Parsnip Arctic Grayling Abundance and Critical Habitats 2018-2022 Final Report

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EXECUTIVE SUMMARY

Following construction of the W.A.C. Bennett dam in 1967 and the formation of Williston Reservoir, Arctic Grayling populations were devastated in the flooded portions of the Parsnip River, Finlay River, and Peace River watersheds. The Parsnip River valley upstream of the Williston Reservoir's footprint is home to one of the upper Peace Basin's few surviving populations. This report presents the results of a 2018-2022 study that used snorkeling surveys and environmental DNA sampling to evaluate the distribution and abundance of Arctic Grayling in the Parsnip core area (conservation unit). Key study results have been used to update the conservation status for the core area, improve the delineation of critical habitat segments, and identify locations where conservation actions are needed.

Because of conservation concern and high value for humans, the Arctic Grayling is a priority species for the Fish and Wildlife Compensation Program – Peace Region (FWCP), collaborators BC Ministry of Forests (MOF), Ministry of Water, Land, and Resource Stewardship (WLRS), and project supporters McLeod Lake Indian Band (MLIB) and Polar Coachman Fly Fishers (PCFF). Historically, from 1995-2007 FWCP periodically monitored adult Arctic Grayling abundance and trend in the Parsnip River watershed using replicated snorkeling counts during August, in four index reaches of the Anzac River and two index reaches of the Table River. Abundance and trend are the two most important indicators of a fish population's conservation status, which identifies whether it still exists and how likely it is to become extinct in the future.

In 2018, after an 11-year hiatus, monitoring in these long-term index sections was resumed. At the same time an important new study component was added: single-pass snorkeling surveys to estimate the distribution of critical summer rearing habitats and adult abundance in other sub-basins of the Parsnip River watershed. This report compiles results from the five-year study program, which addresses Action #9 of FWCP's *Rivers, Lakes, and Reservoirs Action Plan*:

> Conduct research and monitoring of Arctic Grayling to obtain data related to conservation status, critical habitats, and key limiting factors.

A key area of focus for our study since 2019 has been the application of models to estimate detection probability and abundance from replicated count data in snorkeling reaches. Over the 1995-2022 period, a total of 48 surveys have taken place in the six long-term index sections of the Anzac and Table rivers in which at least two replicate counts were made. Variation among replicate counts at individual sites was analyzed to estimate the accuracy of snorkeling counts and site-level abundance using a binomial-likelihood model framework. The best model included a single predictor of detection probability: stream wetted width. Average detection probability estimates ranged from 0.52-0.71 among long-term index sections, with highest estimates associated with the smaller reaches at the top of the Arctic Grayling distribution in each system.

After adjusting snorkeling counts in long-term index sections to account for variable detection probability, analysis of population trend using a linear mixed-effects model indicated significant population growth ($P < 0.001$) over the 1995-2022 period. The linear mixed effects model output

suggested an annual population growth rate of 3.9% for the Parsnip core area, which equates to an increase of approximately 98% over a 25-year time frame. The total number of Arctic Grayling counted in long-term index sections in 2022 was the highest ever recorded.

Between 2018-2022, single-pass reconnaissance snorkeling surveys were completed in potential adult/subadult Arctic Grayling summer rearing habitats throughout the Parsnip River watershed including: previously-unsurveyed sections of the Anzac River (2018), Missinka River (2019), Hominka River (2018), Wichcika Creek (2020), Reynolds Creek (2021), Colbourne Creek (2021), Misinchinka River (2022), and Wooyadilinka Creek (2022). Surveyed streams were subdivided into reaches (if necessary) based on patterns of abundance and sampling intensity, then snorkeling count data were analyzed within a Poisson-mixture model framework to estimate reach-scale abundance. The results make it clear that the Anzac and Table watersheds together are the core of the Arctic Grayling distribution in the Parsnip watershed. Outside of these two systems, the most productive summer rearing habitats for adult Arctic Grayling are distributed from 36-25 km of the Missinka River and from 48-32 km of the Hominka River. Wichcika Creek, Reynolds Creek, Colbourne Creek, and Misinchinka River provide summer rearing habitat for very few adults. Total adult/subadult Arctic Grayling abundance in the Parsnip core area was estimated to be roughly 5,600, with the Anzac and Table populations together comprising 87% of this total.

Despite the positive population trend, uncertainty remains about limiting factors and threats and how they may affect the future status of the Parsnip core area. To address these uncertainties, we have made several recommendations intended to ensure effective conservation management:

1. Share study results and critical habitat information with MOF, WLRS, and First Nations whose territories overlap the Parsnip River watershed, in forms that are useful for land use planning and stewardship.
2. Develop watershed-scale land use objectives (e.g., Fisheries Sensitive Watershed designations) to protect critical habitats against water temperature and hydrological changes known to be detrimental to Arctic Grayling.
3. Initiate new studies to improve knowledge of limiting factors and threats, e.g., the roles of water temperature and other physical habitat variables in limiting Arctic Grayling distribution and abundance now and in the future.
4. Effectiveness monitoring to evaluate whether watershed-scale land use objectives and other stewardship actions are adequate for the long-term conservation of Arctic Grayling in the Parsnip River watershed.
5. Study(s) of life history, habitat use, and population structure focused on lower Parsnip River sub-basins (downstream of Anzac River) to support/guide future habitat enhancement actions.

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1 INTRODUCTION

In the upper Peace Basin, the Arctic Grayling (*Thymallus arcticus*) is a fluvial (stream-dwelling) species. Following construction of the W.A.C. Bennett dam in 1967 and the formation of Williston Reservoir, Arctic Grayling populations were devastated in the flooded portions of the Parsnip River, Finlay River, and Peace River watersheds (Stamford et al. 2017). The Parsnip River valley upstream of the Williston Reservoir's footprint is home to one of the upper Peace Basin's few surviving grayling populations. This report documents the results of a five-year study of Arctic Grayling distribution and abundance, which utilized the methods of snorkeling surveys and environmental DNA (eDNA) sampling. Key study results are utilized to identify the population's current conservation status and delineate the distribution of critical habitats where conservation actions are needed.

Remnant populations of Arctic Grayling in the Williston Reservoir watershed face threats not only from persistent impacts of the reservoir's footprint, but also from other human-caused ecological and physical habitat changes. Parsnip Arctic Grayling appear to be demographically and genetically isolated from other remnant populations (Stamford and Taylor 2005; Shrimpton et al. 2012). Their persistent absence in Parsnip and Peace reach tributaries suggest the presence of the reservoir restricts their dispersal and habitat use beyond Parsnip River (Pillipow and Langston 2002; Sebastian et al. 2003; Clarke et al. 2005; Stamford et al. 2022). The Arctic Grayling is also a species that is sensitive to land use-related habitat degradation (Armstrong 1986; Northcote 1993; Walker 2005; USFWS 2010; Cahill 2015). In the Parsnip River watershed, major increases in industrial activities pose new threats to the Arctic Grayling population. Construction of the Coastal GasLink Pipeline Project through the Anzac River watershed has been ongoing since 2019. Pipeline construction has greatly increased human activity and site disturbance in the watershed which has resulted in charges of non-compliance and fines (e.g., https://projects.eao.gov.bc.ca/api/public/document/63c9c4c9be238b0022d190cc/download/CGL_2022-11-2%20to%204_Inspection%20Record_IR2022-054_Final.pdf). Major increases in forestry activities have encroached in to previously pristine areas of the Parsnip watershed and are slated to continue in future, as a mitigation strategy related to the outbreak of spruce beetle *Dendrontonus rufipennis* (e.g., Thomas 2020). These developments threaten Arctic Grayling habitat suitability, as well as providing increased access for anglers to remote areas where angling regulations are difficult to enforce. Key mechanisms of habitat degradation affecting Arctic Grayling include increases in sediment transport and stream flow variation and water temperature, and decreased water temperature stability (de Bruyn and McCart 1974; Tack 1974; Birtwell et al. 1984; McLeay et al. 1987; Reynolds et al. 1989; Clark 1992; Deegan et al. 1999; Cowie and Blackman 2003; Hawkshaw et al. 2013; Hawkshaw and Shrimpton 2014; O'Connor 2023). Situated at the southern margin of the species' range in British Columbia, Parsnip Arctic Grayling are also threatened by thermal habitat changes from climate warming (Hawkshaw and Shrimpton 2014; O'Connor 2023).

The Arctic Grayling is valued by First Nations of the Williston watershed as a delicacy and as a nutritious food fish (Pearce et al. 2019; Arlene Solonas, pers. comm. January 2022), and is also prized by northcentral British Columbia's recreational anglers. Because of conservation concern and high value for humans, the Arctic Grayling is a priority species for the Fish and Wildlife Compensation Program – Peace Region (FWCP), which was established to conserve and enhance fish and wildlife impacted by the creation of the Williston and Dinosaur Reservoirs (FWCP 2020: *Peace Region Rivers, Lakes, and Reservoirs Action Plan*). To facilitate this aim for Arctic Grayling populations, FWCP conducted a major study to evaluate the existing knowledge base relative to key strategic objectives for species conservation and enhancement. The resulting *Arctic Grayling Synthesis Report* (Stamford et al. 2017) identified key knowledge gaps limiting FWCP's ability to initiate conservation and enhancement actions (Stamford et al. 2017). Highest priority knowledge gaps and monitoring needs were summarized in the companion document *Arctic Grayling Monitoring Framework* (Hagen and Stamford 2017).

This five-year snorkeling study, which was initiated in 2018, was conducted for FWCP by consultants John Hagen and Associates in collaboration with the Fisheries Section of the British Columbia Ministry of Forests (MOF), BC Ministry of Water, Land, and Resource Stewardship (WLRS), and the University of Northern British Columbia's Freshwater Fish Ecology Laboratory (FFEL), with support from McLeod Lake Indian Band (MLIB) and the Polar Coachman Fly Fishers (PCFF). Our study was designed to directly address two of the highest priority information gaps identified within the FWCP reports for Arctic Grayling in the Parsnip River watershed. The first of these was the lack of adult abundance and population growth rate (trend) data since 2007. This information is essential for assessing the conservation status of the Arctic Grayling population (Table 1, ID #1 *in* Hagen and Stamford 2017).[1] Adult abundance and trend are the two most important indicators of conservation status, and therefore, are also the most important indicators of whether current species conservation measures are working (McElhany et al. 2000; O'Grady et al. 2004; USFWS 2010).

The second of these two key information gaps for Parsnip Arctic Grayling was the lack of information about abundance and the distribution of critical adult rearing habitats outside of the Table and Anzac sub-basins (Table 1, ID #2 *in* Hagen and Stamford 2017). Critical habitats are those necessary for the species to persist and thrive, and in which limiting factors regulate growth and survival to the adult life stage (Rosenfeld and Hatfield 2006; Richardson et al. 2010; Hagen and Stamford 2017). Knowledge of critical habitat locations, and the relative importance of these habitats, is instrumental to 1) initiate effective habitat conservation actions, 2) identify threats from human land use, other species, and climate change, and 3) identify locations for habitat restoration and enhancement actions.

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[1] The conservation status of a group of organisms is an estimate of the viability of the group: whether it still exists and how likely it is to become extinct in the future (McElhany et al. 2000; IUCN 2012).

In 2022, our snorkeling study had two key components to address these information gaps. The first of these was the fifth consecutive year of Arctic Grayling abundance monitoring in 6 long-term index sections of the Anzac and Table rivers, using replicated snorkeling surveys, after a hiatus of more than 10 years between 2007 and 2018. These data are analyzed in this report to estimate population trend over the 1995-2022 period. The second study component was comprised of single-pass snorkeling surveys paired with environmental DNA (eDNA) sampling to delineate critical adult/subadult rearing habitat and estimate abundance in the previously-unsurveyed Misinchinka River. The single-pass snorkeling and eDNA data augment similar data collected during reconnaissance surveys of: 1) Anzac River in 2018 (Hagen et al. 2019), 2) Missinka River in 2019 (Hagen and Gantner 2020), 3) Hominka River and Wichcika Creek in 2020 (Hagen and Stamford 2021); and 4) Reynolds Creek and Colbourne Creek in 2021 (Hagen and Stamford 2022). In this report, we integrate the 2018-2022 data with results of other studies past and present to delineate, at the watershed scale, stream segments providing critical habitats for Parsnip Arctic Grayling.

Downstream snorkeling surveys are an attractive abundance monitoring method for stream-dwelling, subadult and adult salmonids including Arctic Grayling. Relative to other methods such as electrofishing or seine netting, snorkeling surveys are non-invasive, relatively rapid, and can be utilized within a variety of habitats. Up to 10 km of stream habitat can be surveyed in a day (Hagen and Baxter 2005), meaning that the sampling fraction within stream reaches can be high and extrapolation errors minimized relative to other methods. However, the accuracy of snorkeling counts may vary widely among systems, with species differences, underwater visibility, instream cover, and observer experience being potential variables that can affect snorkeling detection probability (Northcote and Wilkie 1963; Schill and Griffith 1984; Slaney and Martin 1987; Zubik and Fraley 1988; Young and Hayes 2001; Hagen and Baxter 2005; Mollenhauer and Brewer 2017). A key area of focus for our study since 2019 has been the application of models that analyze replicated count data to estimate detection probability and abundance in snorkeling reaches (Olkin et al. 1981; Royle 2004; Mollenhauer and Brewer 2017), thereby improving the accuracy of estimates of total abundance and trend. These advances in the analysis of the snorkeling count data have been made in collaboration with the University of Northern British Columbia's Freshwater Fish Ecology Laboratory (FFEL) as an in-kind FFEL contribution to this study (e.g., Dowd 2021).

Fausch et al. (2002) pointed out that the typical focus on a limited number of index sites within a stream network, such as the long-term surveys of 6 index sections in two streams of the Parsnip River watershed, may leave many key features affecting abundance and the distribution of critical habitats out of view. Examples of potential features affecting Arctic Grayling distribution and abundance are waterfalls or high gradient sections limiting access, unsuitable thermal regimes, or key geomorphology attributes affecting habitat suitability. They argue that a more continuous view of the entire, spatially heterogenous river environment, which they term the 'riverscape,' is essential for effective research and conservation of fishes and other aquatic biota

(Fausch et al. 2002). Landscape-scale mapping and prioritization of critical Arctic Grayling habitat in the Parsnip River watershed is urgently needed by FWCP and collaborators, given expected cumulative effects from major increases in industrial activities and the need for mitigation of potential threats through habitat conservation actions. To achieve a more continuous view of Arctic Grayling abundance and distribution in the Parsnip River watershed, we increased our spatial coverage during reconnaissance surveys by conducting only a single snorkeling pass, instead of the 2-4 replicates applied in the long-term index reaches of the Anzac and Table Rivers.

Following the 2021 field season, we recommended a watershed-scale habitat stewardship and monitoring framework for Parsnip Arctic Grayling (Hagen and Stamford 2022). We therefore conclude this report by discussing information needs identified in the framework that are addressed by the 2018-2022 study results, what additional information is required to augment study results and enable improved analyses of limiting factors, and what conservation actions are justified in critical habitats based on integrated results of this study and other key FWCP studies occurring over the same period.

2 GOALS AND OBJECTIVES

The FWCP is partnership between BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and public stakeholders. In the Peace Region, FWCP's aim is to conserve and enhance fish and wildlife impacted by the construction of the W.A.C. Bennett and Peace Canyon dams on the Peace River, and the subsequent creation of the Williston and Dinosaur Reservoirs (FWCP 2020).

The first goal of the Parsnip Arctic Grayling snorkeling study is to enable conservation actions that maintain or improve the status of the population and the productivity of its critical habitats. The second goal is to work with study collaborators and supporters to achieve effective fish and fish habitat stewardship in the Parsnip River watershed. As such, these goals are aligned with overarching strategic objectives of FWCP's *Rivers, Lakes, and Reservoirs Action Plan* (FWCP 2020).

The study had the following specific objectives in 2022:

1. Conduct replicated snorkeling counts of Arctic Grayling and other species in long-term index sites located in the Anzac and Table rivers, using a snorkeling survey method consistent with past surveys.
2. Estimate detection probability and abundance of Arctic Grayling in long-term index sites and utilize the estimates to improve the analysis of trend over the 1995-2022 period.
3. Acquire counts of Arctic Grayling and other species in the Misinchinka River using a single-pass snorkeling survey method, to estimate abundance and identify critical summer rearing habitats.

4. Conduct eDNA sampling to i) expand the spatial scope of reconnaissance surveys in the Misinchinka River by including areas that could not be snorkeled, and ii) evaluate the accuracy of single-pass snorkeling surveys and the potential presence of either very low densities of grayling or juvenile life stages undetectable by snorkeling.
5. Utilize the population data generated by this study to update the conservation status assessment for the Parsnip Arctic Grayling core area.
6. Utilize distribution, abundance, and movement data from this and other studies to delineate and prioritize critical habitats for Arctic Grayling in the Parsnip River watershed.
7. Identify key information needs addressed by the 2018-2022 study results, additional information needs which remain, and conservation actions which are justified based on integrated results of this study and other FWCP Arctic Grayling studies occurring over the same period.

These study objectives address Action #9 of the Rivers, Lakes, and Reservoirs Action Plan (FWCP 2020):

> "Conduct research and monitoring of Arctic Grayling to obtain data related to conservation status, critical habitats, and key limiting factors" (p. 12).

3 STUDY AREA

The Parsnip River watershed lies within the traditional territory of the McLeod Lake Indian Band, and the Anzac River and Table River watersheds and their natural resources are of critical community interest (Hagen et al. 2015; Pearce et al. 2019). The mouths of the Anzac River, Table River, and Misinchinka River are located approximately 40 km, 60 km, and 15 km southeast and east of the community of McLeod Lake, respectively (Figure 1). Arctic Grayling of the Table and Anzac rivers are also prized by the recreational angling community in northcentral BC.

Historically, the Parsnip River flowed roughly 280 km along the Rocky Mountain Trench from Arctic Lake to its confluence with the Finlay River, where the two rivers joined to form the Peace River. The 183 m high W.A.C. Bennett Dam is located on the Peace River approximately 110 km downstream of this confluence (Hirst 1991). Construction of the dam was completed in 1967, which resulted in the formation of Williston Reservoir. The Reservoir reached full pool in 1972 and flooded the lower 110 km (approximately) of the Parsnip River.

The post-impoundment Parsnip River system is a 6th order stream that has a watershed area of 5,600 km^2 (Table 1). Major sub-basins of the Parsnip (Misinchinka, Colbourne, Reynolds, Anzac, Table, Hominka, Missinka, Upper Parsnip), range from 290 km^2 to 1,000 km^2 and drain mountainous terrain in the Hart Ranges of the Rocky Mountains, lying to the east of the trench.

In contrast, smaller sub-basins on the west side of the Parsnip (95 km^2 to 182 km^2) drain lower elevation areas of the Nechako Plateau (Figure 1; Table 1).

Streamflow is snowmelt driven, with peak discharge occurring, on average, in late-May to early-June in the Parsnip River watershed (Water Survey of Canada Station 07EE007 *Parsnip River above Misinchika River*). Much of the watershed drains higher elevation, mountainous areas. Consequently, sediment load is relatively high among sub-basins, as evidenced by turbid water flows in spring, wide channels relative to stream size, and extensive bar development (Bruce and Starr 1985). Substantial glacial influence occurs only within the Upper Parsnip sub-basin (Figure 1). Consequently, in most years water clarity is relatively high in watershed sub-basins throughout much of the year, and by late summer the Parsnip mainstem itself becomes relatively clean in areas downstream of the Missinka River (Anonymous 1978).

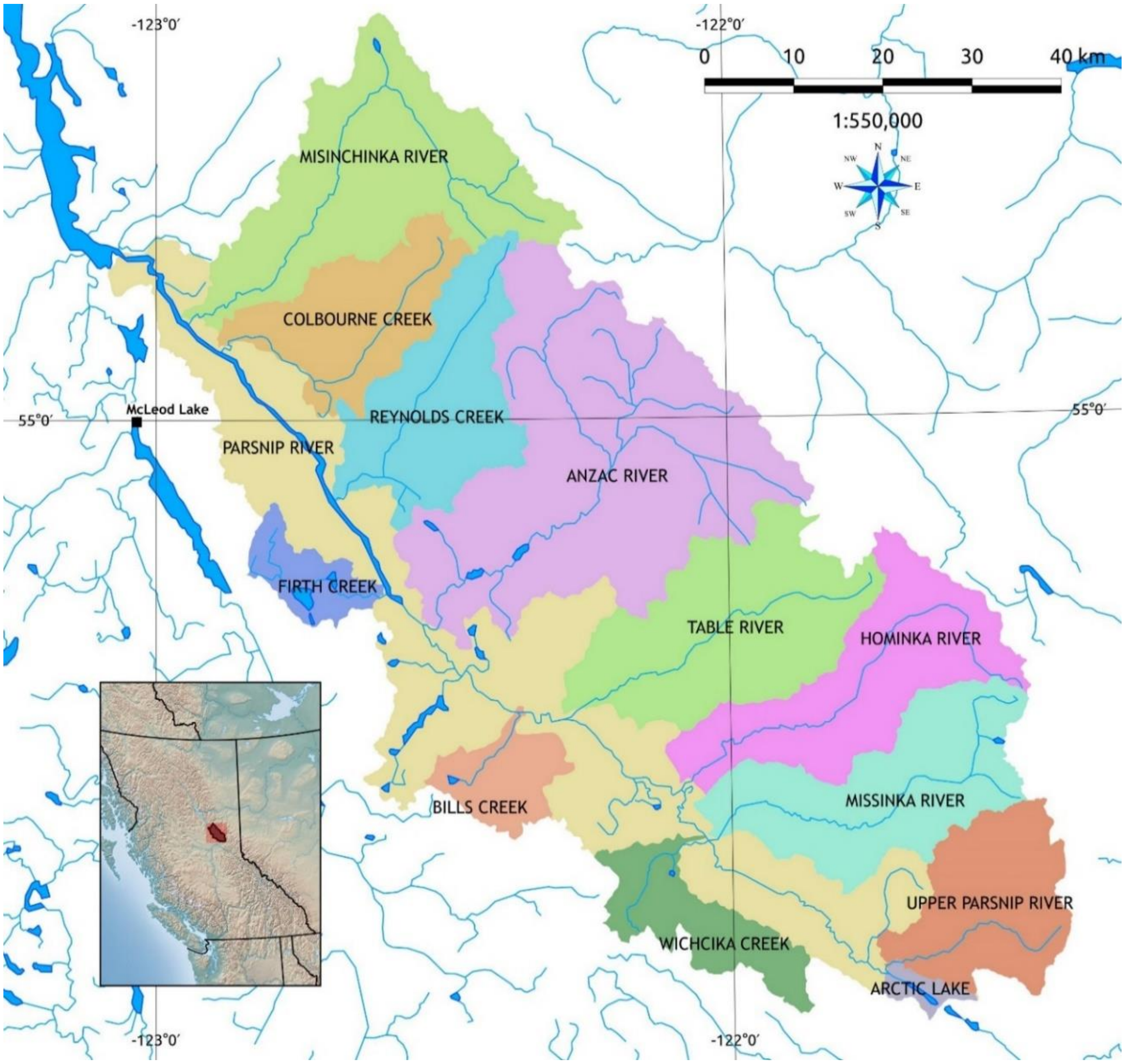

Figure 1. Sub-basins of the Parsnip River watershed potentially utilized by Arctic Grayling.

Table 1. Biophysical characteristics of sub-basins potentially utilized by Arctic Grayling within the Parsnip River watershed.

Watershed	**Sub-basin**	**Watershed area (km^2)**	**Stream order**	**Fish species present**
Parsnip	Parsnip total	5,612	6	GR, EB, BT, BB, KO, LKC, LT, LW, CSU, LNC, LSU, MW, NSC, PCC, CAS, PW, RB, RSC, CCG, WSU
Parsnip	Misinchinka River	595	4	GR, BT, BB, LSU, MW, RB, CCG
Parsnip	Colbourne Creek	289	4	GR, BT, CSU, LSU, MW, RB, CCG
Parsnip	Reynolds Creek	366	5	GR, BT, BB, LKC, CSU, LNC, LSU, MW, RB, RSC, CCG
Parsnip	Firth Creek	95	3	GR, BB, LKC, LW, LNC, LSU, MW, RB, CCG
Parsnip	Anzac River	1,044	5	GR, BT, BB, LKC, LT, LW, LSU, MW, PCC, CAS, RB, RSC, CCG
Parsnip	Tacheeda Lakes	95	4	BT, KO, LT, LW, LNC, LSU, MW, NSC, PCC, CAS, PW, RB, RSC, WSU
Parsnip	Bill's Creek	122	5	GR, BB, MW, RB, CCG
Parsnip	Table River	504	5	GR, BT, BB, LW, CSU, LSU, MW, NSC, RB, CCG, WSU
Parsnip	Hominka River	433	5	GR, BT, BB, LSU, MW, PCC, RB, CCG, WSU
Parsnip	Missinka River	434	5	GR, BT, BB, LKC, CSU, LNC, LSU, MW, NSC, RB, RSC, CCG
Parsnip	Wichcika Creek	182	5	GR, BT, BB, MW, RT, CCG
Parsnip	Arctic Lake	31	-	GR, BT, KO, LT, LW, LSU, MW, NSC, RB, RSC, WSU
Parsnip	Upper Parsnip	303	-	GR, BT, BB, KO, LT, LW, CSU, LSU, MW, NSC, RB, RSC, CCG, WSU

4 METHODS

4.1 Survey Conditions

The feasibility and safety of snorkeling surveys in mountain streams depend on good water clarity and low-to-moderate stream flows. Advance knowledge of stream flows and precipitation at the field site can help minimize unanticipated costs from aborted field days due to high, dirty water and safety concerns.

Water Survey of Canada (WSC) Station 07EE007 *Parsnip River above Misinchinka River* is located on the Parsnip River near its mouth. It is the only WSC flow monitoring station for the Parsnip River watershed. In August 2022, this WSC station provided real time stream discharge and precipitation data that was utilized to assess the potential safety and feasibility of snorkeling surveys.

4.2 Snorkeling Methods

In 2022, we conducted replicated snorkeling surveys in all six long-term index sections of the Parsnip River watershed, including Anzac River section 16-12 km (Figure 2) on August 11, Anzac 47-45 km and Anzac 43-39 km (Figure 2) on August 12, Table River section 35-31 km (Figure 3) on August 13, Anzac 34-30 (Figure 2) on August 14, and Table 26-22 km (Figure 3) on August 16.

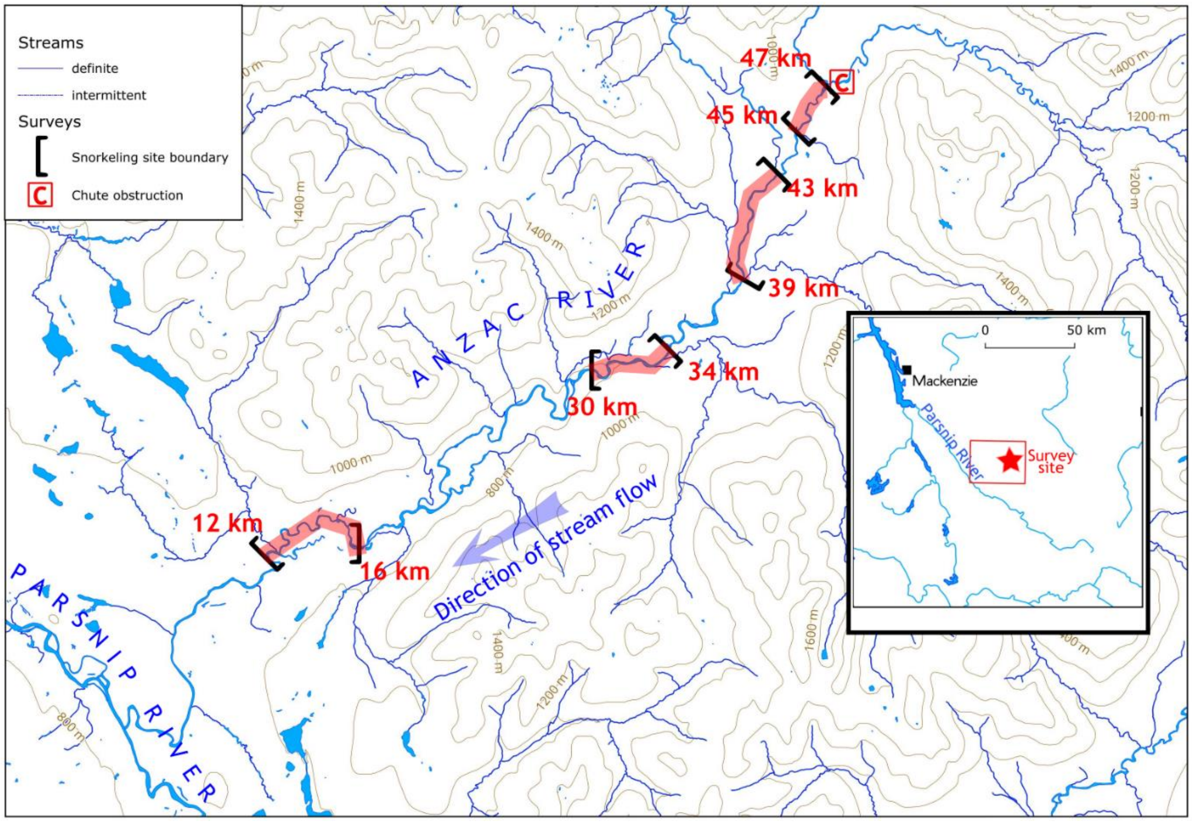

Figure 2. Index sections of the Anzac River used for snorkeling surveys to monitor Arctic Grayling abundance, 1998-2022.

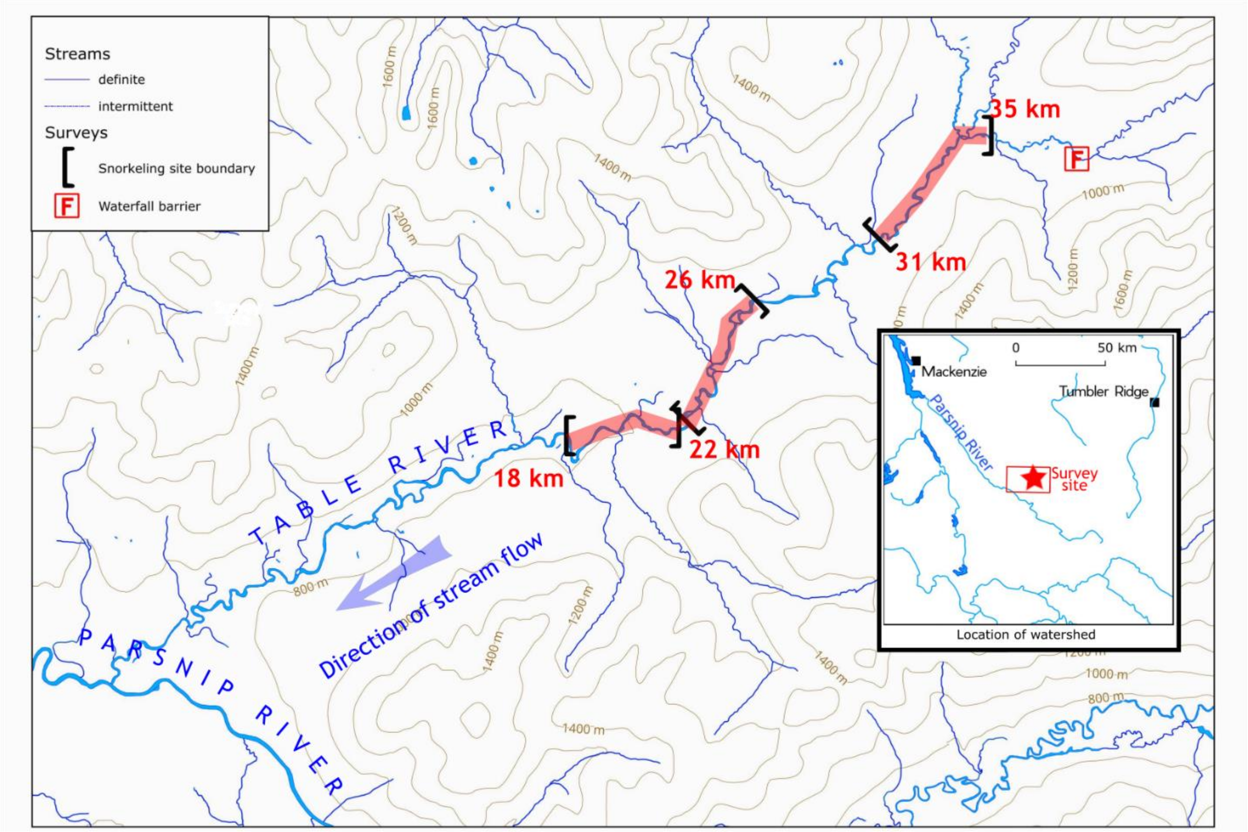

Figure 3. Index sections of the Table River utilized for snorkeling surveys to monitor Arctic Grayling abundance, 1995-2022. Site 22-18 km was surveyed until 2007, then replaced by site 26-22 km in 2018 due to the presence of a natural clay slump at 22 km that created unsuitable visibility conditions downstream.

Single-pass snorkeling surveys of 5 reconnaissance sections of the Misinchinka River watershed were conducted over the August 14-15 period (Figure 4). A reconnaissance survey of a single, 1-km section of lower Wooyadilinka Creek (not mapped) was conducted on August 16.

Replicate snorkeling surveys in the long-term index sections of the Table and Anzac rivers were conducted by two independent, three-person crews. A minimum one-hour delay was utilized between surveys to allow the sections to recover from disturbance. Single-pass reconnaissance sections in the Misinchinka River and Wooyadilinka Creek were surveyed by one crew, but were otherwise surveyed using the same method.

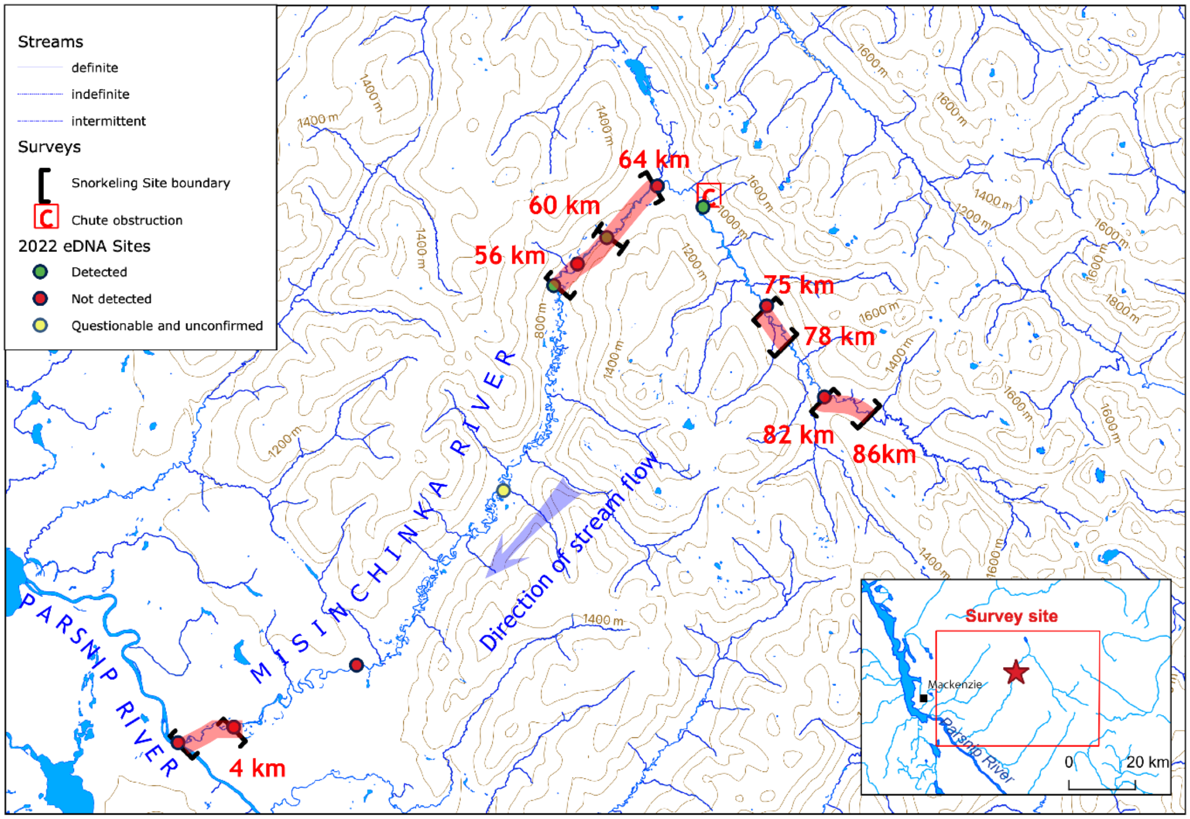

Figure 4. Reconnaissance sections of the Misinchinka River used for snorkeling and environmental DNA (eDNA; see sections 4.3) surveys to monitor Arctic Grayling abundance and presence, respectively, August 2022.

Snorkeling passes were conducted by three-person crews comprised of two observers in drysuits accompanied by a Swiftwater Rescue Technician paddling an inflatable kayak (Figure 5). During snorkeling surveys, observers surveyed lanes of width determined by underwater visibility conditions and habitat suitability (i.e., usable wetted width for subadult and adult Arctic Grayling – see Blackman 2004 for microhabitat preferences of subadult/adult grayling). In narrow stream sections observers counted fish in a lane extending in front of them and to one side only. When the usable wetted width of the stream exceeded the width of 2 lanes surveyed in this manner, one or both swimmers extended their lane widths and looked both ways. If fish moved in reaction to observers, frequent communication ensured that double counting did not occur. Observed fish (e.g., Figure 6) were classified to species and assigned to size categories in 10-cm increments.

Figure 5. Three-person snorkeling team in Anzac River section 43-39 km, August 2020.

Figure 6. Table River Arctic Grayling, August 2020.

Reliable counts require a disciplined effort to organize divers in lanes across the stream, and regular communication among divers to avoid overcounting or missed areas of suitable habitat (Northcote and Wilkie 1963; Schill and Griffith 1984; Slaney and Martin 1987; Hagen and Baxter 2005). In our study, this was facilitated by the use of crews led by biologists with >20 years of snorkeling survey experience.

Two physical habitat attributes potentially affecting snorkeling detection probability were also monitored within surveyed stream sections. These were: 1) underwater visibility and 2) wetted stream width. We measured underwater visibility in snorkeling survey sections in two ways: 1) horizontal underwater Secchi disk visibility (Figure 7), and 2) horizontal underwater distance at which the species identity of a 30 cm Arctic Grayling model could no longer be discerned. We estimated wetted stream width using a laser range finder. Visibility was typically measured twice per reach, at the beginning and at the end of the survey, while wetted stream width was measured at approximately 10 locations within each reach.

Figure 7. Black-and-white Secchi disk used to estimate horizontal underwater visibility, August 2021.

4.3 Environmental DNA Sampling

During August 2019, 2020, 2021, and 2022, eDNA samples were collected concurrently with reconnaissance snorkeling surveys and the data were analyzed to evaluate detection rates of the Arctic Grayling eDNA assay and sampling technique in five watersheds as a component of FWCP Project PEA-F21-F-3198 (see Figure 7 in Stamford et al. 2022). The eDNA assay was consistent in all five watersheds in detecting Arctic Grayling eDNA when snorkeling located adults (as few as a single individual) within 1.5 km upstream (Stamford et al. 2022). In turn, the eDNA sampling complemented the snorkeling data by 1) describing habitat use beyond the range of snorkeled reaches (e.g., tributaries, headwater areas; Hagen and Stamford 2022), and 2) indicating the potential presence of Arctic Grayling that avoided snorkel observations (e.g., rare adults or life stages undetectable by snorkeling). These improvements in our study data were the reason for including eDNA sampling in our study proposal for 2022.

In 2022, eDNA samples were collected during reconnaissance surveys in the Misinchinka River (Figure 4) and Wooyadilinka Creek (not mapped), and opportunistically in Mischinsinlika Creek (not mapped) which is also a stream of interest. We also partnered with FWCP Project PEA-F23-F-3636, a study of eDNA degradation, which provided additional data to inform our Arctic Grayling distribution analyses.

All field, laboratory, and analysis methods are detailed in Stamford et al. (2020, 2022). Briefly, in the field the method involves filtering DNA molecules suspended in the water column, which originate from organisms living upstream that continually slough DNA as part of life’s processes (Figure 8). To avoid contamination, eDNA was always sampled from a downstream to upstream direction and in most cases collected from snorkeled sections in the morning before snorkelers entered the water (see Stamford et al. 2020). At one Misinchinka River site, logistics in the field prevented sampling prior to snorkeling. The eDNA sample was instead collected 48 hours after the snorkel survey had been completed, which was assumed to provide sufficient time to clear potential eDNA contamination from snorkelers’ suits, yet soon enough after snorkel observations that Arctic Grayling had not moved from their previously observed locations. Collected samples were preserved in alcohol and delivered to the University of Northern BC for analysis.

Figure 8. Filter cup through which water is pumped to acquire an environmental DNA sample.

4.4 Analyses

4.4.1 Site-Level Abundance.

Approach. The 1995-2022 snorkeling time series is characterized by missing data from some index sections in almost half of the study years. Because significant variation in abundance exists among index sections (Hagen and Gantner 2020), missing data could result in inaccurate estimates of population trend. A linear mixed-effects model can account for missing data by treating index section ID as a random effect (Section 4.4.2), but to enable this analysis approach index section-specific estimates of Arctic Grayling abundance were required.

Binomial likelihood model. Since 2019 (Hagen and Gantner 2020), we have utilized a binomial-likelihood model to estimate detection probability and abundance (Olkin et al. 1981; Royle 2004) within index sections from replicated snorkeling count data. In this report, we update the model by fitting it to improved data for covariates of detection probability collected during the 2021 and 2022 field seasons.

We assumed for all years 1995-2022 that the population of Arctic Grayling in each index reach was closed with respect to movement, mortality, etc. between the start and finish of all replicate surveys, which happen on the same day. We further assumed that replicated counts of Arctic Grayling n_{ir} were binomially-distributed random variables from the distribution

$$n_{ir} \sim \text{Binomial}\ (N_{it}, p)$$

where i is the site, r the replicate (among R replicated surveys), N_{it} the population size at index section i and year t and p is the detection probability. The likelihood statement for data from a site is detailed in Royle (2004), and represented in simplified form here as:

$$L(N_{it}, p|\{n_{i1}, \dots, n_{iR}\}) = \prod_{r=1}^{R} Binomial(n_{ir}; N_{it}, p) \tag{1}$$

Joint likelihood across all sites of interest is given by the product of the site-specific likelihoods (Royle 2004). In 2022, we updated estimates of N_{it} and p by searching for parameter values that maximized the joint likelihood across all the index section/year possibilities for the 1995-2021 period (e.g., an index section with replicated count data in four separate years would yield four index section- and year-specific likelihoods conditional on the four N_{it} and one p).

Covariate effects on detection probability. A question of key interest to us has been whether predictions of abundance and detection probability p could be improved using logistic regression models for p, for use within the binomial-likelihood model framework described above. Exploratory statistical analysis in 2019 and 2020 (Hagen and Gantner 2020; Hagen and Stamford 2021) indicated that two factors in particular affect snorkeling detection probability: 1) the stream wetted width in index reaches, which is related to the cross-sectional area to be searched by snorkelers, and 2) horizontal underwater visibility. To update the analysis in 2022, we identified a series of candidate models representing different hypotheses about the effects of these 2 factors on p, then compared these models using an information-theoretic approach (Burnham and Anderson 2002). Unfortunately, stream width data have been extremely sparse among years (Cowie 2021). In 2021 and 2022, we were able to substantially improve our knowledge of index section widths, by basing our estimates in all snorkeling reaches on 10 systematically spaced measurements. Because of our concern for unreliable or missing estimates prior to 2021, stream width values used in our analysis for each index section are average values and not year specific.

Model selection was performed using replicated count data from all years 1995-2022. We used the Akaike information criterion corrected for small sample size (AIC_c) for the comparisons among models. We computed the strength of evidence for each candidate model being the best in the set by computing the likelihood of each model given the data $\mathrm{L}(g_i|x)$, then normalizing these likelihoods as a set of Akaike weights w_i (Burnham and Anderson 2002).

4.4.2 Population Trend

We assessed the trend in Arctic Grayling abundance over time for the Parsnip River watershed in two ways: 1) using modeled estimates of abundance N_{it} in long-term index sites (Section 4.4.1), to account for the effects of variable detection probability, and 2) using raw count data. Binomial-Likelihood Model estimates of N_{it} (see Section 4.4.1) and raw snorkeling count data (average across all replicates) were analyzed within a linear mixed effects analysis, performed using the Stata statistical analysis program (StataCorp, 2009) and the ‘xtmixed’ function (Rabe-Hesketh and Skrondal 2008). Natural log-transformed N_{it} or mean counts were entered into the model as a fixed effect, along with observation year. As random effects, we had intercepts for sites nested within streams.

Trend, i.e., population growth rate, is a key indicator of conservation status and risk, and of potential effects of limiting factors (see Section 1.0). From the linear mixed effects model output, we computed population growth rate using the formula:

$$Population\ growth\ rate = \frac{N_t - N_0}{N_0 * (t)} \tag{2}$$

Where N_0 is the predicted initial population size and N_t is the predicted population size after time interval t years.

4.4.3 Reach-Level Abundance

Delineating reaches. In addition to evaluating trend in long-term index sections, a further objective of the 2018-2022 snorkeling study was to estimate abundance of Arctic Grayling in adult summer rearing habitats across the whole Parsnip River watershed, to facilitate delineation of critical habitats and improve estimates of conservation status (Section 2).

N-Mixture Model. Over 2018-2022 period, single-pass snorkeling surveys were conducted in 42 reconnaissance sections of 8 streams. As the first step in generating stream- and watershed-scale abundance estimates, accessible lengths of these streams were divided into reaches based on consistent patterns of Arctic Grayling abundance and sampling intensity, to reduce concerns about overdispersion of the count data.

The model for detection probability p and reach-scale abundance N that we utilized was the Poisson-Mixture Model of Royle (2004), an N-mixture model which is related to Equation (1) from Section 4.4.1 but which treats the N_{it} as independent random variables distributed according to a specified prior distribution for N. Prior parameters for the Poisson distribution are the N_{it} and λ_t (λ_t = both mean and variance for abundance N_t), which are estimated after integrating Equation (1) over the prior distribution for N_t (Royle 2004). In simplified form, the integrated likelihood from Royle (2004) for a given year t is:

$$L(p, \lambda_t | \{n_{ir}\}) = \prod_{i=1}^{I} \left\{ \sum_{N_{it} = \max n_{ir}}^{\infty} \left(\prod_{r=1}^{R} Binomial(n_{ir}; N_{it}, p) \right) * Poisson(N_{it}; \lambda_t) \right\} \tag{3}$$

This model was utilized in a two-stage process to estimate detection probability and reach-scale abundance. In the first stage, the model for detection probability was created by fitting the Royle

(2004) Poisson-mixture model to replicated count data in long-term index sites of the Anzac and Table rivers. We computed the joint likelihood across all study years of 1995-2022 period as the product of the year-specific likelihoods. In searching for values of λ_t and p that maximized the joint likelihood across all years for the 1995-2020 period, λ_t was year-specific but p was not. Similar to the binomial likelihood model for site-specific estimates of abundance (Section 4.4.1), we identified a series of candidate logistic regression models representing different hypotheses about the effects of wetted width and horizontal underwater visibility on p, then compared these models using an information-theoretic approach (Burnham and Anderson 2002).

In the second modeling stage, the best Poisson-mixture model for p and λ_t was applied to count data in our specified reaches to generate the maximum likelihood estimate of N_t (standardized as density) for that reach. The difference relative to fitting the model in the first place was that the logistic regression parameters for p were no longer estimable parameters in the model, having been fixed during the first modeling stage.

Limits of 95% confidence for N_t were estimated using the deterministic approximation to the method of likelihood profile (Haddon 2001):

$$LL(N_t) = LL(N_t)_{max} - \frac{\chi^2_{1,1-\alpha}}{2} \tag{4}$$

where $\chi^2_{1,1-\alpha}$ is the (1-α)th quantile of the χ^2 distribution with 1 degree of freedom (e.g., for 95% CL's $\alpha = 0.95$, $1-\alpha = 0.05$, and $\chi^2_{1,1-\alpha} = 3.84$).

4.5 Delineating and Prioritizing Critical Habitats

Critical habitat types. We delineate two types of critical habitats in stream segments of the Parsnip River watershed: 1) adult/subadult rearing, and 2) juvenile rearing. In doing so, we focus on key life stages thought to limit population productivity, but also attempt to encompass habitat use through the entire Arctic Grayling life cycle.

Evidence for the importance of adult/subadult summer rearing habitats for Arctic Grayling growth and survival is in several forms, including: 1) consistent observations of annual returns to the same stream locations potentially suggesting the migratory behaviour in Arctic Grayling is adapted to local watershed conditions (e.g., Buzby and Deegan 2000; Blackman 2002; Gryska 2019; Vøllestad and Primmer 2019; Martins et al. 2022); 2) observed linkages between habitat degradation in summer rearing habitats and population declines (Armstrong 1986; Northcote 1993; Walker 2005; USFWS 2010; Cahill 2015); 3) direct and indirect effects of high summer stream temperatures on Arctic Grayling growth, survival, and habitat use (Lohr et al. 2000; Deegan et al. 1999; O'Connor 2023) and the expectation that these will increase with climate warming, and 4) evidence for high vulnerability to overexploitation in accessible summer rearing habitats (Northcote 1993; Alberta Sustainable Resource Development 2005).

In the Parsnip River watershed, young-of-year (YOY) and older juvenile (post-YOY) Arctic Grayling may share the same habitats (Murphy and Blackman 2012; Mackay and Blackman 2012), and these in turn often overlap with stream sections utilized by adults during the overwinter period (Blackman 2002; Martins et al. 2022) and during spawning in spring (Blackman 2002; Martins et al. 2022). Therefore, critical juvenile rearing habitat may be used at different times of the year by all life stages. Evidence for the importance of juvenile rearing habitats in population productivity include: 1) loss of entire populations in the Williston Reservoir watershed where juvenile and overwintering habitats were flooded (Stamford et al. 2017); 2) high vulnerability of juvenile Arctic Grayling and alevins to greater variation in stream discharge and temperatures (Tack 1974; Clark 1992; Hawkshaw et al. 2013) and to elevated sediment (Birtwell et al. 1984; McLeay et al. 1987; Reynolds et al. 1989); and 3) adaptation of Arctic Grayling to local watershed conditions which may change significantly as a result of watershed development or climate warming (Kaya and Jeanes 1995; Haugen and Vøllestad 2000, 2001; Vøllestad and Primmer 2019).

Delineating critical habitats. For this report, we conducted a significant update to the table of Parsnip Arctic Grayling critical habitats listed in Stamford et al. (2017). Key, new sources of information included: 1) results of 42 single-pass reconnaissance snorkeling surveys conducted as part of this study; 2) eDNA results refining the distribution of critical habitats and indicating low abundance or undetected life stages (e.g., Hagen and Stamford 2021, 2022); and 3) other studies conducted by FWCP since 2017, particularly PEA-F22-F-3388 *Spatial Ecology of Arctic Grayling in the Parsnip Core Area* (Martins et al. 2020, 2022). Past sources of information have included: 1) BC Government Fish Observations and Fish Obstacles GIS layers populated from the BC Geographic Warehouse (BCGW), 2) written documents warehoused in the Ecological Reports Catalogue for British Columbia, in various government databases (see Stamford et al. 2017), or in the personal libraries of the authors, and 3) personal discussions with knowledgeable persons.

Records of sampled Arctic Grayling were utilized to evaluate whether the presence of the species within the watershed sub-basins also indicated the presence of critical habitats, and for which life stage. Indicators of critical habitats for Arctic Grayling YOY and post-YOY juveniles (also overwintering and spawning) included: 1) the presence of grayling < 100 mm and < 200 mm, respectively, and 2) a relatively high frequency or expectation of occurrence. Adult and subadult grayling critical summer rearing habitats were indicated by: 1) a relatively high frequency of occurrence of fish > 200 mm when sampling frequency was deemed adequate and when suitable sampling techniques were employed.

Prioritizing critical habitats. We also prioritized among critical habitat segments by assigning rankings of 1-3, with a ranking of ‘1’ indicating the highest priority for conservation and enhancement investments, a ranking of ‘2’ indicating additional targets for conservation actions although perhaps not at the same level of investment, and ‘3’ indicating a situation of marginal Arctic Grayling presence and/or where additional information is required before substantial conservation investments are considered. Two key principles guiding the prioritization of critical habitats are presented in the recommended Parsnip Arctic Grayling Stewardship Framework of Hagen and Stamford (2022; Section 6.2.1). These are:

1. Conservation of critical habitats in good ecological condition and expected to be resilient to future climate change is more urgent and cost-effective than restoration and enhancement of already-degraded habitat (Roni et al. 2002; Chapin et al. 2015).
2. More productive sub-basins in the Parsnip River watershed supporting higher abundances of Arctic Grayling are candidates for greater investments in habitat preservation. These populations are likely to have the highest long-term viability.

5 RESULTS

5.1 Survey Conditions

The August 11-17 snorkeling and eDNA surveys were conducted during a period of dry weather and discharge in the Parsnip River watershed was below the long-term average level (Figure 9). Consequently, August 2022 was well-suited to snorkeling surveys, with suitable visibility and safe swimming conditions. Snorkeling surveys occur between August 10-24 each year, a period that occurs well after adults have completed their spring migrations from spawning areas to preferred summer rearing areas, and which is also well before dropping water temperatures in the fall trigger emigration to overwintering areas (Northcote 1993; Blackman 2002; Martins et al. 2022).

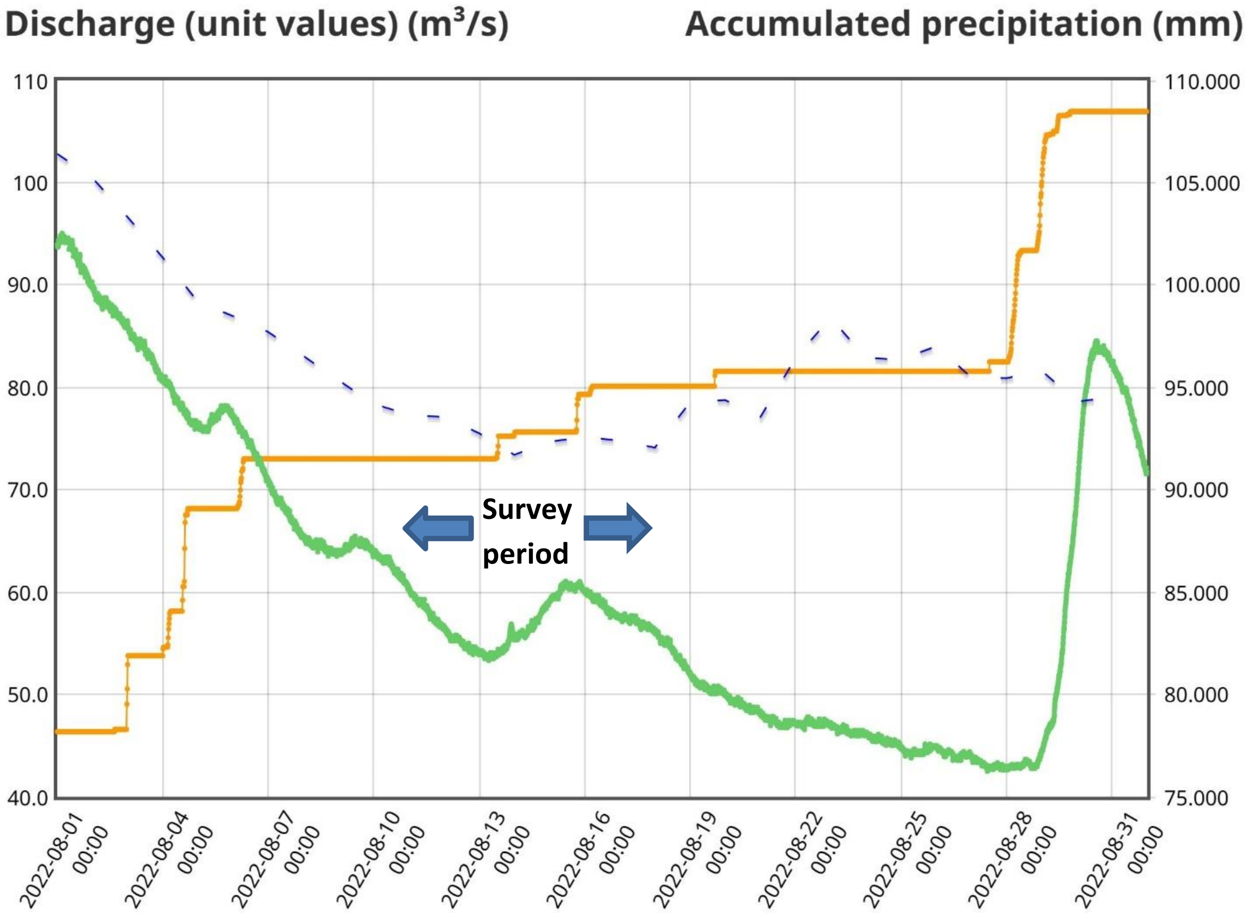

Figure 9. Estimated discharge (solid green line), long-term average discharge (dashed line), and accumulated precipitation (solid orange line) in the lower Parsnip River during August, 2022, Water Survey of Canada (WSC) Station 07EE007 (Parsnip River above Misinchinka River).

Table River site 26-22 km replaced the previous long-term index site 22-18 km in the Table River, due to a major natural clay slump into the river at 22 km, for the fifth consecutive year. The extensive nature of the slump indicates that visibility in the formerly-surveyed reach may be compromised for years to come (Figure 10).

Figure 10. Rapid decline of visibility to < 2 m at Table River 22 km, the top boundary of former long-term index section Table 22-18 km.

Secchi disk visibility in August, 2022 ranged from 6.0-10.0 m in the four long-term index sections of the Anzac River, from 7.5-7.8 m in two long-term index sections of the Table River, and from 3.6-10 m in five reconnaissance sections of the Misinchinka River (Table 2). Misinchinka River visibility declined downstream of 75 km due to a clay slump, but remained sufficient for snorkeling observations (i.e., > 3 m minimum). Further downstream beginning at 54 km, visibility dropped below 3 m and, consequently, a long section of low gradient meanders was not snorkeled. Visibility recovered somewhat downstream, enabling a snorkeling survey near the mouth (4-0 km; Table 2).

Visibility for discernment of Arctic Grayling models ranged from 4.7 m to 6.7 m in Anzac River long-term index sections, from 5.2-5.6 m in the Table River, and from 3.0-7.6 m in reconnaissance sections of the Misinchinka River (Table 2). Fish model visibility levels in long-term index sections were within historic 1995-2021 ranges for all sites except Anzac 43-39, which had the greatest fish visibility ever recorded at this location.

Table 2. Horizontal underwater visibility in snorkeling survey sections of the Parsnip River watershed, August 2022.

	Table River		Anzac River				Misinchinka River				
Snorkeling section (km)	26-22	35-31	16-12	34-30	43-39	47-45	4-0	60-56	64-60	78-75	86-82
Date	16-Aug	13-Aug	11-Aug	14-Aug	12-Aug	12-Aug	17-Aug	17-Aug	15-Aug	15-Aug	15-Aug
Secchi disk visibility (m)	7.8	7.5	8.4	7.0	10.0	6.0	3.6	3.6	3.6	8.1	10
Fish model visibility (m)	5.6	5.2	5.3	5.1	6.7	4.7	3.0	3.3	3.3	5.1	7.6
1995-2021 range (fish)	3.0-7.7	3.5-7.0	3.8-7.0	3.0-9.5	4.3-5.7	3.0-6.6	-	-	-	-	-

5.2 Detection Probability and Abundance in Long-Term Index Sections

Over the 1995-2022 period, a total of 48 surveys have taken place in the seven long-term index sections of the Anzac and Table rivers in which at least two replicate counts were made (Table 3). These data were analyzed within the binomial-likelihood model framework described in Section 4.4.1, which estimates site-specific abundance N_{it} and snorkeling detection probability p conditional on the replicated count data. Detection probability is a parameter of key interest to us given the logistical challenges and investment required to estimate p by other means (e.g., mark-resight studies). We were particularly interested in two variables potentially influencing p and subsequent inferences about population status: 1) mean site wetted width *WIDTH*, and 2) horizontal underwater visibility at which Arctic Grayling could be discriminated *FISH_VIS* (Table 2).

Within the binomial-likelihood model framework, we evaluated a candidate set of four logistic regression models for p using AIC$_c$ (Table 4). The best model contained only *WIDTH* as a predictor, with the expected negative relationship. Application of this model to the replicated count data resulted in a significant improvement in model likelihood relative to the constant-p model (Chi-square P <0.001). The logistic model was:

$$p = \frac{1}{1 + \exp\{-(1.816 - 0.0580 * WIDTH)\}}$$

The probability of this model being the best, as indicated by its Akaike weight w_i, was 0.641 (Table 5). Support for the model with *WIDTH* and *FISH_VIS* as predictor variables with the expected signs (negative for *WIDTH*, positive for *FISH_VIS*) was also high, with only marginally-lower $w_i = 0.359$. The *WIDTH* + *FISH_VIS WIDTH* logistic model was:

$$p = \frac{1}{1 + \exp\{-(1.752 - 0.0582 * WIDTH + 0.0136 * FISH\_VIS)\}}$$

Table 3. Replicated snorkeling counts of Arctic Grayling >20 cm in long-term index sites of the Anzac and Table rivers, 1995-2022.

		Replicate Counts of Arctic Grayling >20 cm						
Site	**Year**	**R1**	**R2**	**R3**	**R4**	***FISH_VIS***	***WIDTH (mean)***	**Predicted N_{it}**
Anzac 16-12	1998	13	3			6.5	28.5	16
	2001	6	15			4.0	28.5	20
	2003	18	30	22		4.5	28.5	42
	2005	26	31			4.5	28.5	52
	2007	44	50			3.8	28.5	87
	2021	46	53			7.0	28.5	93
	2022	39	75			5.3	28.5	106
Anzac 34-30	1998	116	96			9.5	30.1	188
	2001	48	55			3.0	30.1	101
	2003	54	68	41		3.5	30.1	102
	2005	98	56	82		4.0	30.1	155
	2007	34	83	67		3.7	30.1	122
	2018	138	93	111		6.0	30.1	219
	2019	82	67	68		5.0	30.1	140
	2021	149	154			4.8	30.1	313
	2022	175	182			5.1	30.1	365
Anzac 43-39	1998	167	114	127		4.5	26.3	241
	2001	73	96			4.5	26.3	147
	2003	144	181	172		4.5	26.3	291
	2005	99	83			4.3	26.3	158
	2018	173	187	185		4.6	26.3	315
	2019	140	149	167		5.1	26.3	264
	2021	135	128			5.7	26.3	223
	2022	146	209			6.7	26.3	302
Anzac 47-45	1998	157	171			4.5	17.4	232
	2000	69	67			3.0	17.4	97
	2001	15	25			3.0	17.4	31
	2003	62	80	92		3.5	17.4	117
	2019	110	85	77		4.4	17.4	135
	2021	140	152			6.6	17.4	211
	2022	203	213			4.7	17.4	298
Table 22-18	1998	54	79			5.5	22.5	104
	2000	39	30	40	38	4.0	22.5	59
	2001	35	48			3.0	22.5	67
	2003	75	62	72		4.5	22.5	110
	2007	39	57	42		4.0	22.5	75
Table 26-22	2022	206	304			4.9	21.9	404
Table 35-31	1995	107	115			5.0	15.6	153
	1998	137	135			5.5	15.6	186
	2000	101	111	136	145	7.0	15.6	184
	2001	80	102			3.5	15.6	128
	2003	139	138	134		3.5	15.6	194
	2005	96	104	94		3.6	15.6	137
	2007	124	103	112		3.7	15.6	158
	2018	191	230	209		5.7	15.6	294
	2020	139	164			4.7	15.6	218
	2021	221	235			6.5	15.6	312
	2022	246	287			5.2	15.6	371

Table 4. Comparison among predictors of detection probability p estimated within a binomial-probability model framework (see text) from replicated count data in the Parsnip River watershed. Symbols K, Log (L), AIC_c, Δi, $L\ (g_i|x)$, *and* w_i, *denote 1) the number of estimable parameters, 2) model log-likelihoods, 3) the Akaike information criterion values adjusted for small sample size, 4) the difference in* AIC_c *values between each model and the model with the lowest* AIC_c *score, 5) the likelihood that the candidate model is the best among the set, and 6) Akaike weights, respectively.*

Model	K	Log($\mathcal{L}$)	AIC_c	Δ_i	$\mathcal{L}\ (g_i \| x)$	w_i
Constant-only	2	-583.99	1172.24	45.22	1.51504E-10	9.70E-11
WIDTH	3	-560.24	1127.02	0.00	1	0.64
VIS	3	-583.43	1173.40	46.38	8.49336E-11	5.44E-11
WIDTH+VIS	4	-559.623	1128.18	1.16	0.561195134	0.36
$MinAIC_C$			1127.02			
n	48					

Support for *VIS*-only model was almost non-existent (Table 4), despite the obvious need during snorkeling surveys for adequate underwater visibility. This is likely due to three factors: 1) the lack of informative contrast in the underwater visibility among sites (Table 2, Table 3); 2) the lack of documentation of methods for estimating underwater visibility prior to 2018; and 3) species-specific holding behaviour – snorkelers observed that Arctic Grayling tend to maintain their positions longer before reacting to the approaching swimmers relative to other species (although similar to Westslope Cutthroat Trout).

Estimated detection probability among long-term index sections of the Anzac and Table rivers can be compared by applying the *WIDTH* model to mean wetted width data for each section (Table 5). Detection probability estimates show a consistent pattern of highest detection probability in the smaller reaches at the top of the Arctic Grayling distribution in each system. Mark-resight data to validate the detection probability estimates are available for only one index section in one year. In 2019, 12 of 15 acoustic-tagged Arctic Grayling estimated to be present in Table River section 35-31 km were observed by the snorkeling team, equating to a detection probability estimate of 0.80 (80% confidence interval: 0.61-0.92). This suggests that the relatively high model estimate of detection probability for this section of 71% (Table 5) is plausible. In Table River section 35-31 km, two swimmers can easily cover the entire wetted width in most locations.

Table 5. Predictions of mean snorkeling detection probability (p) in long-term index sections of the Parsnip River watershed, computed at average values of wetted width (WIDTH).

	Anzac River				Table River	
Site	16-12	34-30	43-39	47-45	22-18	35-31
WIDTH	28.5	30.1	26.3	17.4	22.5	15.6
Binomial-Likelihood Model Estimated *p*	0.54	0.52	0.57	0.69	0.62	0.71

5.3 Population Trend

Counts of Arctic Grayling >20 cm in long-term index sections over the 1995-2022 period, averaged across replicates for each year, are depicted in Figure 11. In Table River section 35-31 km, counts over the 2018-2022 period are clearly higher than those made over the 1995-2007 period. Counts in Table River section 26-22 km are also much higher than in 22-18 km. To date, 26-22 km has been treated as a replacement for 22-18 km in the time series and their pooled data have been entered in to the linear mixed-effects model. Because of the rather large discrepancy in counts between these sections, beginning in 2022 their data were treated as two independent locations in the analysis of trend.

In the Anzac River, counts over the 1998-2022 time series also indicate an increasing trend, which is most clear in the lower two sites 34-30 km and 16-12 km. In the upper, canyon sections of the Anzac River 47-45 km and 43-39 km, variability among years is higher, particularly for 47-45 km which is located above a 2-km section of rapids that may affect upstream movements in some years. In 2022, more Arctic Grayling were counted in total across index sections than in any other year to date.

To account for the unwanted effects of variable detection probability, our preferred analysis of trend used model estimates of site-level abundance (see previous section), although we also repeated the analysis using the raw count data. The site- and year-specific population estimates (N_{it}) from the binomial-likelihood model (Table 3), analyzed using a linear mixed-effects model in which N_{it} and *YEAR* were fixed effects and *STREAM* and *SITE* nested random effects, confirm a significant increase in the abundance of Arctic Grayling >20 cm in the Parsnip River watershed ($P < 0.001$) over the 1995-2022 period. A benefit of the linear mixed-effects model framework was that it was compatible with missing site data which occurs in nearly half of the sampling years. This estimate of positive trend was corroborated when the analysis was repeated on raw count data ($P < 0.001$).

The linear mixed effects model output suggests an annual population growth rate of 3.9% for Arctic Grayling in the Parsnip River watershed, which equates to an increase of approximately 98% over 25 years.

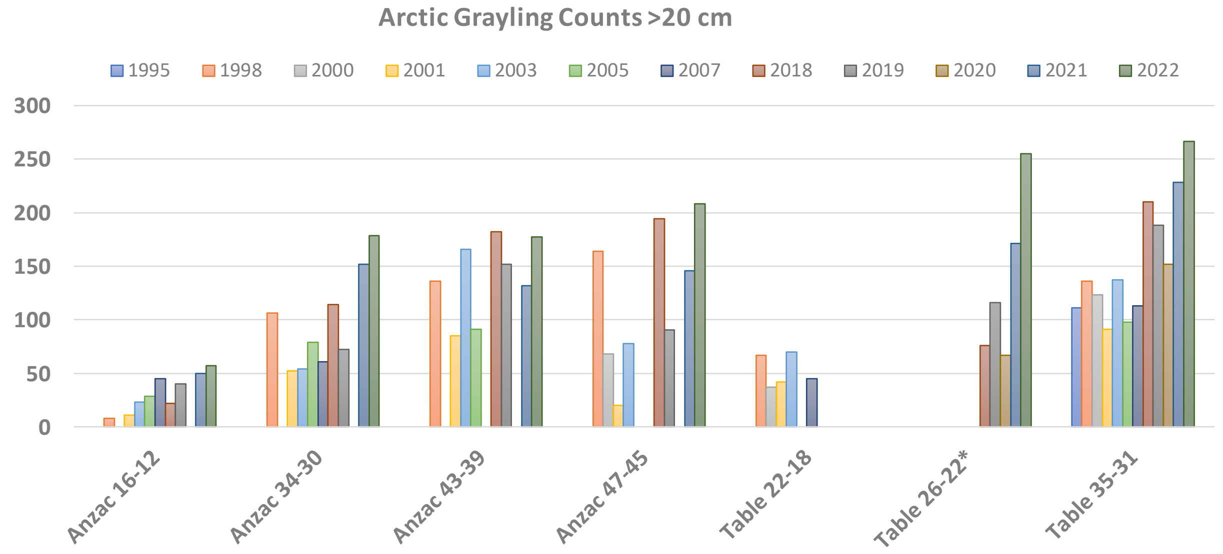

*Figure 11. Unadjusted snorkeling counts of Arctic Grayling > 20 cm in index sites of the Anzac River and Table River watersheds 1995-2022. Values are averages of replicate counts. *Beginning in 2018, Table River section 26-22 km was substituted for 22-18 km which was affected by a major clay slump at 22 km.*

5.4 Misinchinka River Reconnaissance Surveys

5.4.1 Single-Pass Snorkeling Surveys

Over the 2018-2022 study period, single-pass snorkeling reconnaissance surveys in the Parsnip core area included: 1) Anzac River in 2018 (Hagen et al. 2019), 2) Missinka River in 2019 (Hagen and Gantner 2020), 3) Hominka River and Wichcika Creek in 2020 (Hagen and Stamford 2021); 4) Reynolds Creek and Colbourne Creek in 2021 (Hagen and Stamford 2022), and 5) Misinchinka River and Wooyadilinka Creek in 2022 (Table 6; Figure 12).

Table 6. Counts of salmonids >20 cm during 2018-2022 reconnaissance snorkeling surveys.

Stream	Section	Arctic Grayling	Bull Trout	Rainbow Trout	Mountain Whitefish	*WIDTH*	*FISH_VIS*
Anzac River	55.6-52.6	35	22	9	52	15.6	7.5
2018	52.6-49.5	49	17	9	27	15.6	7.5
	49.5-47.7	31	1	5	29	15.6	5.5
	47.7-45	206	76	10	716	17.6	4.7
	45-41.8	183	114	20	913	21.9	4.6
	41.8-37	188	76	2	415	26.3	6.5
	37-31.8	168	56	5	133	28.2	6.0
	31.8-28.3	107	46	46	1224	30.1	5.4
	28.3-23	185	35	17	2689	30.1	5.4
	23-16.6	188	37	44	444	30.1	4.6
	16.6-11	30	8	12	618	28.5	7.7
	11-9.8	33	4	14	25	29.3	5.9
	9.8-5.8	69	10	8	135	29.3	5.9
Missinka River	33-29	34	5	11	211	19.0	5.0
2019	25-22	3	0	1	84	22.3	4.8
	8-4	1	6	0	28	28.0	4.4
Hominka River	60-56	0	14	0	162	15.0	8.0
2020	56-53	0	10	0	150	19.4	10.2
	53-48	2	26	0	220	24.7	10.2
	48-44	42	30	0	381	16.3	11.6
	44-41	23	2	0	144	20.8	8.5
	39-35	29	5	1	343	25.2	4.0
	32-29	4	3	0	28	37.0	2.6
	10-7	1	1	0	41	26.2	4.5
Wichcika Creek	27.5-24	1	0	1	16	13.9	3.5
2020	22.5-20.5	2	3	8	62	15.0	3.6
	15-11	5	0	17	65	17.7	4.6
	10-7	0	0	4	9	16.3	3.9
	5-1	1	2	14	4	18.4	4.6
Reynolds Creek	36-34	0	3	2	0	11.8	7.2
2021	30-26	2	42	70	79	10.5	7.0
	20-16	0	5	24	0	16.6	5.2
	12-10	0	2	39	0	14.5	3.4
	6-2	6	9	39	9	22.2	5.0
Reynolds 2020	30-26	6	0	1	121	16.6	2.7
Colbourne Creek	17-13	0	0	22	3	14.1	4.8
2021	9-5	0	9	31	326	14.3	5.0
	3-1	0	1	0	0	14.3	3.0
Misinchinka River	4-0	0	6	2	263	13.875	3.5
2022	60-56	1	32	32	582	15	3.6
	64-60	1	24	33	956	17.7	4.6
	78-75	0	12	26	627	16.3	3.9
	86-82	0	51	20	581	18.4	4.6
Wooyadilinka Creek 2022	1-0	0	2	4	0	5.0	5.1

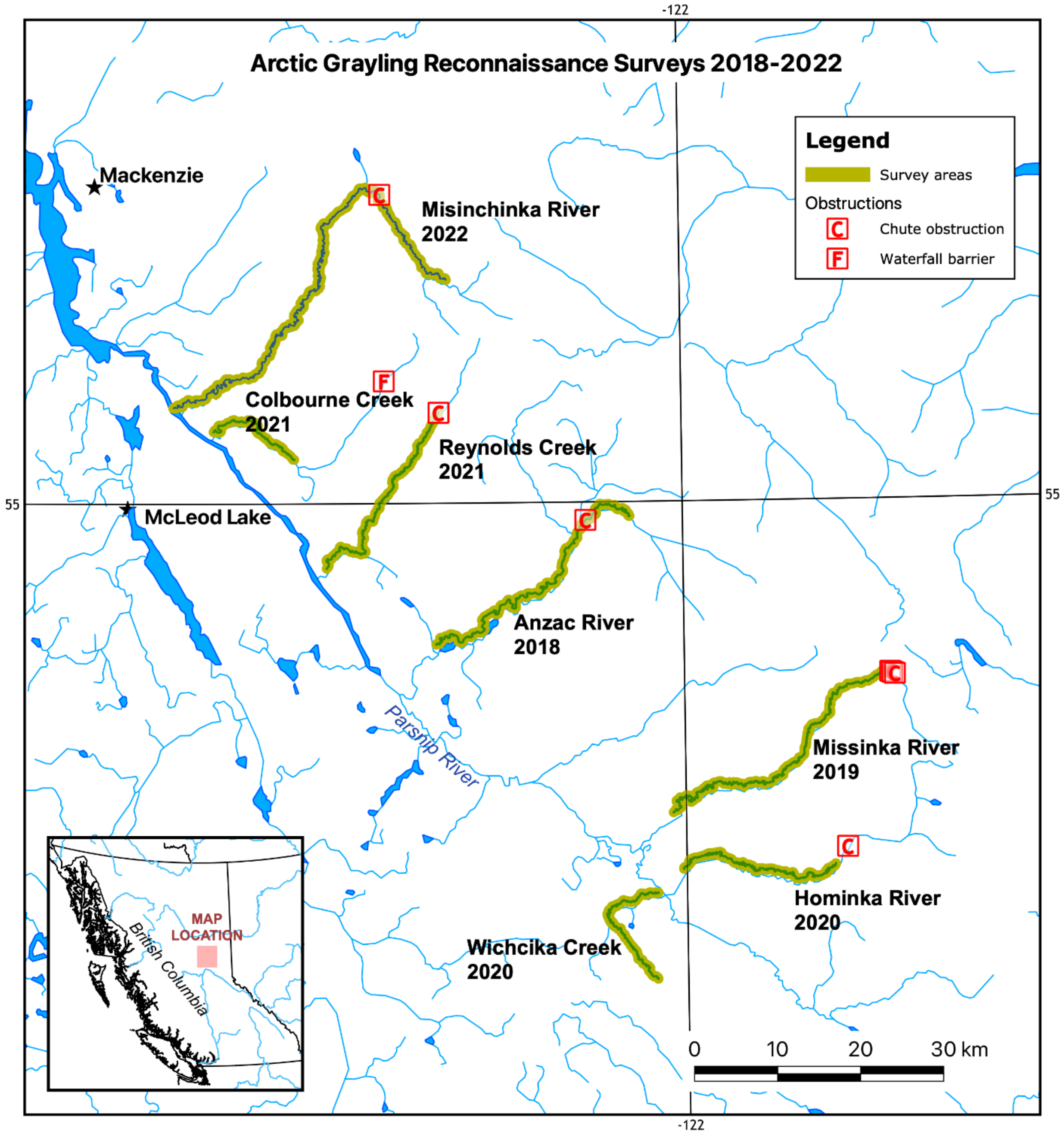

Figure 12. Stream sections of the Parsnip River watershed (green survey areas) sub-sampled using single-pass snorkeling surveys to estimate distribution and abundance of adult/subadult Arctic Grayling.

The use of the Misinchinka River by adult Arctic Grayling was confirmed in 2022 during single-pass snorkeling surveys of 5 reconnaissance sections (Figure 4, Figure 13, Figure 14). We are not aware of any other post-flooding records for the adult life stage in this system (Stamford et al. 2017). However, Arctic Grayling abundance was probably at the margin of detectability with just two adults observed, one in each of the 64-60 km and 60-56 km reconnaissance sections (Table 6). Two juvenile Arctic Grayling 10-20 cm in length were also observed < 500 m upstream of the mouth in the 4-0 km section (not included in Table 6). No Arctic Grayling were observed in two reconnaissance sections of the upper Misinchinka River (86-82 km, 78-75 km; Figure 4), which were utilized by good numbers of Bull Trout > 20 cm (Table 6). Juvenile Bull Trout were also relatively common in the upper two sites. The Misinchinka is known to be one of the most important Bull Trout streams of the Parsnip River watershed, based on annual redd surveys conducted in the upper watershed since 2006 (Hagen and Weber 2019). Rainbow Trout and especially Mountain Whitefish were common in all reconnaissance sections except for the 4-0 km section. Underwater visibility was inadequate for snorkeling surveys downstream of 54 km, which includes a 38-km long reach of low gradient, silty meanders.

Figure 13. Misinchinka River holding pool, located approximately 60 stream km from the mouth, where an adult Arctic Grayling was observed by snorkeling. This and one other holding pool at 56 km were the only locations where adult Arctic Grayling were observed in August 2022.

Figure 14. Reconnaissance snorkeling survey of Misinchinka River section 4-0 km.

No Arctic Grayling were observed in an opportunistic survey of the lowest 1 km of Wooyadilinka, conducted on August 16, 2022. Few salmonids > 20 cm were observed (Table 6), although juvenile Bull Trout, Rainbow Trout, and Mountain Whitefish were present.

5.4.2 Environmental DNA

Among the 20 eDNA samples collected in 2022, Arctic Grayling DNA was definitively detected at seven sites, potentially detected (unconfirmed low positive) at three sites, and not detected at 10 sites (Table 7; Appendix 1). The laboratory analyses found no signs of degradation (i.e., ubiquitous eDNA was present in all samples), no significant PCR inhibition, and no blanks or extraction controls amplified, which indicates proper safeguards successfully guarded against contamination in the field and laboratory (Appendix 1).

Sample sites were distributed among five Parsnip River tributaries[2] including 11 sites situated at the downstream end of snorkel sections, five sites situated between snorkel sections, and four sites not associated with snorkel observations being situated upstream of snorkel sections in Wooyadilinka Creek (n=2) and in Mischinsinlika Creek (n=2) which was not snorkeled (Table 7; Figure 4).

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[2] Anzac River and Table River eDNA results were opportunistic and resulted from collaboration with FWCP Project PEA-F23-F-3636.

Among the seven eDNA positives, Arctic Grayling adults were observed by snorkelers closer than 1.5 km upstream at six sites. Sampling at 58 km of the Misinchinka River, where the nearest upstream snorkel observation of an Arctic Grayling was 2.1 km upstream, failed to detect Arctic Grayling eDNA. These observations corroborate the previous observation that the presence of adult Arctic Grayling within 1.5 km is detectable by eDNA (Stamford et al. 2022).

The eDNA results for the Misinchinka River suggest that Arctic Grayling were potentially distributed as far upstream as 70 km in August 2022, based on positive results at 56 km, 60 km, and 68 km. Furthermore, Arctic Grayling distribution appears to be very limited and abundance low in the watershed, as evidenced by negative eDNA assays at the remainder of locations (unconfirmed low positive at 36 km). There may be a greater risk of underestimating the distribution of Arctic Grayling when juveniles are the life stage present and densities are low, however. The negative eDNA assay at the stream mouth was collected just a few hundred meters downstream of a location where two juvenile Arctic Grayling <20 cm (the only other Arctic Grayling observed by snorkeling) were observed just a few hours later. Having so few Arctic Grayling present in the lower reaches of the Misinchinka River appears to have resulted in a false negative eDNA result at site 0 km.

Previous juvenile Arctic Grayling-oriented sampling has had mixed results but also indicated marginal presence. Electrofishing by FWCP in 2005 (PWFWCP 2005 unpublished) failed to detect Arctic Grayling anywhere in the Misinchinka system, which raised doubt about whether Arctic Grayling continued to utilize the system post-flooding (Stamford et al. 2017). In contrast, juvenile-oriented electrofishing by Hawkshaw and Shrimpton (2014) captured Arctic Grayling in 2010 in a site located at approximately 16 km. Arctic Grayling eDNA was not detected at Misinchinka 16 km in August 2022 (Table 7).

The concentration of eDNA in samples is difficult to relate to fish density because the concentration in the water column is influenced by so many potential variables, including different hydrological and chemical conditions among sites, and vastly different rates in which DNA is sloughed among individual fish (Jane et al. 2015; Carim et al. 2016). More broadly, however, the trends among paired eDNA and snorkeling observations since 2019 suggest that false negative rates among eDNA samples increase as abundance decreases in snorkeling sections (Stamford et al. 2022). Similarly, the laboratory analyses of our 2022 samples found concordance in the number of amplified Arctic Grayling DNA copies and the abundance observed in snorkel surveys: highest DNA copies were amplified among Anzac and Table rivers samples while all positives in Misinchinka River required further testing to confirm detections (Appendix 1). Clearly, the false negative at 0 km in Misinchinka River failed to capture the rare Arctic Grayling DNA present in the water column. Three unconfirmed eDNA positives in 2022, located at 36 km in the Misinchinka River, 0 km in Wooyadilinka Creek, and 0 km in Mischinsinlika Creek (Table 7) may indicate sparsely distributed Arctic Grayling juveniles similar to the Misinchinka mouth, but confirmation of habitat use requires follow up sampling.

Juvenile Arctic Grayling were captured in the lower reaches of Wooyadilinka Creek, Mischinsinlika Creek, and Misinchinka River by Hawkshaw and Shrimpton (2014) in 2010.

Table 7. Distribution of eDNA sample sites relative to snorkeling sections and locations of Arctic Grayling observations in Parsnip River tributaries during August 2022.

Watershed	eDNA Sample	Site Location *	Snorkel Section	Snorkel GR Count	eDNA Result**	Nearest Upstream GR Observed***
Table River	Table 31	31.0	35-31 km	287	1	0
Table River	Table 22	22.0	26-22 km	304	1	0
Wooyadilinka Creek	Wood-0	0.0	1-0 km	0	Low?	na
Wooyadilinka Creek	Wood-54	1.0	na	na	0	na
Wooyadilinka Creek	Wood-57	6.0	na	na	0	na
Anzac River	Anzac 12	12.0	16-12 km	75	1	0
Anzac River	Anzac 45	45.0	47-45 km	213	1	0
Misinchinka River	Miss_0	0.0	4-0 km	2	0	0.15
Misinchinka River	Miss_4	4.0	na	na	0	53
Misinchinka River	Mis_16	16.0	na	na	0	41
Misinchinka River	Mis_36	36.0	na	na	Low?	21
Misinchinka River	Mis_56	56.0	60-56 km	1	1	1.09
Misinchinka River	Mis_58	58.0	60-56 km	1	0	2.08
Misinchinka River	Mis_60	60.0	64-60 km	1	1	0.15
Misinchinka River	Mis_64	64.0	na	na	0	na
Misinchinka River	Mis_68	68.0	na	na	1	na
Misinchinka River	Mis_75	75.0	79-75 km	0	0	na
Misinchinka River	Mis_82	82.0	86-82 km	0	0	na
Mischinsinlika Creek	MISCH-0	0.0	na	na	Low?	na
Mischinsinlika Creek	MISCH-U	17.0	na	na	0	na

* Kilometers upstream from stream mouth

** 1= Arctic Grayling Positive; 0=Arctic Grayling Negative; Low?=Unconfirmed Positive

*** Kilometers

Generally, among all 36 trials between 2019 and 2022 where eDNA sampling was paired with snorkeling observations in seven streams, the distributions of presence/absence data from the two methods are significantly associated (Chi Squared *P* [no association] = 0.01). Snorkeling observations agreed with eDNA detections of Arctic Grayling in 75% of trials (Table 8). As mentioned above, a key factor in the failure of eDNA to detect snorkeler-observed Arctic Grayling are proximities > 1.5 km. The noteworthy exception to this generalization was observed during this study at 0 km of the Misinchinka River, which is a testament to a remarkable efficiency for using single pass reconnaissance snorkeling to evaluate adult and even juvenile habitat uses, given that eDNA detection probability has been estimated as high as 99% (Wilcox et al. 2016). Among the five instances where eDNA detected Arctic Grayling presence and snorkeling failed, three occurred in Colbourne Creek in 2021 where Arctic Grayling presence has never been confirmed with other fish sampling efforts, and one each in Reynolds Creek (2021) and Wichcika Creek (2020) where adults were observed in other snorkeling sections (Hagen and Stamford 2021, 2022). These discrepancies may indicate these sites provide habitat for juveniles undetected by snorkeling. In particular, the recurrent eDNA detections in Colbourne Creek suggest the whole lower 18 km provides habitat for juvenile life stages of Arctic Grayling. Alternatively, eDNA in the water column originates from Arctic Grayling in unsampled tributaries. Follow up fish sampling is needed to distinguish between these alternatives.

Table 8. Contingency table comparing the distributions of eDNA detections and snorkeling observations among all paired sample events carried out between 2019 and 2022 in six Parsnip River tributaries and Ingenika River. Data include only those eDNA samples collected within snorkeling sections.

eDNA	Arctic Grayling Snorkel Survey		Total
	Observed	Not Observed	
Detected	20	5	25
Not Detected	4	7	11
Total	24	12	36

5.5 Basin-Wide Estimates of Arctic Grayling Abundance

Since the initiation of this five-year study in 2018, single-pass snorkeling surveys have been conducted in 42 reconnaissance sections distributed among 8 streams of the Parsnip River including: 1) Anzac River in 2018 (Hagen et al. 2018), 2) Missinka River in 2019 (Hagen and Gantner 2020), 3) Hominka River and Wichcika Creek in 2020 (Hagen and Stamford 2021), 4) Reynolds Creek and Colbourne Creek in 2021 (Hagen and Stamford 2022), and Misinchinka River and Wooyadilinka Creek in 2022 (Table 6). These sections can be organized into reaches of comparable Arctic Grayling density and sampling effort, and count data analyzed to estimate reach abundance within the Poisson-mixture model framework described in Section 4.4.3. This modeling framework assumes abundances N_{it} within sampling sections are Poisson-distributed independent random variables conditional on detection probability p and N_t, the mean and variance of abundance across all sites in the reach at time t.

The first step in developing population estimates from the reconnaissance data was to identify the best model for detection probability p. As described in Section 5.2, we were particularly interested in the potential effects of two variables on p: 1) mean site wetted width *WIDTH*, and 2) horizontal underwater visibility at which Arctic Grayling could be discriminated *FISH_VIS*. Within the Poisson-mixture model framework, we evaluated a candidate set of four logistic regression models for p by fitting them to the to 1995-2022 replicated count data from long-term index sections, then discriminated among them using AIC_c (Table 9). The model with the lowest AIC_c score contained both *WIDTH* and *FISH_VIS* as predictors, with the expected negative relationship for *WIDTH* but with a surprising negative relationship with *FISH_VIS*. This may have been an artefact of the lack of informative contrast in horizontal underwater visibility among sites (Table 5) and is non-logical. Therefore, we chose as the best model the *WIDTH* - only model, which still resulted in a significant improvement in model likelihood relative to the constant-*p* model (Chi-square P <0.001). The probability of this model being the best, as indicated by its Akaike weight w_i, was 0.40 which is not greatly lower relative to the 0.60 for the *WIDTH* + *FISH_VIS* model. The *WIDTH* -only logistic model for *p* was:

$$p = \frac{1}{1 + \exp\{-(2.510 - 0.1044 * WIDTH)\}}$$

We utilized this model to predict detection probability for 41 reconnaissance sections distributed among 13 reaches and for 4 long-term index sections in 2 reaches of the Table River (the Table River had not received reconnaissance snorkeling in previously-unsurveyed reaches[3]), then computed maximum-likelihood estimates of mean abundance (standardized as density) for each reach within the Poisson-mixture model framework (Table 10).

Table 9. Comparison among predictors of detection probability in index sections of the Parsnip River watershed, estimated within a Poisson-mixture model framework. Symbols K, Log (L), AIC_c, Δi, $L\ (g_i|x)$, *and* w_i, *denote 1) no. of estimable parameters, 2) model log-likelihoods, 3) the Akaike information criterion values adjusted for small sample size, 4) the difference in* AIC_c *values between each model and the model with the lowest* AIC_c *score, 5) the likelihood that the candidate model is the best among the set, and 6) Akaike weights, respectively.*

Model		K	Log($\mathcal{L}$)	AIC_c	Δ_i	$\mathcal{L}\ (g_i\|x)$	w_i
Constant-only		2	-731.61	1467.49	208.61	5.02559E-46	3.01E-46
WIDTH		3	-626.57	1259.69	0.80	0.669586703	0.40
VIS		3	-728.99	1464.53	205.64	2.21236E-45	1.33E-45
WIDTH+VIS		4	-624.98	1258.89	0.00	1	0.60
	$MinAIC_C$			1258.89			
	n	48					

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[3] The choice of which years' data to select for the Table River reaches was problematic. We chose average counts for the 2019-2021 period for the Upper Table reach represented by sections 26-22 km and 35-31 km, because reliable water temperature modeling data exist for this period facilitating future limiting factors analysis (O'Connor 2023). Snorkeling of Lower Table sections 9-5 km and 22-18 km has not occurred in the recent period, however (due to visibility problems downstream of the 22 km clay slump), so we chose average counts across the 1995-2007 period for this reach. Given the significant positive trend in abundance within long-term index sections, this choice for the Lower Table reach may have resulted in underestimation bias.

Table 10. Estimated August density and total abundance (N) of Arctic Grayling >20 cm in reaches of the Parsnip River watershed during the 2018-2022 period.

Stream	Reach	Length (km)	Year	No. Sites	Density/km	Density 95% LCL	Density 95% UCL	*N*	*N* 95% LCL	*N* 95% UCL
Anzac	Headwater	7.9	2018	3	21.3	16.1	27.6	169	127	218
Anzac	Upper	31.1	2018	7	93.7	85.8	102	2914	2667	3175
Anzac	Lower	16.6	2018	3	37.3	29.3	46.5	619	487	772
Missinka	Upper	11.4	2019	1	13.6	7.16	22.9	155	82	261
Missinka	Lower	25.0	2019	2	0.91	0.06	3.46	23	1	86
Hominka	Headwater	12.0	2020	3	0.28	0.05	0.86	3	1	10
Hominka	Upper	16.0	2020	3	14.6	10.3	19.9	234	165	318
Hominka	Lower	32.0	2020	2	2.40	0.42	6.39	77	14	205
Wichcika	Mainstem	27.5	2020	5	0.50	0.08	1.51	14	2	41
Reynolds	Mainstem	36.0	2021	5	0.59	0.12	1.64	21	4	59
Colbourne	Mainstem	17.0	2021	2	0.00	0.00	0.34	0	0	6
Misinchinka	Upper	16.0	2022	2	0.00	0.00	0.40	0	0	6
Misinchinka	Lower	70.0	2022	3	0.28	0.05	0.85	19	3	59
Table	Upper*	15.6	2019-2021	2	69.9	57.8	83.4	1090	902	1301
Table	Lower**	22.0	1998-2007	2	13.6	8.64	20.0	299	190	441

* Table River did not receive a reconnaissance survey; upper reach estimates are based on long-term index site data from 2019-2021.
**Table River did not receive a reconnaissance survey; lower reach estimates are based on long-term index site data from 1998-2007.

Among 15 reaches utilized by adult/subadult Arctic Grayling in the Parsnip River watershed, estimated abundance levels range over a remarkable 4 orders of magnitude (Table 10). These estimates confirm earlier conclusions (e.g., Hagen and Stamford 2021, 2022) about the importance of the Anzac and Table rivers as a hub of Arctic Grayling abundance within the Parsnip core area, with the two streams accounting for 87% of the roughly 5,600 adult/subadult individuals present in the core area. Within these two systems, the upper reaches are of especially high importance.

Among other tributary reaches in the Parsnip River watershed, only the Hominka and Missinka have substantial numbers of summer rearing adult/subadult Arctic Grayling (Table 10). Small streams Wichcika Creek, Reynolds Creek, and Colbourne Creek have Arctic Grayling but at very low adult densities (detectable only by eDNA in the case of Colbourne Creek; Hagen and Stamford 2022). In the case of the Misinchinka River, the mouth of which lies closest to the footprint impact of Williston Reservoir, Arctic Grayling are present at barely-detectable levels despite more than 100 km of accessible length.

5.6 Critical Habitats

For Arctic Grayling to be viable within a core area, critical habitats must be present in good ecological condition and provide diverse habitats that support key life history stages. On the basis of new abundance and habitat use data collected since the FWCP Arctic Grayling Synthesis Report of 2017 (Stamford et al. 2017), we have been able to improve our understanding of critical habitat locations within the Parsnip core area.

In 2022, we delineated a total of 24 stream segments as critical habitats for adult and/or juvenile Arctic Grayling life stages (Table 11; Figure 15). A total of 11 ‘Adult’ segments were identified providing rearing habitat for Arctic Grayling > 20 cm, while 13 'Juvenile' segments were identified providing habitat for juvenile rearing, overwintering (all life stages), and spawning (Table 11; Figure 15). Within the Parsnip River watershed, adult and juvenile segments frequently overlap, although adult summer rearing habitats as a rule are distributed further upstream. In the Table and Anzac Rivers, the distribution of critical adult rearing habitat has been broken into several segments reflecting observed patterns of habitat use and abundance (Table 11; Figure 15). This was done to facilitate a finer scale for future prioritization and assessments given the importance of these two watersheds.

Among the 11 critical adult rearing segments, 4 were assigned Priority 1 status identifying them as the highest priority for significant investments in habitat conservation and enhancement (Table 11). These segments comprise the majority of the adult summer rearing distribution of the Anzac and Table rivers and account for an estimated 87% of August total adult abundance within the Parsnip core area (Table 10). A total of 6 segments were assigned Priority 2 status identifying them as candidates for conservation and enhancement actions, although perhaps at lower levels of investment in some cases. A single segment located along the Misinchinka River mainstem appears to be marginal relative to adult summer rearing elsewhere and was assigned Priority 3 status. More information is probably required before Arctic Grayling-focused conservation and enhancement actions can be rationalized in Priority 3 segments.

Table 11. Critical habitats delineated for Arctic Grayling in stream reaches of the Parsnip core area. Sampling methods EF, SN, SW, AN, TY, and eDNA refer to electrofishing, seine netting, swim counts, angling, telemetry, and eDNA, respectively.

ID	Gazetted name	Reach	UTM bottom; UTM top	Life stage	Sampling methods	Conservation priority	Key reference(s)
A1	Parsnip River	Mainstem	10 U 507156 6102960 10 U 573748 6038729	Adult	SN, EF, TY	2	Blackman 2002; Martins et al. 2020, 2022
	Critical habitat comments : Adult overwintering, migration, and spawning occurs in Parsnip mainstem and side channels from Colbourne Creek to above Missinka; spawning habitat is encompassed by juvenile segments; key habitat conservation action may be managing basin-wide cumulative hydrological effects						
J2	Parsnip River	Mainstem	10 U 496205 6113471 10 U 573748 6038729	Juvenile	SN, EF	2	Anonymous 1978; Murphy and Blackman 2012; Mackay and Blackman 2012; Hawkshaw and Shrimpton 2014; Martins et al. 2020, 2022
	Critical habitat comments : Parsnip River mainstem from reservoir to above Missinka R; delineated based on extensive juvenile sampling especially below Table R and springtime telemetry ranges of adults from FWCP spatial ecology study 2018-2021; YOY fry and post-YOY juveniles co-occur in some sites						
A3	Misinchinka River	Mainstem	10 U 520763 6126366 10 U 528373 6130453	Adult	SW, eDNA	3	Langston and Blackman 1993; Stamford et al. 2017; this study
	Critical habitat comments : Moderate gradient section upstream of 38-km reach of low-gradient meanders; 2022 snorkeling found adult presence in Misinchinka mainstem at extreme low density; overall density estimate for Misinchinka mainstem below 70 km = 0.28 fish/km						
J4	Misinchinka River	Mainstem	10 U 502675 6106277 10 U 511225 6109991	Juvenile	EF, SW, eDNA	3	PWFWCP 2005 unpublished (Parsnip GR fry survey); Langston and Blackman 1993; Hawkshaw and Shrimpton 2014; Stamford et al. 2017; this study
	Critical habitat comments : Juveniles present at top of this reach, located below extensive section of low gradient meanders, in 2010 but negative eDNA at this location and at mouth in 2022; juveniles not found higher in system despite significant effort in 2005						
J5	Colbourne Creek	Mainstem	10 U 507156 6102960 10 U 517455 6099846	Juvenile	EF, SW, eDNA	3	PWFWCP 2005 unpublished (Parsnip GR fry survey); Hawkshaw and Shrimpton 2014; Hagen and Stamford 2022
	Critical habitat comments : Although 8 km of snorkeling in 2021 did not detect any adult grayling, GR eDNA consistently detected at five locations distributed throughout the snorkel reach including a tributary at the top boundary; juvenile-oriented sampling in 2005 and 2010 was negative, however, suggesting follow-up is necessary						
A6	Reynolds Creek	Mainstem	10 U 519970 6085783 10 U 532086 6101786	Adult	SW, eDNA	2	PWFWCP 2005 unpublished (Parsnip GR fry survey); Hawkshaw and Shrimpton 2014; Hagen and Stamford 2021, 2022
	Critical habitat comments : Adult GR observed near top of critical habitat in both 2020 and 2021, but at very low density; adults and YOY juveniles also present in lower end; GR eDNA detected in between but not by snorkelling, suggesting extreme low densities or juvenile life stage; overall segment density estimate = 0.59 fish/km;						
J7	Reynolds Creek	Mainstem	10 U 525931 6092203 10 U 532086 6101786	Juvenile	EF, SW, eDNA	2	PWFWCP 2005 unpublished (Parsnip GR fry survey); Hawkshaw and Shrimpton 2014; Hagen and Stamford 2021, 2022
	Critical habitat comments : Presence indicated from substantial electrofishing effort in 2005 (7 sites) and 2010 and visual observations of fry in 2021; 2021 eDNA also consistently positive even in sites where snorkeling did not detect adults; Chuyazega Creek also positive eDNA requiring follow-up						
J8	Firth Creek	Mainstem	10 U 525792 6076619 10 U 524399 6076112	Juvenile	EF	2	PWFWCP 2005 unpublished (Parsnip GR fry survey); Stamford et al. 2017
	Critical habitat comments : Seven grayling records including juveniles 68-90mm collected and far enough upstream (2.6 km) to indicate spawning occurred in stream; lake headed tributary and warm summer temperatures predicted (O'Connor 2023)						

Table 11, continued.

ID	Gazetted name	Reach	UTM bottom; UTM top	Life stage	Sampling methods	Conservation priority	Key reference(s)
A9	Anzac River	Headwater	10 U 552245 6092871 10 U 557591 6093397	Adult	SW	1	Hagen et al. 2019
	Critical habitat comments : Use by adult GR documented during reconnaissance swims in 2018, a year of low stream flow; modeled GR density estimate (estimated in this report) of 21.4 fish/km during reconnaissance survey in 2018						
A10	Anzac River	Upper	10 U 537238 6078204 10 U 552245 6092871	Adult	SW	1	Blackman and Hunter 2001; Blackman 2004; Cowie and Blackman 2012; Hagen et al. 2019
	Critical habitat comments : Highest estimated adult GR density to date anywhere in the Williston Reservoir watershed: 93.7 fish/km (estimated in this report) during reconnaissance swims in 2018						
A11	Anzac River	Lower	10 U 552245 6092871 10 U 526238 6075638	Adult	SW	1	Blackman and Hunter 2001; Blackman 2004; Cowie and Blackman 2012; Hagen et al. 2019; Hagen and Stamford 2023
	Critical habitat comments : Reduced adult GR density relative to upper Anzac beginning at 16 km, potentially associated with increased braiding and reduced gradient; 37.3 fish/km (etimated in this report) during reconnaissance swims in 2018						
J12	Anzac River	Mainstem	10 U 552245 6092871 10 U 550698 6090074	Juvenile	SN, EF, eDNA	1	Blackman and Hunter 2001; Blackman 2004; Cowie and Blackman 2012; Hawkshaw and Shrimpton 2014;
	Critical habitat comments : Post-YOY juveniles much more rare than YOY fry, suggesting importance of mainstem Parsnip for post-YOY; fry also present in lower reaches of tributaries including North Anzac and Crocker Creek (Hawkshaw and Shrimpton 2014); follow-up surveys of tributary use warranted						
J13	Bill's Creek	Mainstem	10 U 540561 6062762 10 U 540158 6061619	Juvenile	EF	2	Anonymous 1978; PWFWCP 2005 unpublished (Parsnip GR fry survey)
	Critical habitat comments : Only one fry collected in 2005 but far enough upstream (1.7 km) to indicate spawning may have occurred in stream; no adult sampling effort (e.g. AN) found; numerous YOY fry oberved (10 U 539146 6059930) rearing near crossing in 2021 (M. Stamford personal observation)						
A14	Table River	Upper	10 U 558559 6067656 10 U 567666 6073730	Adult	SW	1	Cowie and Blackman 2012; Stamford et al. 2017; this study
	Critical habitat comments : Upper Table River mainstem from GR migration barrier at 37.6 km to clay slump at 22 km; second-highest estimated adult GR density in the Parsnip watershed: 69.9 fish/km (estimated in this report) based on observed densities in 2 long-term index sites						
A15	Table River	Lower	10 U 545547 6061836 10 U 558559 6067656	Adult	SW	1	Cowie and Blackman 2012; Stamford et al. 2017; this study
	Critical habitat comments : Lower Table River mainstem from clay slump at 22 km to mouth; Clay slump at 22 km has not allowed snorkeling observations in lower Table River since 2007: follow-up surveys highly advised; 13.6 fish/km (estimated in this report) based on pre-2007 densities in 2 long-term index sites						
J16	Table River	Mainstem	10 U 545547 6061836 10 U 560114 6070554	Juvenile	EF, SN	1	Mathias et al. 1998; Zemlak and Langston 1998; Blackman and Hunter 2001; Hawkshaw and Shrimpton 2014
	Critical habitat comments : Lower 26 km of Table River mainstem; delineated based on relatively extensive PWFWCP sampling; post-YOY juveniles relatively rare in watershed but encountered in lower portion of segment						
A17	Hominka River	Upper	10 U 573671 6061485 10 U 581191 6069185	Adult	SW, eDNA, TY	2	PWFWCP unpublished 2005; Hagen and Stamford 2021
	Critical habitat comments : Snorkeling observations suggest decent densities of fairly large adult grayling over 16 km of river channel; 14.6 adult GR/km estimated in this report vs. 2.4 fish/km in lower Hominka; 2005 sampling captured two adults only by AN						

Table 11, continued.

ID	Gazetted name	Reach	UTM bottom; UTM top	Life stage	Sampling methods	Conservation priority	Key reference(s)
J18	Hominka River	Mainstem	10 U 558750 6054109 10 U 572137 6061263	Juvenile	EF	3	PWFWCP 2005 unpublished (Parsnip GR fry survey); Hawkshaw and Shrimpton 2014
	Critical habitat comments : Low-gradient, meadery section of lower Hominka River; only one fry collected in 2005 but far enough upstream (>20 km) to indicate spawning occurred in stream; significant sampling effort suggests low abundance						
A19	Missinka River	Mainstem	10 U 577380 6050157 10 U 583975 6053680	Adult	SW, AN, TY	2	Triton 1999; Martins et al. 2020, 2022; O'Connor 2023; Hagen and Gantner 2020
	Critical habitat comments : Missinka River mainstem between a chute at 36.4 km to top of low-gradient meanders at 25 km; 13.6 fish/km (estimated in this report) during 2019 reconnaissance vs. 0.91 fish/km in lower river suggest low gradient meanders are not a preferred summer rearing option						
J20	Missinka River	Mainstem	10 U 561781 6048217 10 U 583229 6052577	Juvenile	EF	2	Triton 1999; Hawkshaw and Shrimpton 2014; Stamford et al. 2017
	Critical habitat comments : Many fry collected well upstream from mouth; indicates spawning in stream						
A21	Wichcika Creek	Mainstem	10 U 561783 6048207 10 U 564032 6037426	Adult	SW, eDNA	2	Hagen and Stamford 2021
	Critical habitat comments : Very low adult GR densities: 0.50 fish/km during 2020 reconnaissance (estimated in this report), but detected in most sites in lower 28 km of Wichcika Creek						
J22	Wichcika Creek	Mainstem	10 U 561783 6048207 10 U 555396 6044115	Juvenile	EF, eDNA	2	Anonymous 1978; PWFWCP 2005 unpublished; Hawkshaw and Shrimpton 2014
	Critical habitat comments : Lower 12 km of Wichcika Creek based on 2005 Parsnip GR fry study (PWFWCP unpublished 2005); 2020 eDNA sampling around 7 km (where no adults observed upstream) corroborates fry study and suggest juvenile rearing areas in lower river						
J23	Wooyadilinka Creek	Mainstem	10 U 536695 6064553 10 U 540595 6069125	Juvenile	EF, eDNA, SW	3	Hawkshaw and Shrimpton 2014; this study
	Critical habitat comments : Segment is based on presence of GR fry during 2010 EF survey; eDNA at 3 locations (including mouth) plus snorkel survey of lower 1 km failed to detect GR presence in 2022 (this study)						
J24	Mischinsinlika Creek	Mainstem	10 U 495525 6115371 10 U 504042 6122787	Juvenile	EF, eDNA	3	Hawkshaw and Shrimpton 2014; this study
	Critical habitat comments : Segment is based on presence of GR fry during 2010 EF survey; eDNA at 2 locations (including mouth) failed to detect GR presence in 2022						

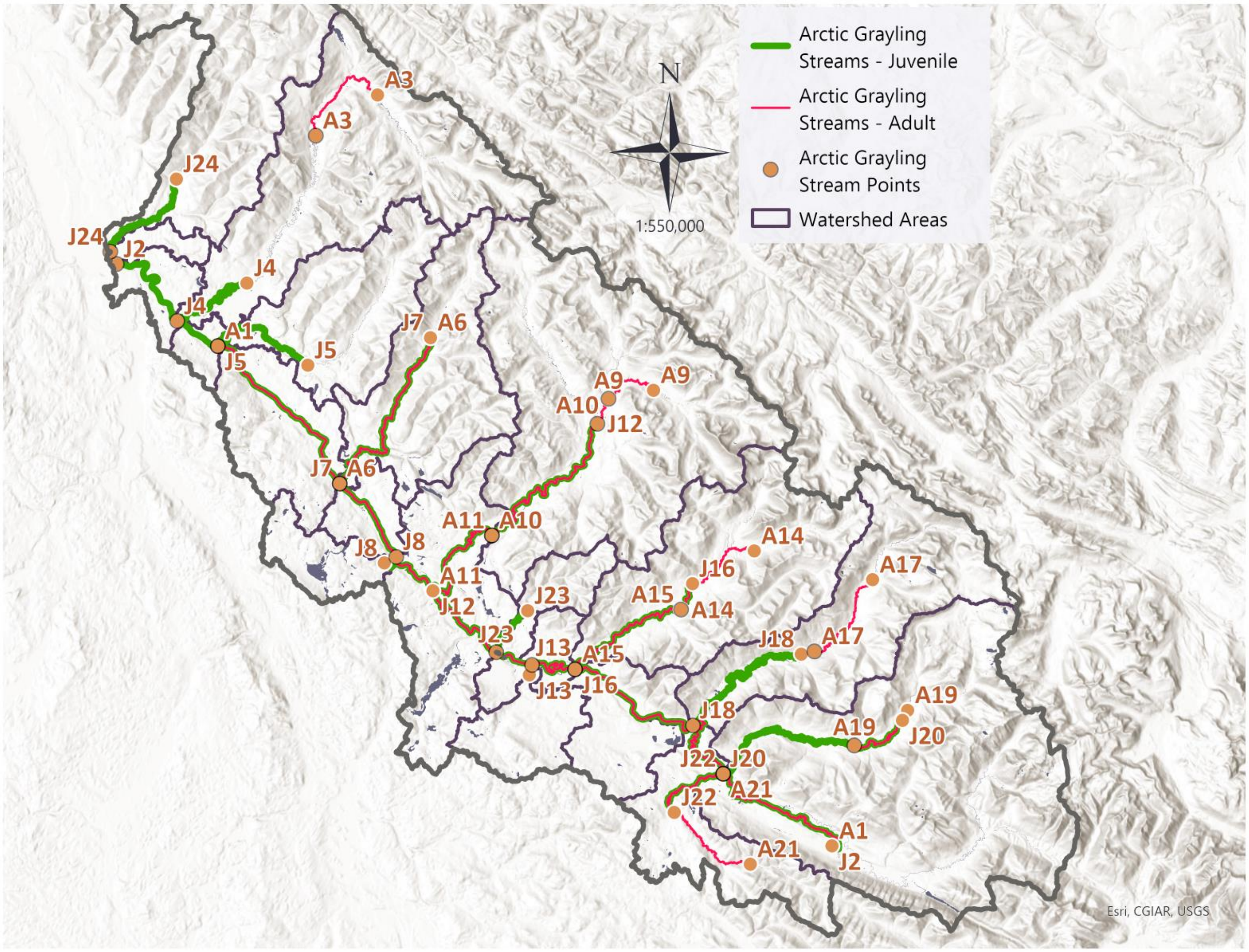

Figure 15. Critical stream habitats for Arctic Grayling in the Parsnip River watershed. Labeled stream points are endpoints for critical adult (thin red segments) and juvenile (thick green segments) habitat segments delineated in Table 11.

Among 13 juvenile segments, 2 were assigned Priority 1 status (Table 11). These are relatively well-studied mainstem reaches of the Anzac and Table rivers which encompass the known YOY juvenile distribution. A total of 6 segments were assigned Priority 2 status, implying we are relatively confident in the potential for these segments to provide important juvenile rearing (and/or spawning and/or overwintering) habitat within the Parsnip core area. A total of 5 segments were assigned Priority 3 status because the juvenile distribution has not been verified (e.g., positive eDNA results in lower Colbourne Creek have not been linked to a life stage), or past sampling efforts have captured very few juvenile Arctic Grayling (or no grayling in some years) implying low densities.

The 2022 critical habitat update has been delivered to study collaborators BC Ministry of Forests (MOF) and Ministry of Water, Land, and Resource Stewardship (WLRS), where it will be used to create a GIS spatial layer to be shared within MOF, WLRS, McLeod Lake Indian Band and other interested parties as a mutually agreed outreach product of this study. Attributes of watershed contributing areas, including potential limiting factors and threats, will be summarized in table form and linked to the critical habitat segments.

5.7 Other Species

In our study, Arctic Grayling were the highest priority for snorkeling observations and our focus for analyses of abundance and trend. However, Bull Trout, Rainbow Trout, and Mountain Whitefish were also counted simultaneously in long-term index sections of the Anzac and Table rivers (Table 12, Figure 16, Figure 17, Figure 18).

Bull Trout. Bull Trout counts in index sites are highly variable among years (Table 12, Figure 16). It has been previously noted that this may potentially indicate an effect of stream conditions on pre-spawning migration and staging behaviour. For example, counts of Bull Trout in 2018 were above long-term averages at most index sites, but this may be an artefact of record low water conditions reducing the suitability of spawning tributaries for staging prior to spawning (Hagen et al. 2019). A more reliable methodology for monitoring Bull Trout abundance in the Parsnip River watershed is through counts of gravel nests, or ‘redds’ following the completion of spawning (Hagen et al. 2015). However, Bull Trout spawner abundance monitoring using redd counts occurs only infrequently in the Table and Anzac rivers, which contain some of the most important critical habitat for the species within the Parsnip River watershed (Hagen et al. 2015; Hagen and Weber 2019). Although imprecise, Bull Trout snorkeling count data may be of increasing value in future if redd count surveys are not conducted to monitor the effects of increased forestry and pipeline development in the Table and Anzac systems on this sensitive species. Therefore, Bull Trout counts should remain a priority during snorkeling surveys. Their abundance is generally low, and reliable counts can be made without sacrificing counts of Arctic Grayling, our target species. Reliable snorkeling counts of Bull Trout in August also indicate vulnerable pre-spawning aggregations of fish. Knowledge of these staging areas can be incorporated during the planning of Bull Trout habitat conservation.

Rainbow Trout. Snorkeling counts of Rainbow Trout (Table 12, Figure 17) are also highly variable among years. The time series of snorkeling count data indicates that Rainbow Trout have rarely been abundant at index sites, but the 2021 and 2022 counts are relatively high in index sites of the Table River (especially Table 26-22 km) and in the lowest Anzac River site (Figure 17). Rainbow Trout counts are of interest because of potential interspecific competition with Arctic Grayling and Bull Trout, with Rainbow Trout expected to become increasingly more prevalent as systems warm (Parkinson and Haas 1996; Parkinson et al. 2012; Hawkshaw and Shrimpton 2014). Currently, Arctic Grayling are numerically dominant over Rainbow Trout throughout most of the Table and Anzac long-term index sections, and there is not obvious evidence for a fish community shift at this point in time.

Mountain Whitefish. Counts of Mountain Whitefish (Table 12, Figure 18) are especially high and variable. Mountain Whitefish are far too numerous to count reliably and for this reason have always been assigned the lowest priority during our snorkeling surveys. Therefore, Mountain Whitefish counts should be considered of low precision and accuracy relative to the other three species. This prioritization was obviously in place during previous surveys also: Mountain Whitefish counts for 2005 are missing altogether. Given their imprecise nature, Mountain Whitefish counts may not be a good indicator of conservation status for the species. However, high abundance of the species currently in the mountainous Parsnip River watershed do not currently indicate cause for conservation concern. Despite the challenge in acquiring them, Mountain Whitefish counts are important as potential indicators of watershed health, changing ecological conditions, and levels of interspecific competition with Arctic Grayling.

Table 12. Unadjusted mean counts of Arctic Grayling, Bull Trout, Rainbow Trout, and Mountain Whitefish in long-term index sections of the Table and Anzac rivers, 1995-2022.

		Anzac River Sites				Table River Sites		
Year	Species	Anzac 16-12	Anzac 34-30	Anzac 43-39	Anzac 47-45	Table 22-18	Table 26-22*	Table 35-31
1995	GR							111
	BT							20
	RB							12
	MW							
1998	GR	8	106	136	164	67		136
	BT	10	13	17	29	17		127
	RB	42	37	6	5	69		83
	MW	1	8	426	170	105		894
2000	GR				68	37		123
	BT				16	6		30
	RB				8	30		11
	MW				217	82		636
2001	GR	11	52	85	20	42		91
	BT	5	10	7	1	1		3
	RB	10	11	5	3	10		10
	MW	458	1272	700	161	315		991
2003	GR	23	54	166	78	70		137
	BT	18	6	60	8	12		28
	RB	29	7	6	4	18		19
	MW	340	641	277	333	320		1341
2005	GR	29	79	91				98
	BT	20	12	19				8
	RB	14	3	5				4
	MW							
2007	GR	45	61			45		113
	BT	20	16			14		21
	RB	29	8			18		15
	MW	600	616			394		1415
2018	GR	22	114	182	194		76	210
	BT	6	42	89	76		14	75
	RB	9	25	7	8		69	12
	MW	458	692	433	705		711	730
2019	GR	40	72	152	91		116	188
	BT	3	5	27	11		10	30
	RB	27	9	6	13		46	17
	MW	821	522	1111	383		1160	1246
2020	GR						67	152
	BT						3	7
	RB						7	21
	MW						516	1128
2021	GR	50	152	132	146		171	228
	BT	25	45	22	14		38	50
	RB	51	20	9	16		83	58
	MW	1021	1962	1438	645		1109	1404
2022	GR	57	179	178	208		255	267
	BT	33	30	66	50		26	69
	RB	40	44	11	13		146	34
	MW	1596	1850	1395	678		1673	1179

*replacement for Table River section 22-18 beginning in 2018.

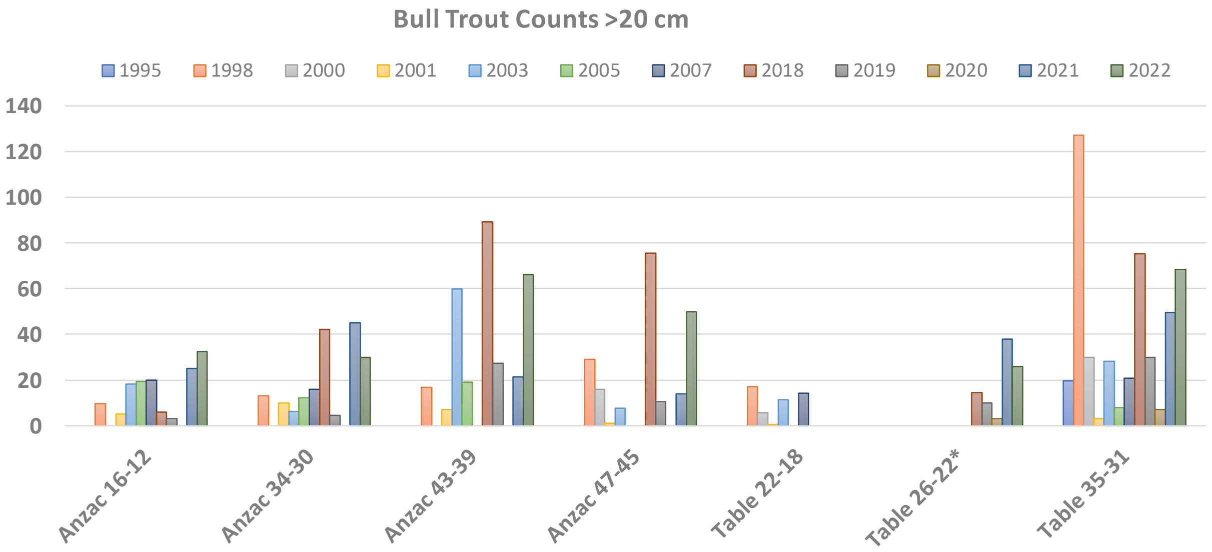

*Figure 16. Counts of Bull Trout >20 cm in sections of the Anzac and Table River watersheds, 1995-2022. *Table 26-22 is a replacement for Table 22-18 beginning in 2018.*

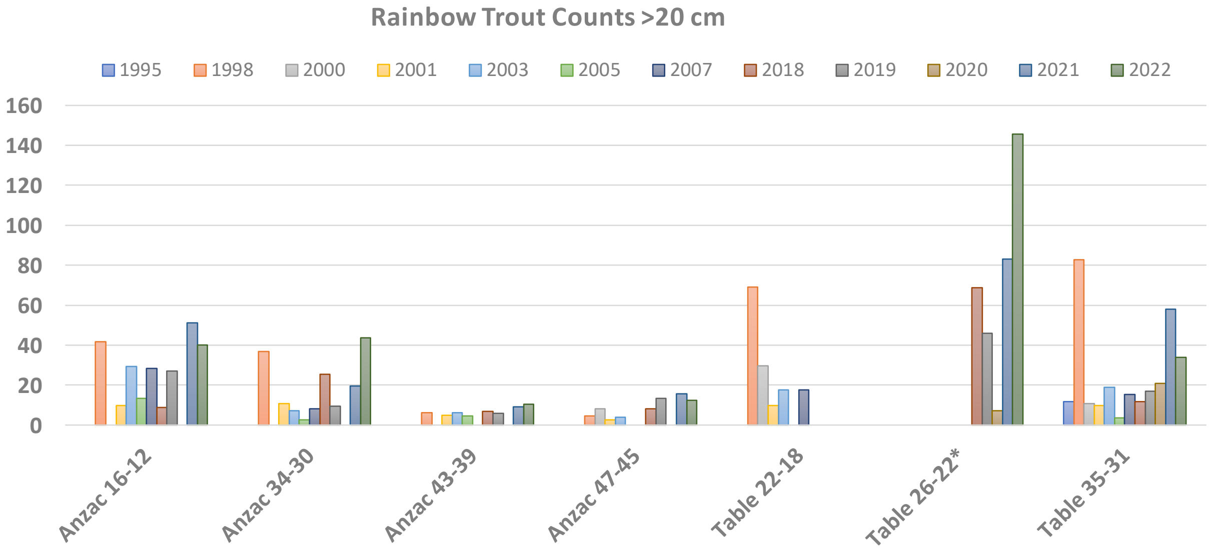

*Figure 17. Counts of Rainbow Trout >20 cm in sections of the Anzac and Table River watersheds, 1995-2022. *Table 26-22 is a replacement for Table 22-18 beginning in 2018.*

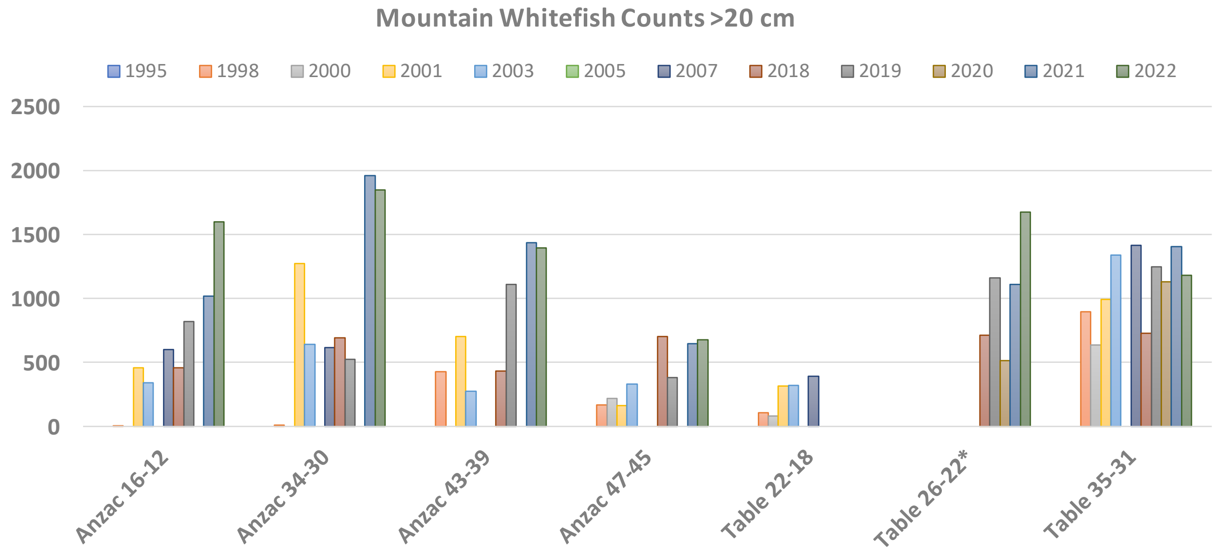

*Figure 18. Counts of Mountain Whitefish >20 cm in sections of the Anzac and Table River watersheds, 1995-2022. *Table 26-22 is a replacement for Table 22-18 beginning in 2018.*

6 DISCUSSION

6.1 Arctic Grayling Conservation Status

6.1.1 Defining Conservation Status.

Following the 2021 field season, we discussed key information needs and conservation actions necessary for effective stewardship of Parsnip Arctic Grayling (Hagen and Stamford 2022). The 'conservation status' for a group of organisms (e.g., an Arctic Grayling core area) indicates whether the group still exists and if so, how likely the group is to become extinct in the near future. Up-to-date information about conservation status is necessary to identify stewardship actions and to evaluate their effectiveness, so this was one of the key information needs identified for Parsnip Arctic Grayling.

The lack of any population data since 2007 with which to evaluate the status of the Parsnip Arctic Grayling core area was a major motivator for this study (Section 1). The compilation of abundance and distribution data over the five-year study period has enables a significant improvement to the conservation status assessment for the core area, which was last conducted in 2017 (Stamford et al. 2017).

6.1.2 Conservation Status Indicators

In the past, we have utilized an indicator-based approach (e.g., McElhany et al. 2000; IUCN 2012; USFWS 2005) for assessing conservation status of Arctic Grayling conservation units (core areas). Adult abundance and trend are the two most important indicators of conservation status (McElhany et al. 2000; O'Grady et al. 2004; USFWS 2010). Extirpation risks posed by demographic stochasticity, inbreeding depression, long-term genetic losses and genetic drift are magnified greatly at very small adult population sizes (Franklin 1980; Nunney and Campbell 1993). Caughley (1994) has suggested that population growth rate, or trend, should be considered an even more important indicator of population viability. Unless the external factors driving negative population growth in the first place (often overharvest and habitat destruction in salmonid populations) can be identified and corrected, extirpation may be a likely outcome.

The distribution and diversity of a group of organisms are also indicators of its conservation status (McElhany et al. 2000; IUCN 2012). Widely distributed groups of organisms consisting of multiple, connected sub-populations are generally more resilient to environmental stochasticity (variability in environmental conditions) and other forces driving population declines, because different genetically-based life history traits often associated with different habitat types are affected differently by environmental changes (Simberloff 1988; McElhany et al. 2000; IUCN 2012). Consequently, adult abundance can remain high among migratory metapopulations if differential levels of survival among natal streams compensate the response to changing seasonal patterns (e.g., Hilbourne et al. 2003).

Threats are limiting factors that are anthropogenic in nature and known to be capable of driving population declines and extirpation. Threats are also an important indicator of conservation status. For example, increased water temperature beyond well-known thresholds is a reliable indicator of reduced viability for populations of cold water-adapted Bull Trout (Parkinson and Haas 1996; Parkinson et al. 2012).

6.1.3 Conservation Status and Risk for Parsnip Arctic Grayling

The indicator-based method used to assess the status of Williston Arctic Grayling populations in 2017 (Stamford et al. 2017) is the *Core Area Conservation Status and Risk Assessment* method originally developed by the US Fish and Wildlife Service (USFS 2005) for Bull Trout, which has been applied throughout the range of that species (Rodtka 2009; Hagen and Decker 2011; USFWS 2015). Conservation status and risk rankings are based on categorical estimates for four indicator classes: 1) distribution 2) abundance (total number of adult individuals including non-reproducing individuals), 3) trend, and 4) threats. In order to compute the rankings, alphabetical scores corresponding to categorical estimates for each indicator are converted to numerical values with positive or negative signs. The numerical values are summed across categories and added to a baseline value (USFWS 2005). The resulting total is then compared to the range of values corresponding to each of four conservation status/risk ranks (C-ranks) to assign a rank to the core area. The C-ranks are *C1-High Risk*, *C2-At Risk*, *C3-Potential Risk*, and *C4-Low Risk*.[4] Details of how the computations are made are presented in Stamford et al. (2017). In applying the method to Arctic Grayling populations, we assume that small population sizes, negative trends, limited distribution, and elevated threats act equivalently on extirpation risk for the two

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[4] Core area status and risk is described by 4 C-ranks, rather than NatureServe's 5 subnational S-ranks, with the conversion between them being: C1-High Risk=S1, C2-At Risk=S2, C3-Potential Risk=S3, and C4-Low Risk=S4 or S5.

species (Stamford et al. 2017). The important attribute of the method is that it can be applied in a consistent, repeatable manner, providing a basis for monitoring changed status even if the accuracy of the risk rankings has not been evaluated specifically for Arctic Grayling.

As introduced in Section 1, acquiring reliable data about abundance, trend, distribution, and threats at spatial scales relevant to human activities has been a challenge for scientists. For instance, the typical focus on a limited number of index sites within a stream network may fail to identify key landscape attributes, both natural and human-caused, affecting the distribution and abundance of organisms (Schlosser 1991; Fausch et al. 2002; Lowe 2002; Jacobs et al. 2021). In this study, we have addressed this challenge by dividing our snorkeling effort into monitoring at a restricted spatial scale but long temporal scale in long-term index sections, and also watershed-scale monitoring using the more rapid method of single-pass snorkeling surveys. The former addresses the need for trend information (Figure 11), and the latter addresses the need for total abundance and distribution information (Table 10). Distribution information in turn can be utilized to indicate threats. Critical habitats delineated as part of this study are for the most part located adjacent to an expanding road network. As described in Section 1, land use activities potentially threatening Arctic Grayling include forestry, pipelines, and road building, by means of increased sediment transport, greater stream flow variation, water temperature changes, and increased accessibility for anglers.

Categorical estimates for conservation status indicators abundance, trend, and distribution can be derived directly from the Section 5 results. Total abundance of Arctic Grayling > 20 cm, estimated as the sum of the estimates of N among reaches (Table 10), was roughly 5,600 for the entire Parsnip core area. A conservative estimate would be that more than half of these are mature adults, given that most observed Arctic Grayling have been greater than 30 cm (Ballard and Shrimpton 2009). Trend over the past 25 years suggests positive population growth of approximately 98% in the size of the Arctic Grayling population (Section 5.3). The total distribution of Arctic Grayling in the Parsnip River watershed, extrapolated from Table 10 and Table 11, is roughly 500 km.

Threats (Section 6.2) to remaining Arctic Grayling in the Parsnip core area (i.e., disregarding past losses in reaches flooded by Williston Reservoir) cannot be directly estimated from our study data. However, road density has long been known to be a good general indicator of the cumulative effects on natural ecosystems associated with land use and human access (Eaglin and Hubert 1993; Forman and Alexander 1998; Baxter et al. 1999; Trombulak and Frissel 2000). In this study, we used road density data and criteria currently in use by Omineca Region MOF for aquatic ecosystems for assigning categorical estimates of threats severity:

Low: <1.2 km/km^2
Moderate: 1.2-2.1 km/km^2
High: >2.1 km/km^2

Based on these criteria and road density data provided by collaborators MOF and WLRS, the most appropriate characterization of the anthropogenic threat to Arctic Grayling in the Parsnip core area was: "Moderate-to-high severity threat for low proportion of population, occurrences, or area" (category E; USFWS 2005).

The estimates for the four conservation status indicators, when factored together using the USFWS (2005) *Core Area Conservation Status and Risk Assessment* method, corresponded to a ranking of *C3-Potential Risk*. According to this ranking, Arctic Grayling of the Parsnip core area are "potentially at risk because of limited and/or declining numbers, range, and/or habitat even though the species may be locally abundant in some areas of the core area" (USFWS 2005). The most influential indicators in the scoring process were the relatively limited distribution and level of threats mitigated by the positive population growth. It should be noted that population data acquired during our five-year study has resulted in a changed assessment of conservation status, from the more severe *C2-At Risk ranking* presented in Stamford et al. (2017). The most important reason for the change in ranking was the greatly improved population data since 2007, which resulted in a higher estimate of Arctic Grayling abundance and a positive estimate of trend.

6.2 Threats and Limiting Factors

6.2.1 Threats (Anthropogenic)

Improved conservation status of Arctic Grayling in the Parsnip core area suggests on the surface that human practices in the watershed since 1995 have been sustainable. However, the potentially complex interaction of various limiting factors is not well understood. Factors driving the population increase are not known, nor are the effects of current, rapid increases in human land use activities, given that there is likely a lag of several (perhaps many) years before degraded rearing environments result in reduced numbers of adult Arctic Grayling. Improving understanding of limiting factors and threats should be a top priority objective in the coming years.

On the one hand positive and substantial population growth suggests that catch-and-release recreational fisheries and First Nations subsistence fisheries have been sustainable over the 1995-2022 period. In fact, the existence of a reliable monitoring framework along with relatively secure conservation status indicate conditions where potential opportunities for increased human use of fish (i.e., permitting a recreational harvest) should be evaluated (e.g., in a management experiment). However, the introduction of catch-and-release regulations in 1995 did not immediately result in obvious population growth (Figure 11) and other factors may also be operating. Relatively common observations of homing behaviour including to adult rearing areas and natal areas suggest that Arctic Grayling adapt their migratory behaviour to improve survival in their local watersheds (e.g., Kaya and Jeanes 1995; Buzby and Deegan 2000, 2004; Haugen and Vøllestad 2000, 2001; Gryska 2019; Martins et al. 2022). Rapid change in stream habitats following flooding had obvious negative effects on Arctic Grayling in direct tributaries to the reservoir where many populations were extirpated (Stamford et al. 2017), and negative effects may also have extended to populations that managed to survive flooding. Considering we identified 13 distinct juvenile critical habitat segments (Table 11) these probably also include differently adapted life history traits and it is entirely plausible that certain genotypes have emerged or proliferated that are better able to avoid the new risks and take advantage of the changed, post-flooding watershed conditions.

On the other hand, rapid increases in industrial activity (and associated angler access) in the Parsnip River watershed, in the forms of increased forestry and pipeline initiatives, are potential threats to Arctic Grayling population viability as described in Section 1. In August 2020, which came at the end of a wet summer, peak flow conditions and elevated sediment levels (Figure 19) resulted in our inability to survey any sites at all in the Anzac River. While monitoring the situation, we learned that stream visibility in the mainstem dropped rapidly below suitable levels after the stream entered the industrialized zone of the watershed, where simultaneous, intensive pipeline and forestry road construction was occurring (Hagen and Stamford 2021). As cumulative effects mount with continued watershed development, more serious potential consequences include elevated peak flows, landslides, increased sediment delivery, and increased water temperature (see Section 1).

Among threats mechanisms associated with habitat degradation, high sediment transport is one of particular importance to Arctic Grayling. Rearing adults can avoid turbid conditions and move to different feeding locations, but young-of-year (YOY) and post-YOY juveniles are more restricted in their ability to move, and survival depends on rapid growth and associated adapted traits fine-tuned to local natal streams. In streams impacted by elevated sediment loads, surviving YOY juveniles have exhibited reduced growth and signs of stress (Birtwell et al. 1984; McLeay et al. 1987). At spawning locations affected by elevated sediment, alevins have suffered increased mortality, starvation, and reduced growth rates during early rearing (Birtwell et al. 1984; Reynolds et al. 1989). Greater variation in discharge following watershed development may be another threats mechanism of particular importance to Arctic Grayling populations. YOY juveniles are highly susceptible to high flows for two weeks after hatch, a time when spring freshets cause unpredictable flood events. Tack (1974) noted absence of a whole year class in Chena River following a flood event and Clark (1992) found a significant correlation between recruitment levels and stream flows over a 14-year period. Large numbers of grayling YOY have also been observed stranded in pools isolated from the mainstem after water levels dropped (de Bruyn and McCart 1974; Cowie and Blackman 2003). The importance of this early critical period in Parsnip Arctic Grayling population dynamics, and the factors which affect juvenile recruitment variation in the watershed, are unknown. Given that Parsnip Arctic Grayling likely do not mature until age-5 and may live at least as long as 8 years (Ballard and Shrimpton 2009), the effects of habitat degradation may not show in the time series of adult/subadult snorkel counts for many years, like the observed population crash that occurred 10 years after the flooding of Williston Reservoir (Blackman 1992).

Figure 19. Lower Anzac River on August 22, 2020.

Snorkeling count data in long-term index sections provide an important measure of potential effects of increased land disturbance on the productivity of Parsnip Arctic Grayling. The ability of these data to discriminate potential effects will be improved by collating them with information about changes in water temperature, sediment delivery, and hydrology. These potential factors affecting Arctic Grayling are now under study by collaborators UNBC (e.g., Martins et al. 2022; O'Connor 2023; FWCP project no. PEA-F23-F-3632) and MOF. Therefore, we recommend a long-term role for the snorkeling study within a stewardship and monitoring framework for the Parsnip River watershed (Section 7). Combined results will provide important effectiveness monitoring data for the most important habitat conservation initiative undertaken so far in the Parsnip River watershed: the establishment of Fisheries Sensitive Watershed (FSW) legislation for the Anzac, Table, Hominka, and Missinka sub-basins, which was approved during this five-year study (Sandra Sulyma, Omineca Region WLRS, pers. comm. March 2022).

6.2.2 Natural Limiting Factors

The accumulation of single-pass snorkeling count data from 42 reconnaissance sections, distributed among 8 streams, provides a picture of sharp contrast among these sections in their relative importance for adult/subadult Arctic Grayling rearing. These data therefore provide a good opportunity for evaluating natural factors limiting Arctic Grayling distribution and abundance in the Parsnip core area in addition to the anthropogenic factors identified in the preceding section. Examples include:

1. <u>Water temperature and its variation</u>: Adults prefer cold water and can show higher growth rates during years when summer water temperatures are lower (e.g., approaching 10°C; Lohr et al. 1996; Deegan et al. 1999; O'Connor 2023) while juveniles, especially YOY, require warmer temperatures (~16^{o}C) that promote rapid growth and improved survival (Deegan et al. 1999; Buzby and Deegan 2004). Juvenile Arctic Grayling are consequently likely to be present in large river habitats and lake headed tributaries that provide relatively warm and stable summer temperatures (Hawshaw and Shrimpton 2014).
2. <u>Watershed size</u>: Included in best models of juvenile Arctic Grayling presence for Hawkshaw and Shrimpton (2014). The Anzac River is the Parsnip watershed's largest sub-basin and has the strongest population size.
3. <u>Stream gradient</u>: Our observation is that streams of the Parsnip River watershed with extensive sections of low-gradient meanders have fewer adult grayling.
4. <u>Hydrological variables</u>: Excessively high peak flows and sediment delivery are known to affect productivity for juvenile and adult life stages (Section 6.2.1).
5. <u>Past land use</u>: The Anzac River has until recently escaped industrial developments in the upper watershed.
6. <u>Distance from the reservoir</u>: Included in best models for predicting juvenile Arctic Grayling presence in Hawkshaw and Shrimpton (2014).
7. <u>Distance from overwintering habitat</u>: I.e., from the Parsnip River mainstem (Martins et al. 2022).
8. <u>Stream channel geomorphology</u>: E.g., Anzac River adult Arctic grayling occupy the deepest water at or near the edge of the thalweg, usually where the river is narrow (Blackman 2004).
9. <u>Site-level physical habitat attributes</u>: e.g., pool depth, bed material particle size, e.g., Blackman (2004) found preferred adult grayling depth is 1.36 m and 0.36 m/sec velocity.

The process of compiling these data and designing an analysis has begun. However, completion of the analysis and reporting is not anticipated until winter 2024 at the earliest depending on future study funding.

6.3 Critical habitats

6.3.1 Adult/Subadult Summer Rearing Habitats

At the time of the 2017 FWCP Arctic Grayling information synthesis (Stamford et al. 2017), there was a great deal of uncertainty about the distribution and abundance of adult and subadult Arctic Grayling in the Parsnip River watershed outside of the Anzac and Table sub-basins. The single-pass, reconnaissance snorkeling surveys have been effective at addressing this information gap. We now know that Arctic Grayling are present and widely distributed in other parts of the Parsnip River watershed, particularly upstream of the Table River (Table 6). Adult Arctic Grayling utilize at least 36 km of the Missinka River (Hagen and Gantner 2020), 48 km of the Hominka River (Hagen and Stamford 2021), 27.5 km of Wichcika Creek (Hagen and Stamford 2021), and 30 km of Reynolds Creek (Table 6). Wichcika Creek and Reynolds Creek appear to be somewhat marginal for adult Arctic Grayling, with very low densities which are numerically dominated by Rainbow Trout (Table 6). The Misinchinka River, situated closest to Williston Reservoir, has only extreme low densities of Arctic Grayling at the margin of detectability even for eDNA (Table 6, Table 7). The most productive summer rearing habitats for adult Arctic Grayling outside of the Table and Anzac rivers are in the upper Parsnip River watershed distributed from 36-25 km of the Missinka River (Hagen and Gantner 2020) and from 48-32 km of the Hominka River (Hagen and Stamford 2021).

Based on the improved knowledge since 2018, in the forms of eDNA assays, single-pass reconnaissance snorkeling, and acoustic telemetry surveys, critical habitat segments (Table 11) have been delineated with greater precision and confidence relative to the last major update of 2017 (Stamford et al. 2017). Most importantly, this critical habitat information can be utilized to locate conservation and enhancement actions for Arctic Grayling in the Parsnip River watershed.

Following completion of the basin-wide reconnaissance snorkeling surveys, it is abundantly clear that the most important of the Parsnip River's sub-basins are the Anzac and Table systems (Table 10). Given the disproportionate importance of these two systems, future reconnaissance in the Table River watershed similar to the spatially-continuous survey of the Anzac River in 2018 (Hagen et al. 2019) is warranted. The Table River has not been a target for reconnaissance surveys during this study mostly because of poor visibility below the slump at 22 km. The potential recovery of water clarity downstream of this slump should be investigated during fieldwork in future years, and other methods (e.g., angling catch per effort, snorkeling mark-resight, and/or telemetry) could also be considered for application in the lower Table River.

6.3.2 Habitat Conservation and Enhancement Priorities

To enable our vision of the long-term conservation of Arctic Grayling in the Parsnip River watershed at abundance levels that support recreational and subsistence fisheries, we have previously recommended six principles for implementation of conservation and enhancement actions (Hagen and Stamford 2022):

1. Watershed-scale land use objectives will have a greater chance of conserving habitat productivity for both adult and juvenile life stages.
2. Local communities and First Nations whose territories overlap with the Parsnip River watershed should have a central role in stewardship of watersheds they are close to, care about, and depend on for subsistence needs and livelihoods (Bennett et al. 2018). The Parsnip watershed is of critical community interest to McLeod Lake Indian Band in particular.
3. Achieving FWCP strategic objectives for conservation, sustainable use, and community engagement (FWCP 2020) will require collaboration with BC Government agencies, First Nations, and communities of the Williston Reservoir watershed. This is because critical habitats for Arctic Grayling exist on Crown land and First Nations territories where FWCP itself may have limited opportunities for habitat conservation.
4. Conservation of critical habitats in good ecological condition and expected to be resilient to future climate change is more urgent and cost-effective than restoration and enhancement of already-degraded habitat (Roni et al. 2002; Hilbourne et al. 2003; Chapin et al. 2015).
5. More productive sub-basins in the Parsnip River watershed supporting higher abundances of Arctic Grayling are candidates for greater investments in habitat preservation. These populations are likely to have the highest long-term viability.
6. Application of the Precautionary Principle implies that when scientific confidence is lacking, decisions should be sufficiently risk averse to avoid serious or irreversible harm.

Critical habitats for adult/subadult Arctic Grayling and juvenile life stages identified in this report are distributed across relatively long stream segments. Indeed, this was the intended resolution of our study, which was designed to estimate information deficiencies at the watershed scale. Maintaining the productivity of Arctic Grayling populations overall will require that conservation actions encompass as high a proportion of these critical habitats as possible, so watershed-scale land use objectives which maintain the hydrological integrity and thermal regimes of Arctic Grayling streams should be the top priority of a habitat stewardship framework (Principle #1). For FWCP to enable effective, watershed-scale conservation of habitat, collaboration with the BC Government and First Nations is essential because these agencies are responsible for land use decisions (Principles #2, #3). The Parsnip watershed is of critical community interest to McLeod Lake Indian Band in particular. The key role that FWCP has played has been to conduct the scientific information gathering that enables effective conservation at this watershed scale. An important tool to facilitate use of the scientific data by collaborators McLeod Lake Indian Band and BC Ministry of Water, Land, and Resource Stewardship is a GIS spatial layer that depicts critical habitat segments and physical watershed attributes linked to them. This key outreach product of the study is under preparation by WLRS personnel and will be shared with FWCP and MLIB.

While land stewardship objectives and land use decisions are ultimately the responsibilities of First Nations and the BC Government, additional scientific investigations by FWCP into the effects of limiting factors and threats on Arctic Grayling may produce new information that improves conservation outcomes. An example of such a study currently ongoing is the multi-scale modeling study of current and future water temperatures in the Williston Reservoir watershed (FWCP project no. PEA-F23-F-3632), which has been conditionally funded for 2023. Other key physical attributes linked to Arctic Grayling productivity have been discussed previously in this report and include factors such as variation in stream flow and fine sediment delivery. More detailed studies of these attributes and their linkages with land use activities could improve confidence that watershed-scale objectives will be effective in conserving the productivity of critical habitats.

Table 11 delineates watersheds where land use objectives are needed to limit the cumulative effects of human land use and natural disturbance on watershed hydrology and water temperature. To maximize conservation of Arctic Grayling productivity in critical habitats, such objectives should: 1) be increasingly risk-averse as the priority level (Priority 1 higher, Priority 3 lower) of the critical habitat segment increases (Principle #4), 2) focus on protecting key habitat attributes in good ecological condition (e.g., areas of low land use) (Principle #5), and 3) be risk-averse when information adequacy is low (Principle #6).

An example of a tool for establishing watershed-scale land use objectives within British Columbia's *Government Actions Regulation* (GAR) is the 'Fisheries Sensitive Watershed' (FSW) designation (GAR Section 14). Designated FSWs for the Anzac, Table, Hominka, and Missinka rivers were approved during the five-year study period. These correspond well to high-priority stream segments identified in this report (Table 11). Objectives included in the FSW orders include: 1) the general objective of preventing cumulative hydrological effects that would have a Material Adverse Effect (MAE) on fish values, 2) the requirement to maintain Equivalent Clearcut Area (ECA) below thresholds specified for each watershed, 3) the requirement to minimize fine sediment increases through managing road crossings, road locations, and cutblock placements, 4) the requirement to maintain riparian function by retaining undisturbed leave strips, retaining mature windfirm forests on floodplains and fans, and excluding livestock from riparian areas and stream channels, and 5) the requirement to maintain fish passage at stream crossings by maintaining natural stream width, roughness, depth, and slope (Sandra Sulyma, BC FLNRORD, pers. comm. 2020).

Habitat conservation at the landscape scale is not solely the task of the BC Government, with the role of First Nation-led stewardship initiatives increasing rapidly. Examples of increased collaboration between First Nations and governments in land stewardship are Environmental Stewardship Initiative (ESI) engagements in BC.

The watershed-scale land use objectives and other stewardship actions have been or will be designed to protect against hydrological and water temperature changes in critical habitats, but uncertainty will remain about whether they are enough to ensure long-term conservation of Arctic Grayling. Therefore, regular monitoring of abundance using snorkeling surveys in index sections will be necessary to evaluate their effectiveness. This is especially important in the near-term to ensure that potential cumulative effects from rapid increases in land use in sub-basins of the Parsnip River watershed (Section 6.1.3) do not go undetected. Consequently, we recommend that replicated snorkeling surveys in the long-term index sections of the Anzac and Table rivers be continued in future at periodic intervals (Section 7).

It is important to recognize that Arctic Grayling have a complex life history and critical habitats for adult rearing, spawning, overwintering, and juvenile rearing are often in distinct locations and often separated by substantial migration distances (Armstrong 1986, Northcote 1993, Stamford et al. 2017). Therefore, critical summer rearing locations for adult Arctic Grayling identified during this study (Table 10, Table 11) do not comprise a complete picture of the distribution of critical habitats for the species in the Parsnip River watershed. Fortunately, a substantial amount of past FWCP sampling targeting juvenile life stages of Arctic Grayling has occurred in the Parsnip watershed. We have interpreted these data to delineate critical habitats for other life stages (Table 11) and the results should also be considered when identifying targets for watershed-scale stewardship actions. It should be noted, however, that data from some Parsnip sub-basins is very limited and follow-up studies targeting juvenile life stages are recommended. These systems include: 1) Misinchinka River, 2) Colbourne Creek, 3) Reynolds Creek (including Chuyazega Creek), 4) Firth Creek, 5) Bill’s Creek, 6) Hominka River, 7) Wichcika Creek, and 8) tributary sub-basins within the Anzac and Table watersheds. Positive eDNA results in areas where adult Arctic Grayling were not observed snorkeling suggest that additional eDNA assays combined with juvenile-oriented sampling methods could be a powerful combination for identifying elusive juvenile rearing and spawning habitats.

In our view, the most urgent priority is for the conservation of existing critical habitats that are productive and in good ecological condition (Principle #4, Principle #5), rather than enhancements of degraded or unproductive habitats. Given rapid increases in land use within the Parsnip River watershed, these actions are the most time sensitive. However, the observations of marginal or very small populations, particularly in the lower Parsnip River watershed (Table 10), intriguingly suggests these streams as potential enhancement targets. If successful, enhancements could make a significant difference for the abundance and viability of these marginal populations. To be successful, they would require an understanding (or at least a theory worthy of an experiment) of what factors currently limit distribution and abundance in these streams. Demonstrating such an understanding with a successful enhancement experiment may even have implications for recolonizing some of the lost range of Arctic Grayling in other parts of the Williston Reservoir watershed. A first step may be to propose a study focused on the lower Parsnip River watershed, which seeks to learn about 1) distinct genetic attributes associated with some populations and whether there are demographic connections with stronger populations, 2) patterns of life history and movement, and 3) finer-scale observations of critical habitats across life history stages.

7 RECOMMENDATIONS

In the Parsnip River watershed, critical habitats in the core of the Arctic Grayling distribution in the Anzac and Table rivers remain productive, as indicated by the positive abundance trend. Therefore, we recommend conservation of habitats currently in good ecological condition as the most reliable and cost-effective stewardship approach. However, uncertainty remains about limiting factors and threats and how they may affect the future status of the Parsnip Arctic Grayling population. Therefore, existing habitat conservation actions in the form of watershed-scale land use objectives (e.g., Fisheries Sensitive Watershed designations and associated objectives) are experiments with somewhat uncertain outcomes. To enable conservation actions for Parsnip Arctic Grayling now, while addressing these uncertainties, we have made recommendations and identified information needs throughout this report. Highest-priority recommendations and information needs for the near term are:

1. *Share study results and critical habitat information with First Nations whose territories overlap the Parsnip River watershed*. First Nations also have the goal of long-term Arctic Grayling conservation, and have personnel and programs dedicated to land use stewardship. Representatives of these Nations have identified that sharing of spatial layers depicting critical fish habitat would be an especially valuable outreach product. Critical fish habitat layers for the Parsnip River watershed are being prepared in collaboration with BC Ministry of Water, Land, and Resource Stewardship, Omineca Region. Following completion, they will be distributed to collaborating First Nations (the Parsnip River watershed is of critical community interest to McLeod Lake Indian Band in particular).

2. *Watershed-scale land use objectives to protect against water temperature increases and hydrological hazards in watersheds that provide summer rearing habitat for adult Arctic Grayling*. Watershed-scale habitat protection measures designed to protect against changes in hydrology and water temperature are necessary, because Arctic Grayling are vulnerable to increased peak flows, increased fine sediment in streams, and changes in water temperature regimes (Section 6.2). These objectives should include limits to watershed disturbance (e.g., ECA) to protect against elevated peak flows, wider buffers on all stream reaches to protect against fine sediment inputs and water temperature increases, and road building practices that maintain fish passage at stream crossings, reduce fine sediment inputs, and avoid easy angler access to vulnerable aggregations of fish. Designing and implementing watershed-scale land use objectives may be led by McLeod Lake Indian Band's Land Stewardship in consultation with licensees and their own forestry operators, and also by BC Government's Ministry of Water, Land, and

Resource Stewardship. FSWs have already been approved by the BC Government for the Anzac, Table, Hominka, and Missinka sub-basins.

3. *Studies to improve knowledge of limiting factors and threats*. Factors driving Arctic Grayling abundance trends in long-term index sections are not known, nor are the effects of the current, rapid increases in human land use activities, given that there is likely a lag of several years before degraded environments result in reduced numbers of adult Arctic Grayling. Improving the understanding of limiting factors and threats should be a top priority objective in the coming years. Key areas of uncertainty are: i) whether increased opportunities for human use of fish are sustainable, ii) effects of land use-related changes in water temperature, sediment delivery, and hydrology on the productivity of Parsnip Arctic Grayling, and iii) effects of natural limiting factors on the distribution and abundance of Arctic Grayling at the scale of the whole Parsnip River watershed, which may have implications for designing enhancements in areas that have marginal or small populations. We specifically recommend that snorkeling count studies in long-term index sections continue on a periodic basis[5] and that the data be paired with data from ongoing studies of water temperature, sediment delivery, and hydrology conducted by FWCP and MOF. We also recommend that the basin-wide estimates of Arctic Grayling distribution and abundance generated by the single-pass reconnaissance snorkeling surveys be analyzed in relation to watershed physical attributes to better understand natural limiting factors.

4. *Effectiveness monitoring of existing habitat conservation actions*. As identified above, FSWs have already been approved by the BC Government for the Anzac, Table, Hominka, and Missinka sub-basins. These designations have been informed in large part by data collected during FWCP-funded studies. Despite this legislated protection, rapid watershed developments in these sub-basins since FSW approval include increased forestry and major pipeline construction. The effectiveness of current FSW land use objectives at preventing cumulative effects of these developments on Arctic Grayling productivity can only be evaluated with continued monitoring within long-term index sections, particularly in the near-term (on a periodic basis as recommended in #3 above), given that effects may disproportionately affect juvenile life stages and it may take several years for them to be reflected in the adult abundance estimates.

5. *Study(s) focused on lower Parsnip River sub-basins (downstream of Anzac River)*. Sub-basins of the lower Parsnip River watershed provide habitat for few adult Arctic Grayling. Enhancements in these systems could make a significant difference for the abundance and viability of these marginal adult habitats and possibly promote range

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[5] e.g., 3-year study duration followed by a 3-year hiatus in which other parts of the Williston Reservoir watershed are prioritized for snorkeling surveys (e.g., tributaries to the reservoir’s west shore).

expansion for the Parsnip core area overall. To be successful, enhancement actions would require additional information about what factors current limit distribution and abundance in these streams. To evaluate the potential for such an enhancement, we recommend a study to i) evaluate potentially distinct genetic attributes associated with these streams (e.g., locally adapted fry; straying phenotypes) and possible demographic connections with other streams, 2) identify patterns of life history, habitat use, and movement, and 3) provide finer-scale observations of critical habitats across life history stages.

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10 APPENDIX 1: Environmental DNA laboratory report

Final report on eDNA analysis of water samples from the Parsnip Core Area for the presence of Arctic grayling (*Thymallus arcticus*), 2022 Season

31 January 2023

Summary

The previously validated grayling real-time PCR assays were utilized to assess the presence of Arctic grayling eDNA in water filter samples from the Parsnip Core Area. A digital PCR platform was used to test for both signals simultaneously. The assays were previously validated and optimized with both tissue and synthetic GBlock DNA. The accompanying spreadsheet contains a summary of the data obtained from the analysis of water filter samples for the presence of Arctic grayling. Two elutions of each filter isolate were first tested, and the corresponding quantities (copy number per reaction well) of each replicate are reported, as well as the number of detections used to calculate the copies. Additionally, an internal control (bacterial lambda DNA) and ePlant assay were used to confirm sample quality. If testing of the internal controls indicated PCR inhibition (either a decreased signal or failed amplification), the second elution ("ii") of a sample was retested. Second elutions were also retested if there was a low copy number result, to confirm positive amplifications. Arctic grayling eDNA was detected in samples from the following sites: Anzac River (12 km, and 45 km), Table River (22 km [above the clay slump] and 31 km), Misinchinka River (56 km, 60 km, 68 km). Other sites had low signals that we were unable to confirm as positive: Misinchinka River (36 km), Mischinsinlinka Creek (0 km), and Wooyadlinka Creek (0 km).

Methods

Two published TaqMan assays for Arctic grayling were used for this work (Rodgers et al., 2018; Carim et al., 2016). These assays were designed for populations on the North Slope of Alaska, and Montana, respectively, and were tested against other salmonids and non-salmonids by the authors. We previously (2018 study) checked the specificity and sensitivity 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). 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. The same reporter dyes are used for the inhibitor test duplexes for LAMBDA and ePlant. Digital droplet PCR (ddPCR) successfully amplified the GBlock. Briefly, samples were run in 24 uL reactions using mastermix for probes (no UTP). Droplets were generated using the AutoDG and run on a QX200

Droplet Reader (Bio-Rad Laboratories). GRAY1 and GRAY2 were additionally tested across a temperature gradient to determine the range of optimal annealing temperatures.

Twenty-four samples and field control blanks were submitted for analysis. 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) following a modified protocol for water filters. For each filter, an “i” and “ii” eDNA elution was collected. 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, both elutions for the presence of Arctic grayling DNA, with the “ii” sample analyzed again if samples showed signs of PCR inhibition or low signal.

Positive control lambda DNA was added to a master mix to test all first elutions. An advantage of ddPCR is that copy numbers can be directly compared, making identification of outliers more clear (rather than a simple pass/fail system). These tests suggest partial assay inhibition as results show lambda DNA presence at a lower quantitative amount than the other samples, despite being spiked with the same amount of control DNA. Any samples that failed, or where lambda appeared lower than controls, were retested using the second elute. The ePlant amplifications were all consistently high. Any reactions that appeared to have a lower peak florescence were also retested using the “ii” elutions (“ii” elutions tend to be more dilute in terms of both eDNA and contaminants, and are thus cleaner). A few samples were also retested if initial round of testing was below cutoff of 4 droplets.

Results

Previously, a dilution series was used to evaluate the performance of the ddPCR platform with a GBlock containing grayling sequence (Figure 1). The dilution series ranged from 1,000 to 1 copies per reaction. Duplicates were within range of each other and decreased an order of magnitude with each dilution. Viewing the data from left to right, as concentration decreases data points become more dispersed for GRAY1 (Figure 1). GRAY2 exhibited the same pattern (Figure 2). A temperature gradient experiment was used to determine the optimal temperature at which both assays would amplify the highest (not shown). With retesting, results corresponding to at least 4 detections (combination of both GRAY1 and GRAY2 detections) had to be obtained for a site to be considered positive. Positive results below this cutoff are denoted as possible “low”; additionally, coamplification of GRAY1 and GRAY2 still indicated specificity despite the low levels. Any sample that had amplifications below this cutoff, and no agreement of the two assays, could not be confirmed and was determined to be negative.

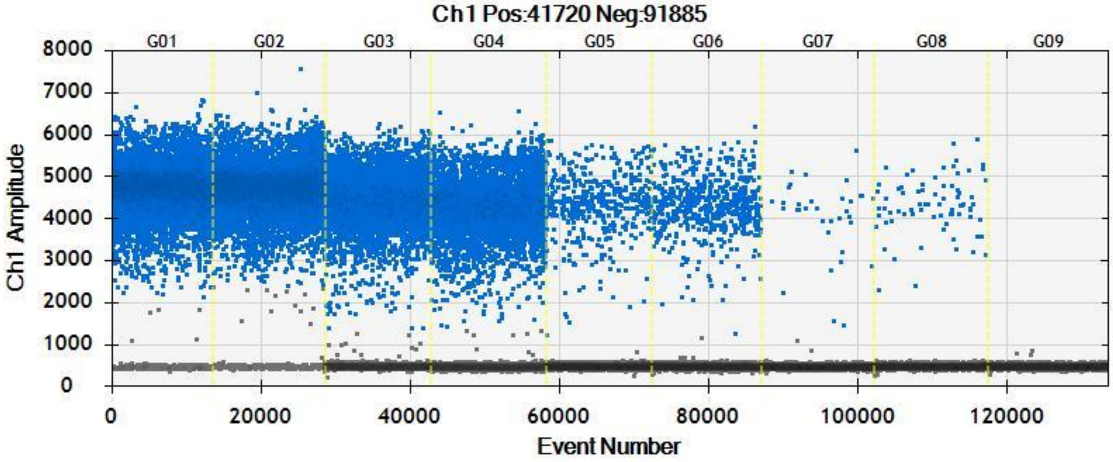

Figure 1 – GRAY1 Amplification Plot of dilution series of Arctic grayling DNA from GBlock.

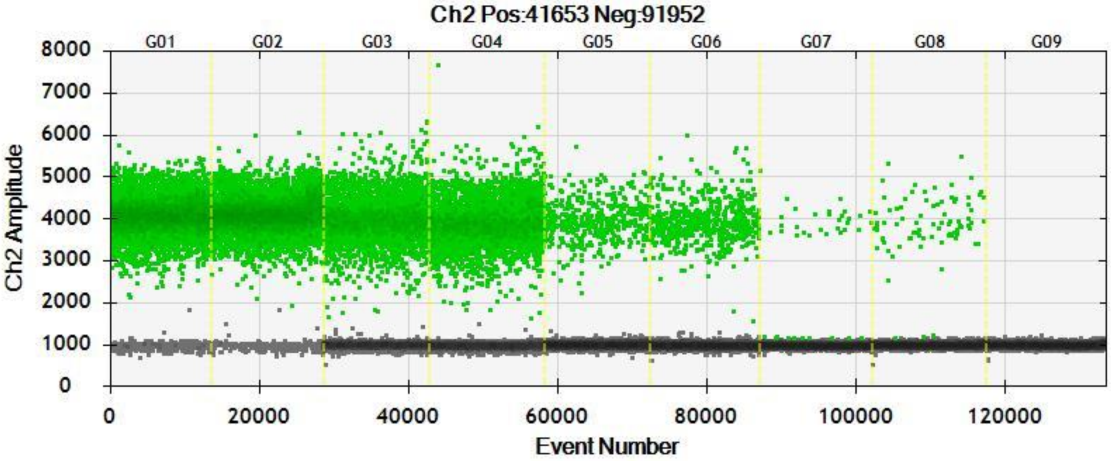

Figure 2 – GRAY2 Amplification Plot of dilution series of Arctic grayling DNA from GBlock.

Filters from the Anzac and Table River sites had the highest number of grayling DNA copies per reaction (Figures 3 and 4, and “Summary” in the attached spreadsheet). Other sites had less target eDNA, and required further testing to confirm at least 4 detections occurred. All sites had consistent ePlant amplification and no indication of DNA degradation (subset shown in Figure 5). The bacterial lambda test (subset shown in Figure 6) did not indicate any significant inhibition. Mischinsinlika Creek (0 km) had one amplification from each assay that were also coamplifications (within the same droplet). This suggests high specificity, but could not be confirmed with additional testing.

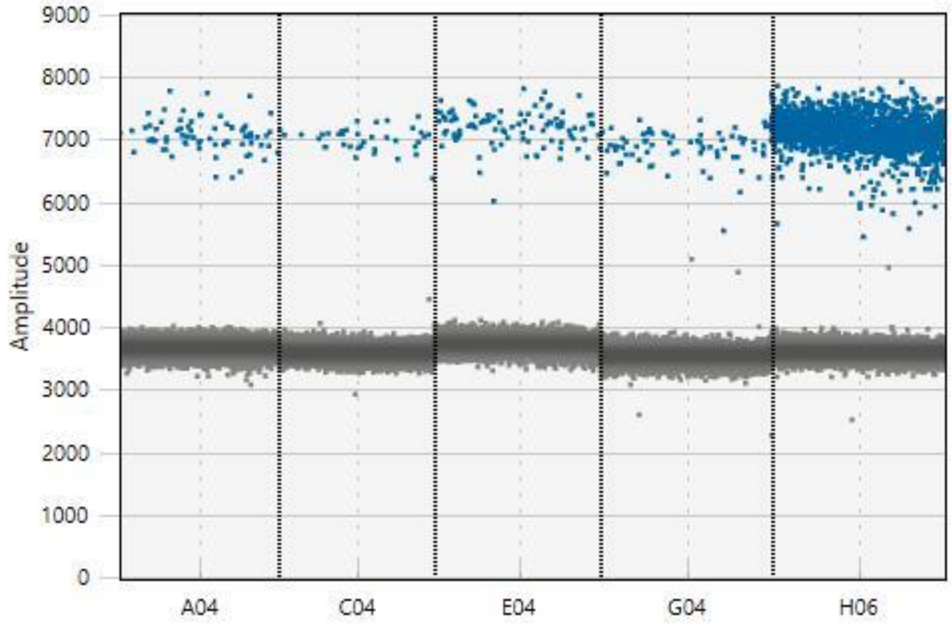

Figure 3 – Positive droplets (blue) for GRAY1 assay. Left to right: Anzac 12 km, Anzac 45 km, Table 22 km, Table 31 km, positive control (Gblock).

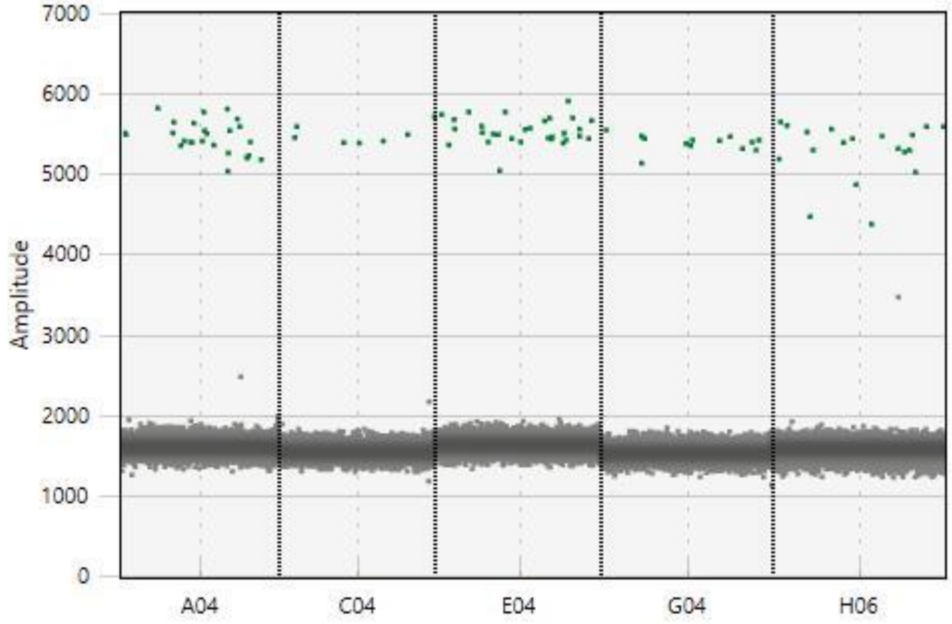

Figure 4 – Positive droplets (green) for GRAY2 assay. Left to right: Anzac 12 km, Anzac 45 km, Table 22 km, Table 31 km, positive control (Gblock).

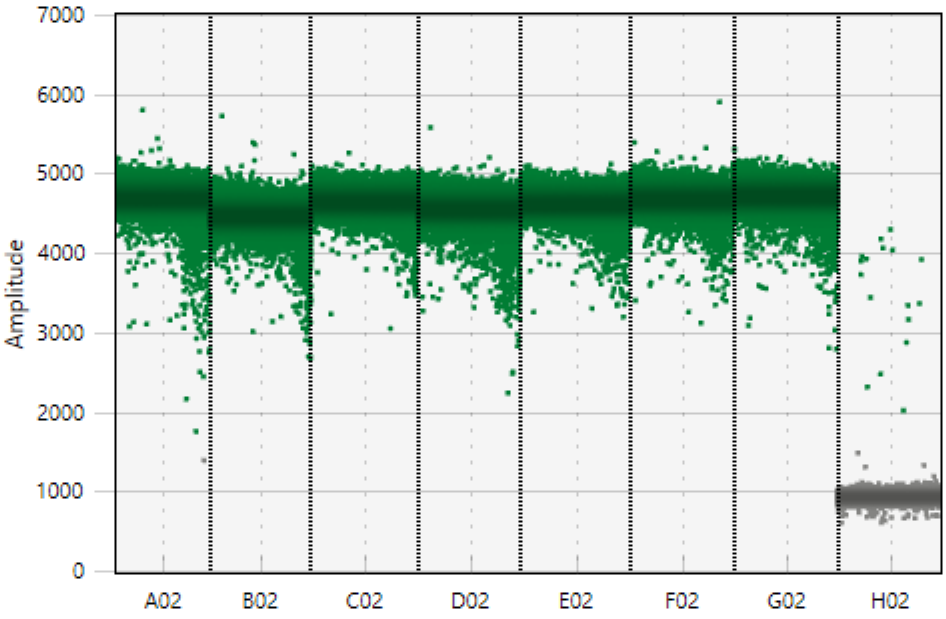

Figure 5 – Subset showing droplet amplitude of ePlant test (positive droplets in green). Lane H02 is a blank water sample.

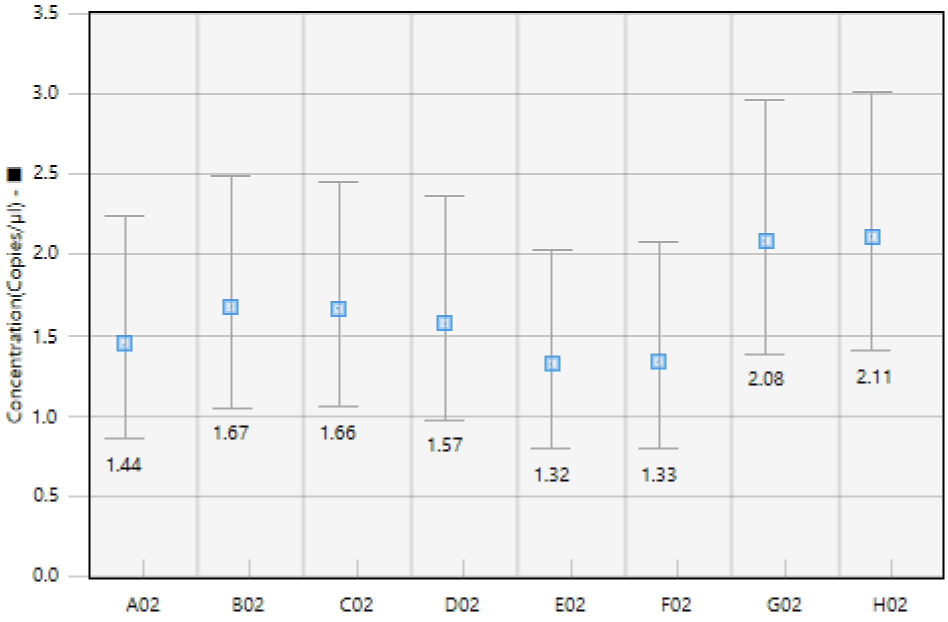

Figure 6 – Subset of concentration results for lambda control reactions. Lane H02 is the positive control lane.

The spreadsheet “Summary” shows all sites that had a positive result, and those that were reanalyzed after detection of PCR inhibition. Sites are designated “positive”, “low”, or “negative” for those that were too low and could not be confirmed with retests. In the event of a failure of part of a duplicate, the quantity for the failed reaction is listed as “0”. Rationale behind retesting is listed in the comments. Raw Quantification data from each plate are in the other spreadsheets. No blank or extraction controls amplified, indicating proper safeguards against field, extraction and assay contamination.

Please let us know if you require further clarification of the result and interpretations present here.

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