1. Arctic Grayling core areas of the Williston watershed which are thought to exist as metapopulations (*From* Stamford et al. 2017).

2 Goals and Objectives

The original three proposed objectives, which included fish sampling to validate eDNA detections in 2018 data, were changed due to uncertainties in the false negative rates for our Arctic Grayling assay. Funding for the fish sampling objective was denied so we instead designed sampling to estimate the detection probability and false negative rate for our Arctic Grayling eDNA assay. A prioritized list of 102 eDNA sites was developed to answer the objectives but also allowed field crews to respond to unforeseen variables (e.g. weather, increased sampling times per site) and still collect informative data without going over the project budget (Table 2.1). As a result of difficult access to some sites, uncharacteristic weather that terminated snorkel counts, and higher sample times required per site, eDNA was collected from fewer sites than planned (n=47) but the objectives were successfully addressed and within budget.

Table 2.1: Project objectives for 2019 eDNA study showing number of sites planned and number of sites sampled to complete objectives.

	Objective	No. Sites Planned	No. Sites Sampled	Comment
1	Expand Scope	40	20	Collected from priority sites.
2	Tributary Use	42	13	Poor weather and collected from priority sites.
3	Detection Probability and Methods Comparison	20	14	Poor weather stopped snorkel surveys; added more eDNA sites

2.1 Expanding the scope of eDNA sampling

2.1.1 Anzac, Manson, and Ospika rivers re-sampling

Negative Grayling results in 2018 were unexpected in the Anzac and Manson Rivers, given recent observations at these sites (Hawkshaw and Shrimpton 2014; Hagen et al. 2019). Possibly influences from deep pools and low gradient glides located immediately upstream from the eDNA sites might have facilitated rapid settling of eDNA from the water column and Arctic Grayling were actually present upstream. Alternatively, Arctic Grayling might be rare in Manson River and the lower reaches of Anzac River[2], and were beyond the detection distance for our assay during sampling in 2018. To examine the hypothesis that slow current reduced the detection distance for eDNA in the lower Anzac River, we re-sampled the same site except in riffle habitat upstream from the pool. In Manson River we resampled

[2] Arctic Grayling are often abundant upstream in Anzac River and less abundant downstream. The lower reaches where eDNA was sampled in Anzac and Manson rivers might serve primarily as migration corridors to access other habitats (e.g. Parsnip River, small west Parsnip tributaries).

the same 2018 expected positive reach with three sites and added six sites upstream in the mainstem and two tributaries to examine the hypothesis that Arctic Grayling are rare in rearing habitats. Recent observations suggest Arctic Grayling fry and adults might continue to use habitats in Manson River (Hawkshaw and Shrimpton 2014; Stamford et al. 2017).

There were no positive results in 2018 from the smaller streams draining the western slopes of Parsnip Arm (Blackwater, Fries, Strandburg creeks), which were sampled opportunistically each at a single road crossing site. Additional samples were required to address the hypothesis of habitat use. Previous records suggest that yearling juveniles (100mm size range) might be distributed in the extensive low gradient habitats located further downstream from where they were sampled in 2018. We therefore collected from three sample sites in the low gradient downstream habitats in both Fries and Blackwater creeks.

Ospika River and its complex and extensive network of tributaries was thought to be devoid of Arctic Grayling prior to the positive mainstem eDNA samples collected in 2018. Therefore, a primary goal of 2019 sampling was to assess the species' summer rearing distribution in the lower 50km of the Ospika mainstem from the mouth at Williston Reservoir, north to McCusker Creek, including several sampling events at the mouths of tributaries (Stevenson, Gauvreau, Aley, Unnamed, Balden, McCusker creeks).

2.2 Tributary use in Lower Finlay, Ingenika, and Williston core areas.

2.2.1 Ingenika River

Absence of recent tributary use in Ingenika River Arctic Grayling was interpreted by the most recent conservation assessment to potentially signify a range contraction, which suggests the population might be declining (Stamford et al. 2017). The only negative eDNA result in 2018 from fluvial habitats in Ingenika River came from Swannell River below falls where in 1996 adults were observed rearing in deep pools (Stamford et al. 2017). Grayling absence at this site and its close proximity to linear developments suggest that exploitation might have resulted in a range contraction and supports the recent conservation assessment. The positive 2018 eDNA result downstream in Swannell River, however, is the first record of Arctic Grayling presence since the mid 1970's in the lower reaches of Ingenika tributaries, despite significant electrofishing and angling efforts (e.g. Cowie and Blackman 2004, 2012). In 2019 we chose to further examine tributary use higher in the Ingenika watershed, and planned to sample the lower reaches of five tributaries, including Wrede Creek where (similar to Swannell River) electrofishing and angling efforts in 2004 failed to verify a mid 1970's observation of Arctic Grayling presence (Bruce and Starr 1985; Cowie and Blackman 2004). Helicopter access to these tributaries and sampling personnel were kindly provided by the Ingenika River Arctic Grayling snorkel survey project.

2.2.2 Finlay River and Williston Core Area

Sampling in 2018 detected Arctic Grayling eDNA in three locations in lower Finlay River, including one site in the reservoir, which suggests Arctic Grayling may rear in the flooded river mouth and possibly disperse to other tributaries[3]. Numerous fry records identify natal areas in lower Finlay River that potentially provide recruitment for a large river ecotype uniquely adapted to complex migrations that potentially include numerous small tributaries (Stamford et al. 2017). Such a life history might also explain the positive 2018 eDNA results in five eastern slope tributaries that currently drain into flooded sections of Finlay Arm. If dispersal to numerous tributaries describes the migratory behaviour of lower Finlay River Arctic Grayling, then other tributaries might also provide habitats required to complete the life history of this potential ecotype. Alternatively, the population structure is more complex and other independent metapopulations complete their life history in other streams (e.g. Ospika River). Sampling in 2019 was designed to begin addressing these alternative hypotheses with more intensive sampling in tributaries where Arctic Grayling presence is unknown, including two entering lower Finlay River and five tributaries of Ospika River.

2.3 Detection Probability and methods comparison

2.3.1 Calibration of eDNA results using Snorkel Observations

Uncertainties associated with the detection rate of our eDNA assay were addressed for streams in 2019 by pairing eDNA sample collections with direct observations from snorkel surveys in Ingenika River. The aim was to determine a false negative rate for our assay within a standard stream distance of 1.5 kilometers; a distance used in other studies to successfully resolve boundaries around habitat use areas for stream dwelling salmonids (e.g. Laramie et al. 2015; McKelvey et al. 2016). Although observation probability is unknown for these snorkel surveys, they are probably higher further upstream where smaller discharge and stream widths provide fewer opportunities for fish to avoid the snorkellers. For our study, eDNA was hypothesized to have equivalent to, or higher, detection probability relative to snorkel surveys. eDNA was predicted to detect Arctic grayling presence at all sites where snorkel observations confirmed presence upstream. Alternatively, capture efficiency (detection rate) of our Arctic Grayling assay might be shorter than 1.5 kilometers or be inconsistent due to influences from other variables (e.g. water chemistry, turbulence, discharge) and fail to detect eDNA when Arctic Grayling presence is confirmed upstream. Possibly higher discharge in downstream reaches dilutes eDNA in the water column and diminishes capture efficiency; or perhaps Arctic Grayling life history improves capture efficiency in downstream sites (regardless of increases in discharge) where younger life history stages often accumulate and therefore increase eDNA concentrations (Cowie and Blackman

[3] Arctic Grayling eDNA was not detected in seven other reservoir samples located near the mouths of other Finlay Reach streams (Strohm et al. 2019).

2004, 2012). For instance, eDNA results in 2018 revealed presence in three downstream locations in Ingenika River where Arctic Grayling have consistently avoided detection in two years of snorkel surveys (Hagen et al. 2019). Electrofishing data suggest fry are patchily distributed in the lower reaches of Ingenika River (Cowie and Blackman 2004, 2012).

To save costs, we partnered with another FWCP funded study that provided helicopter support, field personnel to collect eDNA, and direct observations of Arctic Grayling upstream of eDNA sites.

2.3.2 Collection method comparison and temporal repeatability.

The eDNA sampling needed to be rapid to collect from multiple sites on the same morning as but before snorkel surveys in Ingenika River, and the Bottle Filling Method (Section 3.1.3) was most appropriate. The Instream Filtering Method (Section 3.1.2) was used to collect eDNA at all other sites in 2018 and 2019 (See Section 3 for detailed methods), however, and it was not certain how much the different water volumes and filter pore sizes used between these methods would affect the eDNA results. Smaller pore sized filters (e.g. 0.45um MCE) are often recommended when sampling smaller volumes of water (e.g. 1-L replicates) assuming they yield higher quantities of eDNA (Helbing and Hobbs 2019). Alternatively, significant quantities of eDNA might exist as larger particles in the water column and some studies suggest using larger pore sized filters (1.5um glass fiber) to collect from large volumes of water (e.g. 5 Liters) will improve detection of the target organism, especially in streams (Wilcox et al. 2016; Carim et al. 2016). To distinguish between these alternatives, we conducted laboratory trials to determine eDNA capture rates between the two types of filters (i.e. 0. 45um pore sized MCE versus 1.5um Glass Fiber). To determine if the different sampling methods affected the detection rate in water collected from the same sites, we also paired both collection methods at four sites, including Chowika Creek (n=1), Davis River (n=1), and Ospika River (n=2). Arctic Grayling eDNA was detected at all four sites using the instream filtering method and therefore expected to be detected again in 2019 (Strohm et al. 2019).

2.3.3 Viability of eDNA samples, and sensitivity of eDNA detection

Following the analysis of 2018 samples, it was suggested that an additional assay be incorporated into the laboratory workflow to ensure the presence of viable eDNA in samples. This is especially important for the bottle filling method of sample collection since bottles must be bleached between sampling events. Therefore, the ePLANT assay was chosen to detect the presence of intact chloroplast DNA, which is ubiquitously present in aquatic environments within algal communities (Helbing and Hobbs 2019).

To refine our judgment of the quantity of eDNA present in samples, and the sensitivity of detection, we chose to switch from a quantitative PCR (qPCR) based workflow (used in 2018), to digital droplet (ddPCR; used in 2019). Briefly, qPCR monitors the accumulation of target DNA during cycles of enzymatic amplification. This is done by quantifying the intensity of florescent signal associated with DNA amplification, that intensity is then compared to a reference with a standard curve to calculate the

original abundance of template DNA. DdPCR separates all DNA in a sample into thousands of individual oil droplets which are analyzed individually for their florescent signal, and the absolute number of successful amplifications (copy numbers) can be calculated without the use of standard curves. In addition, ddPCR has been found to be more effective at detecting eDNA in low concentrations, and is less sensitive to inhibitors present in environmental samples (Doi et al. 2015).

3 Methods

3.1 Sample collection

We conducted field sampling between August 5 – 16, 2019 and used the Instream Filtration Method (Section 3.1.2) to collect eDNA at all sites except for those in Ingenika River. The Bottle Filling Method (Section 3.1.3) was used to pair eDNA sampling with snorkel surveys in Ingenika River, and also four sites in other streams where both methods were used (see Section 2.3.2). Generally, sampling effort was focused on the low gradient and low elevation reaches of rivers that provide Arctic Grayling critical habitats in Williston Core Area (Stamford et al. 2017). To expand the scope of sampling and identify presence in tributaries (Objectives 2.1 and 2.2), sites were selected in the downstream reaches of tributaries and low gradient mainstems of larger rivers where fry often accumulate to rear during summer (Figure 3.1). Environmental DNA often settles rapidly from the water column (Goldberg et al. 2015), so sampling was focused where more individuals were expected to be present. To examine the detection probability and methods comparisons (Objective 2.3), sites with expected Arctic grayling presence or absence were selected based on previous sampling results.

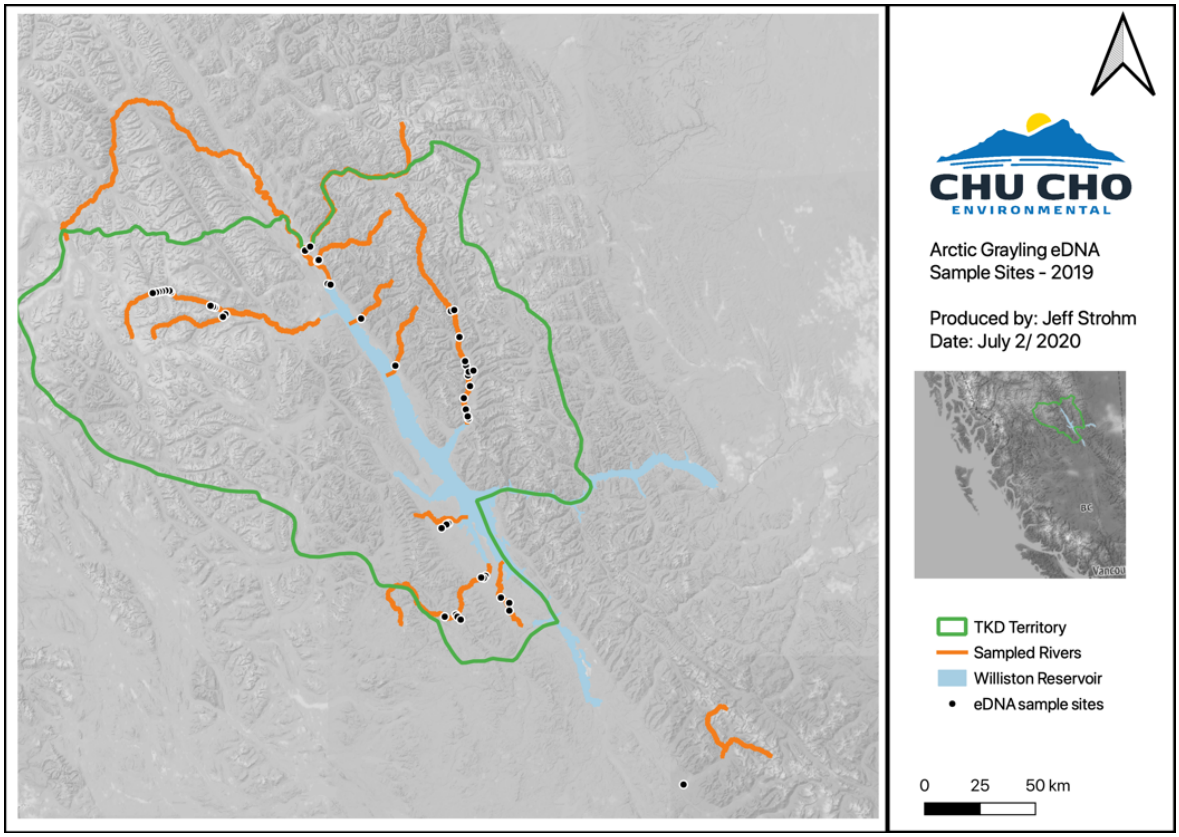

Figure 3.1 Spatial distribution of Arctic Grayling eDNA sample collection in 2019.

3.1.1 Control sampling

Two control samples were collected each sampling day to detect equipment 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 sampled water bottles to avoid direct contamination. Each control sample consisted of 1 L of distilled water filtered through the assembly, folded the filter into quarters (filtrate side in) and stored in an ethanol-filled cryovial, which was stored at -20 °C until laboratory analyses.

3.1.2 Instream Filtering Method

Sample collection methods in 2019 were consistent with 2018 and are described by Carim et al. (2016) and used by Jane (2015), McKelvey et al. (2016), and Wilcox et al. (2016) to estimate eDNA capture probability in streams. The method, which captures eDNA on 1.5um pore size 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). We conducted in-field water filtering and sample collection using a portable peristaltic pump to draw water through a filter assembly fitted with 1.5um pore sized glass fiber filters. The filter assembly was placed directly into the stream at sites with sufficient flow near the stream bank and estimated to draw water from far upstream. At sites where stream margins had minimal flow and sites in the reservoir, sterile bottles were used to collect from flowing water or some distance offshore to bring water to the filter apparatus.

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 liters of water directly from the stream through 1.5um pore sized glass fiber filters. If a filter became clogged before pumping 3 L of water, it was replaced with new filter to complete the total 5 L water volume filtered. 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 kept cool and dark until delivery to the lab. Cryovials were stored in the laboratory at -20°C until analyses. Multiple filters (maximum 3) from a single site were stored in separate cryovials but all were combined and analysed as a single replicate in the laboratory.

All equipment was carried in a 50 L backpack in the field (Table 3.1). Site kits were assembled in 4 L resealable bags prior to deployment and each contained enough supplies to collect one sample. Items 1, 2, 3 and 4 (Table 3.1) 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 3.1, Figure 3.2). 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).

Table 3.1: Equipment required for in-field collection of eDNA samples.

a. Site kit	b. Filtering apparatus	c. Other equipment
1. nitrile gloves 2. filter assembly 3. glass filters 4. steel forceps	5. peristaltic pump 6. pump battery (with backup battery) 7. power cord 8. tubing with funnel adaptor 9. 5 L outflow bucket	10. 1 L bottles 11. garbage bags for used supplies 12. battery charger 13. ethanol-filled cryovials for sample storage 14. clean sample bags 15. binder with datasheets 16. permanent markers, pencils 17. GPS unit

Figure 3.2. The filter apparatus for eDNA sample collection consisted of the peristaltic pump (5), pump battery (6), power cord (7), tubing with funnel adaptor (8), filter assembly (2), and 5L outflow bucket (9).

3.1.3 Bottle filling method

3.1.3.1 Sample Collection

Samples were collected from flowing water in triplicate 1L HDPE bottles. 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.

3.1.3.2 Sample filtration

Both small pore sized (0.45um MCE as recommended by Helbing and Hobbs 2019) and large pore sized (1.5um glass fiber filters; GF) were used to capture eDNA from the one-liter replicate bottle samples. Small pore size MCE were the first choice for consistency and used at all sites in the clear flowing Ingenika River and Chowika Creek. The MCE were problematic, however, because suspended sediments rapidly clogged the small pore size in other tributaries of Finlay Reach. Therefore MCE were replaced with GF to improve sampling efficiency. Filters were placed in the funnel assembly on top of a vacuum flask, and a GAST pump was used to draw the full 1L sample from each replicate through the filter. The filter was then placed in a cryovial containing 95% ethanol, and stored at -20 °C. Forceps were then cleaned with 50% bleach and rinsed thoroughly with distilled water. Two negative control blanks were run following sample filtering whereby 1L of distilled water was processed and handled in the same manner as eDNA samples.

3.2 Detection Probability and Methods Comparison

To examine the ability for our assay to detect eDNA when Arctic Grayling are present upstream (false negative rate), and possibly gain insight into the distribution of difficult to sample juvenile life history stages, eDNA sample sites were distributed 1.5 kilometers apart within snorkel survey sections in the Ingenika River. Snorkel survey data from 2004 and 2018 (Cowie and Blackman 2012; Hagen at al. 2019) were used to ensure that eDNA sites were selected below snorkel sections expected to have both absent and present observations of Arctic grayling upstream. The original sampling design drew from methods used by Laramie et al. (2015) and included 15 sites distributed among snorkel surveys in Upper, Middle, and Lower reaches of Ingenika River (Figure 3.3). The snorkel survey was terminated early in 2019 because heavy rains caused high turbidity. Only seven eDNA sites were paired with snorkel observations in the Upper and Middle reaches including both expected Arctic grayling present and expected absent sites. Additional sampling was needed to improve the rigour of analyses, however, so the number of sites with expected presence (n=9) and expected absence (n=7) for Arctic Grayling from Ingenika and other surrounding watersheds (Table 3.2).

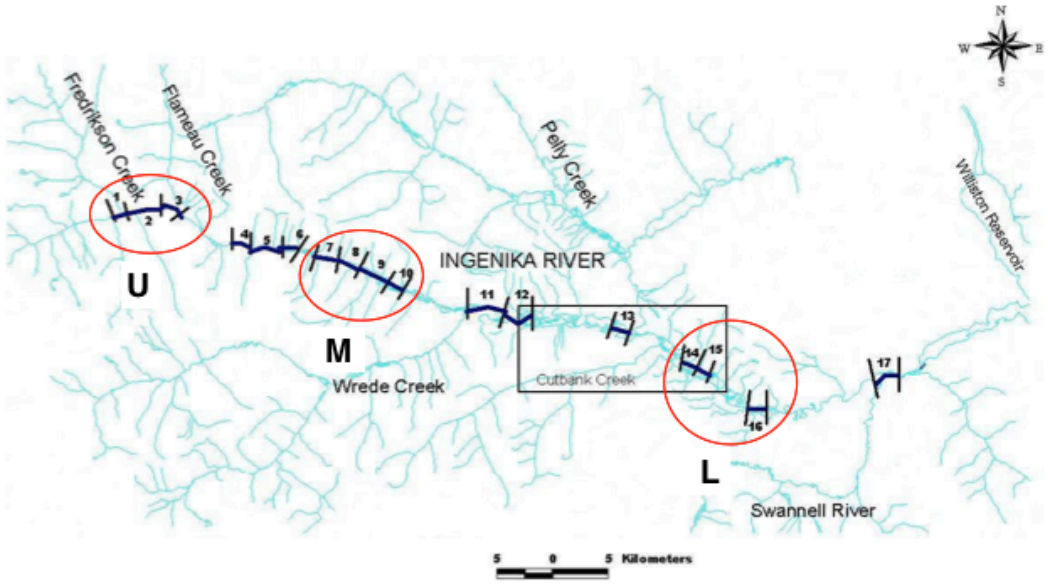

Figure 3.3: Ingenika River showing the original sampling design for paired eDNA and snorkel observation data. Snorkel sections enclosed in red ovals are Upper, Middle, and Lower survey sections (each and labelled U, M, and L, respectively). Snorkel survey reaches labelled with numbers between vertical lines along the river. Rectangle estimates the hub of Arctic grayling distribution as estimated from the first 2004 snorkel survey (Cowie and Blackman 2012).

Table 3.2 Distribution of sampling in Ingenika River and surrounding watersheds to investigate false negative rate for our eDNA assay during summer 2019. Sites designated as expected to indicate Arctic grayling present (n=9) and expected absence (n=7) in eDNA samples were determined from previous sampling records, including snorkel observations and other sampling efforts.

Watershed	eDNA Sites	Paired Snorkel Surveys	No. eDNA sample sites		Objective
			Expected GR Present	Expected GR Absent	
Ingenika	HW1, HW2	NA	0	2	Investigate GR distribution upstream of snorkel surveys; Sites HW1 and HW2 expected GR absent (LRDW; Cowie and Backman 2012).
Ingenika	U1, U2, U3, U4, U5	U1, U2, U3, U4, U5	2	3	SN sites consecutive and include expected GR present (SN2, 3) and expected GR absent (SN1) sections (Cowie and Blackman 2012; Hagen et al. 2019).
Ingenika	M1, M2, M3	M2, M3	3	0	Sites consecutive and all GR present. M1 was not snorkelled but GR expected present. Possible upstream limit of fry distribution (Cowie and Blackman 2012).
Ingenika	Wrede 1, Wrede 2	NA	0	2	Investigate tributary use; expected GR absent (Cowie and Blackman 2012).
Chowika	Chow-1	NA	1	0	Compare methods; examine repeatability; expected GR present (2018 eDNA result).
Davis	Davis-1	NA	1	0	Compare methods; examine repeatability; expected GR present (2018 eDNA results)
Ospika	Osp-Main1, Osp-Main2	NA	2	0	Compare methods; examine repeatability; expected GR present and expected GR absent (2018 eDNA results; Williamson and Zimmerman 2004).

3.2.1.1 Sampling sites

Sampling for eDNA in the Ingenika watershed, Chowika Creek, Davis River, and Ospika River occurred between August 5th and 9th, 2019 eDNA using the Bottle Filling Method (Section 3.3.1). Sampling in Ingenika River was successfully paired with snorkel observations in five upper sites (U1, U2, U3, U4) and two middle sites (M1, and M2) and evaluated both presence and absence of Arctic Grayling in 1.5km sections (Table 3.2, Figure 3.3). Five additional sites in Ingenika watershed included one expected present (M3), two expected absent sites in Wrede Creek, and two expected absent sites upstream of snorkel surveys (HW1, HW2). Four expected present sites were situated in Chowika Creek (n=1), Davis River (n=1), and Ospika River (n=2) and used in the analysis of detection probability and methods comparison (Table 3.2). Expected presence/absence of Arctic Grayling was determined based on previous sampling (e.g. Bruce and Starr 1985; Cowie and Blackman 2004, 2012; Hagen et al. 2019; Strohm et al. 2019).

3.2.1.2 Sample collection

During the snorkel survey reconnaissance flight for the index section to be swum that day, samples were collected at 1.5 km intervals working from a downstream to upstream direction. Additional sites upstream (HW1, HW2) and downstream (M1) of snorkelled sections were similarly spaced 1.5km apart in Ingenika River. Two sites in Wrede Creek were selected from the air to be adjacent to potential Grayling habitats located roughly 3 and 4.5km upstream from the mouth.

Sites in other watersheds included Chowika Creek (n=1), Davis River (n=1), and Ospika River (n=2) were sampled using both the Bottle Filling Method and Direct Filtering Method, and all sites were expected to detect presence of Arctic Grayling as did the 2018 eDNA results (Strohm et al. 2019). All replicates collected from Ingenika River, Chowika Creek, and one replicate from Davis River were filtered using 0.45um MCE filters. Two replicates from Davis River, and all six replicates collected from Ospika River were filtered using GF filters.

3.2.1.3 Snorkel surveys

For a detailed description of the Ingenika snorkel study, see Strohm et al. (2020). Concurrent with the collection of fish abundance and size data for that study, we noted the total number of Arctic Grayling observed within 1.5km upstream of each eDNA sampling sites. Surveys were conducted by three experienced biologists snorkelling left, middle, right lanes of the river. Constant communication was maintained, and a detailed plan was devised to effectively survey the available habitat whenever the river morphology changed. Such organization was key to maintain a line of swimmers to reduce the ability for fish to evade detection.

3.2.1.4 Experimental comparison filter types

Laboratory trials using known quantities of DNA were conducted to compare the capture efficiency of small (0.45µm) and larger (1.5µm) pore sized filters. Both types of filters were used to examine the distribution of Arctic grayling in this study and understanding the influences of filter pore size on eDNA yield is key to interpreting the data describing the distribution of Peace Basin Arctic grayling. Possibly,

eDNA is captured equally by both small (0.45µm) and larger (1.5µm) pore sizes if eDNA clumps are commonly larger than 1.5µm (e.g. Wilcox et al. 2016). Alternatively, smaller pore sizes yield higher quantities of eDNA and are required for smaller volumes composed in triplicates (e.g. Helbing and Hobbs 2019). See Appendix I for more detailed methods.

3.2.2 Data analyses

We used a chi square test to compare the distributions of eDNA detection/not detection with the known (expected) distribution based on direct observations (snorkel surveys) and inferred presence/absence (most recent records). Detection probability was calculated as the average site detection rate ± SE to compare between snorkel sites with confirmed presence (Arctic Grayling observed upstream), inferred presence, and inferred absence of Arctic Grayling. Individual site detections were the number of 1L 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 needed per site to determine presence of Arctic Grayling, among sites where confirmed presence was indicated by detection of Arctic Grayling eDNA. Thus, 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.

Figure 3.4: Snorkel survey crew demonstrating the organization needed for effective fish counts during the 2019 Ingenika swims, and eDNA sampling.

3.3 Molecular methods

3.3.1 eDNA Sample Processing

Filters were processed with an extraction control for each extraction batch. Environmental DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen) following a modified protocol for water filters. Sites that required multiple filters had their extracts pooled on the column loading step, after lysis. For each filter, an "i" and "ii" eDNA elution was collected (50 μL each). After the initial sample "i" eDNA was eluted from its purification column, a second volume 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 "ii" sample analyzed only when the "i" sample showed signs of assay inhibition or low signal.

A published TaqMan assay for Arctic Grayling was used to detect Arctic Grayling eDNA (Rodgers et al. 2018). This assay was designed for populations on the North Slope of Alaska and was tested against other salmonids and non-salmonids by the authors. We previously (Stamford et al. 2018) checked the specificity and sensitivity of the assay against Arctic Grayling DNA isolates from Northern BC, as well as other co-occurring fish species (Whitefish, Rainbow Trout, Northern Pikeminnow, Sockeye Salmon, Dolly Varden, Brook Trout). Digital droplet PCR (ddPCR, BioRad QX200 AutoDG) successfully amplified the gBlock and known positive samples, as well as a previously inhibited sample from last year (Anzac). Briefly, samples were run in 24 μL reactions using Master Mix for probes (no UTP), nuclease-free water, 6 μL of sample extract, and PrimeTime probe mix. Droplets were generated by an automated droplet generator using 20 μL of the above reaction mix (5 μL – 1/10 of sample elution), amplified with a BioRad C1000 Touch thermocycler, and read on a QX200 Droplet Reader (Bio-Rad Laboratories).

Positive control lambda DNA was added to the master mix to test all first elutions for PCR inhibition. This was run as a duplex (both assays run in the same reaction) with the ePlant (i.e. IntegritE-DNA) assay (Hobbs et al. 2019). The dual assay was run under the same conditions as the samples, but with only 2 μL of sample. An advantage of ddPCR is that copy numbers can be directly compared, making identification of outliers clearer (rather than a simple pass/fail system). These tests suggest partial assay inhibition by results showing lambda DNA presence at a lower quantitative amount than control samples, despite being spiked with the same amount of control DNA template. Any samples that failed, or where lambda appeared lower than 80 copies/μL (of 20 μL reaction volume), were retested using the second elution volume. Any ePlant reactions that appeared to have a lower peak florescence were also retested using the second elution volume. The "ii" elutions of these samples were then tested ("ii" elutions are suspected to be more dilute in terms of both eDNA and contaminants, and are thus cleaner). In addition to the second elution, any sample exhibiting possible inhibition from either lambda or ePlant testing had the remaining "i" and "ii" elution volumes pooled and cleaned using a PCR inhibitor removal column (ZYMO Research. Irvine, CA, U.S.A). These cleaned elutions were also run in duplicate.

3.3.2 ddPCR Assay Sensitivity

A dilution series was used to evaluate the performance of the ddPCR platform with a gBlock consisting of Grayling sequence. The gBlock is a synthesized piece of target DNA and provided a more precise estimate of the number of mitochondrial DNA (mtDNA) copies that the assays can detect. The dilution series ranged from 60,000 to 0.6 copies per reaction. Copy numbers estimated from each duplicate were within the same range of each other and decreased by an order of magnitude with each dilution, confirming that the ddPCR platform detected quantities of template, with this particular assay, as expected. At the highest end of the range (60,000 copies), the limit of quantifiable resolution is reach due to the saturation of positive droplets (i.e., all 15-20,000 droplets yield positive results (see Supplementary Information for details).

4 Results and Discussion

4.1 ddPCR Grayling eDNA Results

One hundred twenty-four individual filters, representing one hundred six sample sites and field control blanks, were submitted for analysis. Arctic Grayling eDNA was not detected in any field distilled water controls (n=23). Generally, the ddPCR improved detection of Arctic grayling eDNA, relative to the qPCR used in 2018 in spite of thick sediment deposits on many filters, which often also increase PCR inhibition (see Appendix I for details).

4.2 Experimental filter comparison

The results from the laboratory trials (see Appendix I for details) found that the 1.5um pore sized glass fiber (GF) were able to filter significantly larger volumes of water before saturation and outperform 0.45um pore sized (MCE) filters after filtering roughly 700ml of water. When filtered to saturation the GF yield gave a roughly 2X stronger signal than MCE filters (Figure 4.2). These results are supported by other studies, which also found GF significantly outperformed MCE when both were filtered to saturation (Lacoursiere-Roussel et al. 2016) but MCE had higher eDNA yield when smaller volumes (e.g. 500ml) were filtered (Hinlo et al. 2017).

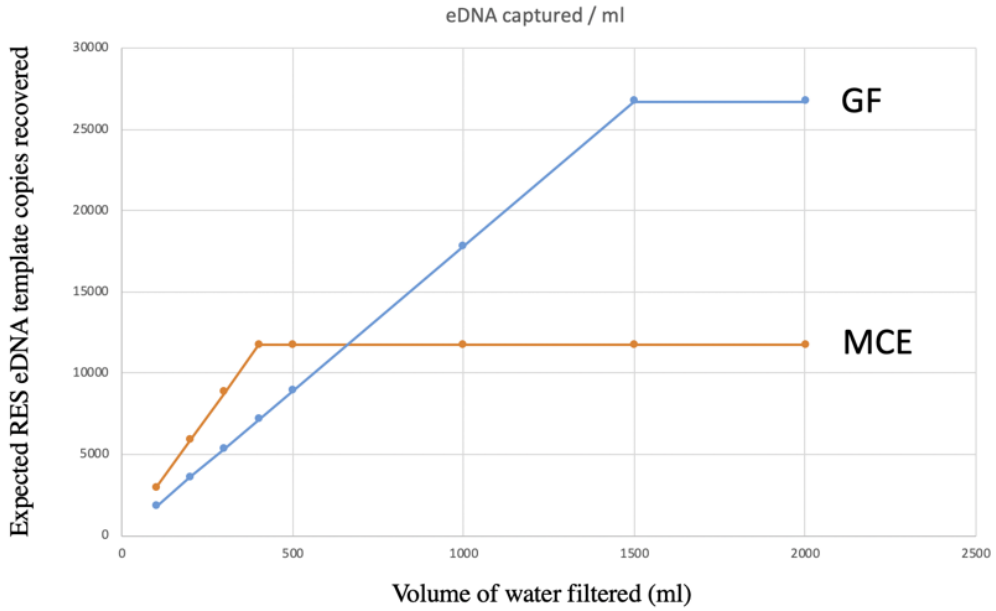

Figure 4.1: Predicted relationship between copies detected and volume of water filtered for both MCE (orange) and GF (blue) filters. Based on estimated mean values for copies/ml and water filtered (see Appendix I for details).

4.3 Methods Comparison

Among the four sites where two eDNA collection methods were used, triplicate water sampling detected Arctic Grayling eDNA more frequently than did the direct filtering method (Figure 4.2, Table 4.1). A single 1L replicate collected from Chowika Creek was a false negative (positive direct filtering), but all sites assessed with three replicates contained at least one positive and the paired direct filtering method failed to detect Arctic Grayling eDNA at all three sites. The higher false negative rate for direct filtering was surprising considering larger water volumes were filtered relative to the bottle dunk method and both methods used the same GF filters (Table 4.1). Direct filtering also detected Arctic Grayling eDNA at all of these sites in 2018 (Strohm et al. 2019) so it is difficult to explain the failure to detect in 2019.

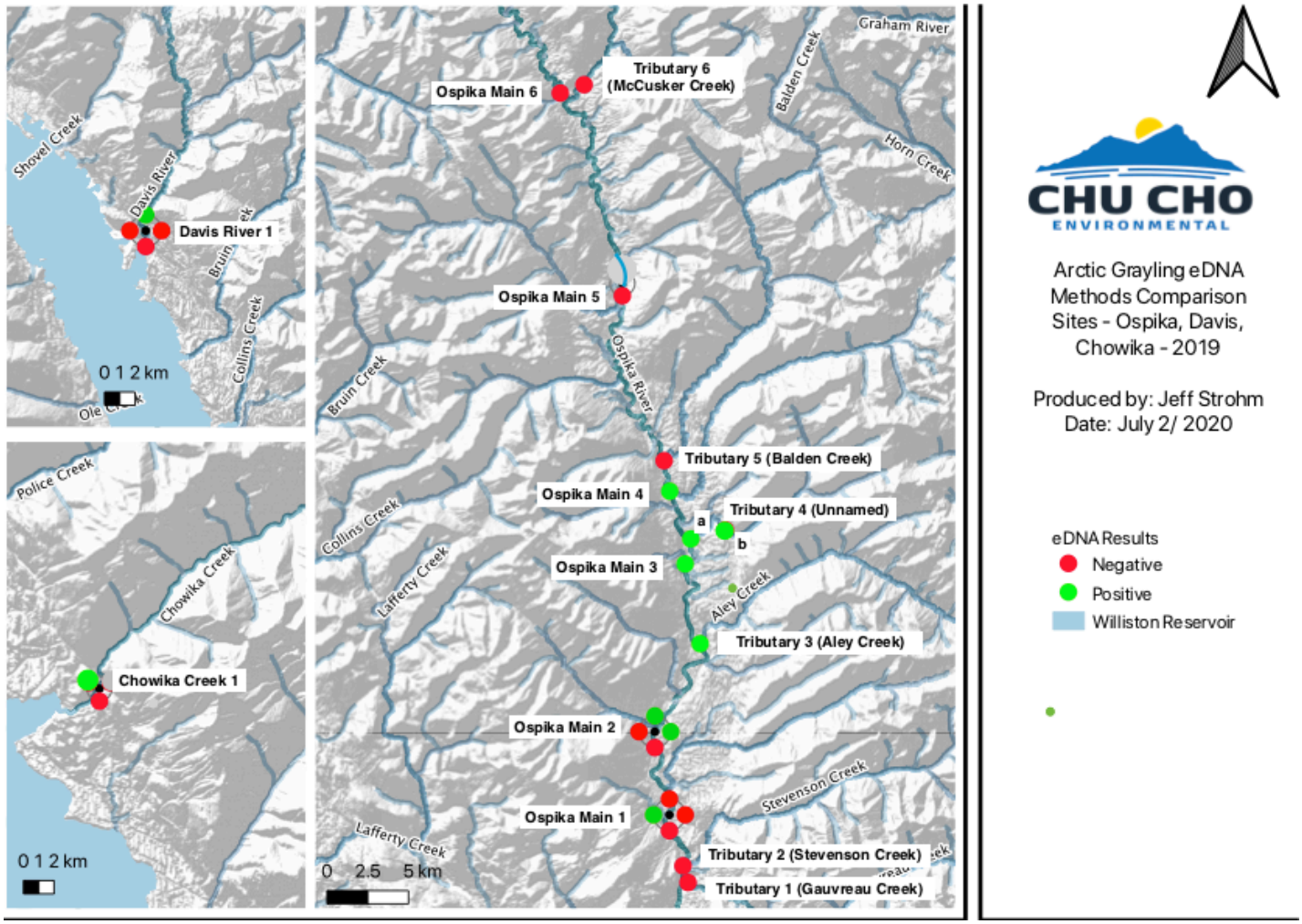

Figure 4.2: Arctic Grayling eDNA sampling results from the Ospika River (right) Davis River (top left), and Chowika Creek (lower left). Small black dots are eDNA sites and surrounding coloured dots are replicates (n=3) or paired direct filtering (n=1). Red=eDNA not detected; Green=eDNA detected. (see Table 4.1 for details).

Consulting the field notes suggest, however, that filtering directly from the stream edge in the larger river sites in Ospika and Davis rivers exposed the filter apparatus to slow moving current. Furthermore, both rivers were in flood conditions at the time of sampling, which suggests the stream margins were only recently watered. Consequently, eDNA may have become entrained into the thalweg and perhaps settled from the water column before reaching the funnel intake far on the flooded shore. In contrast, the bottle dunk method included wading out into the current to collect 1-L replicates, where Arctic Grayling eDNA appears to have remained suspended in the water column and was consistently detected in smaller water volumes (i.e. 1-L replicates). These results highlight the importance of ensuring eDNA is collected from flowing waters where eDNA is more likely to remain suspended in the water column and likely to originate from further upstream. Another difference from the 2018 sampling, however, was the higher turbidity due to heavy rains in 2019. Arctic Grayling eDNA appears to be present in low quantities at these sites in Davis and Ospika rivers and possibly the sediment deposited on the filters inhibited the detection of eDNA where concentrations appear to be lower along the stream margins.

Table 4.1: Results from four sites where eDNA was collected using two methods: Three 1L replicates filtered at the end of the day, and direct filtering on site. Glass fiber filters were used for all samples except for a 1L replicates from Chowika Creek and two 1L replicates from Chowika Creek and two 1L replicates from Davis River.

Site	eDNA result			
	Three replicate eDNA 1=detected; 0=not detected	Volume Filtered (L)	Direct Filtering eDNA 1=detected; 0=not detected.	Volume Filtered (L)
Chowika Creek	0	1.0	1	4.5
Davis River	1	3.0	0	5.0
Ospika main 1	1	3.0	0	3.0
Ospika main 2	1	3.0	0	4.0

4.4 Capture Probability among triplicate samples

A total of 16 sites and 44 replicates were sampled for eDNA using the bottle collection method. Two sites received a single 1-L replicate because other replicates were lost during sampling at site U3 in Ingenika River and site Chow2019-01 in Chowika Creek. Both failed to detect Arctic Grayling eDNA (Figure 4.2, 4.3). Absence of Grayling in snorkel observations upstream suggest the single replicate from U3 was a negative and paired direct filtering detected Arctic Grayling eDNA in Chowika Creek both in 2018 and 2019, which suggests the single 1-L bottle was a false negative.

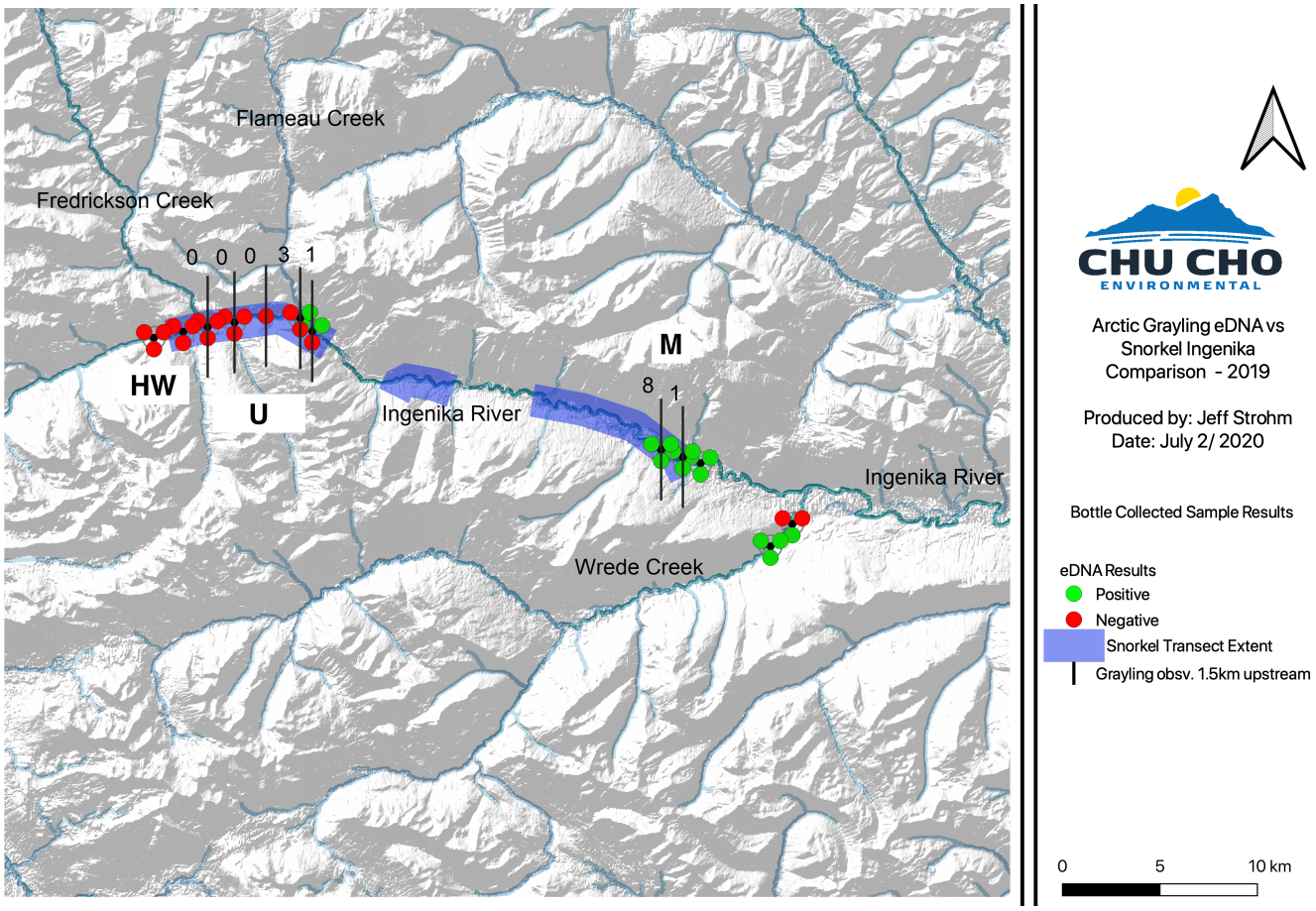

Figure 4.3: Distribution of eDNA samples among snorkel survey sections in Ingenika River during August 2019. Small black dots indicate eDNA sample sites and surrounding coloured dots indicate replicates; Red = Arctic Grayling eDNA not detected; Green=Arctic Grayling eDNA detected. Purple shading highlight snorkel sections and numbers above are Grayling counted within 1.5km long stream sections (between vertical lines). Upper and Middle snorkel reaches and eDNA samples upstream are labelled with bold letters: 'U', 'M', and HW, respectively.

Arctic Grayling were expected to be present in Chowika Creek and the single replicate is the one expected positive that failed to detect eDNA in Table 4.2. Arctic Grayling eDNA was detected in 62.5% of sites, including two sites in Wrede Creek where they were expected to be absent based on previous sampling, and the three sites in Davis, and Ospika rivers that were filtered with GF (Table 4.2). Including the false negative in Chowika Creek, the eDNA detection of Arctic Grayling was similar to the known (expected) distribution (Chi Sq. 6.112, DF 1, p (*no association*) =0.013). Even though the results expanded the known range of habitat use into Wrede Creek most sites agreed with the expected presence or absence based on other sampling, which make the data appropriate for evaluating the detection rate of our assay.

Table 4.2: August 2019 eDNA results from triplicate one-liter water sampling in Ingenika River, Chowika Creek, Davis River, and Ospika River. Known distribution includes seven sites with direct snorkel survey observations and five sites with inferred presence/absence.

Known Distribution	eDNA Sampling (1Liter bottles)		
	Detected	Not detected	Total
Present	8	1	9
Absent	2	5	7
Total	10	6	16

Both snorkel observations and eDNA detections found complete agreement among snorkel reaches, which confirmed Arctic Grayling presence within 1.5km upstream among four eDNA sites (M2, M3, U1, U2) and absence in three consecutive upstream reaches (U3, U4, U5; Figure 4.3). Including two eDNA sites upstream of the snorkel survey (HW1, HW2), a total of five sites failed to detect Arctic Grayling eDNA and consecutively mark a 7.5km section of Ingenika River where Arctic Grayling were absent both in snorkel observations and eDNA detections (Figure 4.3). There are no obvious habitat features that might restrict the upstream movements of adult Arctic Grayling beyond this known distribution and we found no previous records indicating presence upstream of site U3. Possibly, Arctic Grayling avoid the predation risk from the numerous Bull Trout observed during snorkel surveys in this pristine upper section (Cowie and Blackman 2012; Hagen et al. 2019). The Arctic grayling appear to accumulate in the deep pool below passable cascades situated between reaches U and M and only a few individuals move upstream into the lower 3km of reach U where both snorkel observations and eDNA detections identified their presence (Figure 4.3; Hagen et al. 2019).

Arctic Grayling eDNA was detected at all three sites located downstream of snorkel surveys including site M1 in Ingenika (expected present) and two sites in Wrede Creek where they were expected to be absent (Figure 4.3). Among the four eDNA sites with confirmed Arctic Grayling presence upstream in snorkel observations, the detection probability was 27% higher relative to sites with expected presence inferred from other sampling efforts and 42% higher than sites with expected absence (Figure 4.4). Sites with 100% individual detections (all three replicates positive) were distributed in the hub of the known Ingenika Arctic Grayling distribution but also included one site upstream in Wrede Creek. All remaining sites had lower detection rates among replicates included two furthest upstream in Ingenika River, one downstream in Wrede Creek, and all four sites in other watersheds (Figure 4.2, 4.3). This trend suggests that lower site detection (i.e. presence of false negative replicates) might illustrate the margins of habitat areas. For instance, tributaries that provide natal habitats might be expected to have higher eDNA detection rates closer to the mouth, since fry tend to accumulate downstream in natal habitats. Conversely, tributaries that provide only adult rearing habitats might be expected to have higher eDNA detection rates upstream since adults often migrate upstream to rear.

A general tendency to migrate upstream and strong site fidelity for summer rearing habitats among adults appear to influence a remarkable trend observed among Arctic Grayling populations with larger

fish upstream (Hughes 1999, Buzby and Deegan 2000; Baccante 2010). Larger adults not only displace smaller individuals from optimal foraging space (Hughes and Dill 1990) but also have greater swimming ability to navigate further upstream (Hughes 1999). As a result, smaller individuals migrate shorter distances to find summer rearing habitats closer to natal areas (Hughes 1999). Such a trend of larger fish upstream is also true for Arctic grayling in Ingenika River (Hagen et al. 2019) and possibly this upstream dispersal from natal areas in the middle river also include tributaries. Wrede Creek, for instance, might provide adult rearing habitat since eDNA detection rates were higher upstream than downstream. In contrast, the 2018 direct filtering results in Swannell River suggest that Arctic Grayling were absent upstream but present downstream, which might suggest natal areas also include Swannell River. Fish sampling is needed to address these intriguing hypotheses that suggest different life histories might be present in these tributaries. Currently, however, eDNA sampling has successfully expanded the known range of summer habitat use by Arctic Grayling to include tributaries in Ingenika river and supports mid 1970's observations of adults in Wrede Creek (Bruce and Starr 1985). Potentially tributaries provide more optimal rearing habitats for Arctic grayling during periods when higher discharge decrease habitat quality in the mainstem (e.g. during Spring freshet). Fewer Arctic Grayling were observed during snorkel surveys in 2019, relative to 2018 and 2004 (Strohm et al. 2020) possibly because more of the population had remained in the tributaries during higher discharge of 2019. Possibly as discharge decreases in the watershed the Arctic grayling adjust their distribution into the mainstem Ingenika River with larger fish upstream as observed in 2018 and 2004 (Hagen et al. 2019)

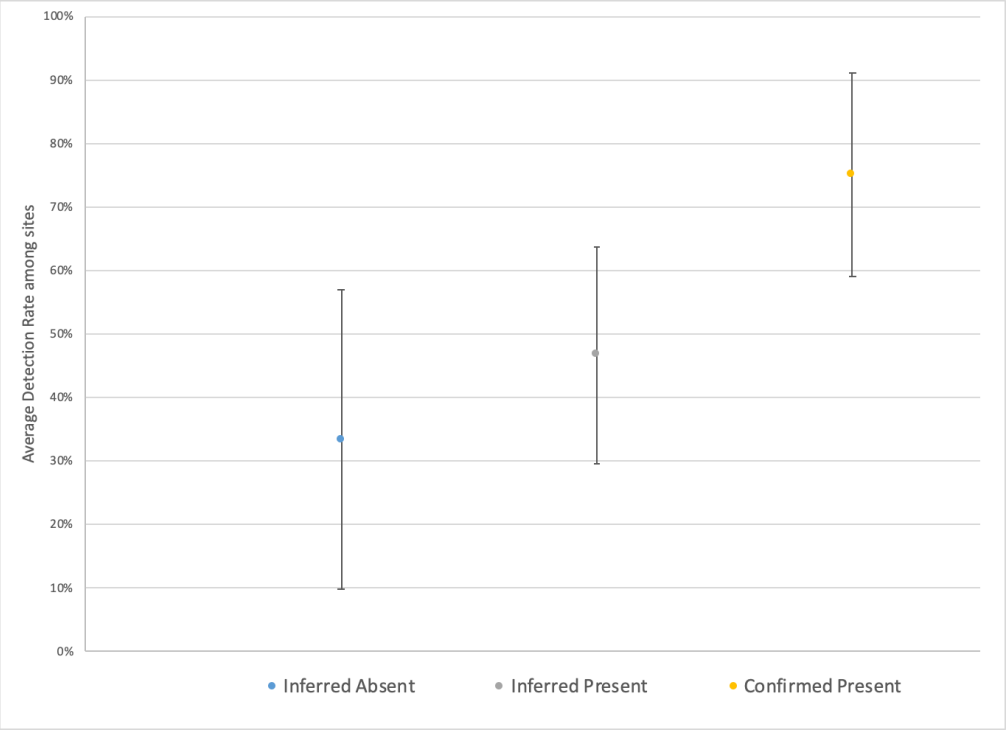

Figure 4.4: Average eDNA detection rate (+/-SE) for Arctic Grayling among sites sampled using the bottle dunk method to collect three 1L replicates. "Inferred Absent" and "Inferred Present" drew from post flood sampling records; "Confirmed Present" are sites with direct upstream observations of Arctic Grayling in snorkel surveys.

Arctic Grayling eDNA was not detected in any field controls (n=23 distilled water), showing no evidence that false positives arose from contamination. Among the 44 1-L bottles collected there were four non-ordered outcomes and the assay failed to detect Arctic Grayling eDNA in 23 replicates, including the false negative collected from Chowika Creek (Table 4.3). Among the 12 replicates collected from snorkel positive sites, three failed to detect Arctic Grayling eDNA and suggests a 24% false negative rate. Including all 30 replicates collected from Arctic Grayling positive sites (eDNA was detected in at least one triplicate), 10 were negative and suggests a 30% false negative rate overall. Sites with triplicate negative results ('000') and triplicate positives ('111') consistently pair with respective confirmed absence and presence of Arctic Grayling in snorkel surveys, and false negative rates increase near the margins of habitat use in Ingenika River. A false negative rate of 30% suggests that a minimum of three liters of water filtered per site is sufficient to detect presence of Arctic Grayling near the margins of their habitat areas, or in sites where they are rare in streams.

Table 4.3: Percentage of sites with one of four eDNA detection outcomes with triplicate 1L water sampling at 16 sites in Ingenika River, Chowika Creek, Davis River, and Ospika River. A single replicate collected at site U3 in Ingenika River and one site in Chowika Creek ('0') were assumed negative and grouped with the '000' outcomes. 1=Grayling eDNA detected; 0=Grayling eDNA not detected.

Results sequence	SUM	Percent of sites
000, 0	6	38%
100, 010, 001	4	25%
110, 011, 101	2	13%
111	4	25%
Total	16	100%

4.5 Williston Arctic Grayling Distribution

4.5.1 Eastern slope of Finlay Reach

Repeat sampling at four sites in 2018 and 2019 detected Arctic Grayling eDNA in both years and suggests temporally consistent habitat use in Chowika Creek, Davis River, and lower Ospika River mainstem (Figure 4.2). Replicates at all four sites included false negatives, however, which suggests the sample sites mark the margins of the Arctic Grayling habitat use, assuming trends in Ingenika can apply to other streams (Figure 4.2, 4.3). The results support the hypothesis that individuals from other core areas migrate to summer rearing sites in the lower reaches of Chowika Creek and Davis River. Further support for this hypothesis comes from 2018 sampling in Davis River, which failed to detect Arctic Grayling eDNA upstream (Strohm et al. 2019). Unfortunately, the steep sided terrain in Chowika Creek prohibited further sampling upstream by helicopter in 2019. Nonetheless, the eDNA results support previous observations that adult Arctic Grayling rear in the lower reaches of Chowika Creek (no records were found upstream) and has expanded the known range of habitat use to include lower Davis River

(Stamford et al. 2017). Further investigations are needs to confirm the life histories present and determine associations with surrounding watersheds (e.g. locations of natal areas).

Similar to Wrede Creek, the false negative rates in Ospika River mainstem suggest the lower reaches are the margins of the Arctic Grayling habitat area since detection rates increased further upstream (Figure 4.2). Arctic Grayling eDNA detection among sites using direct filtering further upstream in Ospika River suggests the hub of their distribution is in the middle river and associated with at least two tributaries (Aley Creek and unnamed Tributary 4; Figure 4.2). The distribution of eDNA is remarkably similar to Ingenika River and challenges the current core area structure identified for Peace Basin Arctic Grayling (i.e. Stamford et al. 2017). Possibly, Ospika River might contain an independent metapopulation, but further investigations are needed to determine which life histories are present, what are the movements among habitats, and associated genetic associations among surrounding watersheds. Tributary use also needs further investigation, especially since eDNA was detected upstream in the unnamed Tributary 4 where cascades were observed both upstream and downstream of the eDNA sample site. The eDNA results suggest these high gradient cascades do not restrict upstream movements. Alternatively, a resident population exists upstream, which might suggest periodic headwater exchange with Halfway River watershed.

4.5.2 Lower Finlay

Failure to detect eDNA in the reservoir supports other studies that suggest Arctic Grayling avoid rearing in Williston Reservoir (Clarke et al. 2005). Arctic Grayling eDNA was detected, however, at the same site on the western shore of Finlay Arm both in 2018 and 2019 and this temporal consistency suggests that individuals might return to this location to rear. Alternatively, eDNA drifted from the Finlay River and remained suspended at detectable concentrations in the water column for at least two kilometers (Table 4.4). Considering how rapidly eDNA settles from the water column in flowing water (e.g. 750m [Jane et al. 2015]; 1.5 km [Goldberg et al. 2015]), and that no current was visible at the sample site, the second alternative seems unlikely. Furthermore, sampling at eight other reservoir sites between 2018 and 2019 failed to detect Arctic Grayling eDNA, even though each site was between one and 12 km from a stream where Arctic Grayling eDNA was detected at the same time (Table 4.4). This suggests that eDNA does not remain in the water column for long distances in Williston Reservoir and something unique about the west side of Finlay Arm attracts Arctic Grayling eDNA. Alternatively, Arctic Grayling are present in low densities in the reservoir, but low detection probability in slack water environments require more intensive sampling with strategically placed sites close to potential habitats (e.g. Sigsgaard et al. 2015). Reservoir site selection in this study was more opportunistic and influenced more by its proximity to adjacent streams and road access, instead of presence of certain habitat variables that might attract Arctic Grayling. Consequently, many samples might be false negatives. Arctic Grayling might disperse from other core areas (e.g. Finlay River) to rear in other streams (e.g. Chowika Creek, Davis River), and move through the west side of Finlay Arm past our sample site. A sampling strategy designed to rigorously distinguish between these alternative explanations would require multiple sites and potentially

a specialized sampling technique. Such a strategy has successfully detected presence of rare fish species previously thought extinct in other lakes (e.g. Sigsgaard et al. 2015).

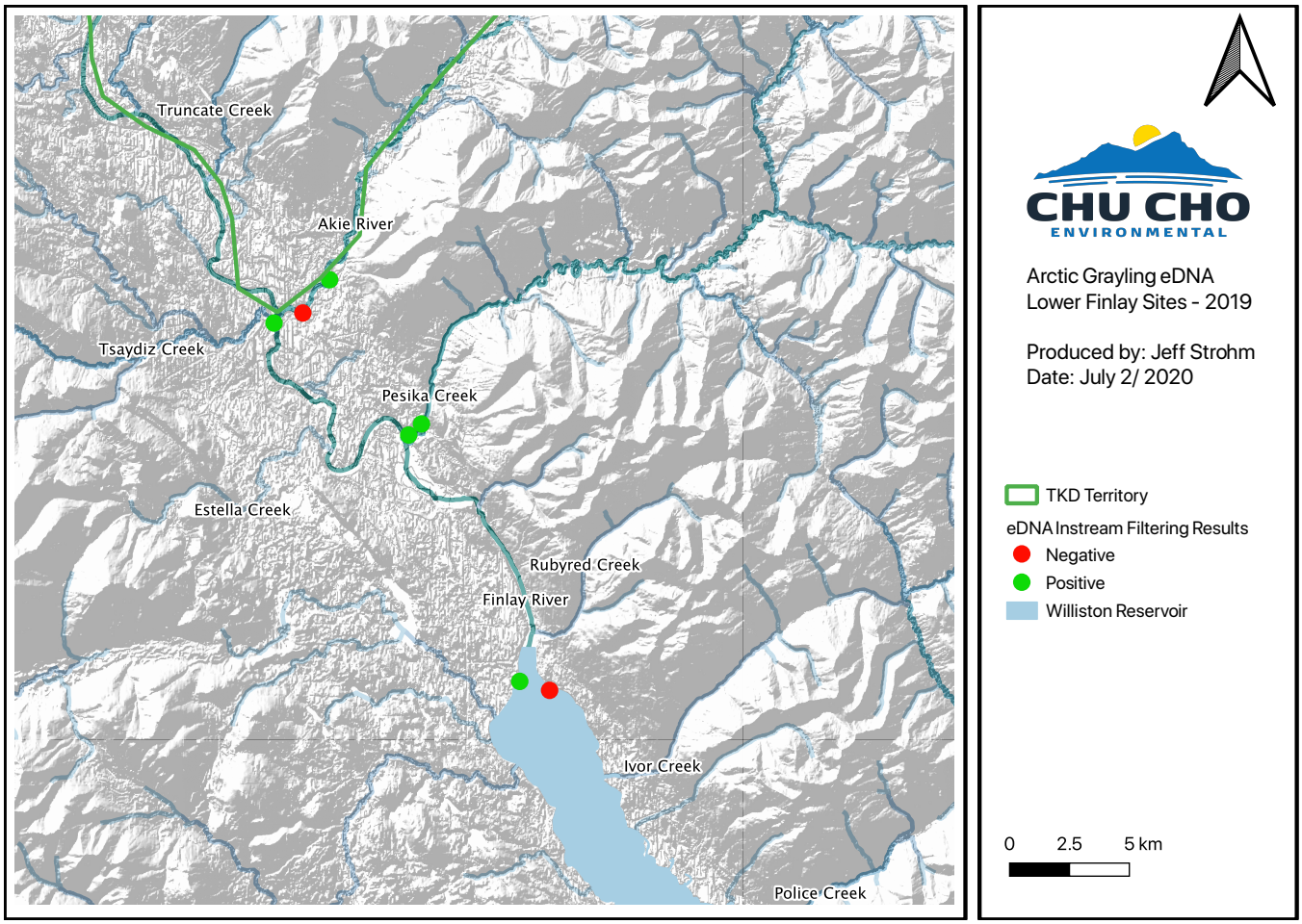

Figure 4.5: Arctic Grayling eDNA sampling results from the lower Finlay River watershed.

Table 4.4 Summary of eDNA results from sampling in Williston Reservoir and adjacent streams in 2018 and 2019. "+" Arctic Grayling eDNA detected; '-'Arctic Grayling eDNA NOT detected. Comment refers to the year sampling took place.

Sampling Area	Distance from positive stream mouth (km)	eDNA Sampling		Comment
		Stream	Reservoir	
Ingenika Arm	7	+	-	2018
Finlay Arm west side	2	+	+	2018 and 2019 same result
Finlay Arm east side	2	+	-	2019
Chowika Bay	2	+	-	2018
Davis Bay	3	+	-	2018
Collins Creek Bay	1	+	-	2018
Lafferty Creek Bay	2	+	-	2018
Ospika Arm	12	+	-	2018

Detection of Arctic Grayling eDNA at two locations each in Pesika River and Akie River has expanded the known range of Arctic Grayling habitat use in lower Finlay River (Stamford et al. 2017). This is the first record indicating Arctic Grayling presence in Pesika River, and the first record since 1963 showing Arctic Grayling presence in Akie River. Both tributaries appear to have extensive low gradient habitats upstream, which suggests they might support resident populations. Alternatively, habitat use is linked to movements among distant tributaries required to complete different stages of their life history. Further sampling aimed at identifying which life histories are present in these streams and tracking movements among habitats will help distinguish between these hypotheses. Also, the migratory behaviours might include movements through the reservoir and might explain the positive results in the reservoir and other streams draining the east side of Finlay Reach (i.e. lower Chowika Creek, lower Davis River, Collins Creek, Lafferty Creek, Ospika River; Strohm et al. 2019). Such a migratory might include multiple core areas, given the evidence in other large rivers where adults found rearing together in the same stream originated from multiple natal tributaries often situated hundreds of kilometers away (Earthtone and Mainstream 2013; Stamford et al. 2020)

4.5.3 Western-Slope Williston Tributaries

After sampling 10 sites in Manson River, four sites each in Blackwater and Fries creeks, and one site in Strandburg Creek between 2018 and 2019, Arctic Grayling eDNA has never been detected (Figure 4.6). This despite an early 2000's record in Fries Creek that suggest juveniles (100+ size) might rear in the extensive low gradient areas drained by these streams. Also, records of adults and fry in Manson River was not supported by our eDNA result even though the reach where fry were observed (Hawkshaw and Shrimpton 2014) was sampled both in 2018 and 2019, including three sites spaced 1.5km apart. Possibly Arctic Grayling are both rare and highly mobile in this low gradient watershed and avoid capture in these meandering watersheds even by eDNA samples; this supported by periodic observation of adult Arctic grayling in snorkel surveys (see Stamford et al. 2017). The negative eDNA results in two tributaries of Manson River agree with Arctic Grayling absence determined previously by fish sampling and habitat

modelling, but eDNA results disagree in the mainstem where juveniles were captured in 2010 (Hawkshaw, 2011).

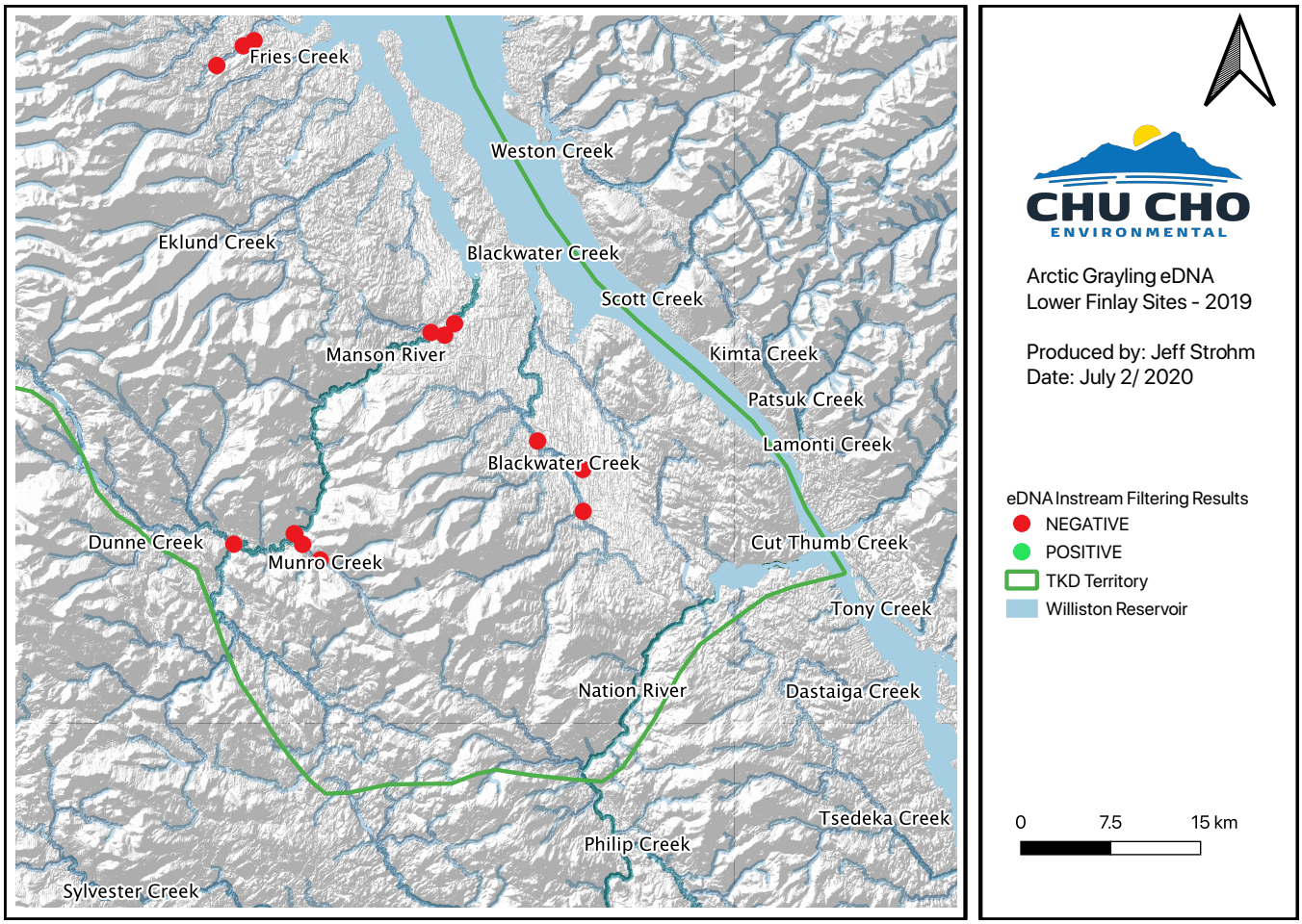

Figure 4.6: Distribution of sample sites in west Parsnip Arm where eDNA was collected during August 2019.

4.5.4 Parsnip Arm – Anzac River

The Anzac River sample collected from riffle habitat using direct filtering yielded a strong positive signal with the highest number of copy numbers observed (56, and 40 for the 1st elution). The result suggests that sampling from the margin of a pool was a false negative possibly because eDNA rapidly settled from the water column. Alternatively, Arctic Grayling were absent in lower Anzac during September 2018 and present during August 2019. Unfortunately, we did not collect from the same pool site in 2019, which would have helped distinguish between these alternatives.

4.5.5 Conclusions and recommendations

1. The ddPCR improved the detection of Arctic grayling eDNA relative to the qPCR used in 2018 even though numerous 2019 filters contained large quantities of sediments often associated with PCR inhibitors; see Appendix 1 for details.
2. Laboratory comparison of the larger pore sized GF versus smaller pore size MCE filters suggest that GF should outperform the MCE using the water volumes filtered in this study. The choice of using MCE versus GF for eDNA studies may be seen as a consideration in the design of eDNA surveys for different aquatic systems. In cold clear mountain streams were 1 or 2 L of water can be filtered through 0.45 um MCE filters prior to saturation, their increased capture/ml of water filtered may make them the best choice for studies which are based on collecting water at source and filtering in a lab. For systems which reach saturation below 500-600 ml, or for those using in-stream filter to saturation studies, GF filters will likely outperform MCE filters.
3. Methods comparison and repeatability in the field found the triplicate bottle filling method had higher success detecting Arctic Grayling at positive sites than the direct filtering method. Both methods used GF due to high sediment load in the rivers. Higher detection success in the bottle filling method appears to result from collecting water from higher flows near the thalweg. False negatives in the direct filtering method occurred at sites where flows were slow and recently watered near the stream margin where samples were collected. These false negative results highlight the importance of ensuring water is collected where stream flows keep eDNA suspended in the water column from further upstream.
4. The eDNA detections were similar to the expected presence and absence among sites but also expanded the known range to include tributaries. Complete agreement with direct observations upstream in snorkel surveys suggest both methods have similar capture probabilities along 1.5km stream section of Ingenika River. False negatives in triplicate sampling appears to illustrate the margins of habitat areas and inform further sampling.
5. The eDNA results have provided valuable habitat distribution data for Ingenika that inform abundance estimates and helped interpret snorkel survey results. Further pairing eDNA sampling with snorkel surveys in other watersheds is recommended to improve estimates of distribution and capture probability for both sampling methods.
6. Detection of Arctic grayling eDNA in Williston Reservoir and tributaries draining the eastern slopes of Finlay River and Finlay Reach support the hypothesis that Arctic grayling migrate through the reservoir to find summer rearing habitats. Further fish sampling is recommended to determine life histories present, estimate their abundance, and determine natal origin.
7. Sampling at 19 sites over two years failed to detect Arctic Grayling eDNA along the west Parsnip Arm. The post flood observations of Arctic Grayling in these streams can be explained by rare occurrence and a highly mobile life history adapted to these slow meandering streams, which might enable the fish to avoid capture by eDNA.

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6 Appendices

Please see eDNA supplementary Methods.