Site C Clean Energy Project

Fisheries and Aquatic Habitat Monitoring and Follow-up Program

Fish Genetics Study
2023 Status Report for Bull Trout, Arctic Grayling, Rainbow Trout, and Redside Shiner

Construction Year 10 (2024)

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EXECUTIVE SUMMARY

BC Hydro's Site C Clean Energy Project (the Project) near the town of Fort St. John in northeastern British Columbia is the Peace River's third hydroelectric dam. BC Hydro developed the Site C Fisheries and Aquatic Habitat Monitoring and Follow-up Program (FAHMFP) in accordance with Provincial Environmental Assessment Certificate Condition No. 7 and Federal Decision Statement Condition Nos. 8.4.3 and 8.4.4 for the Project. To date, Mon-1b, Task 2c (Site C Reservoir Tributaries Fish Population Indexing Survey), Mon-2, Task 2a (Peace River Large Fish Indexing Survey), Mon-2, Task 2b (Peace River Fish Composition and Abundance Survey), the Contingent Fish Capture and Transport Program, and the Temporary Upstream Fish Passage Facility (TUF) have collected tissue samples from Bull Trout (*Salvelinus confluentus*), Arctic Grayling (*Thymallus arcticus*) and Rainbow Trout (*Oncorhynchus mykiss*), and three small-bodied species found in the Local Assessment Area (LAA), Slimy Sculpin (*Cottus cognatus*), Longnose Dace (*Rhinichthys cataractae*), and Redside Shiner (*Richardsonius balteatus*).

The first phase of a Site C Fish Genetics Study was conducted between 2018 and 2021 by the laboratory of Eric Taylor at the University of British Columbia (UBC) where we: (a) determined levels and patterns of population structure in Bull Trout, Arctic Grayling and Rainbow Trout in the Peace River and its tributaries, (b) developed genotyping assays for genetic monitoring of the system, and (c) deployed those assays for samples collected in the Peace River from 2016 to 2020. That project was extended until the end of December 2025 with the following activities: Activity 1) population assignment of Bull Trout, Arctic Grayling and Rainbow Trout samples collected in the

Peace River from 2021 to 2024, Activity 2) development and deployment of medium sized genotyping panels (200 to 300 loci) for Bull Trout and Rainbow Trout for demographic analyses, and Activity 3) generation of genome-wide sequence data for three small-bodied fish species for analyses of patterns and levels of population structure in the LAA prior to river diversion. Here, we report on the progress of the Site C Fish Genetics Study from January 1, 2024 to December 31, 2024. Previous results and findings can be found in Geraldes and Taylor (2020, 2021, 2022, 2023, 2024).

For Activity 1, samples of Bull Trout, Arctic Grayling and Rainbow Trout for population assignment were collected in the Peace River in sampling year 2023 and 478 samples were received at UBC where they were stored and catalogued.

For Bull Trout, 257 samples were collected in the Peace River in 2023, their DNA was extracted, and they were genotyped at six loci previously developed for population assignment to either of two genetic groups detected in the LAA: one genetic group consists of samples that spawn upstream of the Project (UP) in the Halfway River, and the other consists of samples that spawn downstream of the Project (DP) in the Pine River (Geraldes and Taylor 2020). Of the 257 Bull Trout samples collected in 2023 (including 84 sampled from the TUF), the vast majority of samples were assigned to UP (N=228, 88.7% of all samples) and a small number were assigned to DP (N=16, 6.2% of all samples). Of the 84 Bull Trout sampled at the TUF in 2023, only 3 were assigned to DP (3.6%) and 77 (91.2%) were assigned to the UP group. Overall, (5.1%) of fish could not be assigned to one of the two groups with more than 95% confidence (N=4 in the TUF and N=9 elsewhere).

For Arctic Grayling, 98 samples were collected in the Peace River in 2023, their DNA was extracted, and they were genotyped at 11 loci previously developed for population assignment (Geraldes and Taylor 2021). Geraldes and Taylor (2021) found that four distinct population groups of Arctic Grayling are found in the LAA, each one corresponding to a single tributary where they are known to spawn: the Halfway River and the Moberly River (located UP) and the Pine River and the Beatton River (located DP). Only 3 fish (3%) were not assigned to the UP group (two could not be assigned with more than 95% confidence to either the UP or DP group and one was assigned to the DP group). No samples from the TUF were assigned to DP. More specifically, 89 samples were assigned to the Moberly River (90.8%) and one (1%) was assigned to the Pine River, while 8 could not be assigned to a specific tributary with more than 95% confidence.

Finally, for Rainbow Trout, 123 samples were collected in the Peace River in 2023, their DNA was extracted, and they were genotyped at six loci previously developed for population assignment (Geraldes and Taylor 2022). Geraldes and Taylor (2022) found that population structure for Rainbow Trout in the LAA was more complex but two genetic groups, largely corresponding to ancestry from populations spawning UP and ancestry from groups spawning DP (plus hatchery ancestry), were identified. Of the 123 samples subject to assignment tests in 2024, 62 (50.4%) were assigned to the UP group, 45 (36.6%) to the DP group, and 16 (13.0%) could not be assigned with at least 95% confidence. The results for fish sampled at the TUF were similar, of 37 fish analyzed, 18 were assigned UP (48.6%), 14 were assigned DP (37.8%), and 5 (13.5%) could not be assigned to either with at least 95% confidence.

Activity 2 consists of the development and deployment of medium size single nucleotide polymorphism (SNP) panels for Bull Trout and Rainbow Trout. In 2024 we developed and tested a panel of 219 loci for Rainbow Trout and we genotyped 3,610 Bull Trout samples collected up to 2022 with a 190 SNP panel previously developed (Geraldes and Taylor 2024). Both panels have high genotyping rate and accuracy. Results for Bull Trout revealed that approximately half the samples were female and that for approximately 9% of samples a female parent could be identified and for 5% a male parent could be identified. Once samples born after river diversion are analyzed we will be able to determine if fish that were passed through the upstream fish passage facility are contributing progeny to the system.

An additional 1,022 samples of the three salmonid species, collected in Peace River tributaries in the LAA in 2023, were received at UBC and catalogued. Among these samples, extraction and quality control of DNA were performed for 624 Bull Trout and 452 samples of Rainbow Trout; no additional samples of Arctic Grayling (46) were processed. All samples of Bull Trout and Rainbow Trout will be used for demographic inference (Activity 2) in 2025.

For Activity 3, Geraldes and Taylor (2023) used reduced representation genomic DNA sequencing with genotyping-by-sequencing (GBS) to generate sequence data for 612 samples of Slimy Sculpin, Longnose Dace and Redside Shiner, and examined population structure with the resulting SNP data for Slimy Sculpin (Geraldes and Taylor 2023) and Longnose Dace (Geraldes and Taylor 2024). Briefly, two distinct genetic groups of each species were identified in the LAA, one comprising mostly samples from the Moberly River and the other samples from the Peace River. Genetic differentiation

between the two regions was low and many samples bore signs of admixture between the two groups. No genetic differentiation was detected between sampling years, nor between sampling sections of the Peace River suggesting that enough genetic exchange occurs to prevent genetic differentiation between sites upstream and downstream of the project. In 2024 we examined population structure in the LAA for Redside Shiner, which is the first species in our work for which a suitable reference genome for sequence mapping and SNP discovery is not available. Instead, we used the Redside Shiner sequencing reads generated in 2022 to build a de-novo reference onto which to map the sequences for each fish and identify polymorphisms. The resulting SNP data was used to determine that population structure for Redside Shiner in the LAA is similar to that of Slimy Sculpin and Longnose Dace; two genetic groups identified, one more common in the Moberly River and one more common in the Peace River mainstem with abundant admixture between them.

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LIST OF ACRONYMS AND ABBREVIATIONS

BE	Beatton River
BP	Base pair
CVE	Cross Validation Error
DNA	Deoxyribonucleic Acid
DP	Downstream of the Project
FAHMFP	Fisheries and Aquatic Habitat Monitoring and Follow-up Program
F_{ST}	Fixation index is a measure of genetic differentiation owing to population subdivision among localities (S) relative to total variation (T)
GBS	Genotyping-by-sequencing
GT-seq	Genotyping-in-Thousands by sequencing
HA	Halfway River
K	Number of genetic groups in the Admixture analysis
LAA	Local Assessment Area
LD	Linkage Disequilibrium
LX	Lynx Creek
MO	Moberly River
PCA	Principal components analysis
PCR	Polymerase chain reaction
PI	Pine River
PR	Peace River
QC	Quality control
SNP	Single nucleotide polymorphism

TUF	Temporary Upstream Fish Passage Facility
UBC	University of British Columbia
UP	Upstream of the Project

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INTRODUCTION

The Site C Clean Energy Project (the Project) near the town of Fort St. John in northeastern British Columbia (hereafter referred to as the Local Assessment Area, LAA) is the third hydroelectric dam on the Peace River operated by BC Hydro. Between 2018 and 2021, BC Hydro and the laboratory of Eric Taylor at the University of British Columbia (UBC), Department of Zoology, collaborated to apply genomic techniques to facilitate aspects of the mitigation and monitoring plan for the LAA. The work covered by that agreement focused on three large bodied salmonid fishes: Bull Trout (*Salvelinus confluentus*), Arctic Grayling (*Thymallus arcticus*), and Rainbow Trout (*Oncorhynchus mykiss*) that are common in the LAA (see Geraldes and Taylor 2020, 2021, 2022).

Since September 2021 a new four-and-one half year agreement between the lab of Eric Taylor and BC Hydro has been underway to: (1) continue the population assignment work for Bull Trout, Arctic Grayling, and Rainbow Trout from 2021 sample years onwards, (2) develop and deploy medium sized (200 to 300 loci) genomic assays to monitor critical demographic parameters of Bull Trout and Rainbow Trout (e.g., effective population size), and (3) complete descriptive population genetic structure work for three small bodied species of fish also found in the LAA, Slimy Sculpin (*Cottus cognatus*), Longnose Dace (*Rhinichthys cataractae*), and Redside Shiner (*Richardsonius balteatus*), in support of Mon-15 (Site C Small Fish Translocation Monitoring Program).

Our work relates directly to the Site C Fisheries and Aquatic Habitat Monitoring and Follow-up Program (FAHMFP) that BC Hydro developed in accordance with Provincial Environmental Assessment Certificate, Schedule B, Condition No. 7 and

Federal Decision Statement Condition Nos. 8.4.3 and 8.4.4 for the Project. Further, the analyses illustrate BC Hydro's use of multiple lines of evidence to better understand the population structure, migration, and movement patterns of key fish species in the Peace River and its tributaries. For examples, data from otolith and fin ray microchemistry, radio telemetry, fish distribution, and genetics are being used to test hypotheses developed to answer management questions posed in the FAHMFP.

Purpose and Objectives

The Site C Fish Genetics Study involves three main activities: (1) population assignment of samples of Bull Trout, Arctic Grayling and Rainbow Trout collected in the mainstem of the Peace River and from the Temporary Upstream Fish Passage Facility (TUF), (2) develop and deploy genotyping assays for genetic monitoring and demographic analysis of Bull Trout and Rainbow Trout in the LAA, and (3) determine levels and patterns of genetic structure of Slimy Sculpin, Redside Shiner and Longnose Dace prior to river diversion as a baseline for future monitoring.

Work Conducted Prior to 2024

Geraldes and Taylor (2020, 2021, 2022, 2023, 2024) reported on the results of genetic work contributing to the FAHMFP, focusing on the use of genotyping-by-sequencing (GBS) across the genomes of Bull Trout, Arctic Grayling, and Rainbow Trout to resolve differences among samples collected from tributaries of the Peace River. For Bull Trout, the Halfway, Moberly and Pine rivers were the focus of study. For Arctic Grayling, samples from the same three rivers plus the Beatton River were examined. In Rainbow Trout, samples were examined from the Halfway, Moberly and Pine rivers, a few smaller tributaries of the Peace River (Farrell, Lynx and Maurice creeks), the Dinosaur Reservoir (created by Peace Canyon Dam upstream of the Project), and three hatchery

strains known to be used for stocking of fish in the area (Pennask Lake, Blackwater River, and Fraser Valley Domestic).

Geraldes and Taylor (2020, 2021, 2022, 2023) reported strong genetic differences amongst geographic groups that were exploited to develop six (Bull Trout), six (Rainbow Trout), and 11 (Arctic Grayling) TaqMan™ genotyping assays that differentiated samples collected from the mainstem Peace River in terms of whether an individual fish belonged to a spawning population located upstream of the Project (UP, i.e., Halfway River or Moberly River) or downstream of the Project (DP, i.e., Pine River or Beatton River).

Overall, about 94% of the 1,405 Bull Trout were assigned to UP and about 3% to DP between 2016 and 2022; only about 3% of mainstem Peace River samples of Bull Trout could not be assigned to either the UP or DP spawning groups with more than 95% confidence. No Bull Trout sampled at the TUF were assigned DP, 30 were assigned UP (93%) and two (7%) could not be assigned to either the UP or DP spawning groups with more than 95% confidence.

Of the 344 Arctic Grayling sampled from the mainstem Peace River between 2016 and 2022, 95% were assigned to UP and about 4% to DP; about 1% of the Arctic Grayling samples could not be assigned to either the UP or DP spawning groups with more than 95% confidence. For Arctic Grayling, population assignment showed that about 88% of fish were assigned to the Moberly River (located UP), 4% to the Pine River (located DP), less than 1% to the Halfway River (located UP) and none were assigned to the Beatton River (located DP). About 7% of Arctic Grayling could not be assigned to individual tributaries with over 95% confidence. All 57 Arctic Grayling

sampled at the TUF were assigned UP, 95% (N=54) were assigned to the Moberly River, one was assigned to the Halfway River and two could not be assigned to a specific tributary with more than 95% confidence.

The majority of the 684 LAA samples of Rainbow Trout from 2018 to 2022 were assigned to UP (55% vs 28% DP), but there was a high percentage (about 18%) of samples that could not be assigned to UP or DP groups with 95% or higher confidence.

In 2022, we used reduced representation genomic DNA sequencing with genotyping-by-sequencing (GBS) to generate sequence data and genetic variant discovery (single nucleotide polymorphisms, SNPs) for 612 samples of Slimy Sculpin, Longnose Dace and Redside Shiner, and examined population structure in Slimy Sculpin (Geraldes and Taylor 2023) and Longnose Dace (Geraldes and Taylor 2024). Briefly, for each species, one genetic group was mainly associated with fish from the Moberly River and one with fish from the Peace River mainstem (but many fish had evidence of admixture between those groups). For Longnose Dace (Geraldes and Taylor 2024) two samples from the Moberly River had close genetic affinity to Longnose Dace from Eastern Canada and a few were identified as being potentially admixed with that Eastern Lineage. In both species, genetic differentiation between sampling sites upstream and downstream of the project was low suggesting that enough genetic exchange occurs to prevent genetic differentiation.

Finally, we developed a Genotyping-in-thousands (GT-seq, Campbell et al. 2014) panel for Bull Trout in the LAA. The panel has the ability of genotyping 190 SNP loci for thousands of samples in a single lane of next-generation sequencing technology. The panel has 4 categories of loci, (i) one locus is for identifying the sex of the sample, (ii)

17 loci are for species identification (to distinguish between Bull Trout, Arctic Char/Dolly Varden, Lake Trout and Brook Trout), (iii) 15 loci are for assignment to the UP and DP genetic groups of the LAA and, (iv) 157 loci are for demographic analyses (e.g. for parentage assignment to assess if fish are the progeny of fish that were passed by the TUF in previous years). The panel was tested with a small number of samples and showed a high genotyping rate, correct sex identification, correct species identification and ability to assign samples to the UP and DP genetic groups.

Work Conducted Over The Past Year (2024)

The current report summarizes the work performed in 2024 on the three main project activities. For Activity 1, Bull Trout, Arctic Grayling, and Rainbow Trout population assignment work for samples collected in the mainstem of the Peace River in 2023 and provides a summary for all sample years between 2016 and 2023.

For the demographic analyses within Activity 2, DNA extractions of Bull Trout and Rainbow Trout from all sampling sites in the LAA were completed. For Bull Trout we used to the GT-seq 190 SNP panel (Geraldes and Taylor 2024) to genotype 3,588 samples of Bull Trout from the LAA and used the data generated to i) determine that the genotyping accuracy of the panel is very high (99.6% determined by comparing the genotypes of 27 samples that were submitted for genotyping in duplicate), ii) determine the sex of the samples genotyped (51.5% were female) and iii) establish parentage relationships among samples (319 samples were the progeny of 92 female parents and 157 were the progeny of 99 male parents). For Rainbow Trout we developed a GT-seq panel with 219 SNP loci using an approach similar to the one used to develop the Bull Trout GT-seq panel (Geraldes and Taylor 2024). The panel has 3 categories of loci, (i)

one locus is for identification of the sex of the sample, (ii) 28 loci are for assignment to the genetic groups of Rainbow Trout found in the LAA and, (iv) 190 loci are for demographic analyses (e.g. for parentage assignment to assess if fish sampled in the region are the progeny of fish that were passed by the TUF in previous years). Testing of the SNP panel with 95 samples revealed that the panel has a high genotyping rate (96.1% overall and 97.1% per locus), a high accuracy rate (99.98% of the genotypes for 40 samples submitted in duplicate were similar) and was able to generate a pattern of population structure similar to the one observed with GBS sequencing (Geraldes and Taylor 2022).

For Activity 3, we report on the analysis of population structure of the samples of Redside Shiner with GBS data generated in 2022 (Geraldes and Taylor 2023). We observed a pattern of population structure in the LAA similar to that of Slimy Sculpin and Longnose Dace with no genetic differentiation within the Peace River and moderate differentiation between two genetic groups, one associated with the Moberly River and one associated with the Peace River mainstem, and extensive admixture between the two groups.

ACTIVITY 1: BULL TROUT

Materials and Methods

A total of 881 Bull Trout genetic samples were collected from the LAA in 2023 (Table 1). Subsequent DNA extraction and quality control (QC) of all 881 samples followed Geraldes and Taylor (2020). A total of 257 of these samples were used in population assignments (Activity 1); the 624 samples collected in the LAA outside the mainstem of the Peace River (Table 1) were also extracted and will be genotyped in the future with the SNP panel developed (see below) to monitor demographic parameters in Bull Trout populations of the LAA (Activity 2).

Table 1. Bull Trout samples available for genetic work for Study Year 2023 and across all Study Years (2016-2023). Indicated are numbers of samples received (UBC), with DNA extracted (DNA) and genotyped at ancestry informative SNPs (TaqMan).

		Study Years 2016-2023			Study Year 2023 Only		
Watershed	**River/SectionID**	**UBC**	**DNA**	**TaqMan**	**UBC**	**DNA**	**TaqMan**
All	All	5467	5464	1845	881	881	257
Peace River	TUF	116	116	116	84	84	84
Peace River	Section 1	326	326	326	24	24	24
Peace River	Section 3	517	517	517	58	58	58
Peace River	Section 5	453	453	453	49	49	49
Peace River	Section 6	187	187	187	24	24	24
Peace River	Section 7	111	111	111	11	11	11
Peace River	Section 9	47	47	47	7	7	7
Halfway River	Chowade River	1521	1521	16	245	245	0
Halfway River	Colt Creek	48	48	13	8	8	0
Halfway River	Cypress Creek	1444	1444	13	241	241	0
Halfway River	Fiddes Creek	612	612	12	123	123	0
Halfway River	Halfway River	7	7	6	0	0	0
Halfway River	Halfway River	1	1	0	0	0	0
Halfway River	Kobes Creek	3	2	0	2	2	0
Halfway River	Turnoff Creek	40	40	4	0	0	0
Moberly River	Moberly River	11	11	8	0	0	0
Peace River	Dry Creek	10	10	10	0	0	0
Peace River	Maurice	13	11	6	5	5	0

We used six TaqMan™ assays designed from the GBS data as described by Geraldes and Taylor (2020) to efficiently genotype six ancestry informative SNPs (i.e., loci showing large levels of genetic differentiation between UP and DP genetic groups) and assign 257 Peace River Bull Trout samples collected in 2023 in the Peace River mainstem, including from the TUF (full methods in Geraldes and Taylor 2020 and 2021). Briefly, using the analytical procedure of Rannala and Mountain (1997) as implemented in the program GeneClass2 (Piry et al., 2004), samples were considered assigned to UP or DP if they had 95% or higher chance of being from one of those respective groups and considered unassigned if the chance of belonging to either group was lower than 95%.

Results

In 2023, 173 Bull Trout were collected in six sections of the Peace River and an additional 84 samples were collected from the TUF (Table 1). All 257 samples were successfully genotyped at six ancestry informative loci with TaqMan™ assays. As in previous years, most samples collected in the Peace River mainstem were assigned to the UP group (N=228, 88.7%; Table 2 and Appendix I), only 16 were assigned to the DP group (6.2% of all samples), and 13 could not be assigned to either group (i.e., assignment confidence was below 95%; 5.1% of all samples). Results for the 84 samples collected in the TUF were similar to the results from all samples, with 91.7% of samples assigned UP (N=77), 3.6% assigned DP (N=3) and 4.8% (N=4) could not be assigned to either group with at least 95% confidence (Table 2).

Table 2. Number of Bull Trout samples caught in the Peace River (PR) or the Temporary Upstream Fish Passage Facility (TUF) assigned (% of total) to the UP (upstream of the Project) or DP (downstream of the Project) groups with more than 95% confidence based on genotypes at six SNPs.

Location	Year	Total	UP	DP	Unassigned[1]
All Samples	2023	257	228 (88.7%)	16 (6.2%)	13 (5.1%)
	2016-2022	1500	1405 (93.7%)	49 (3.3%)	46 (3.1%)
	All years	1757	1633 (92.9%)	65 (3.7%)	59 (3.4%)
PR Section 1	2023	24	22 (91.7%)	1 (4.2%)	1 (4.2%)
	2016-2022	302	290 (96.0%)	7 (2.3%)	5 (1.7%)
	All years	326	312 (95.7%)	8 (2.5%)	6 (1.8%)
PR Section 3	2023	58	54 (93.1%)	1 (1.7%)	3 (5.2%)
	2016-2022	459	428 (93.2%)	13 (2.8%)	18 (3.9%)
	All years	517	482 (93.2%)	14 (2.7%)	21 (4.1%)
PR Section 5	2023	49	37 (75.5%)	9 (18.4%)	3 (6.1%)
	2016-2022	404	372 (92.1%)	16 (4.0%)	16 (4.0%)
	All years	453	409 (90.3%)	25 (5.5%)	19 (4.2%)
PR Section 6	2023	24	20 (83.3%)	2 (8.3%)	2 (8.3%)
	2016-2022	163	148 (90.8%)	12 (7.4%)	3 (1.8%)
	All years	187	168 (89.8%)	14 (7.5%)	5 (2.7%)
PR Section 7	2023	11	11 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	100	98 (98.0%)	0 (0.0%)	2 (2.0%)
	All years	111	109 (98.2%)	0 (0.0%)	2 (1.8%)
PR Section 9	2023	7	7 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	40	39 (97.5%)	1 (2.5%)	0 (0.0%)
	All years	47	46 (97.9%)	1 (2.1%)	0 (0.0%)
TUF	2023	84	77 (91.7%)	3 (3.6%)	4 (4.8%)
	2016-2022	32	30 (93.8%)	0 (0.0%)	2 (6.3%)
	All years	116	107 (92.2%)	3 (2.6%)	6 (5.2%)

[1]Samples that cannot be assigned to either UP or DP with over 95% confidence.

ACTIVITY 1: ARCTIC GRAYLING

Materials and Methods

A total of 144 Arctic Grayling samples were collected in 2023 from the LAA (Table 3). Subsequent DNA extraction and QC of all 98 samples collected in the Peace River itself, including 87 from the TUF, followed Geraldes and Taylor (2020). Forty-six samples collected in the Moberly River (Table 3) were catalogued but were not extracted or analyzed.

Table 3. Arctic Grayling samples available for genetic work for Study Year 2023 and across all Study Years (2016-2023). Indicated are numbers of samples received (UBC), with DNA extracted (DNA) and genotyped at ancestry informative SNPs (TaqMan).

		Study Years 2016-2023			Study Year 2023 Only		
Watershed	**River/SectionID**	**UBC**	**DNA**	**TaqMan**	**UBC**	**DNA**	**TaqMan**
All	All	852	616	487	144	98	98
Peace River	TUF	144	144	144	87	87	87
Peace River	Section 1	5	5	5	0	0	0
Peace River	Section 3	104	104	104	1	1	1
Peace River	Section 5	112	112	112	8	8	8
Peace River	Section 6	43	43	43	1	1	1
Peace River	Section 7	29	29	28	1	1	1
Peace River	Section 9	6	6	6	0	0	0
Beatton River	Beatton River	37	37	3	0	0	0
Beatton River	Bratland Creek	54	53	15	0	0	0
Beatton River	La Prise Creek	39	39	13	0	0	0
Beatton River	Unnamed Creek 1	1	1	1	0	0	0
Halfway River	Colt Creek	4	1	1	0	0	0
Halfway River	Kobes Creek	3	0	0	0	0	0
Moberly River	Moberly River	271	42	12	46	0	0

We used the 11 TaqMan™ assays designed from the GBS work described by Geraldes and Taylor (2021) to genotype the 78 Arctic Grayling samples collected in 2023 from the Peace River and to assign them to UP or DP, as well as to each of the

four spawning tributaries using the methods described above for Bull Trout (see also Geraldes and Taylor 2021).

Results

All 98 samples were successfully genotyped at 11 ancestry informative loci with TaqMan™ assays. All but three samples were assigned to the UP group (Table 4; Appendix II), only one was assigned DP and two could not be assigned to either group with at least 95% confidence. Of the 87 samples collected in the TUF, 85 (97.7%) were assigned UP and two (2.3%) could not be assigned to either group with at least 95% confidence.

Table 4. Number of Arctic Grayling samples collected in the Peace River (PR), including the Temporary Upstream Fish Passage Facility (TUF), and assigned (% of total) to the UP (upstream of the Project) or DP (downstream of the Project) groups with more than 95% confidence based on genotypes at 11 SNPs.

Location	Year	Total	UP	DP	Unassigned[1]
All Samples	2023	98	95 (96.9%)	1 (1.0%)	2 (2.0%)
	2016-2022	344	325 (94.5%)	15 (4.4%)	4 (1.2%)
	All years	442	420 (95.0%)	16 (3.6%)	6 (1.4%)
PR Section 1	2023	0	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	5	4 (80.0%)	1 (20.0%)	0 (0.0%)
	All years	5	4 (80.0%)	1 (20.0%)	0 (0.0%)
PR Section 3	2023	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	103	103 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	104	104 (100.0%)	0 (0.0%)	0 (0.0%)
PR Section 5	2023	8	8 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	104	104 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	112	112 (100.0%)	0 (0.0%)	0 (0.0%)
PR Section 6	2023	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	42	32 (76.2%)	7 (16.7%)	3 (7.1%)
	All years	43	33 (76.7%)	7 (16.3%)	3 (7.0%)

Location	Year	Total	UP	DP	Unassigned[1]
PR Section 7	2023	1	0 (0.0%)	1 (100.0%)	0 (0.0%)
	2016-2022	27	22 (81.5%)	4 (14.8%)	1 (3.7%)
	All years	28	22 (78.6%)	5 (17.9%)	1 (3.6%)
PR Section 9	2023	0	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	6	3 (50.0%)	3 (50.0%)	0 (0.0%)
	All years	6	3 (50.0%)	3 (50.0%)	0 (0.0%)
TUF	2023	87	85 (97.7%)	0 (0.0%)	2 (2.3%)
	2016-2022	57	57 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	144	142 (98.6%)	0 (0.0%)	2 (1.4%)

[1]Samples that cannot be assigned to either UP or DP with over 95% confidence

As in previous years, when samples are assigned to each of the four spawning tributaries, a larger proportion of samples cannot be assigned with more than 95% confidence to one population (N=8, 8.2%; Table 5 and Appendix II) compared to the proportion of samples that cannot be assigned as either UP or DP (N=2, 2.0%). One sample was assigned to the Pine River population group and 89 samples to the Moberly River (Table 5).

Table 5. Number of Arctic Grayling samples collected in the Peace River (PR), including the TUF (Temporary Upstream Fish Passage Facility), and assigned (% of total) to the Halfway River (HA), Moberly River (MO), Pine River (PI) and Beatton River (BE) with more than 95% confidence based on genotypes at 11 SNPs.

Location	Year	Total	HA	MO	PI	BE	Unassigned[1]
All Samples	2023	98	0 (0.0%)	89 (90.8%)	1 (1.0%)	0 (0.0%)	8 (8.2%)
	2016-2022	344	2 (0.6%)	304 (88.4%)	14 (4.1%)	0 (0.0%)	24 (7.0%)
	All years	442	2 (0.5%)	393 (88.9%)	15 (3.4%)	0 (0.0%)	32 (7.2%)
PR Section 1	2023	0	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	5	0 (0.0%)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)
	All years	5	0 (0.0%)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)
PR Section 3	2023	1	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Location	Year	Total	HA	MO	PI	BE	Unassigned[1]
	2016-2022	103	1 (1.0%)	96 (93.2%)	0 (0.0%)	0 (0.0%)	6 (5.8%)
	All years	104	1 (1.0%)	97 (93.3%)	0 (0.0%)	0 (0.0%)	6 (5.8%)
PR Section 5	2023	8	0 (0.0%)	8 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	104	0 (0.0%)	98 (94.2%)	0 (0.0%)	0 (0.0%)	6 (5.8%)
	All years	112	0 (0.0%)	106 (94.6%)	0 (0.0%)	0 (0.0%)	6 (5.4%)
PR Section 6	2023	1	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	42	0 (0.0%)	31 (73.8%)	6 (14.3%)	0 (0.0%)	5 (11.9%)
	All years	43	0 (0.0%)	32 (74.4%)	6 (14.0%)	0 (0.0%)	5 (11.6%)
PR Section 7	2023	1	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	27	0 (0.0%)	19 (70.4%)	4 (14.8%)	0 (0.0%)	4 (14.8%)
	All years	28	0 (0.0%)	19 (67.9%)	5 (17.9%)	0 (0.0%)	4 (14.3%)
PR Section 9	2023	0	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2016-2022	6	0 (0.0%)	2 (33.3%)	3 (50.0%)	0 (0.0%)	1 (16.7%)
	All years	6	0 (0.0%)	2 (33.3%)	3 (50.0%)	0 (0.0%)	1 (16.7%)
TUF	2023	87	0 (0.0%)	79 (90.8%)	0 (0.0%)	0 (0.0%)	8 (9.2%)
	2016-2022	57	1 (1.8%)	54 (94.7%)	0 (0.0%)	0 (0.0%)	2 (3.5%)
	All years	144	1 (0.7%)	133 (92.4%)	0 (0.0%)	0 (0.0%)	10 (6.9%)

[1]Samples that cannot be assigned to any single population with over 95% confidence.

ACTIVITY 1: RAINBOW TROUT

Materials and Methods

A total of 575 Rainbow Trout genetic samples were collected in 2023 from the LAA (Table 6). Subsequent DNA extraction and QC of all samples followed Geraldes and Taylor (2020). A total of 123 samples were collected in the Peace River mainstem and used in population assignments (Activity 1). The remaining 452 samples collected in the LAA outside the mainstem of the Peace River (Table 6) were also extracted and will be genotyped in the future with a GT-seq panel for demographic monitoring (Activity 2).

Table 6. Rainbow Trout samples available for genetic work for Study Year 2023 and across all Study Years (2016-2023). Indicated are numbers of samples received (UBC), with DNA extracted (DNA) and genotyped at ancestry informative SNPs (TaqMan).

		Study Years 2016-2023			Study Year 2023 Only		
Watershed	**River/SectionID**	**UBC**	**DNA**	**TaqMan**	**UBC**	**DNA**	**TaqMan**
All	All	2272	2272	899	575	575	123
Peace River	TUF	44	44	44	37	37	37
Peace River	Section 1	333	333	333	40	40	40
Peace River	Section 3	301	301	301	33	33	33
Peace River	Section 5	84	84	84	4	4	4
Peace River	Section 6	21	21	21	7	7	7
Peace River	Section 7	23	23	23	2	2	2
Peace River	Section 9	1	1	1	0	0	0
Halfway River	Chowade River	25	25	14	4	4	0
Halfway River	Colt Creek	302	302	12	69	69	0
Halfway River	Cypress Creek	41	41	14	8	8	0
Halfway River	Kobes Creek	400	400	11	84	84	0
Halfway River	Halfway River	1	1	0	0	0	0
Peace River	Dry Creek	7	7	7	0	0	0
Peace River	Farrell Creek	389	389	23	109	109	0
Peace River	Maurice Creek	300	300	11	178	178	0

We used the six TaqMan™ assays described by Geraldes and Taylor (2022) to genotype the 123 Rainbow Trout genetic samples collected in 2023 from the Peace

River, following the methods described above for Bull Trout and Arctic Grayling (see also Geraldes and Taylor 2022).

Results

In 2023, 123 Rainbow Trout were collected from the Peace River mainstem including 37 samples collected from the TUF (Table 6). All were successfully genotyped at six ancestry informative loci with TaqMan™ assays. Half of all samples were assigned to the UP group (N=62, 50.4%; Table 7; Appendix III), 45 samples were assigned to the DP group (36.6% of all samples) and 16 (13.0%) could not be assigned to either group with at least 95% confidence. These values are in close agreement with those of previous years as are assignment results for the 37 samples collected from the TUF (Table 7).

Table 7. Number of Rainbow Trout samples collected in the Peace River (PR), including the TUF (Temporary Upstream Fish Passage Facility), and assigned (% of total) to the UP (upstream of the Project) or DP (downstream of the Project) groups with more than 95% confidence based on genotypes at six SNPs.

Location	Year	Total	UP	DP	Unassigned[1]
All Peace River	2023	123	62 (50.4%)	45 (36.6%)	16 (13.0%)
	2018-2022	712	391 (54.9%)	197 (27.7%)	124 (17.4%)
	All years	835	453 (54.3%)	242 (29.0%)	140 (16.8%)
PR Section 1	2023	40	22 (55.0%)	12 (30.0%)	6 (15.0%)
	2018-2022	293	160 (54.6%)	69 (23.5%)	64 (21.8%)
	All years	333	182 (54.7%)	81 (24.3%)	70 (21.0%)
PR Section 3	2023	33	19 (57.6%)	10 (30.3%)	4 (12.1%)
	2018-2022	268	165 (61.6%)	62 (23.1%)	41 (15.3%)
	All years	301	184 (61.1%)	72 (23.9%)	45 (15.0%)
PR Section 5	2023	4	0 (0.0%)	3 (75.0%)	1 (25.0%)
	2018-2022	80	60 (75.0%)	32 (40.0%)	16 (20.0%)
	All years	112	60 (53.6%)	35 (31.3%)	17 (15.2%)

Location	Year	Total	UP	DP	Unassigned[1]
PR Section 6	2023	7	2 (28.6%)	5 (71.4%)	0 (0.0%)
	2018-2022	14	0 (0.0%)	14 (100.0%)	0 (0.0%)
	All years	21	2 (9.5%)	19 (90.5%)	0 (0.0%)
PR Section 7	2023	2	1 (50.0%)	1 (50.0%)	0 (0.0%)
	2018-2022	21	2 (9.5%)	18 (85.7%)	1 (4.8%)
	All years	23	3 (13.0%)	19 (82.6%)	1 (4.3%)
PR Section 9	2023	0	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2018-2022	1	0 (0.0%)	1 (100.0%)	0 (0.0%)
	All years	1	0 (0.0%)	1 (100.0%)	0 (0.0%)
TUF	2023	37	18 (48.6%)	14 (37.8%)	5 (13.5%)
	2018-2022	7	4 (57.1%)	1 (14.3%)	2 (28.6%)
	All years	44	22 (50.0%)	15 (34.1%)	7 (15.9%)

[1]Samples that cannot be assigned to either UP or DP with over 95% confidence.

ACTIVITY 2: BULL TROUT

Materials and Methods

For the demographic analyses within Activity 2, DNA extraction and quality control were performed for all Bull Trout collected in 2023 in the LAA outside of the Peace River mainstem (Table 1, N=624). Samples from the Peace River mainstem were extracted for Activity 1 and reported above.

In 2023, we developed a Genotyping-in-Thousands by sequencing (GT-seq, Campbell et al. 2014) SNP panel with 190 loci: one sex identification locus, 17 species specific loci, 15 UP/DP ancestry informative loci and 157 loci for demographic inference.

This panel was used to genotype a total of 3,610 samples (3,588 samples were Bull Trout from the LAA) throughout 38 plates (one of which was for panel development and testing). To test the accuracy of the genotyping panel, 27 samples were submitted in duplicate and the genotypes of the two replicates were compared.

We tested two programs/algorithms for estimating familial relationships across samples. Sequoia (Huisman, 2017) is an R package that implements a maximum likelihood algorithm for pedigree reconstruction which uses SNP data to establish sibship and parental relationships in large numbers of genotyped individuals. The algorithm takes advantage of a life history file with metadata on the sex of each sample and birth year, or an estimate of latest or earliest birth year, for the identification of parent/offspring relationships between pairs of individuals. Colony (Jones and Wang, 2010) is another program for parentage and sibship inference from multilocus genotype data that implements a different likelihood approach to simultaneously infer sibship and

parentage among genotyped individuals. Colony requires the inclusion of three sample lists, one for candidate fathers, one for candidate mothers and one for offspring.

We performed a trial run in Sequoia with all Bull Trout samples and loci to check the data quality and identify the know (27) and potentially unknown sample duplicates. After eliminating all duplicate samples identified (see results below) we kept 3,476 unique Bull Trout samples. We then removed from the dataset all species identification loci (17) and six loci with more than 40% missing genotypes and performed subsequent analysis with 3,395 samples with less than 70% missing data at 167 loci. The sex identification locus was not used in the genotype files but simply to identify the sex of each sample in the life history metadata file. Metadata on birth year was provided by Dustin Ford at WSP. This dataset was run for both Colony and Sequoia to compare the results which were qualitatively similar. The results presented herein are from the Sequoia program, a computationally more efficient algorithm.

Results

The genotyping rate across all 3,610 samples genotyped with the Bull Trout GT-seq panel averaged 90.6% (median is 95.8%). For 228 samples (6.3% of all samples) the genotyping rate was below 70%.

For the 27 samples included in duplicate, we compared the genotypes of the two replicates across all 190 loci and found only two discrepant genotypes (one for each of two replicate pairs) across 4,785 comparisons yielding an accuracy rate of 99.96%.

A trial run with Sequoia revealed that 80 samples in the dataset were included multiple times. Of those, 27 were included in duplicate to test the genotyping accuracy,

but the remaining 53 were not known to be present multiple times. For many of those, close inspection of the metadata revealed that they were in fact tissue samples collected form the same individual at different times because they had the same PIT tag number. A PIT tag is not inserted into all individuals and we therefore assume that the duplicate samples where PIT tag numbers were not present in more than one sample are also repeated samplings of the same fish. For subsequent analyses the genotypes from the different "replicates" were collapsed into a single "synthetic genotype" by keeping the genotypes at the replicate with the least missing data and substituting missing genotypes at particular loci by the genotype at that locus from a different replicate.

A final run of Sequoia was performed with 3,395 samples with less than 70% missing data at 166 loci (Appendix IV). This dataset contained 1,741 females, 1,638 males and 16 samples for which the sex locus did not produce a genotype (Appendix IV). Most samples included in the analysis were from the Halfway River watershed, specifically from the Chowade River and Cypress and Fiddes creeks, and from the Peace River (Table 8). Most samples were from sampling years 2016 to 2021, but a few were from earlier sampling years and some were from 2022 (Table 8).

Table 8. Sampling location and year of the 3395 Bull Trout samples used in the Sequoia parentage analysis. Sampling locations with less than 10 samples were collapsed into larger groups.

Watershed (Tributary/Sampling section)	**N_{all}**	**$N_{2006-2015}$**	**N_{2016}**	**N_{2017}**	**N_{2018}**	**N_{2019}**	**N_{2020}**	**N_{2021}**	**N_{2022}**
All (all)	3395	34	107	723	633	651	323	871	53
Halfway River (Chowade River)	962	0	30	217	212	252	50	201	0
Halfway River (Cypress Creek)	804	0	27	213	120	199	53	192	0
Halfway River (Fiddes Creek)	350	0	0	41	119	48	24	118	0
Halfway River (Turnoff Creek)	40	0	0	40	0	0	0	0	0
Halfway River (Colt Creek)	28	0	0	3	6	5	4	10	0
Halfway River (Halfway River)	13	3	10	0	0	0	0	0	0

Watershed (Tributary/Sampling section)	N_{all}	$N_{2006-2015}$	N_{2016}	N_{2017}	N_{2018}	N_{2019}	N_{2020}	N_{2021}	N_{2022}
Moberly River (Moberly River)	12	1	2	0	2	1	4	0	2
Pine River (all)	30	30	0	0	0	0	0	0	0
Peace River (Section 1)	274	0	8	52	50	46	41	31	46
Peace River (Section 3)	322	0	6	76	60	40	67	72	1
Peace River (Section 5)	296	0	7	35	27	19	36	172	0
Peace River (Section 6)	129	0	9	31	23	20	13	33	0
Peace River (Section 7)	75	0	3	8	10	18	12	24	0
Peace River (Section 9)	27	0	5	7	4	3	7	1	0
Peace River (Others)	18	0	0	0	0	0	12	2	4
Peace River (TUF)	15	0	0	0	0	0	0	15	0

Finally, over all sampling areas, 412 (about 12%; Table 9 and Figure 1) of the 3,391 samples for which an age at sampling could be estimated were of reproductive age (assuming age at first maturity ~ 5 years, COSEWIC 2012) and almost 40% of samples (N=1,339) were less than 1 year old.

Table 9. Fish age at sampling for each sampling location of the 3391 Bull Trout samples used in the Sequoia parentage analysis for which an age could be determined. Sampling locations with less than 10 samples were collapsed into larger groups.

Watershed (Tributary/Sampling section)	N_{all}	N_0	N_1	N_2	N_3	N_4	$N_{5\ or\ older}$
All (all)	3391	1339	871	347	238	184	412
Halfway River (Chowade River)	961	688	260	4	2	1	6
Halfway River (Cypress Creek)	804	487	286	15	4	3	9
Halfway River (Fiddes Creek)	349	137	203	9	0	0	0
Halfway River (Turnoff Creek)	40	11	29	0	0	0	0
Halfway River (Colt Creek)	28	6	11	10	1	0	0
Halfway River (Halfway River)	13	0	8	3	0	1	1
Moberly River (Moberly River)	11	0	1	5	2	3	0
Pine River (all)	30	10	13	2	3	2	0
Peace River (Section 1)	273	0	14	54	76	44	85
Peace River (Section 3)	322	0	21	116	66	48	71
Peace River (Section 5)	296	0	10	43	36	37	170
Peace River (Section 6)	129	0	5	41	25	25	33
Peace River (Section 7)	75	0	5	29	17	10	14
Peace River (Section 9)	27	0	2	7	6	8	4
Peace River (Others)	18	0	3	9	0	2	4

Watershed (Tributary/Sampling section)	N_{all}	N_o	N_1	N_2	N_3	N_4	$N_{5\ or\ older}$
Peace River (TUF)	15	0	0	0	0	0	15

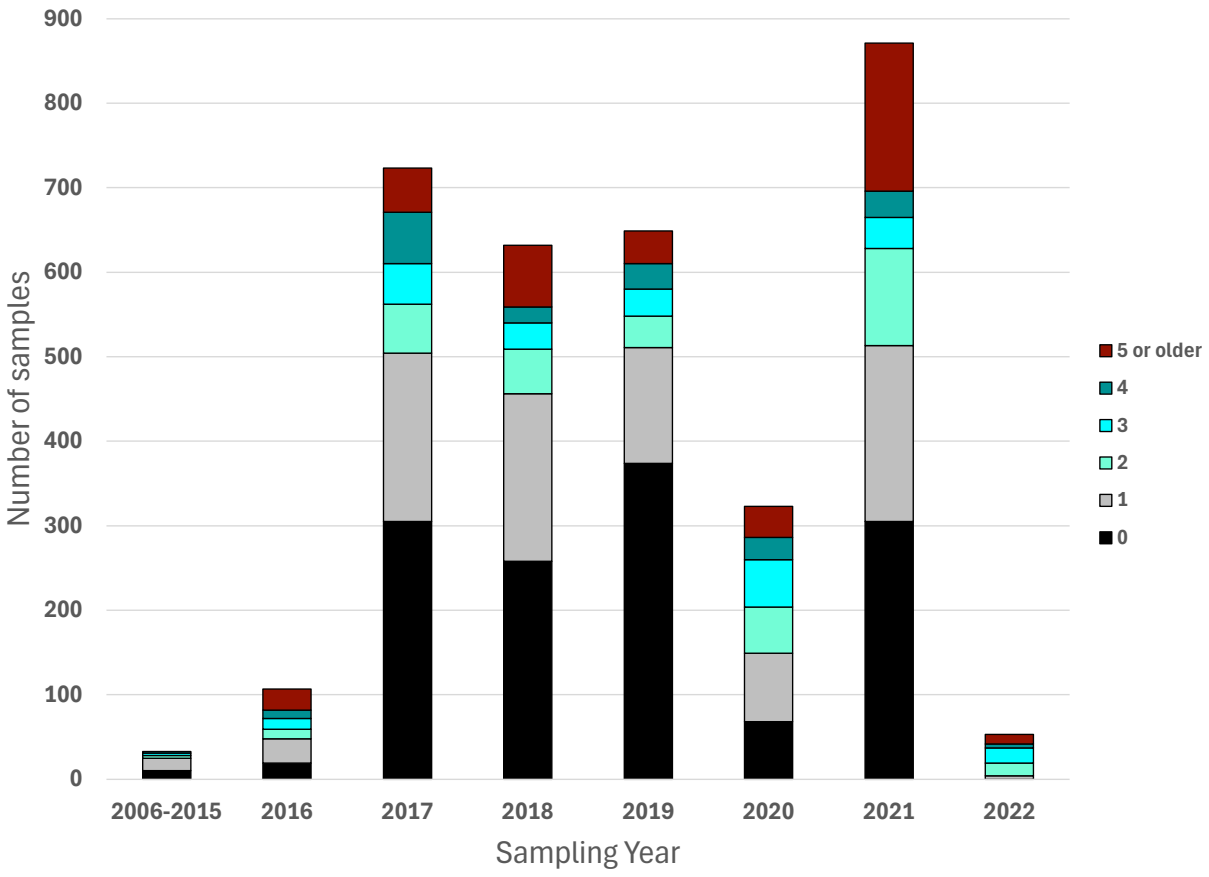

Figure 1. Age distribution of the Bull Trout samples in each sampling year included in the Sequoia analysis.

For 319 samples (9.4% of all samples) a female parent could be assigned (Table 10 and Appendix IV), for 157 samples a male parent could be assigned (4.6% of all samples), and for 9 samples both a female and male parent could be assigned (representing 7 families, 6 families had only one progeny identified each and one had three siblings identified; Figure 2). A total of 92 female parents were identified (Appendix IV) and they had an average of 3.5 progeny identified (maximum progeny

identified for a female parent in the dataset was 27, Figure 2). A total of 99 male parents were identified (Appendix IV) and they had an average of 1.6 progeny identified (maximum progeny identified for a male parent in the dataset was 15, Figure 2). The distribution of progeny for female parents was clearly left skewed with 58% of female parents having only one progeny identified and just 20% having 5 or more. This pattern was even more extreme for male parents with 84% having only one progeny identified and just 5% having 5 or more (Figure 2). For samples born before 2015, less than 4% had a female parent assigned, but for samples born in 2018 or later more than 10% of samples had a female parent assigned (Table 10) The same pattern can be seen for samples with a male parent assigned: the percentage was much higher in recent years (over 6% for samples born in 2018 and later) than in earlier years.

Table 10. Number of Bull Trout samples born each year used Sequoia parentage analysis (N), number of samples born each year that were assigned a female parent (Female Parent N(%)), and number of samples born each year that were assigned a male parent (Male Parent N(%)).

Birth Year	N	Female Parent N (%)	Male Parent N (%)
NA	3	0 (0.0%)	0 (0.0%)
2002-2011	215	0 (0.0%)	0 (0.0%)
2012	75	1 (1.3%)	0 (0.0%)
2013	133	3 (2.3%)	3 (2.3%)
2014	115	5 (4.3%)	0 (0.0%)
2015	187	6 (3.2%)	3 (1.6%)
2016	356	26 (7.3%)	3 (0.8%)
2017	630	43 (6.8%)	23 (3.7%)
2018	493	50 (10.1%)	44 (8.9%)
2019	588	64 (10.9%)	41 (7.0%)
2020	291	58 (19.9%)	19 (6.5%)
2021	309	63 (20.4%)	21 (6.8%)
All years	**3395**	**319 (9.4%)**	**157 (4.6%)**

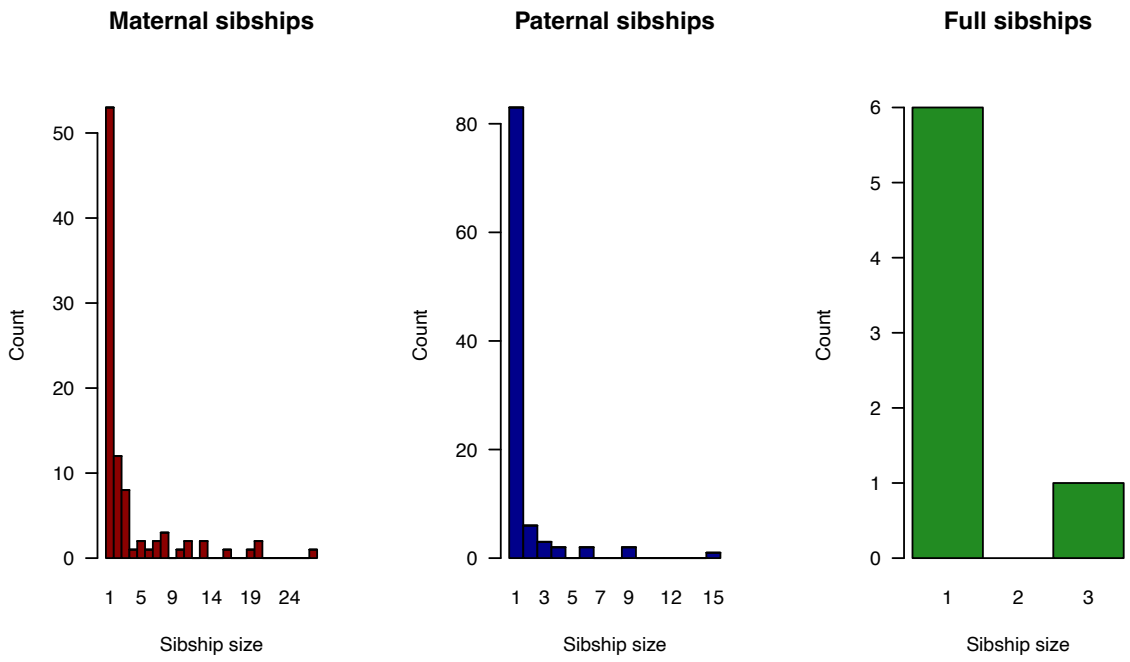

Figure 2. Distribution of number of progeny for female parents (in red), male parents (blue) and sibships with both female and male parents identified (in green) in the Sequoia analysis of 3,395 Bull Trout samples with less than 70% missing data at 166 SNP loci.

ACTIVITY 2: RAINBOW TROUT

Materials and Methods

For the demographic analyses within Activity 2, DNA extraction and quality control were performed for all Rainbow Trout collected in 2023 in the LAA outside of the Peace River mainstem (Table 6, N=452). Samples from the Peace River mainstem were extracted for Activity 1 and reported above.

Here we aimed to develop a medium sized SNP genotyping panel for Rainbow Trout that would serve three purposes: i) identify the sex of each sample, ii) assign ancestry to UP and DP genetic groups, and iii) perform demographic monitoring of the species in the LAA by estimating familial relationships among samples (relatedness).

For the above purposes we relied on previously generated data. The sex-identification locus, purpose i, was provided by Nathan Campbell of GTseek LLC (the company we collaborated with for Activity 2) from previous work. For the remaining purposes (ii, ancestry and iii, relatedness) we relied on GBS data generated in 2021 Geraldes and Taylor (2022) from two GBS libraries with 184 samples, of which 54 were from the three main tributaries of the Peace River in the LAA where Rainbow Trout is known to spawn (Halfway River, Moberly River and Pine River), 28 were from smaller tributaries of the Peace River (Lynx Creek, Maurice Creek and Farrell Creek), 12 were from the Dinosaur Reservoir (created by the Peace Canyon Dam) and 6 were from three strains that are known to have been used for restocking in the area (Blackwater River, Pennask Lake and Fraser Valley Domestic). The remaining 84 samples were not from the LAA and were not used here. Analysis of those data were reported in Geraldes and Taylor (2022) and revealed the existence of four main genetic groups: the Pine

River (located DP) and all six samples from hatchery strains, the Halfway River (located UP), the Moberly River (located UP), and Lynx Creek (located UP). We filtered our catalog of potential genetic variants (over 2.9 million variants in all 184 samples sequenced) to include only 49 samples from the LAA that had 90% or more ancestry in a single group and included only SNPs with average coverage of 8 or more reads, less than 30% missing genotypes (when filtered to include only genotypes with minimum genotype quality of 20 or higher) and where the rare allele had a minimum allele frequency of 10% or higher across the 49 samples (57,302 SNPs were retained). We further filtered the dataset to keep only 29,155 SNPs with less than 30% missing data in each of the four genetic groups and that were in linkage groups anchored to the Rainbow Trout chromosomes and not in unmapped scaffolds. Of those candidates, we selected for primer design 57 SNPs (Table 11) for the ancestry informative group (i.e., to allow for assignment of samples to UP and DP and potentially to each of the four genetic groups in the LAA) by including the loci we have been using in our TaqMan assays (Geraldes and Taylor 2022) and choosing 51 additional loci that had Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984) between any pair of populations higher than 0.8 and were at least 25 M bp from other SNPs in the same category. For the demographic inference group, i.e., to estimate familial relationships among samples (relatedness), SNPs that are common throughout the entire LAA and do not show pronounced allelic frequency differences among population groups are most useful. We selected for primer design for the relatedness group 572 SNPs (Table 11) that had minor allele frequency in each population of at least 10%, Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984) between any pair of populations lower than 0.2 and were

at least 2 M bp from any other SNP in that group. On average there were 20 candidate loci per chromosome and a range of 8-18 loci per chromosome for the relatedness group and 0-2 for the ancestry group (Table 11).

This pool of candidate loci was sent to GTseek LLC for primer design with an optimized pipeline that screens primers for their ability to amplify unique on-target regions of the genome and that through in-silico testing do not interfere with other primers in the mix. The selected primer pairs then underwent four rounds of testing through PCR and sequencing with a set of 55 unique samples from the LAA (40 of which were sent in duplicate). The sequence data generated for each round of testing were analyzed in collaboration with Nathan Campbell of GTseek LLC following a pipeline used in similar projects. This included genotype calling for each sample where it was assumed that a heterozygote genotype would have close to 50:50 reads supporting each of the two alleles and that homozygous genotypes would have most reads supporting only one allele. In each round of testing, loci were dropped if they a) did not amplify, b) interfered too much with the performance of other primers, c) amplified off-target, d) amplified multiple loci, e) amplified too much and generated a disproportionate number of reads in the sequencing run, and f) failed to discriminate the two alleles at the locus. The resulting inferred genotypes from the fourth and final round of testing were used to test the quality of the data generated by the final panel in three ways. First, for the 40 samples for which we included duplicates in the GT-seq test plate, we compared the genotype calls from each to determine the repeatability of the genotypes. Second, we compared the genotype at each locus and sample generated by GT-seq and GBS for all 54 samples for which we had both data sources. Third, we

performed a principal components analyses (PCA) with the R package SNPrelate (Zheng et al. 2012) to summarize the genotype data and verify that the samples clustered in genotype space in a similar manner to the PCA obtained with the GBS dataset.

Results

From the 629 candidate loci for primer design and in-silico testing (57 in the ancestry group and 572 in the relatedness group), a total of 371 primer pairs were designed and selected for PCR and sequencing testing (Table 11) plus one previously developed sex identification primer pair. Primer pairs targeted loci in all Rainbow Trout chromosomes. Four rounds of testing with a set of 55 unique samples led to the retention of 219 loci (Table 11) distributed across all but one (Chromosome 31) chromosomes (average of 7 loci per chromosome). The final set of 219 loci included: one sex identification locus, 28 ancestry loci 190 relatedness loci (Table 11).

Table 11. Genomic distribution of loci in the Rainbow Trout GT-seq panel. For each chromosome (Chr.) we report the number of loci selected for primer design (Candidates), loci with primer designs (Primer Design) and the final loci in the Rainbow Trout GT-seq panel (GT-seq Panel) for the ancestry and the relatedness groups. The sex identification locus was provided by Nate Campbell of GTseek LLC and is not included here.

		Candidates		Primer Design		GT-seq Panel	
Chr.	**Length (bp)**	**Ancestry**	**Relatedness**	**Ancestry**	**Relatedness**	**Ancestry**	**Relatedness**
1	95,772,356	3	23	3	12	3	7
2	103,806,877	2	27	1	13	1	7
3	85,311,031	2	24	2	14	2	9
4	46,841,314	1	11	1	5	1	3
5	100,798,064	3	24	1	15	1	8
6	101,096,859	3	29	1	18	1	11
7	90,918,291	3	21	2	12	1	6
8	91,622,588	2	21	2	14	1	8
9	79,455,637	3	19	3	9	3	5
10	87,811,138	2	23	1	13	0	6
11	86,280,908	3	22	1	17	0	11
12	102,853,256	2	29	1	13	1	5

		Candidates		Primer Design		GT-seq Panel	
Chr.	**Length (bp)**	**Ancestry**	**Relatedness**	**Ancestry**	**Relatedness**	**Ancestry**	**Relatedness**
13	73,332,040	1	20	1	12	1	3
14	43,310,081	1	11	0	8	0	7
15	81,569,517	1	24	1	22	1	13
16	78,541,548	3	22	0	11	0	6
17	95,212,422	1	25	0	15	0	7
18	74,657,750	2	22	2	15	1	6
19	67,237,266	2	19	1	8	1	3
20	46,616,863	1	10	1	5	0	4
21	64,935,962	1	19	1	12	1	8
22	52,474,311	1	14	1	6	1	6
23	62,880,378	2	15	1	7	1	3
24	45,930,806	1	12	1	5	1	5
25	47,542,702	1	12	1	8	1	5
26	51,113,553	1	12	1	10	1	3
27	51,556,237	2	12	0	10	0	8
28	43,716,683	1	8	1	4	1	4
30	46,327,593	2	8	2	3	1	1
31	44,108,611	2	9	0	2	0	0
32	41,837,469	2	11	2	7	1	5
Y	47,748,341	0	14	0	10	0	7
All		**57**	**572**	**36**	**335**	**28**	**190**

For 94 out of the 95 samples included in the test plate, we were able to genotype 85% or more of the 219 loci in the GT-seq panel (one sample had a genotyping rate of 28% only but its duplicate had a genotyping rate of 96%; DNA quantity likely caused the low genotyping rate for one of the replicates) for an average genotyping rate of 96.1%. Considering the 55 unique samples included in the test plate (for each sample with a duplicate we chose the replicate with the highest genotyping rate) the minimum genotyping rate was 91.8% and the average was 97.1%. Average genotyping rate per locus was 97.1%. Only 20 out of 219 loci had a genotyping rate below 90% and 170 out of 219 loci had no missing genotypes.

Comparing the genotypes across duplicates for the 40 samples for which we included duplicates in the GT-seq test plate, we only observed 2 mismatching genotypes out of 8,251 genotypes compared (mismatch rate is 0.02%). The two

mismatches occurred at different loci but always involved the duplicate pair of samples in which one replicate had low overall genotyping rate and in both cases one replicate was homozygous for one allele and the other replicate was heterozygous for that allele and the alternative allele.

We also compared the genotypes at all loci for the 54 samples for which genotypes were available from the GBS dataset and the GT-seq dataset. When the GBS data is filtered so that genotypes with genotype quality below 20 are set to missing data, we found an overall concordance rate between datasets of 96.4% (only 357 genotypes differed out of 10,636 genotypes compared). The concordance between datasets was above 98% or higher for half the loci and was below 90% at only 13 out of the 218 loci compared. When those 13 loci are eliminated, the concordance rate between datasets is 97.7%.

Similar to a PCA with the GBS results (Geraldes and Taylor 2022), a PCA analysis of the GT-seq dataset (219 loci and 55 unique samples), separates along the first PC (explaining 10.5% of the variation in the data) Rainbow Trout samples collected DP (from the Pine River) from samples collected UP (from the Halfway River, Moberly River, Lynx Creek, Farrell Creek and the Dinosaur Reservoir; Figure 2). The second PC (explaining 5.7% of the variation in the data) mostly separates samples from the Halfway River from other samples collected UP. The Lynx Creek samples separate along the third PC axis and the Moberly River samples along the fourth PC axis (not shown).

The sex-locus produced a genotype for 94 of the 95 samples included (genotyping rate of 99%, the sample that failed at this locus only produced genotypes at

28% of all other loci). All duplicates had the same genotype at the sex locus, and 60% of samples had a female genotype (homozygous, i.e. XX) and 40% of samples had a male genotype (heterozygous, i.e. XY).

The ability of the Rainbow Trout GT-seq SNP panel to estimate parentage relationships will be evaluated later this year when all samples collected in the LAA from 2018 to 2024 are genotyped.

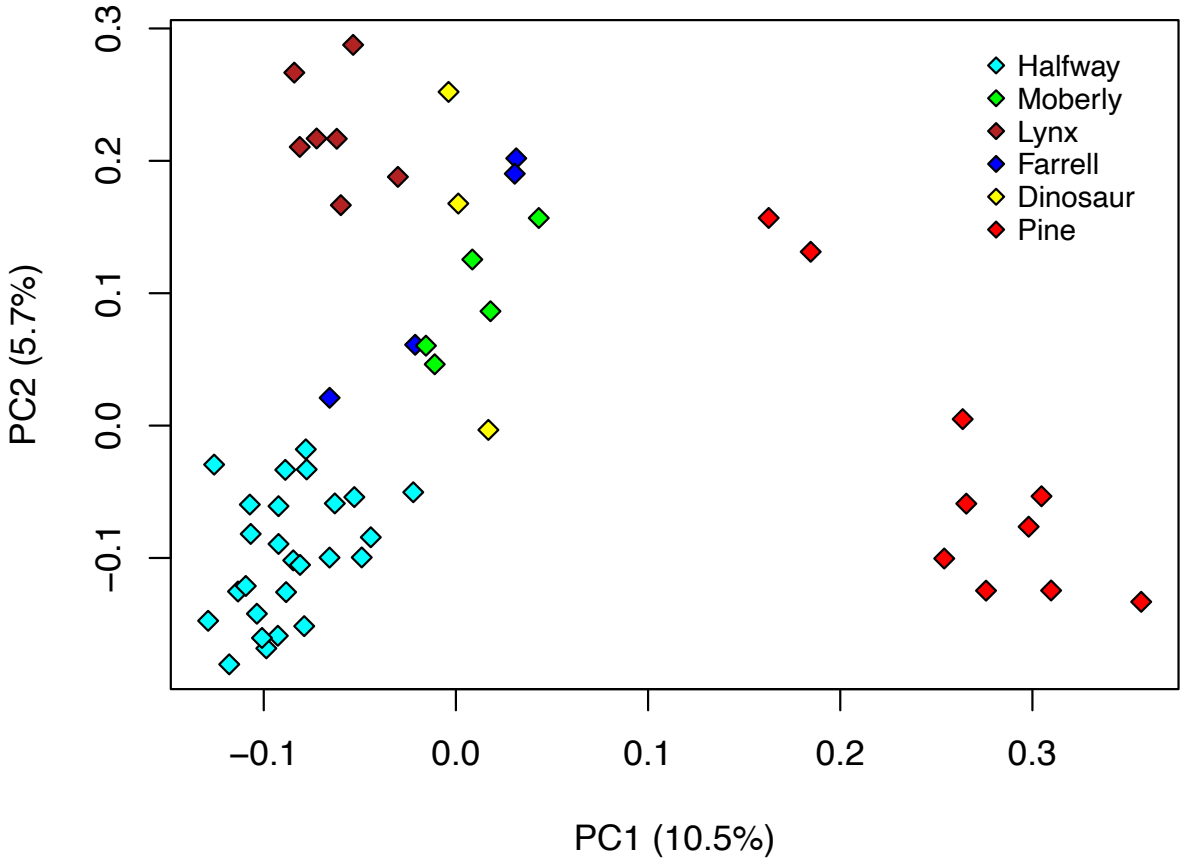

Figure 3. The first two Principal Components of a PCA with the Rainbow Trout GT-seq panel generated genotype data (219 loci). Samples are plotted as diamonds, the colour of which indicate their sampling sites as indicated by the inset: Halfway River (N=26, green), Moberly River (N=5, cyan), Lynx Creek (N=7, dark red), Farrell Creek (N=4, dark blue) and Dinosaur Reservoir (N=3, yellow), all located UP, and Pine River (N=10, red) located DP. The amount of variation explained by each component is shown along each axis.

ACTIVITY 3: Redside Shiner

Materials and Methods

The data for analysis of population structure of Redside Shiner was generated in 2022 in a large genotyping-by-sequencing (GBS) library (Geraldes and Taylor 2023) that included samples of the three non-game fish species (Slimy Sculpin, Redside Shiner and Longnose Dace).

Samples

DNA extraction and QC of all 226 samples of Redside Shiner sampled up to 2020 in the LAA and received at UBC for genetic analysis followed Geraldes and Taylor (2020). Eight samples from the Moberly River collected in 2019 failed QC and were not selected for DNA sequencing (Table 12).

Table 12. Number of samples of Redside Shiner collected in the LAA for which DNA was extracted (UBC), number of samples used for sequencing (GBS), number of samples used for trial parameter search for SNP calling (Trial 43), and number of samples used in population genetic analysis (Pop212).

River/Section(ID)	Year	UBC	GBS	Trial43	Pop212
All	All	226	218	43	212
Lynx Creek (LX)	2006	11	11	4	11
Moberly River (MO)	2018	20	20	4	20
Moberly River (MO)	2019	23	15	4	15
Moberly River (MO)	2020	27	27	4	26
Peace River/Section 3 (S3)	2018	20	20	4	20
Peace River/Section 3 (S3)	2019	20	20	4	20
Peace River/Section 3 (S3)	2020	25	25	4	25
Peace River/Section 5 (S5)	2018	23	23	4	23
Peace River/Section 5 (S5)	2019	21	21	4	20
Peace River/Section 5 (S5)	2020	33	33	4	29
Peace River/Section 7 (S7)	2020	3	3	3	3

Sequencing, read mapping and variant identification

We used reduced representation genomic DNA sequencing with genotyping-by-sequencing (GBS) for sequence data generation and genetic variant discovery (single

nucleotide polymorphisms, SNPs). Detailed descriptions of library preparation and sequencing were reported by Geraldes and Taylor (2023). The DNA library was sequenced using an Illumina NovaSeq 6000 S4 platform with 150 bp paired end reads at the McGill University and Génome Québec Innovation Centre in 2022.

For the pooled DNA libraries sequenced in 2022, we used dual barcoding, i.e. each sample is barcoded with a combination of a well and a plate barcode. Reads were demultiplexed and assigned to individual samples with the function “process_radtags” from the STACKS v2.5 pipeline (Catchen et al. 2013) by analysing the two barcodes present, one in each of the two paired reads. Six of the 218 samples used generated less than 1 M reads and were dropped from further analysis. One sample was from the Moberly River (2020) and five were from Section 5 of the Peace River (four from 2020 and one from 2019, Table 12 and Appendix V). The resulting reads for the remaining 213 samples were trimmed with Trimmomatic-0.39 (Bolger et al. 2014) with options TRAILING:3, SLIDINGWINDOW:4:10, MINLEN:30.

Unlike for the previously studied species in this project (Bull Trout, Rainbow Trout, Arctic Grayling, Slimy Sculpin and Longnose Dace), there is no reference genome available for Redside Shiner to map the demultiplexed reads and identify genetic polymorphisms, so we used a reference free, de novo assembly pipeline - denovo_map.pl - from STACKS2 (Rochette et al. 2019) available at (https://catchenlab.life.illinois.edu/stacks/comp/denovo_map.php). To do so, we first followed the recommendations of Paris et al. (2017) to determine appropriate values for the parameters in the pipeline using a subset of 43 samples from all sampling regions (Trial 43 dataset, Table 12). From ustacks, we varied the m parameter (minimum

number of reads to form a putative allele) between 2 and 6, and the M parameter (number of mismatches between putative alleles to merge them into a putative locus) from 1 to 4, and from cstacks, we varied the n parameter (number of mismatches allowed between putative loci during construction of the catalog) between 1 and 5. We evaluated the different parameter combinations using the same approaches as described below for the complete dataset. Inspection of the results from a PCA, and Admixture analysis, revealed that only parameter combinations that included n=1 made biological sense, i.e., they tended to reflect patterns observed in the LAA for Slimy Sculpin and Longnose Dace that employed reference genomes, and temporal samples within sites tended to be similar to each other. For the remaining parameter combinations, we followed the two recommendations of Paris et al. (2017): i) keeping n=M plus or minus one (in our case as we chose n=1, we only evaluated M=1 and M=2) and ii) the 80% polymorphic(r80) loci rule. The number of polymorphic loci present in 80% of the samples decreased as m increased from 2 to 6, and for each m value, using M=1 or M=2 changed r80 by less than 9%.

To generate the polymorphism data for further analysis with the 212 samples that had more than 1 M reads generated we used the pipeline's default parameter combination of m=3, M=2, and n=1 after examining alternative values and verifying that the default values produced the most consistent results.

Analyses of Population Structure in Redside Shiner

After polymorphism identification we first used a custom script (Owens et al. 2016) to eliminate variants that showed an observed heterozygosity of 0.6 or higher across all retained samples, as these are likely the result of mapping to paralogous regions of the

genome and then, using VCFtools v0.1.11 (Danecek et al. 2011), we filtered our polymorphism file further to arrive at a set of high-quality SNPs to form the basis of subsequent population genetic analysis. Namely, we eliminated: i) insertion/deletion polymorphisms to retain only SNPs, ii) SNPs with more than two alleles, iii) SNPs with genotype quality below 10 (these have a higher than 10% chance of being incorrect genotypes), iv) loci with missing genotypes in more than 30% of samples, and v) low frequency SNPs (SNPs present at a frequency below 5%). We then kept only one SNP from each stack to remove SNPs that were in close linkage disequilibrium (LD) with other SNPs in the dataset as they are not independent data points.

We used two complementary and independent approaches to infer patterns of population structure in Redside Shiner. In the first approach, we ordinated the SNP dataset in “genotype space” using principal components analyses (PCA) with the R package SNPrelate (Zheng et al. 2012) to summarize genetic variation into up to ten successive orthogonal principal components (PCs). In the second approach, we used the program Admixture v1.3.0 (Alexander et al. 2009) to estimate ancestry proportions for each fish. Admixture is a program that models the probability of the observed genotypes using ancestry proportions and population allele frequencies with a maximum likelihood approach to determine the most likely number of genetic groups (i.e., K). In this analysis, individual fish can be composed of more than one of these K genetic groups and the analysis provides an estimate of the proportion of each fish’s genome composed of each of the K groups (i.e., its admixture proportions). To assess the consistency of the results we ran five replicates of Admixture for each K from one to

five and terminated each run when the difference in log-likelihood between successive iterations fell below 1 x 10^{-9}.

Finally, we used VCFtools (Danecek et al. 2011) to estimate per locus Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984) to quantify levels of genetic differentiation between sampling regions and between sampling years within sampling regions. Sampling Section 7 in the Peace River mainstem was excluded from these analyses as only three samples were available which precludes accurate estimation of allele frequencies. This analysis was performed for all SNPs that remained after filtering for population genetics analysis but prior to LD pruning.

Results

Across all 218 samples used for GBS an average of 8.6 M paired reads were generated (range 0.02-15.05; Appendix V). After eliminating 6 samples with less than 1 M paired reads (range 0.02-0.61 M paired reads), the remaining 212 samples had an average of 8.9 M paired reads (minimum 1.6 M reads). We identified 1,487,915 putative genetic variants across these 212 Redside Shiner samples. After filtering the dataset for population genetic analyses, we kept 60,431 SNPs for F_{ST} estimation and 30,943 SNPs after keeping just one SNP per stack for PCA and Admixture analyses.

Results from a PCA (Figure 3; Appendix V) revealed some separation of samples from the Moberly River and all other samples across the first axis of variation (PC1, explaining 4.4 % of variation in the data). The second axis, PC2, explained less than 1% of the variation in the dataset and did not separate samples according to either sampling location or sampling year.

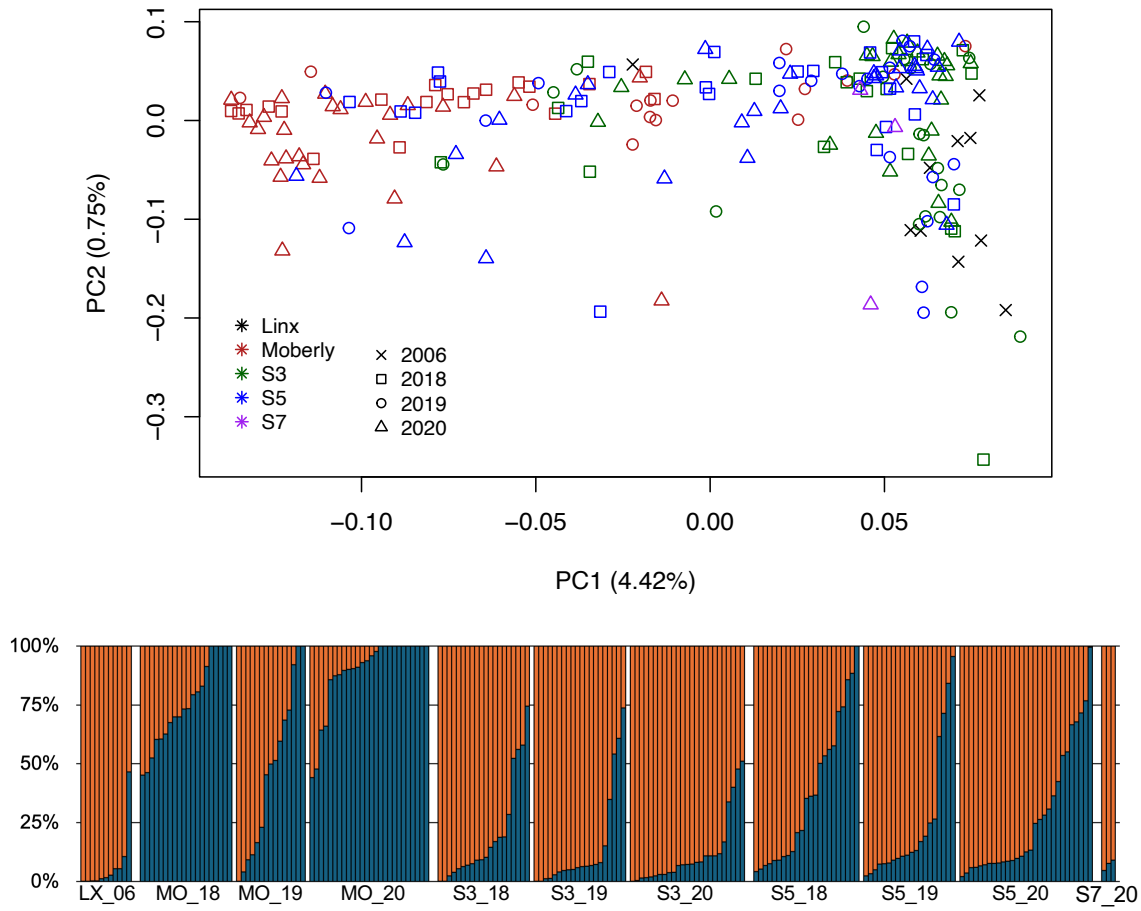

Figure 4. Population structure of Redside Shiner inferred with the Pop212 dataset of 30,943 SNPs unlinked SNPs with minor allele frequency of at least 5%. Samples were collected in Lynx Creek in 2016 (LX_06, N=11), the Moberly River in 2018 (MO_18, N=20), in 2019 (MO_19, N=15) and in 2020 (MO_20, N=26), in the Peace River Section 3 in 2018 (S3_18, N=20), in 2019 (S3_19, N=20) and in 2020 (S3_20, N=12), Section 5 in 2018 (S5_18, N=23), in 2019 (S5_19, N=20) and in 2020 (S5_20, N=29), and Section 7 in 2020 (S7_20, N=3). The top panel shows the position of each sample along the first two axes of variation of a Principal Components Analysis. The sampling location is indicated by different colours (black for Lynx Creek, red for the Moberly River, green for Section 3, blue for Section 5 and purple for Section 7) and the sampling year is indicated by the different symbols (cross for 2006, square for 2018, circle for 2019 and triangle for 2020). The bottom panel shows the results of an Admixture analysis with two genetic groups. Each column represents the genotype of an individual fish, and the different colours represent the proportion of the genome of each fish that is assigned to each genetic (blue for the Moberly River genetic group and orange Peace River and Lynx Creek genetic group).

An Admixture analysis with two genetic groups (K=2) was the best fit to the data (had the lowest cross validation error, CVE) and like the PCA indicated strong differences in the proportion of the two genetic groups in fish from the Moberly River and sites within the mainstem Peace River and Lynx Creek (Figure 3; Appendix V). On

average, samples collected in the Moberly River had 74.5% ancestry in one genetic group (in dark blue in Figure 3) while samples collected in Lynx Creek had 6.7% and samples collected in the Peace mainstem had 22.8% ancestry in that same group. This pattern is quantified in estimates of genetic differentiation between sampling regions with Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984). Weighted average F_{ST} between the Moberly River and the other sampling regions (Table 13) is one order of magnitude higher than between the other sampling regions suggesting some restriction of gene flow between the Moberly and Peace Rivers but not within the Peace River itself. Estimates of genetic differentiation between years within sampling regions were close to zero (Table 14), except for the Moberly River where weighted average F_{ST} between 2019 and the other two sampling years (2018 and 2020) were similar to estimates between the Moberly and other sampling regions (Table 14). While in 2019 just 47% of samples collected in the Moberly River had more than 50% ancestry in the genetic group associated with the Moberly River (in dark blue in Figure 3), that value was 90% in 2019 and 92% in 2020.

Table 13. Weighted average Weir and Cockerham's F_{ST} between sampling regions of Redside Shiner in the LAA.

	Moberly	Lynx	Peace S3
Lynx	0.052		
Peace S3	0.036	0.003	
Peace S5	0.021	0.008	0.002

One key difference in the sampling in the Moberly River across years is that in 2019 all but three fish were sampled very close to the confluence with the Peace River mainstem, while in 2018 and 2020 all samples were collected at least 12 km further upstream in the Moberly River (Figure 5).

Table 14. Weighted average Weir and Cockerham's F_{ST} between sampling years (within sampling regions) of Redside Shiner in the LAA.

Year 1	Year 2	F_{ST}
Moberly_18	Moberly_19	0.0126
Moberly_18	Moberly_20	0.0017
Moberly_19	Moberly_20	0.0262
Peace S3_18	Peace S3_19	0.0000
Peace S3_18	Peace S3_20	0.0002
Peace S3_19	Peace S3_20	-0.0001
Peace S5_18	Peace S5_19	0.0000
Peace S5_18	Peace S5_20	0.0002
Peace S5_19	Peace S5_20	-0.0009

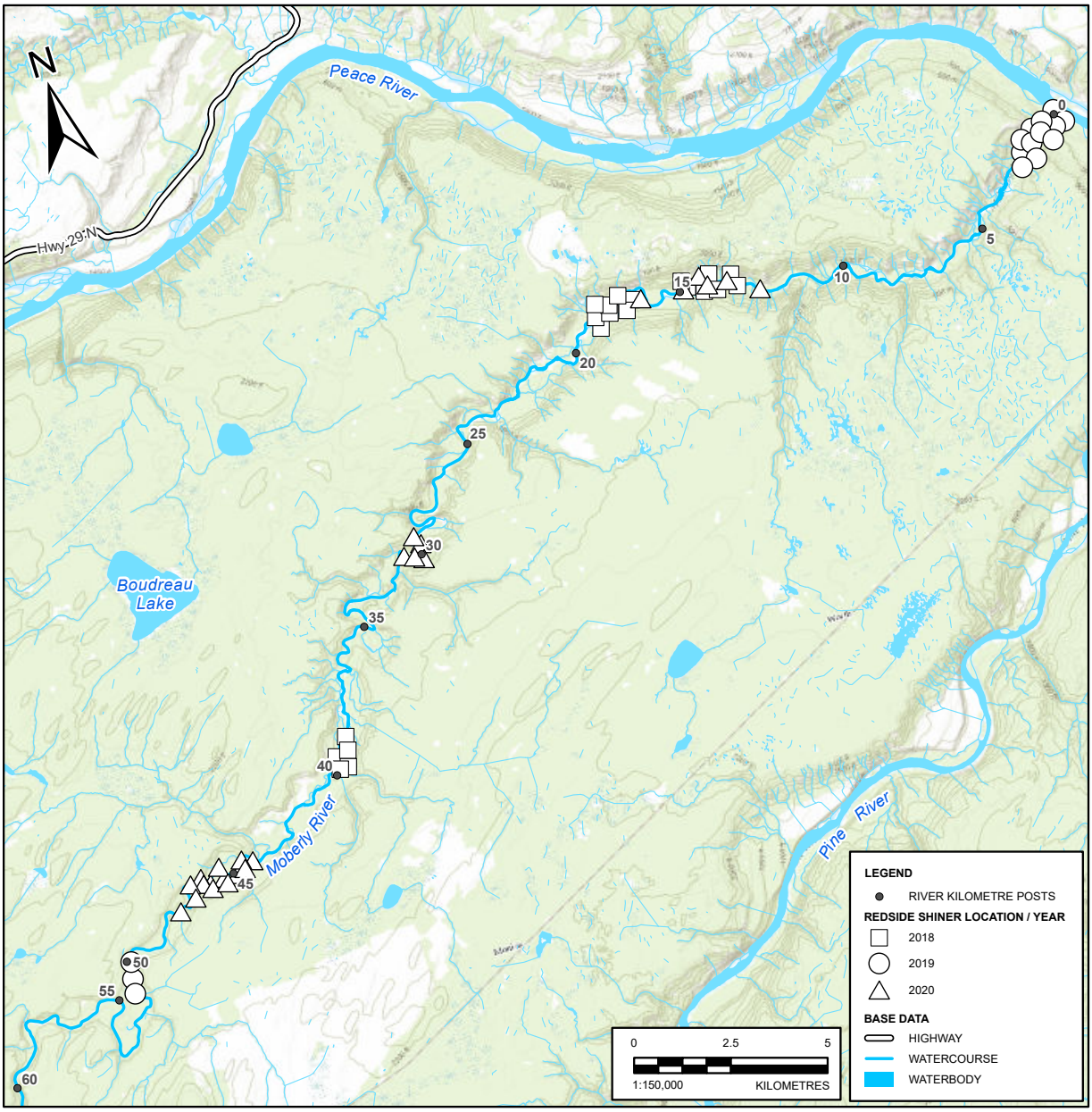

Figure 5. Sampling locations of Redside Shiner in the Moberly River in years 2018 (squares), 2019 (circles), and 2020 (triangles). Numbers next to black dots along the river indicate distance in kilometers (km) from the confluence of the Moberly and Peace Rivers. One Redside Shiner sample was captured at 117 Km from the confluence of the Moberly and Peace River and was omitted from the figure.

DISCUSSION

Our 2024 work continues to demonstrate that most Bull Trout, Arctic Grayling, and Rainbow Trout samples collected from various sections of the Peace River mainstem and the TUF originate from spawning tributaries upstream of the Project. This fundamental result remains most pronounced for Bull Trout and Arctic Grayling and less for Rainbow Trout. Similarly, for 2023 samples, the Halfway and Moberly rivers are key tributaries for the production of Bull Trout and Arctic Grayling, respectively, while the assignment of Rainbow Trout to UP or DP continued to produce the highest percentage of unassigned fish (see discussion in Geraldes and Taylor 2022).

The Bull Trout GT-Seq panel was successful in identifying either female or male parents for hundreds of samples, and in nine samples, both the male and female parents for individual offspring. Most individual putative parents were associated with a relatively small number of offspring produced (< 5) which is probably to be expected given finite sampling of progeny and reproductive adults and where spawning population size may be 200 or more in the Halfway system (Putt et al., 2024). A similar pattern of left-skewed offspring production in Bull Trout (i.e., most parents were assigned few offspring) was reported by Adams and Bernall (2021) in two streams of the Clark Fork River in Montana. In the Montana study, however, a much higher percentage of juveniles (69% - 100% of 143 and 341) were assigned to at least one parent. The higher percentage of fish assigned to at least one parent is likely due to a more complete sampling of adults in the Montana systems (through multiple fish passage facilities, weirs, and by electrofishing). Further, we found that fish that were inferred to have been born later in our study had a higher chance of being assigned to

at least one parent. This likely occurred because: (i) most fish sampled were very young (ages 0 and 1), (ii) most samples were obtained in 2017 or later, and (iii) Bull Trout typically mature at age 4 or older (McPhail 2007). Most of our genotyped samples were collected between 2017 and 2021 and, thus, would have matured in 2020 or later (assuming maturity at age 4 or 5; COSEWIC 2012). Consequently, it is likely that we will be able to assign female and males parents to an even larger percentage of samples in future years as the majority of genotyped samples to date become reproductively mature.

Our work this year completed the analysis of population structure in three non-game, small-bodied fishes with the analysis of Redside Shiner in the LAA. Although the spatial sampling regime was different for the three small-bodied fishes, each showed, to varying degrees, a distinctive population within the Moberly River when compared to the mainstem Peace River samples, with the Longnose Dace showing the least pronounced differentiation.

Finally, although the spatial sampling regime was different for the three small-bodied fishes, each showed, to varying degrees, evidence of a distinctive population within the Moberly River when compared to samples collected from the Peace River. The level of differentiation between fish from the Moberly River and the Peace River is perhaps not surprising given the lithophilic behaviour of at least Slimy Sculpin and Longnose Dace and that such habits may constrain dispersal abilities in freshwater fishes (Leavy and Bonner 2009; Comte and Olden 2018; Gray et al. 2018; Shelley et al. 2021; Zhbiden et al. 2023). By contrast, the Redside Shiner is a mid-water inhabitant and is presumably more mobile that either the Slimy Sculpin or Longnose Dace. Indeed,

between 2020 and 2024, BC Hydro passed 29,544 Redside Shiner, that had entered the TUF, upstream of Site C. Over the same time period, however, the combined total of Slimy Sculpin and Longnose Dace that entered the TUF was fewer than 20 fish (BC Hydro unpubl. data). Thus, despite some evidence of extensive movements of Redside Shiner, they still showed modest differentiation between the Moberly River and the Peace River (indeed, a higher level than shown by Longnose Dace). Interestingly, Slimy Sculpin, like many sculpins, may have planktonic larvae (McPhail 2007) which could facilitate downstream dispersal and gene flow among sites. Such behaviour is consistent with the lack of subdivision observed in Slimy Sculpin among sections of the Peace River (see below), but contrasts with the striking differentiation between the Moberly River and Peace River. Given the distinct nature of the habitats between Moberly and Peace rivers' fish habitats (Mainstream Aquatics 2012), it is possible that natural selection may favour reduced downstream dispersal (e.g., via positive rheotaxis) in the three species of small-bodied fishes from Moberly River, especially Slimy Sculpin. Such differences occur in different populations of various salmonids (e.g., Raleigh 1971; Northcote 1981; Kaya 1989; Taylor 1988). It is also possible that Moberly River species do disperse widely downstream, but that such migrants are selected against in the Peace River environment.

For Slimy Sculpin, Redside Shiner, and Longnose Dace, sites within the mainstem Peace River that spanned the dam site showed little to no differentiation from one another. Such low differentiation suggests that gene flow amongst sites within the mainstem Peace River is substantial and is consistent with low differentiation within these species sampled over comparable or greater distances in other areas (cf. Ruskey

and Taylor 2016; Crispo et al. 2017; Euclide et al. 2018; Gray et al. 2018). Unlike the sampling of the salmonid species, however, we did not collect fish from known breeding localities for the three small-bodied species which may have constrained attempts to resolve population structure within the mainstem Peace River.

Collectively, the results across Slimy Sculpin, Redside Shiner, and Longnose Dace and the three game species (e.g., Geraldes and Taylor 2020, 2021, 2022) indicate that the mainstem Peace River habitats spanning the dam site are important as a movement corridor for these species (see also AMEC and LGL 2008, 2009; Taylor et al. 2014). Our results therefore suggest that efforts to maintain connectivity for these fish, using the existing fish passage program (see BC Hydro 2020; Bradford 2022) will continue to be an important aspect of mitigation during the construction and operation of the Project. In sum and at the largest comparative scale, all species demonstrated a degree of population subdivision reflecting localized use of distinct tributaries and use of the mainstem Peace River as a movement corridor – phenomena that are likely important to the persistence of all species across the project area waterscape.

In conclusion, our work continues to provide genomic assays for efficient and accurate monitoring of population structure and for assignments of all three species to UP or DP and in some cases (Arctic Grayling) for assignment to tributary of origin. We have also resolved significant population structure in Redside Shiner. Over the next few months, we will be i) assigning to UP and DP all samples collected in 2024 in the Peace River mainstem and TUF and, i) using the GT-Seq panels for both Bull Trout and Rainbow Trout for all samples from the LAA sampled up to and including 2024 to

examine demographic characteristics (e.g., effective population size, parentage and cohort replacement rate).

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