Site C Clean Energy Project

Fisheries and Aquatic Habitat Monitoring and Follow-up Program

Fish Genetics Study
2021 Status Report for Bull Trout, Arctic Grayling and Rainbow Trout

Construction Year 7 (2021)

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EXECUTIVE SUMMARY

BC Hydro is currently constructing the Site C Clean Energy Project (the Project) near the town of Fort St. John in northeastern British Columbia which will be the third hydroelectric dam on the Peace River. 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, the 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), Contingent Fish Capture Program and Upstream Fish Passage Facility Program activities of the FAHMFP have collected DNA samples from species of game fish, Bull Trout (*Salvelinus confluentus*), Arctic Grayling (*Thymallus arcticus*) and Rainbow Trout (*Oncorhynchus mykiss*), and three species of non-game fishes also found in the local assessment area (LAA) Slimy Sculpin (*Cottus cognatus*), Longnose Dace (*Rhinichthys cataractae*), and Redside Shiner (*Richardsonius balteatus*). The Site C Fish Genetics Study aims to: (a) determine levels and patterns of population structure for the three species of game fish in the Peace River and its tributaries, (b) develop genotyping assays for genetic monitoring of the system, and (c) deploy these assays in an initial number of samples available for analysis. Here we report on the progress of the Site C Fish Genetics Study from January 1, 2021 to December 31, 2021. The results and status from earlier components of this study can be found in Geraldes and Taylor (2020) and Geraldes and Taylor (2021).

In sampling year 2020, 781 samples were collected from all three species of game fish and a total of 4,052 genetic samples have been collected across all sampling

years (2016 through 2020). These samples were shipped to UBC where they have been stored and catalogued. For Bull Trout, 332 samples were collected in 2020 for a total of 2,612 samples received and catalogued at UBC across all sampling years (2006 through 2020). The DNA was extracted from 2,128 Bull Trout samples. All 194 Peace River Bull Trout samples collected in 2020 in the Peace River mainstem (and Dry and Maurice creeks) were genotyped at six loci previously developed and shown to be sufficient to assign samples with high confidence to two genetic groups identified with genome-wide data: one group consisted of samples that spawn upstream of the Project (UP) in the Halfway River, and the other consisted of samples that spawn downstream of the Project (DP) in the Pine River (Geraldes and Taylor 2020). Genetic analysis allowed most samples collected in 2020 to be assigned to one of the two groups with more than 95% confidence, with the vast majority being assigned to the Halfway group (N=183, 94.3% of all samples) and a small number being assigned to the Pine group (N=6, 3.1% of all samples). Five samples could not be assigned to either UP or DP with 95% confidence (2.6% of all samples).

For Arctic Grayling, 172 samples were collected in 2020 for a total of 494 samples received and catalogued at UBC across all sampling years (2018 through 2020). The DNA has been extracted and quality controlled for 373 of those samples. Previous work (Geraldes and Taylor 2021) found that four distinct population groups of Arctic Grayling can be identified 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 Moberly River (located DP). All 198 samples collected in the Peace River mainstem and additional samples collected in the four tributaries were genotyped at 11 ancestry informative loci developed in Geraldes and Taylor (2021) and

during the current reporting year. Genetic analysis revealed that 170 out of 198 (85.9%) Arctic Grayling samples collected between 2018 and 2020 in the Peace River mainstem belong to Moberly River population group, only one (0.5%) to the Halfway River population group, 13 (6.6%) to the Pine River group and none to the Beatton River group. Fourteen samples (7.1%) could not be assigned with more than 95% confidence to a single tributary population group. Assignment of Arctic Grayling samples to only the UP population group (Halfway or Moberly rivers) and DP population group (Pine and Beatton rivers) resulted in 181 samples (91.4%) being assigned to the UP group and 14 samples (7.1%) being assigned to the DP group; only three samples (1.5%) not being assigned to either population group.

Finally, for Rainbow Trout, 277 samples were collected in 2020 for a total of 946 received and catalogued at UBC across all sampling years (2017 through 2020). The DNA has been extracted and quality controlled for all of those samples. To investigate levels and patterns of genetic differentiation among fish representative of possible provenances of fish caught in the Peace River mainstem, Geraldes and Taylor (2021) selected samples from a) large tributaries of the Peace River (Halfway, Moberly and Pine rivers), b) smaller tributaries of the Peace River (Farrell, Lynx and Maurice creeks), c) the Dinosaur Reservoir (created by Peace Canyon Dam located UP) and d) three hatchery strains known to be used for restocking of fish in the area (Pennask Lake, Blackwater River, and Fraser Valley Domestic) for genome sequencing. Analysis of the genetic data revealed the existence of three genetic groups associated with the Halfway River (located UP), the Moberly River and Lynx Creek (located UP), and the Pine River (located DP). All six samples from hatchery strains grouped together with the Pine River genetic group. Contrary to the results found for Bull Trout (Geraldes and Taylor 2020)

and Arctic Grayling (Geraldes and Taylor 2021), high levels of admixture between samples from these three genetic groups was detected. Of 90 samples from the LAA, only 40 collected UP had more than 90% ancestry in one of the two UP genetic groups and only 10 collected DP had more than 90% ancestry in the DP genetic group. Six assays developed to genotype ancestry informative SNPs were found to be sufficient to assign samples to UP or DP population groups. Genotyping of 433 Rainbow Trout samples collected in the Peace River mainstem and Dry Creek resulted in the assignment of 248 (67.3%) to the UP genetic group and 110 (25.4%) to the DP genetic group; 75 (17.3%) could not be assigned to either with more than 95% confidence.

Taken together, our work revealed that for all three species, there is considerable population structure in the LAA. Patterns of population structure are clearest for Bull Trout and least clear for Rainbow Trout, where high levels of admixture between the different population groups are evident. For Bull Trout, across all sampling years, almost 94% of samples were assigned to the UP population group despite more than 40% being sampled DP. For Arctic Grayling, across all sampling years, over 90% of samples were assigned to the UP genetic group despite more than 50% being sampled DP. In particular, of the 171 Arctic Grayling samples assigned to the UP population group that could also be assigned to a specific tributary population group, 99% were assigned to the Moberly population group and of the 13 Arctic Grayling assigned to the DP population group that could also be assigned to a specific tributary population group, none were assigned to the Beatton population group. For Rainbow Trout, a much higher fraction of samples (17.3%) caught in the Peace River mainstem and Dry Creek could not be assigned to either UP or DP than for Bull Trout (2.4%) or Arctic Grayling (1.5%). Also, for Rainbow Trout, over 25% of samples were assigned to the DP population

group (that value is close to 4% for Bull Trout and 7% for Arctic Grayling) despite less than 10% being sampled DP. This reveals that unlike for Bull Trout and Arctic Grayling, the Pine River is likely an important source of Rainbow Trout sampled in the Peace River mainstem, even in sections of the river located UP.

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LIST OF ACRONYMS AND ABBREVIATIONS

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 the total variation in a sample (T)
GBS	Genotyping-by-sequencing
K	Number of genetic groups in the Admixture analysis
LAA	Local Assessment Area
PCA	Principal components analysis
PCR	Polymerase chain reaction
Pine BB	Pine River drainage: Burnt River and Blind Creek
Pine PW	Pine River drainage: Pine River and Willow Creek
qPCR	Quantitative polymerase chain reaction
QC	Quality control
SNP	Single nucleotide polymorphism
UP	Upstream of the Project

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INTRODUCTION

BC Hydro is currently in the eighth year of construction of the Site C Clean Energy Project (the Project) near the town of Fort St. John in northeastern British Columbia (hereafter referred to as Local Assessment Area, LAA) which will be the third hydroelectric dam on the Peace River. In 2018, BC Hydro and the laboratory of Eric Taylor at the University of British Columbia, Department of Zoology, entered into an agreement to apply genomic techniques to facilitate aspects of the mitigation and monitoring plan for the LAA. This work initially focuses on three important recreational sport fishes: Bull Trout (*Salvelinus confluentus*), Arctic Grayling (*Thymallus arcticus*), and Rainbow Trout (*Oncorhynchus mykiss*) which are common in the LAA. These efforts are directly tied 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. BC Hydro uses multiple lines of evidence to better understand the population structure, migration and movement patterns of these key fish species in the Peace River and its tributaries. Such evidence includes data from otolith and fin ray microchemistry, radio telemetry, fish distribution, and genetics, that are being used to test hypotheses developed to answer management questions posed in the FAHMFP.

Purpose and Objectives

The Site C Fish Genetics Study aims to: (a) determine levels and patterns of population structure for Bull Trout, Arctic Grayling and Rainbow Trout in the Peace River and its tributaries in the LAA, (b) develop genotyping assays for genetic monitoring of the

system, and (c) deploy these assays in an initial number of samples available for analysis.

Geraldes and Taylor (2020, 2021) reported on the results of the first two years of genetic work contributing to the FAHMFP. In those reports, the authors summarized genomic work focused on using genotyping-by-sequencing (GBS) across the genomes of Bull Trout and Arctic Grayling to resolve differences among samples collected from tributaries of the Peace River (Halfway, Moberly, Pine rivers for Bull Trout and those three rivers plus the Beatton River for Arctic Grayling).

Their work revealed pronounced genetic differences between Bull Trout that spawn in tributaries upstream (Halfway River) and downstream (Pine River) of the Project and those genome-wide differences were used to develop a set of six TaqMan™ genotyping assays that differentiated samples collected from the mainstem Peace River (n=664) in terms of whether an individual fish belonged to a spawning population located upstream of the Project (UP, i.e., in the Halfway River; ~94% of all samples) or downstream of the Project (DP, i.e., in the Pine River; ~4% of all samples). Only about 2% of all mainstem Peace River samples of Bull Trout could not be assigned to either the Halfway or Pine river spawning groups with more than 95% confidence.

For Arctic Grayling, the same approach revealed four genetic groups, one for each of the four tributaries of the Peace River sampled. Both the Beatton River (DP) and the Moberly River (UP) genetic groups where well differentiated genetically and showed no (Beatton), or little (Moberly), evidence of genetic admixture with samples from the other genetic groups. The Halfway River group (UP) and the Pine River group (DP) were less well differentiated from one another and roughly one fourth of the samples in each group showed some evidence of recent admixture with other genetic

groups (Geraldes and Taylor 2021). For Arctic Grayling we used seven TaqMan™ assays to genotype seven loci that allowed for the assignment of samples collected from the mainstem Peace River (n=146) to a spawning population located UP (i.e., in the Halfway or the Moberly river; ~84% of all samples) or DP (i.e., in the Pine or the Beatton river; ~8% of all samples). Approximately 8% of all mainstem Peace River samples of Arctic Grayling could not be assigned to either the UP or DP spawning groups with more than 95% confidence.

Geraldes and Taylor (2021) further reported on the initial steps of the work to investigate levels and patterns of population structure in Rainbow Trout, including sample selection, genomic library construction and sequencing results.

The current report summarizes the work during the third year of the study to the end of 2021. Specifically, the report summarizes: (i) Bull Trout population assignment work for samples collected in the mainstem of the Peace River in 2020; (ii) the development of additional TaqMan™ genotyping assays for improved population assignment of Arctic Grayling samples to each of the four tributaries in the LAA and (iii) the improved population assignment of all samples of Arctic Grayling collected in the mainstem of the Peace River between 2018 and 2020; (iv) analysis of levels and patterns of Rainbow Trout population structure in the LAA, (v) development of TaqMan™ genotyping assays to assign Rainbow Trout samples to the detected population groups, and (vi) population assignment of all samples of Rainbow Trout collected in the mainstem of the Peace River between 2018 and 2020.

The fourth year of the study (2022) will focus on population assignment work for all three species of samples collected from the mainstem of the Peace River in 2021. Additionally, we will develop more sophisticated genomic assays to monitor critical

demographic parameters of Bull Trout and Rainbow Trout (e.g., effective population size), and initiate analysis of levels and patterns of population structure for three species of non-game fishes also found in the LAA: Slimy Sculpin (*Cottus cognatus*), Longnose Dace (*Rhinichthys cataractae*), and Redside Shiner (*Richardsonius balteatus*).

BULL TROUT

Materials and Methods

The 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), Contingent Fish Capture Program and Upstream Fish Passage Facility Program activities of the FAHMFP collected 194 Bull Trout genetic samples in 2020 from the Peace River (Table 1), including the Peace River mainstem (N=182), Dry Creek (N=10) and Maurice Creek (N=2). Subsequent DNA extraction and quality control (QC) followed Geraldes and Taylor (2020).

Geraldes and Taylor (2020) used genome wide polymorphism data generated through GBS to investigate levels and patterns of population structure of Bull Trout in the LAA. They determined that there were two population groups in the area, one represented by samples of fish spawning in the Halfway River watershed (located UP) and one by samples of fish spawning in the Pine River watershed (located DP). They developed six TaqMan™ assays that allow for the quick and efficient genotyping of six ancestry informative SNPs (i.e., loci showing large levels of genetic differentiation between UP and DP genetic groups) and the assignment of fish to the UP and DP genetic groups. Here, we used those six TaqMan™ assays to genotype 194 Bull Trout genetic samples collected in 2020 from the Peace River at the six ancestry informative loci, following the methods in Geraldes and Taylor (2020). Those genotype data were used to assign all Bull Trout samples to spawning tributaries UP and DP following Geraldes and Taylor (2021). Briefly, we used the program GeneClass2 (Piry et al., 2004) to assign samples to UP and DP following the method of Rannala and Mountain (1997). 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%.

Table 1. Bull Trout, Arctic Grayling and Rainbow Trout samples available for genetics work for Study year 2020 and across all Study years (2016-2020).

			Study years 2016-2020			Study year 2020 only		
Species	**Watershed**	**River/SectionID**	**UBC[1]**	**DNA[2]**	**TaqMan[3]**	**UBC[1]**	**DNA[2]**	**TaqMan[3]**
All	All	All	4052	3447	1691	781	422	401
Bull Trout	All	All	2612	2128	928	332	214	194
Bull Trout	Halfway River	Chowade River	782	605	16	50	4	0
Bull Trout	Halfway River	Colt Creek	18	18	13	4	4	0
Bull Trout	Halfway River	Cypress Creek	650	404	13	55	4	0
Bull Trout	Halfway River	Fiddes Creek	248	187	12	25	4	0
Bull Trout	Halfway River	Halfway River	7	7	6	0	0	0
Bull Trout	Halfway River	Turnoff Creek	40	40	4	0	0	0
Bull Trout	Moberly River	Moberly River	9	9	6	4	4	0
Bull Trout	Peace River	Dry Creek	10	10	10	10	10	10
Bull Trout	Peace River	Maurice	2	2	2	2	2	2
Bull Trout	Peace River	Section 1	223	223	223	42	42	42
Bull Trout	Peace River	Section 3	290	290	290	70	70	70
Bull Trout	Peace River	Section 5	142	142	142	37	37	37
Bull Trout	Peace River	Section 6	103	103	103	13	13	13
Bull Trout	Peace River	Section 7	58	58	58	12	12	12
Bull Trout	Peace River	Section 9	30	30	30	8	8	8
Arctic Grayling	All	All	494	373	244	172	53	52
Arctic Grayling	Beatton River	Beatton River	37	37	3	0	0	0
Arctic Grayling	Beatton River	Bratland Creek	56	54	15	0	0	0
Arctic Grayling	Beatton River	La Prise Creek	39	39	13	0	0	0
Arctic Grayling	Beatton River	Unnamed Creek 1	1	1	1	0	0	0
Arctic Grayling	Halfway River	Colt Creek	1	1	1	0	0	0
Arctic Grayling	Moberly River	Moberly River	161	42	12	119	0	0
Arctic Grayling	Peace River	Section 1	4	4	4	1	1	1
Arctic Grayling	Peace River	Section 3	93	93	93	19	19	19
Arctic Grayling	Peace River	Section 5	39	39	39	16	16	16
Arctic Grayling	Peace River	Section 6	36	36	36	4	4	4
Arctic Grayling	Peace River	Section 7	21	21	21	8	8	7
Arctic Grayling	Peace River	Section 9	6	6	6	5	5	5

			Study years 2016-2020			Study year 2020 only		
Species	**Watershed**	**River/SectionID**	**UBC[1]**	**DNA[2]**	**TaqMan[3]**	**UBC[1]**	**DNA[2]**	**TaqMan[3]**
Rainbow Trout	All	All	946	946	519	277	155	155
Rainbow Trout	Halfway River	Chowade River	14	14	14	1	1	1
Rainbow Trout	Halfway River	Colt Creek	106	106	12	23	1	1
Rainbow Trout	Halfway River	Cypress Creek	27	27	14	6	4	4
Rainbow Trout	Halfway River	Kobes Creek	150	150	12	34	0	0
Rainbow Trout	Peace River	Dry Creek	7	7	7	7	7	7
Rainbow Trout	Peace River	Farrell Creek	177	177	23	42	5	5
Rainbow Trout	Peace River	Maurice Creek	38	38	11	38	11	11
Rainbow Trout	Peace River	Section 1	205	205	205	68	68	68
Rainbow Trout	Peace River	Section 3	182	182	181	50	50	50
Rainbow Trout	Peace River	Section 5	23	23	23	3	3	3
Rainbow Trout	Peace River	Section 6	6	6	6	1	1	1
Rainbow Trout	Peace River	Section 7	10	10	10	4	4	4
Rainbow Trout	Peace River	Section 9	1	1	1	0	0	0

[1]Number of samples received at UBC

[2]Number of samples for which a DNA extraction was performed

[3]Number of samples for which SNP genotyping with TaqMan™ assays was performed

Results

In 2020, 182 Bull Trout were collected in six sections of the Peace River, with Sections 1 and 3 located UP and Sections 5, 6, 7 and 9 located DP and an additional 12 samples were collected in Dry Creek (N=10) and in Maurice Creek (N=2) both located UP (Table 2 and Appendix I). All 194 samples were successfully genotyped at six ancestry informative loci with TaqMan™ assays. The vast majority of samples were assigned to the UP group (N=183, 94.3%), only six were assigned to the DP group (3.1% of all samples: one in Section 1, four in Section 3 and one in Dry Creek) and five could not be assigned to either group (i.e., assignment probability to either was below 0.95; 2.6% of all samples: one in Section 1, three in Section 3 and one in Section 5). Overall, there was little variability in the proportion of fish assigned to UP and DP between 2020 and all previous years (2016 through 2019; Table 2).

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

Location	Year	Total	UP	DP	Unassigned[1]
All Peace River	2020	194	183 (94.3%)	6 (3.1%)	5 (2.6%)
	2016-2019	664	623 (93.8%)	25 (3.8%)	16 (2.4%)
	All years	858	806 (93.9%)	31 (3.6%)	21 (2.4%)
PR Section 1	2020	42	40 (95.2%)	1 (2.4%)	1 (2.4%)
	2016-2019	181	176 (97.2%)	3 (1.7%)	2 (1.1%)
	All years	223	216 (96.9%)	4 (1.8%)	3 (1.3%)
PR Section 3	2020	70	63 (90.0%)	4 (5.7%)	3 (4.3%)
	2016-2019	220	203 (92.3%)	6 (2.7%)	11 (5.0%)
	All years	290	266 (91.7%)	10 (3.5%)	14 (4.8%)
PR Section 5	2020	37	36 (97.3%)	0 (0.0%)	1 (2.7%)
	2016-2019	105	95 (90.5%)	8 (7.6%)	2 (1.9%)
	All years	142	131 (92.3%)	9 (6.3%)	2 (1.4%)
PR Section 6	2020	13	13 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2019	90	81 (90.0%)	8 (8.9%)	1 (1.1%)
	All years	103	94 (91.3%)	8 (7.8%)	1 (1.0%)
PR Section 7	2020	12	12 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2019	46	46 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	58	58 (100.0%)	0 (0.0%)	0 (0.0%)
PR Section 9	2020	8	8 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2019	22	22 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	30	30 (100.0%)	0 (0.0%)	0 (0.0%)
PR Dry Creek	2020	10	9 (90%)	1 (10%)	0 (0.0%)
	2016-2019	0			
	All years	10	9 (90%)	1 (10%)	0 (0.0%)
PR Maurice Creek	2020	2	2 (100.0%)	0 (0.0%)	0 (0.0%)
	2016-2019	0			
	All years	2	2 (100.0%)	0 (0.0%)	0 (0.0%)

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

ARCTIC GRAYLING

Materials and Methods

The 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), Contingent Fish Capture Program and Upstream Fish Passage Facility Program activities of the FAHMFP collected 53 Arctic Grayling genetic samples in 2020 from the Peace River (Table 1). Subsequent DNA extraction and quality control (QC) followed Geraldes and Taylor (2020).

In 2021, Geraldes and Taylor used genome wide polymorphism data generated through GBS to investigate levels and patterns of population structure of Arctic Grayling in the LAA. They determined that four population groups could be detected in the area, one for each tributary watershed where Arctic Grayling are known to spawn (Halfway River and Moberly River, both located UP, Pine River and Beatton River, both located DP). The Beatton River and Moberly River population groups were well differentiated from each other and the other localities, but differentiation between the Halfway River and Pine River population groups was less pronounced. They reported on the development of seven TaqMan™ genotyping assays targeting seven ancestry informative SNPs (six were highly differentiated between UP and DP spawning groups, i.e., Halfway and Moberly versus Pine and Beatton, and one was highly differentiated between the Halfway group, located UP, and the three others), which resulted in highly successful assignment (91.8%) into UP and DP spawning groups of samples caught in the Peace River. These assays did not, however, allow for assignment of samples into each of the four population groups detected in the study area (corresponding to the Halfway, Moberly, Pine and Beatton rivers).

Here, we developed additional TaqMan™ assays to genotype six more SNPs (Table 3), employing the same methodology as in Geraldes and Taylor (2021). Two SNPs were selected because they showed high genetic differentiation (i.e., F_{ST} [Weir and Cockerham, 1984]) between UP and DP, two were selected because they showed high F_{ST} between the Moberly and the other three spawning tributaries, and two were selected because they showed high F_{ST} between the Pine and the other three spawning tributaries. We followed the approach of Geraldes and Taylor (2021) to test whether each assay showed good qPCR amplification, had high genotyping success and clearly distinguished the three genotypes at each SNP locus. One of the six assays (ag21api2, Table 3) showed low qPCR amplification and genotyping rate and was not used further. The remaining five successful TaqMan™ assays were used to genotype 53 Peace River samples collected in 2020, as well as the 307 Arctic Grayling samples reported in Geraldes and Taylor (2021), including all those collected in the Peace River mainstem in 2018 and 2019 (N=146), as well as 33 samples from the Halfway River (19 reference samples and 14 test samples), 43 from the Moberly River (25 reference samples and 18 test samples), both located UP, 40 samples from the Beatton River (18 reference samples and 22 test samples) and 45 samples from the Pine River (24 reference samples and 21 test samples) both located DP. Reference samples were those from the Peace River tributaries for which we have GBS data that arranged them into four genetic groups, one for each of the four tributaries to which we want to assign samples caught in the Peace River mainstem. Test samples were those collected from the four Peace River tributaries, but that were not used as reference samples: they allow us to test how well the assignment tests perform. These are samples for which we did not have GBS data and hence, we assumed that they belong to the genetic group of the

tributary where they were caught. The 53 Peace River samples collected in 2020 were also genotyped with the seven TaqMan™ assays described in Geraldes and Taylor (2021).

Table 3. TaqMan™ assays tested to genotype Arctic Grayling samples.

TaqMan Assay	SNP name	Rationale [1]	F_{ST}	F_{ST} rank	Reference
ag13b01	CM014997.1:17726527	UP/DP	0.71	1	Geraldes and Taylor (2021)
ag20b03	CM015014.1:28434291	UP/DP	0.64	3	Geraldes and Taylor (2021)
ag01b04	CM015012.1:19976165	UP/DP	0.62	4	Geraldes and Taylor (2021)
ag17a14	CM015005.1:26896876	UP/DP	0.54	14	Geraldes and Taylor (2021)
ag19b18	CM015010.1:15356601	UP/DP	0.51	18	Geraldes and Taylor (2021)
ag15b24[2]	CM015002.1:21619794	UP/DP	0.48	24	Geraldes and Taylor (2021)
ag23aha1	CM015019.1:14738697	Halfway	1.00	1	Geraldes and Taylor (2021)
ag21a25	CM015015.1:18186665	UP/DP	0.48	25	This report
ag13c27	CM014998.1:3845029	UP/DP	0.48	27	This report
ag10amo8	CM014990.1:10151354	Moberly	0.81	8	This report
ag17bmo6	CM015006.1:26638693	Moberly	0.83	6	This report
ag21api2[3]	CM015015.1:9700830	Pine	0.74	2	This report
ag19api3	CM015009.1:21936972	Pine	0.72	3	This report

[1]Describes whether a SNP was selected based on it being a top ranking F_{ST} SNP between tributaries upstream (UP) and downstream (DP) of the Project, or top ranking F_{ST} between a single tributary and all others. F_{ST} and F_{ST} rank refer to the value and rank specific to that rationale.

[2]ag15b24 was used in Geraldes and Taylor (2021) but not in this report because it had poor amplification and did not significantly improve the ability to either assign samples to UP and DP population groups or to each tributary population.

[3]ag21api2 failed to amplify in preliminary tests and was not used further.

We used the same methodology for population assignment described above for Bull Trout, and samples were considered assigned to UP or DP if they had 95% or higher chance of being from the UP or the DP group and considered unassigned if the chance of belonging to either group was lower than 95%. We detected one genetic population group per tributary in Arctic grayling; consequently, we also repeated the

assignments as above, but instead of assigning samples to UP (Halfway River and Beatton River) or DP (Pine River and Beatton River), we assigned samples to each tributary independently.

One of the TaqMan™ assays (ag15b24, Table 3) reported in Geraldes and Taylor (2021) had poor amplification and genotyping success. Assignment of Peace River samples to either UP and DP or to each of the four tributaries with (12 SNPs) or without ag15b24 (11 SNPs) were similar (data not shown) and the assignments presented here were performed with genotype data from 11 SNPs only.

Results

In 2020, 53 Arctic Grayling were collected in six sections of the Peace River during sampling year 2020, with Sections 1, and 3 located UP and Sections 5, 6, 7 and 9 located DP. We genotyped those 53 samples at 12 ancestry informative loci using TaqMan™ assays (Table 1). We also genotyped 307 Arctic Grayling samples (Geraldes and Taylor, 2021) from previous sampling years (146 from the Peace River mainstem and 161 from the four tributaries) with five new TaqMan™ assays. These were developed so that we would be able to assign samples not only to UP and DP, but to each of the four tributary watersheds. One assay developed by Geraldes and Taylor (2021) was dropped from analysis because of poor amplification and because assignment results with and without that locus were similar. For 84.4% of the samples there was no missing data (i.e., we obtained genotypes at 11 out of the 11 SNPs), 13.1% of the samples had only one missing genotype and three samples (0.8%) had fewer than seven genotypes (Appendix 2). Those three samples were not considered further (one was from sampling year 2020 from Peace River sampling Section 7, one

was from the Moberly River and one from the Beatton River). We performed population assignments for the remaining 357 Arctic Grayling samples based on the genotypes at 11 loci.

For Arctic Grayling, all samples used as reference samples for watersheds located UP (19 from the Halfway River watershed and 25 from the Moberly River watershed) were correctly assigned to the UP group. For the DP group reference samples (24 from the Pine River watershed and 18 from the Beatton River watershed), all Beatton River watershed reference samples were assigned to the DP group, and 95.8% of the Pine River watershed samples were assigned to the DP group (one sample could not be assigned with more than 95% confidence; Table 4 and Appendix 2).

All of the Halfway River (N=14) and Moberly River (N=17) Arctic Grayling used as test samples, i.e., those caught in the Halfway River or Moberly River, respectively, for which no GBS data is available, were assigned to the UP group. Similarly, the Beatton River (N=21) test samples were assigned to the DP group. For the Pine River test samples, 20 were assigned to the DP group and one sample (4.8% of all samples) could not be assigned to either group with 95% confidence (Table 4 and Appendix 2). These results (assignment of reference and test samples) are a clear improvement over those in Geraldes and Taylor (2021) where with seven loci, seven out of 161 (4.3%) samples could not be assigned to UP or DP with 95% confidence, while with eleven loci only two samples (1.2%) could not be assigned (Table 4).

Table 4. Comparison of population assignment results of reference and test samples of Arctic Grayling to upstream of the project (UP) and downstream of the project (DP) population groups using the genotype data from the seven TaqMan™ assays from Geraldes and Taylor (2021) and the larger set of eleven assays.

			7 loci (Geraldes and Taylor 2021)			11 loci (This report)		
Use	**Location**	**N**	**UP**	**DP**	**NA[1]**	**UP**	**DP**	**NA[1]**
Reference	Halfway River	19	19	0	0	19	0	0
Reference	Moberly River	25	25	0	0	25	0	0
Reference	Pine River	24	1	21	2	0	23	1
Reference	Beatton River	18	0	18	0	0	18	0
Test	Halfway River	14	14	0	0	14	0	0
Test	Moberly River	17	16	0	1	17	0	0
Test	Pine River	21	0	17	4	0	20	1
Test	Beatton River	21	0	21	0	0	21	0

[1]NA, not assigned: samples that cannot be assigned to either UP or DP with over 95% confidence

With the new set of eleven loci used in this report, the vast majority of samples collected in sampling year 2020 were assigned to the UP group (92.3%), only two were assigned to the DP group (3.8%; both in Section 9) and two could not be assigned to either group (i.e., assignment probability to either was below 95%; both in Section 6). There was some variability in the proportion of fish assigned to DP; over the three sampling years 7.1% of samples were assigned to DP, but that proportion was only 3.8% for sampling year 2020 compared to 8.2% for the years of 2018-2019 (Table 5 and Appendix 2).

Using the same genotype data, we also assigned samples to each of the four tributaries. Most samples used either as reference samples or as test samples could be assigned to their respective sampling location (Table 6). The highest percentage of unassigned fish were those from the test group sampled in the Halfway River where

four out of 14 samples (28.6%) could not be assigned with more than 95% confidence. For any of the other sets of samples, either all samples could be assigned to the correct group or only one could not be assigned with more than 95% confidence.

Table 5. Number of Arctic Grayling samples caught in the Peace River (PR) assigned (% of total) to the UP (upstream of the Project) or DP (downstream of the Project) groups based on genotypes at eleven ancestry informative SNPs with more than 95% confidence.

Location	Year	Total	UP	DP	Unassigned[1]
All Peace River	2020	52	48 (92.3%)	2 (3.8%)	2 (3.8%)
	2018-2019	146	133 (91.1%)	12 (8.2%)	1 (0.7%)
	All years	198	181 (91.4%)	14 (7.1%)	3 (1.5%)
PR Section 1	2020	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	3	2 (66.7%)	1 (33.3%)	0 (0.0%)
	All years	4	3 (75.0%)	1 (25.0%)	0 (0.0%)
PR Section 3	2020	19	19 (100.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	74	74 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	93	93 (100.0%)	0 (0.0%)	0 (0.0%)
PR Section 5	2020	16	16 (100.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	23	23 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	39	39 (100.0%)	0 (0.0%)	0 (0.0%)
PR Section 6	2020	4	2 (50.0%)	0 (0.0%)	2 (50.0%)
	2018-2019	32	25 (78.1%)	6 (18.8%)	1 (3.1%)
	All years	36	27 (75.0%)	6 (16.7%)	3 (8.3%)
PR Section 7	2020	7	7 (100.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	13	9 (69.2%)	4 (30.8%)	0 (0.0%)
	All years	20	16 (80.0%)	4 (20.0%)	0 (0.0%)
PR Section 9	2020	5	3 (60.0%)	2 (40.0%)	0 (0.0%)
	2018-2019	1	0 (0.0%)	1 (100.0%)	0 (0.0%)
	All years	6	3 (50.0%)	3 (50.0%)	0 (0.0%)

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

Table 6. Population assignment results of reference and test Arctic Grayling samples to each of the four tributary population groups using genotype data from eleven TaqMan™ assays

			Assignment				
Use	**Location**	**N**	**Halfway**	**Moberly**	**Pine**	**Beatton**	**Unassigned[1]**
Reference	Halfway River	19	19	0	0	0	0
Reference	Moberly River	25	0	24	0	0	1
Reference	Pine River	24	0	0	23	0	1
Reference	Beatton River	18	0	0	0	18	0
Test	Halfway River	14	10	0	0	0	4
Test	Moberly River	17	0	16	0	0	1
Test	Pine River	21	0	0	20	0	1
Test	Beatton River	21	0	0	0	20	1

[1]Samples that cannot be assigned to any group with over 95% confidence

Assignment of samples collected in the Peace River mainstem to each of the four Peace River tributaries across all sampling years (2018-2020) revealed that the vast majority belong to the Moberly River population group (85.9%, Table 7 and Appendix 2) while only 0.5% of samples (i.e., one sample) were assigned to the other UP population group (Halfway River). No samples were assigned to the Beatton River population group and only 6.6% were assigned to the Pine River population group. Of the 198 samples for which we performed assignments to both UP/DP and each of the four tributaries, three samples could not be assigned with more than 95% confidence to either UP/DP or to a single tributary. Of the 181 samples assigned to UP, 10 could not be assigned to either the Moberly or the Halfway population groups and of the 14 samples assigned to DP, one could not be assigned to either the Pine or the Beatton population groups. In total, we could not assign 7.1% of the samples (14 out of 198 samples) to a tributary population group with more than 95% confidence. While this value (7.1%) is low, it is almost five times higher than the 1.5% percentage of samples

(three out of 198) that could not be assigned to UP and DP with more than 95% confidence.

Table 7. Number of Arctic Grayling samples caught in the Peace River (PR) assigned (% of total) to each of the four tributary population groups based on genotypes at eleven ancestry informative SNPs with more than 95% confidence.

Location	Year	Total	Halfway	Moberly	Pine	Beatton	NA[1]
All Peace River	2020	52	1 (1.9%)	44 (84.6%)	2 (3.8%)	0 (0.0%)	5 (9.6%)
	2018-2019	146	0 (0.0%)	126 (86.3%)	11 (7.5%)	0 (0.0%)	9 (6.2%)
	All years	198	1 (0.5%)	170 (85.9%)	13 (6.6%)	0 (0.0%)	14 (7.1%)
PR Section 1	2020	1	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	3	0 (0.0%)	2 (66.7%)	1 (33.3%)	0 (0.0%)	0 (0.0%)
	All years	4	0 (0.0%)	3 (75.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)
PR Section 3	2020	19	1 (5.3%)	17 (89.5%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
	2018-2019	74	0 (0.0%)	69 (93.2%)	0 (0.0%)	0 (0.0%)	5 (6.8%)
	All years	93	1 (1.1%)	86 (92.5%)	0 (0.0%)	0 (0.0%)	6 (6.5%)
PR Section 5	2020	16	0 (0.0%)	16 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	2018-2019	23	0 (0.0%)	22 (95.7%)	0 (0.0%)	0 (0.0%)	1 (4.3%)
	All years	39	0 (0.0%)	38 (97.4%)	0 (0.0%)	0 (0.0%)	1 (2.6%)
PR Section 6	2020	4	0 (0.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	2 (50.0%)
	2018-2019	32	0 (0.0%)	24 (75.0%)	5 (15.6%)	0 (0.0%)	3 (9.4%)
	All years	36	0 (0.0%)	26 (72.2%)	5 (13.9%)	0 (0.0%)	5 (13.9%)
PR Section 7	2020	7	0 (0.0%)	6 (85.7%)	0 (0.0%)	0 (0.0%)	1 (14.3%)
	2018-2019	13	0 (0.0%)	9 (69.2%)	4 (30.8%)	0 (0.0%)	0 (0.0%)
	All years	20	0 (0.0%)	15 (75.0%)	4 (20.0%)	0 (0.0%)	1 (5.0%)
PR Section 9	2020	5	0 (0.0%)	2 (40.0%)	2 (40.0%)	0 (0.0%)	1 (20.0%)
	2018-2019	1	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
	All years	6	0 (0.0%)	2 (33.3.0%)	3 (50.0%)	0 (0.0%)	1 (16.7%)

[1]Samples that cannot be assigned to any group with over 95% confidence

RAINBOW TROUT

Materials and Methods

The 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), Contingent Fish Capture Program and Upstream Fish Passage Facility Program activities of the FAHMFP collected 213 Rainbow Trout tissue samples in 2020 from the Peace River (Table 1), 126 of which were collected in the Peace River mainstem, seven in Dry Creek, 42 in Farrell Creek and 38 in Maurice Creek. We extracted the DNA from all Peace River mainstem samples, as well as all Dry Creek samples, five Farrell Creek samples and 11 Maurice Creek samples. Subsequent DNA extraction and quality control (QC) followed Geraldes and Taylor (2020).

Geraldes and Taylor (2021) reported on their work to determine levels and patterns of population structure of Rainbow Trout in the LAA. To that end, they selected 100 Rainbow Trout samples collected between 2006 and 2019 (Appendix 3). The majority were from the main Rainbow Trout spawning areas in the LAA: 30 from the Halfway River (located UP), eight from the Moberly River (located UP), and 16 from the Pine River (located DP). They also included samples 28 samples from smaller tributaries located UP (Lynx Creek, N=8, Maurice Creek, N=10, and Farrell Creek, N=10). In addition, they included 12 samples from the Dinosaur Reservoir, located UP (as these may occasionally be entrained downstream through Peace Canyon Dam), and two samples each from three hatchery strains (Blackwater River, Pennask Lake and Fraser Valley Domestic) that are known to have been used for stocking in the area (Freshwater Fisheries Society of BC, 2021). The GBS library preparation and mapping

of reads to the Rainbow Trout reference genome sequence (assembly USDA_OmykA_1.1, retrieved from https://www.ncbi.nlm.nih.gov/assembly/GCF_013265735.2/ on October 9th, 2020) were completed at UBC from sequences generated by Genome Quebec as fully described in Geraldes and Taylor (2021).

Four samples (one from the Halfway River, one from the Moberly River and two from Maurice Creek) were eliminated from further analysis because they had lower amounts of sequence reads which would likely result in large amounts of missing data (Appendix 3). We followed the protocols successfully employed in the Bull Trout (Geraldes and Taylor 2020) and the Arctic Grayling dataset analyses (Geraldes and Taylor 2021) for SNP filtering in the Rainbow Trout dataset. 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 samples (N=184 samples, 88 of which were from a different project in our laboratory and will not be considered here) as these are likely the result of mapping to paralogous regions of the genome. Then we created two datasets: one with only the 52 samples from the Halfway River, Moberly River and Pine River, and one with 96 samples (all LAA samples and the six samples from the hatchery strains used for stocking). We applied several filtering criteria, to each dataset independently, with VCFtools v0.1.11 (Danecek et al. 2011) to arrive at two sets of high-quality SNPs to form the basis of subsequent population genetic analyses. 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) SNPs with missing genotypes in more than 30% of samples, and v) low frequency SNPs (SNPs present at a frequency below 5%). For

analysis of population structure (see below), we used Plinkv1.9 (Chang et al. 2019) to remove SNPs that were in close linkage with other SNPs in the set (option "--indep-pairwise 50 10 0.2" to eliminate SNPs with r^2 greater than 0.2 in overlapping windows of 50 consecutive SNPs moving 10 SNPs at a time between windows) as they are not independent data points.

Following the analysis pipeline previously employed for Bull Trout (Geraldes and Taylor, 2020) and Arctic Grayling (Geraldes and Taylor, 2021), we used two complementary and independent approaches to infer patterns of population structure in Rainbow Trout. 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 seven and terminated each run when the difference in log-likelihood between successive iterations fell below 1×10^{-9}. We chose the K value that minimized the cross-validation error (CVE), i.e., that best fit the data (Alexander et al. 2009), and made one last run with K varying from two to four

using 1,000 bootstraps to estimate the standard error of the inferred admixture proportions for each K.

To select target loci for SNP genotyping to allow for the assignment of Rainbow Trout to the UP and DP genetic groups identified (see results below) we followed the same approach as used for Bull Trout (Geraldes and Taylor 2020) and Arctic Grayling (Geraldes and Taylor 2021 and this report). Namely, we used VCFtools (Danecek et al. 2011) to estimate Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984) for each locus between samples collected UP and DP for which more than 90% of their ancestry in the Admixture analyses could be assigned to UP for samples collected UP, or to DP for samples collected DP. For this analysis we used the Admixture results assuming three genetic groups (K=3) as this was the number of groups for which the cross-validation error was minimized indicating that it was the solution that best fit the data. F_{ST} is a summary statistic that takes a value of one if two groups of samples are fixed for different alleles at one SNP locus and a value of zero if the two groups have the same allele frequencies at that SNP locus. We inspected each SNP in descending order of their F_{ST} rank to determine their suitability for designing custom TaqMan™ (Applied Biosystems; Foster City, CA, USA) SNP genotyping assays. Each TaqMan™ assay uses coloured fluorescent "reporter" dyes (VIC and FAM) to efficiently determine the genotype of each fish at a single SNP amplified by quantitative PCR. Specifically, we only selected SNPs for assay design if they: a) had low missing data even if higher genotype filtering criteria were applied (HaplotypeCaller's genotype quality of 20 instead of 10, i.e. probability of an incorrect genotype call is 1% or lower), b) if we had sequence data for most samples for 30 bp upstream and downstream of the SNP, i.e. the flanking

region, c) if there were no other polymorphisms in the flanking region, and so that d) all selected SNPs were from different chromosomes.

Ten SNPs that passed these criteria were submitted for TaqMan™ assay design using the ThermoFisher online design tool and ordered for testing (Table 8). For assay testing we selected an initial set of 15 Rainbow Trout samples that had been used for the GBS experiment and had amongst them representatives of all three possible genotypes at all loci. Genotyping of Rainbow Trout samples followed the protocol for Bull Trout genotyping (Geraldes and Taylor 2020) and Arctic Grayling (Geraldes and Taylor 2021). One assay, rb08_01 failed to amplify and was not used further (Table 8).

Table 8. TaqMan™ assays tested to genotype Rainbow Trout samples.

TaqMan™ assay	SNP name	F_{ST}	F_{ST} rank	Test	Used in Assignments
rb08_1	NC_048572.1:31515353	0.98	1	Fail	No
rb06_3	NC_048570.1:26151074	0.96	3	Pass	Yes
rb05_9	NC_048569.1:42888801	0.95	9	Pass	Yes
rb16_10	NC_048580.1:76321022	0.93	10	Pass	No
rb24_15	NC_048588.1:21113506	0.93	15	Pass	Yes
rb04_17	NC_048568.1:17726244	0.93	17	Pass	Yes
rb02_19	NC_048566.1:86237336	0.91	19	Pass	Yes
rb28_23	NC_048592.1:7410147	0.90	23	Pass	No
rb17_24	NC_048581.1:35291794	0.90	24	Pass	No
rb18_26	NC_048582.1:70358502	0.89	26	Pass	Yes

We then tested the power of the nine TaqMan™ assays to assign samples to the UP and DP groups. To do so, we genotyped with the nine TaqMan™ assays, 197 Rainbow Trout samples that we divided into two groups: Known Genotype samples and Test samples. Known genotype samples were all 96 samples used for SNP discovery with GBS. Test samples were an additional set of 101 samples collected outside of the Peace River mainstem for which we knew the sampling location, but for which we had

no prior genetic data and hence did not a priori know if they belonged to UP, DP or if they were admixed between UP and DP.

Analysis of the genotyping and assignment data of the known genotype samples and the test samples revealed that: a) for three of the nine TaqMan™ assays (rb16_10, rb28_23 and rb17_24) heterozygote genotypes were sometimes hard to clearly distinguish from homozygous genotypes resulting in increased amounts of missing genotypes and, b) the power to correctly assign samples to UP or DP did not change considerably when using data from nine or six loci (data not shown). Consequently, we decided that the best approach for assignment of Rainbow Trout samples collected in the Peace River mainstem to UP or DP was to genotype samples with six loci only (Table 8).

Finally, all 434 Rainbow Trout samples collected in the Peace River (including seven samples from Dry Creek; Table 1) were genotyped with the six TaqMan™ assays selected above. Assignment of samples to UP and DP followed the same protocols as for Bull Trout (Geraldes and Taylor, 2020) and Arctic Grayling (Geraldes and Taylor, 2021). Namely, we used the program GeneClass2 (Piry et al., 2004) to assign samples to UP and DP following the method of Rannala and Mountain (1997). Samples were considered assigned to UP or DP if they had 95% or higher chance of being from that group. The set of 50 non-admixed samples (40 collected UP and 10 collected DP) used to estimate F_{ST} for each SNP between UP and DP were used as reference samples for the UP and the DP genetic groups in the assignment tests.

Results

Results from a PCA (Figure 1; Appendix 3) on the Rainbow Trout dataset with the 52 samples from the Halfway River (N=29), the Moberly River (N=7) and the Pine River (N=16) genotype dataset (20,420 SNPs) revealed that the first axis of variation (explaining 9% of variation) largely separated most Halfway River samples from others and the second axis (explaining 4.78% of variation) largely separated samples from the Pine River collected in the Burnt River and Blind Creek (Pine BB in Figure 1) from samples collected in the Moberly River and samples collected in the Pine River mainstem and its tributary Willow Creek (Pine PW in Figure 1). The third axis of variation explained nearly the same amount of variation (4.12%) as the second axis and separated samples from the Pine River into the same two groups, yet, on this axis, the samples collected in the Moberly River, separated more clearly from the samples from the Pine River (Figure 1). Results from the Admixture analysis are in good agreement with the PCA. A model with two genetic groups (K=2; Figure 1; Appendix 3) identified one genetic group with most samples collected in the Halfway River and one other with the samples from the Pine River collected in the Burnt River and Blind Creek (Pine BB). In this analysis, some samples from the Halfway River, all samples from the Moberly River, and some samples collected in the Pine River mainstem and its tributary Willow Creek (Pine PW) appeared as highly admixed. A model with three genetic groups (K=3; Figure 1; Appendix 3) also identified some Halfway samples as one group, all the samples from the Pine River collected in the Burnt River and Blind Creek (Pine BB) as another group, and most samples from the Moberly River as a third genetic group. In this analysis, some samples from the Halfway River appeared admixed with the Moberly group and some with the Moberly and Pine BB groups. Samples collected in the Pine

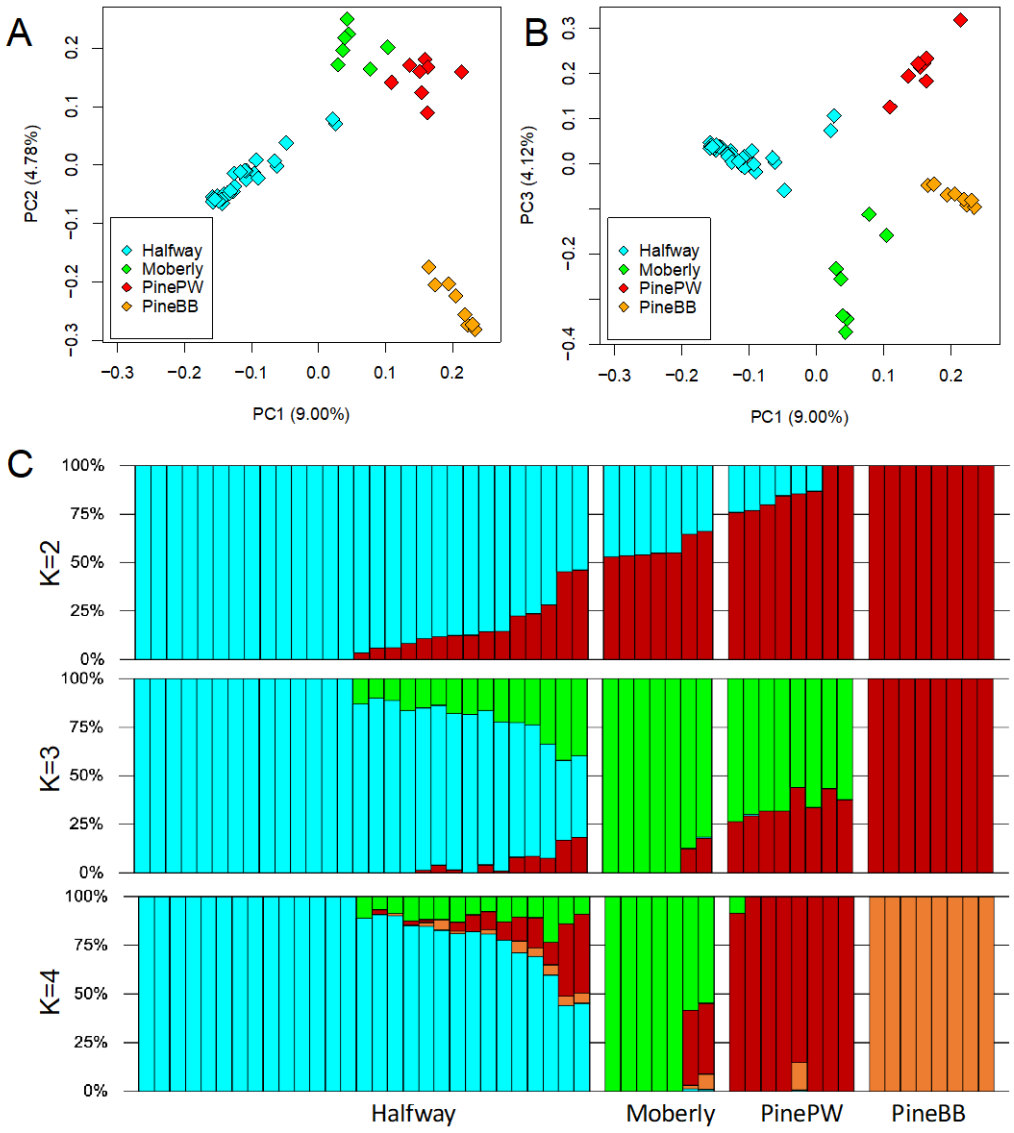

Figure 1- Population structure of Rainbow Trout populations from three tributaries of the Peace River, the Halfway River (N=29), the Moberly River (N=7), and the Pine River (N=16). *Samples from the Pine River are divided into two groups, those sampled in the Pine River mainstem and its tributary Willow Creek (Pine PW, N=8), and those sampled in two tributaries of the Pine River, Blind Creek and Burnt River (Pine BB, N=8). Both the PCA (A) and Admixture (B) analysis were performed on 20,420 SNPs. (A) Each diamond represents a single Rainbow Trout sample. The first two principal components (PC) are plotted on the left panel and the first and third PCs are plotted on the right panel. The percentage of variation in the data explained by each PC is indicated in the axis name. (B) Admixture results are shown for models with two genetic groups, K=2 (top), three genetic groups, K=3 (middle), and four genetic groups, K=4 (bottom). 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 group.*

River mainstem and its tributary Willow Creek (Pine PW) appeared as highly admixed between the Pine BB group and the Moberly group. Finally, a model with four genetic groups (K=4, Figure 1; Appendix 3) maintained the Halfway and Moberly groups and separated the samples from the Pine into the Pine BB and the Pine PW groups. In this

analysis, samples from the Pine River showed little or no admixture, two Moberly River samples appeared as highly admixed with the Pine PW group and many samples from the Halfway showed some admixture with all other three groups.

We repeated the same analyses as above but with the genotype dataset (35,238 SNPs) that included all 96 samples (i.e., all LAA samples and the six samples from the hatchery strains; Appendix 3). The first axis of variation (explaining 7.03% of the variation in the data, Figure 2) again mostly separated most samples collected in the Halfway River at one end of the component axis, from samples collected in the Pine River as well as samples from the three hatchery strains (Fraser Valley, Pennask Lake and Blackwater River) at the other end. Samples collected in the Moberly River and most other sources appeared at an intermediate position along this first principal component axis. Notably, most samples from Lynx Creek appeared closer to the samples collected in the Halfway River, than most other samples. The second axis of variation (explaining 3.84% of variation) separated samples from the Pine River, collected in the Burnt River and Blind Creek (Pine BB in Figure 2), and samples from the Blackwater River and Fraser Valley hatchery strains from samples collected in most other locations (including the Pennask Lake hatchery strain). The third axis of variation (explaining 3.15% of the variation) mostly separated the two samples of the Pennask Lake hatchery strain from the samples collected in Lynx Creek (Figure 2). Overall, samples from most source locations (Dinosaur Reservoir, Farrell Creek, Maurice Creek and Moberly River) appeared in a large group in the centre of the plots suggesting that either they are highly admixed or that there is little information along these axes to separate them as individual genetic groups. Both in the analysis with 52 samples and the analysis with 96 samples, further axes of variation explained much less variation in

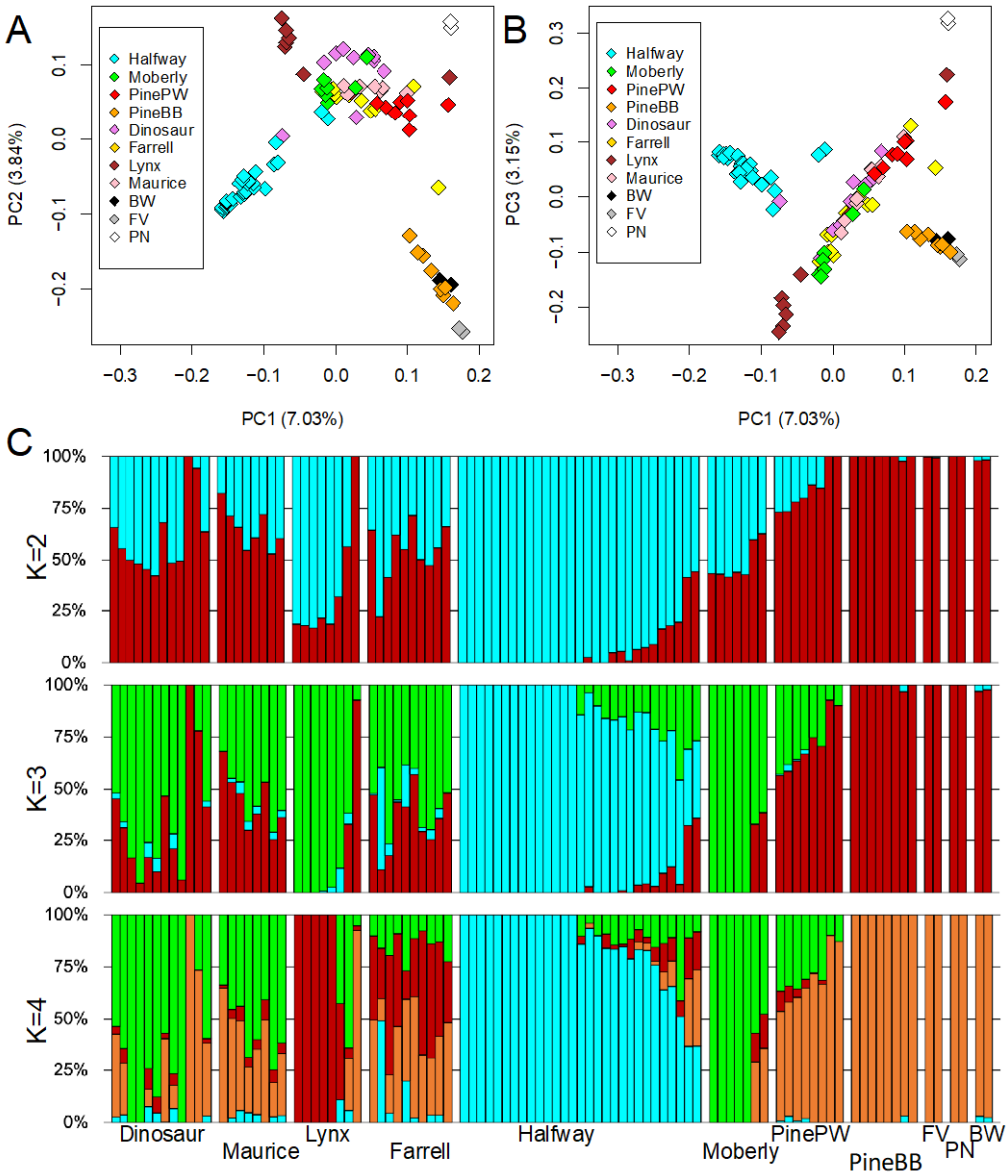

Figure 2 - Population structure of Rainbow Trout in the Peace River. Samples included are form the Halfway River (N=29), the Moberly River (N=7), the Pine River mainstem and it tributary Willow Creek (Pine PW, N=8), two tributaries of the Pine River, Blind Creek and Burnt River (Pine BB, n=8), Lynx Creek (N=8), Maurice Creek (N=8), Farrell Creek (N=10) and Dinosaur Reservoir (N=12). Also included are two samples each from three hatchery strains of Rainbow Trout commonly used for stocking in the area, Fraser Valley Domestic (FV), Pennask Lake (PN), and Blackwater River (BW). *Both the PCA (A) and Admixture (B) analysis were performed on 35,238 SNPs. (A) Each diamond represents a single Rainbow Trout sample. The first two principal components (PC) are plotted on the left panel and the first and third PCs are plotted on the right panel. The percentage of variation in the data explained by each PC is indicated in the axis name. (B) Admixture results are shown for models with two genetic groups, K=2 (top), three genetic groups, K=3 (middle), and four genetic groups, K=4 (bottom). 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 group.*

the data and failed to show samples from Dinosaur Reservoir, Farrell and Maurice creeks as genetically distinct.

The Admixture analysis (Figure 2) at K=2 showed samples from the hatchery strains grouping with samples from the Pine River collected in the Burnt River and Blind Creek (Pine BB) and samples from most other sources as highly admixed, except for most samples collected in the Halfway River. Assuming three genetic groups, i.e., K=3 which is the model that best fits our data (it minimizes the cross-validation error of the analysis), most samples from the Moberly River appeared as a distinct genetic group together with most samples from Lynx Creek. Finally, at K=4 most samples from Lynx Creek appeared genetically distinct from others.

We used the results from the 96 samples dataset at K=3, which identified three genetic groups largely corresponding to i) samples collected in the Halfway River, ii) samples collected in the Moberly River and Lynx Creek, and iii) samples collected in the Pine River, to generate a set of samples with low levels of mixed ancestry representative of UP and DP source populations. Here, we eliminated any samples collected UP, that had at least 10% ancestry in the Pine River group, and any samples collected DP that had less than 90% ancestry in the Pine group. This resulted in the retention of 50 samples and the elimination of 40 samples. Forty samples from the UP group were retained, three from Dinosaur Reservoir, six from Lynx Creek, 26 from the Halfway River and five from the Moberly River and 10 samples from the DP group were retained.

Genotyping of six ancestry informative SNPs with TaqMan™ assays was performed for a total of 631 Rainbow Trout. Genotyping was 99.5% successful (i.e., we obtained 3766 genotypes out of 3786 possible genotypes, 631 samples times 6 loci). One sample from the Peace River failed at every locus, two samples failed at two loci and 10 samples failed at one locus only.

Assignments of samples from the Known Genotype group (i.e., all 96 samples of know genotype from the SNP discovery set) were largely concordant with the Admixture (at K=3) results. All 21 samples with less than 30% UP in the Admixture analysis were assigned to DP with more than 95% confidence (i.e., an assignment score to UP below 5%; Figure 3) and all 50 samples with more than 70% ancestry in UP in the Admixture analysis were assigned to UP with more than 95% confidence (i.e., an assignment score to UP over 95%; Figure 3). There were 25 samples with inferred UP ancestry in the admixture analysis between 30 and 70%. Of those, six were assigned to the DP group (i.e., an assignment score to UP below 5%; Figure 3), 14 to the UP group (i.e., an

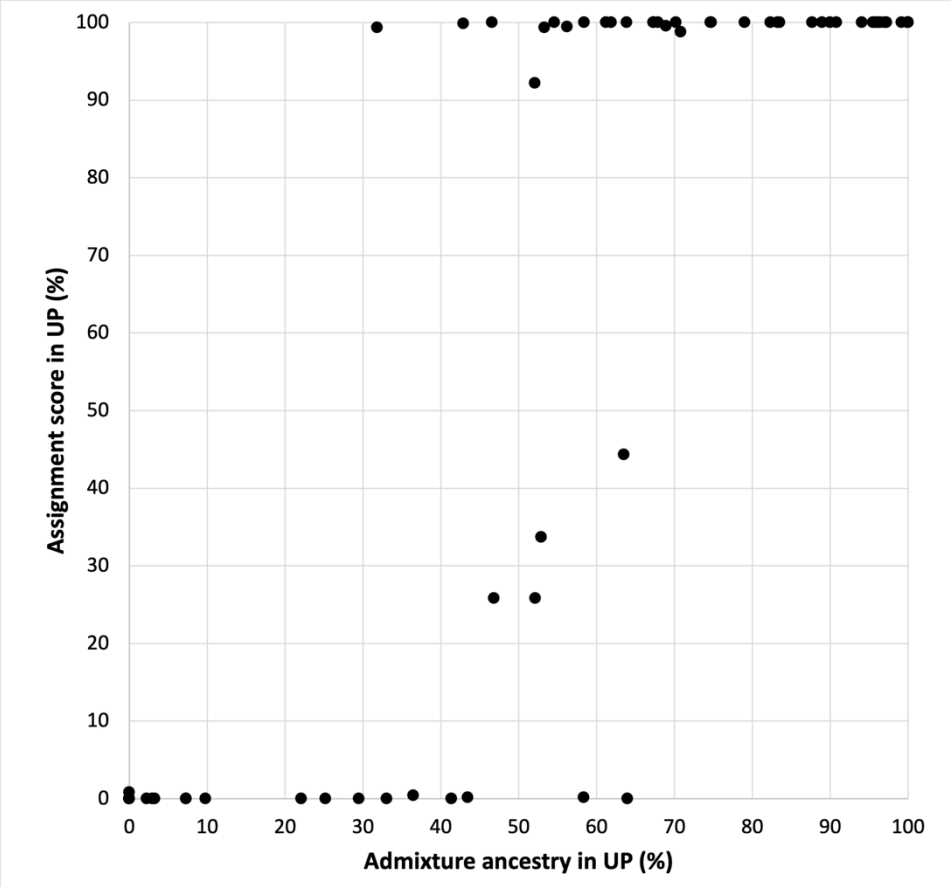

Figure 3 – Assignment score in genetic group UP as a function of Admixture ancestry in genetic group UP for 96 Rainbow Trout samples for which both GBS and TaqMan™ assay genotype data were available.

assignment score to UP above 95%; Figure 3), and five samples could not be assigned to either group with 95% confidence (Figure 3).

Next, we performed assignment tests for the 101 test samples (samples of known sampling location but unknown genotype; Table 9). All samples from the Halfway River (N=34) from the Moberly River (N=5) and Lynx Creek (N=8) were assigned to UP and all samples from the Pine River (N=10) and from the three hatchery strains (N=6) were assigned to DP. These were also the sample locations where less admixture was detected in the PCA and Admixture analysis (Figure 2). Rainbow Trout sampled from the Dinosaur Reservoir, Farrell Creek and Maurice Creek were assigned both UP and DP and some could not be assigned with more than 95% confidence (Table 9). These results are again similar to those of the PCA and Admixture analysis where large proportions of the samples at these three locations were highly admixed and some even had more than 90% ancestry in DP (Figure 2).

Table 9. Population assignment results of reference Rainbow Trout samples to upstream of the project (UP) and downstream of the project (DP) population using genotype data from six TaqMan™ assays.

		Assignment		
Location	**Total**	**UP**	**DP**	**Unassigned[1]**
UP/Halfway River	34	34 (100.0%)	0 (0.0%)	0 (0.0%)
UP/Moberly River	5	5 (100.0%)	0 (0.0%)	0 (0.0%)
UP/Peace River (Dinosaur Reservoir)	12	5 (41.7%)	6 (50.0%)	1 (8.3%)
UP/Peace River (Farrell Creek)	13	6 (46.2%)	3 (23.1%)	4 (30.8%)
UP/Peace River (Lynx Creek)	8	8 (100%)	0 (0.0%)	0 (0.0%)
UP/Peace River (Maurice Creek)	13	8 (61.5%)	3 (23.1%)	2 (15.4%)
DP/Pine River	10	0 (0.0%)	10 (100.0%)	0 (0.0%)
Hatchery strains	6	0 (0.0%)	6 (100.0%)	0 (0.0%)

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

Finally, we performed assignment tests for the 433 Rainbow Trout samples collected in the Peace River (including Dry Creek) for which we obtained genotype data with at least four out of six TaqMan™ assays (one sample out of 433 failed with all TaqMan™ assays). Across all sampling years (2018-2020) and sampling locations, 57.3% of fish in the Peace River were assigned to UP, 25.4% were assigned to DP and we could not assign 17.3% of fish with over 95% confidence (Table 10). Over 50% of fish collected in sampling sections located UP (Sections 1 and 3) were assigned to UP in each year. Conversely, despite very low sample sizes, all fish captured in Section 6 (at the confluence of the Pine and Peace Rivers; N=6 over the three years) were assigned to DP, 90% of fish captured further downstream in Section 7 were assigned to DP (N=10 over the three years), and the one fish captured in Section 9 (near the Alberta border) was also assigned to DP. There was high consistency across sampling years in the proportion of fish assigned to UP and DP (as well as fish that could not be assigned with more than 95% confidence).

Table 10. Number of Rainbow Trout samples caught in the Peace River (PR) assigned (% of total) to upstream of the project (UP) and downstream of the project (DP) population groups based on genotypes at six ancestry informative SNPs with more than 95% confidence.

			Assignment		
Sampling Location	**Year**	**Total**	**UP**	**DP**	**Unassigned[1]**
All Peace River	2018	145	88 (60.7%)	35 (24.1%)	22 (15.2%)
	2019	155	88 (56.8%)	37 (23.9%)	30 (19.3%)
	2020	133	72 (54.1%)	38 (28.6%)	23 (17.3%)
	All years	433	248 (57.3%)	110 (25.4%)	75 (17.3%)
PR Dry Creek	2020	7	3 (42.8%)	2 (28.6%)	2 (28.6%)
PR Section 1	2018	62	40 (64.5%)	12 (19.4%)	10 (16.1%)
	2019	75	43 (57.3%)	15 (20.0%)	17 (22.7%)
	2020	68	35 (51.5%)	18 (26.5%)	15 (22.1%)
	All years	205	118 (57.6%)	45 (22.0%)	42 (20.4%)

			Assignment		
Sampling Location	**Year**	**Total**	**UP**	**DP**	**Unassigned[1]**
PR Section 3	2018	66	45 (68.2%)	11 (16.7%)	10 (15.1%)
	2019	65	42 (64.6%)	12 (18.5%)	11 (16.9%)
	2020	50	31 (62.0%)	13 (26.0%)	6 (12.0%)
	All years	181	118 (65.2%)	36 (19.9%)	27 (14.9%)
PR Section 5	2018	11	3 (27.3%)	6 (54.5%)	2 (18.2%)
	2019	9	3 (33.3%)	4 (44.4%)	2 (22.2%)
	2020	3	2 (66.7%)	1 (33.3%)	0 (0.0%)
	All years	23	8 (34.8%)	11 (47.8%)	4 (17.4%)
PR Section 6	2018	2	0 (0.0%)	2 (100.0%)	0 (0.0%)
	2019	3	0 (0.0%)	3 (100.0%)	0 (0.0%)
	2020	1	0 (0.0%)	1 (100.0%)	0 (0.0%)
	All years	6	0 (0.0%)	6 (100.0%)	0 (0.0%)
PR Section 7	2018	4	0 (0.0%)	4 (100.0%)	0 (0.0%)
	2019	2	0 (0.0%)	2 (100.0%)	0 (0.0%)
	2020	4	1 (25.0%)	3 (75.0%)	0 (0.0%)
	All years	10	1 (10.0%)	9 (90.0%)	0 (0.0%)
PR Section 9	2019	1	0 (0.0%)	1 (100.0%)	0 (0.0%)

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

DISCUSSION

Our analyses of the samples collected in 2020 in the Peace River mainstem continue to find that the strong majority of Bull Trout collected from throughout the various sampling sections of the Peace River mainstem originate from spawning tributaries upstream of the Project area. This dominance of fish assigned to upstream tributaries was despite the observation that 43% of all 2020 fish assayed were collected from sections in the mainstem Peace River located downstream of the Project site (sections 5, 6, 7, and 9). The consistent result observed for samples collected from 2016-2020 clearly suggests that most production of Bull Trout that use the mainstem Peace River comes from tributaries located upstream of the Project. Consequently, the continued passage of mainstem Bull Trout upstream of the Project area would appear to be consistent with promoting the continued high productivity of upstream tributaries. The low percentage of Bull Trout encountered in the mainstem Peace River that were assigned across all years to tributaries downstream of the Project area may also stem from the suggestion (see Taylor et al. 2013) that fish in the Pine River drainage are largely residents and tend not to migrate into the mainstem Peace River.

As indicated in Taylor et al. (2013) and Geraldes and Taylor (2020, 2021) the lower degree of genetic distinction in Arctic Grayling across tributaries in the LAA necessitated the development of additional TaqMan™ assays (from 7 to 11) to reduce the proportion of fish unassigned to UP or DP. The incorporation of the additional SNP assays reduced the percentage of unassigned fish in the reference and test samples by 71% with essentially identical assignments to each of the four tributaries (Halfway, Moberly, Pine, and Beatton rivers). One point to consider is that although using all 11 SNP assays reduced the number of unassigned fish, 92% of all fish could be confidently

assigned with the original seven loci and the additional loci made no material difference to the assignment of fish to UP or DP and no difference in the assignment of reference or test fish to the four individual tributaries (two fish remained unassigned in both cases). Consequently, if the main objective is to assign fish as to UP or DP in origin, some effort and time could be realized if using the original seven loci only. That said, if only seven loci were used, the proportion of fish that are unassignable to the four individual tributaries at 95% confidence would undoubtedly increase (it was 7.1% with 11 loci).

Consistent with the pattern observed for Bull Trout, there is a strong preponderance of fish sampled in the mainstem Peace River (in all years of sampling including 2020) that were assigned to UP. Although there was more variation, year-to-year, in the degree of predominance of UP-assigned fish, much of this variation is likely attributable to considerable variation in sample sizes. For instance, in section 1, sample sizes ranged between only one and three fish for 2020 and 2018-2019, respectively, and comparing percentages of fish assigned as UP or DP between these sample years based on such small samples is clearly problematic. By contrast, when sample sizes are larger and more equitable between years (e.g., section 5), the results are much more consistent (both 100% UP).

Considering only sampling sections of the Peace River mainstem with appropriate sampling sizes across all sampling years (i.e., 15 or more samples; sections 3, 5, 6 and 7), tributary-based assignments suggests that the Moberly River produced most fish sampled. The data also indicate that Arctic Grayling can have wide-ranging movements when in the mainstem; indeed, one fish sampled in section 1 was assigned to the Pine River and two sampled in the most downstream section (9) were assigned to

the Moberly River. No fish sampled in the mainstem Peace River were assigned to the Beatton River (even when sampled from the proximate section 7) suggesting Arctic Grayling from this tributary rarely use the mainstem Peace River as a foraging area or migration corridor.

Patterns of genetic structure in Rainbow Trout were perhaps the most complex of the three species examined to date. In addition, our analysis of Rainbow Trout could not benefit from information gleaned from previous microsatellite DNA for fish from the LAA, information that was available both for Bull Trout and Arctic Grayling (Taylor et al. 2013). Further complicating analysis and interpretation of genomic data for LAA Rainbow Trout is the fact that the area has been subject to numerous stocking events with myriad hatchery strains of Rainbow Trout (summarized by Euchner 2011). Our analyses indicated that Rainbow Trout in the LAA contained a much higher number of fish that were admixed, i.e., individual fish whose genomes consisted of mixtures of two or more genetic groups. This was particularly true of fish from the Pine River proper and one of the Pine River tributaries (Willow Creek) and for some fish within the Halfway River (Figure 1). Relatively high levels of admixture in Rainbow Trout were also observed when further samples were examined (Figure 2) that included fish from Dinosaur Reservoir, Lynx, Maurice, and Farrell creeks, and the three hatchery strains. Interestingly, fish from the three hatchery strains were composed of the same genetic group in the Admixture analysis as fish from two Pine River tributaries (Burnt River and Blind Creek) and this genetic group contributed to much of the admixture found in fish from all other localities. Consequently, either the three hatchery strains are similar genomically to many fish in the LAA or there has been some introgression of hatchery strains into Rainbow Trout in the LAA associated with stocking activities in the past. The

former possibility seems unlikely given the disparate geographic origins of the hatchery strains (two from distant localities in the Fraser River basin and one originally of Californian origin) and given that Rainbow Trout from some localities within the LAA are quite distinct from one another (e.g., Halfway River vs. Moberly River). Although the Admixture analysis did not differentiate well amongst the three hatchery strains (possibility owing to assaying only two fish per strain), the PCA did reveal some distinctions (particularly of the Pennask Lake strain), and clearly indicated similarity between the hatchery strains and some Rainbow Trout with the LAA.

Introgression between hatchery strain and putatively native Rainbow Trout in the LAA is consistent with observation that between 1995 and 2010, thousands of Rainbow Trout of all three strains have been stocked in the Dinosaur Reservoir drainage, although virtually all were triploids (i.e., largely sterile) or all female triploids to reduce the possibility of spawning (Euchner 2011). Further, many such stocked fish were entrained through the Peace Canyon Dam and into the Peace River mainstem, and hatchery-wild introgression is common in other geographic areas (Steimer 2006; Taylor et al. 2007). Considerable levels of *known* stocking events of diploid Rainbow Trout have also occurred in several presumably isolated lakes, downstream of the Project area (FFSBC 2021).

On balance, it seems reasonable to assume that the genetic structure of putatively native Rainbow Trout in the LAA has been influenced by introgression with hatchery strains, and this has at least two consequences for our assignment analyses. First, stocking of hatchery strains in various areas of the LAA and subsequent introgression with native Rainbow Trout has the potential to result in genetic homogenization across localities, i.e., common hatchery alleles are spread across

localities (e.g., Utter 1998), and making identification of diagnostic markers more challenging. Second, and perhaps contributing to potential homogenization, straying may increase in hatchery-produced salmonids under some circumstances and may vary amongst strains (Quinn 1993; Westley et al. 2013), particularly if they are introduced into reservoirs with little opportunity to imprint on individual streams. All of these factors: hatchery stocking, straying, and introgression and potential homogenization almost surely account for the higher degree of Rainbow Trout that could not be assigned as UP or DP with a minimum 95% confidence relative both to Bull Trout and Arctic Grayling (17.3% versus < 3%). Regardless of the higher uncertainty in Rainbow Trout assignments, the > 80% of Rainbow Trout that were assigned with at least 95% confidence indicated a different pattern to the UP vs. DP assignments relative to sample location in the mainstem Peace River compared both to Bull Trout and Arctic Grayling. A clear majority of Rainbow Trout sampled upstream of the Project (sections 1 and 3) were assigned as UP whereas the opposite was true of fish sampled below the Project (sections 5-7, 9) – the clear majority here were assigned as DP. Although the smaller sample sizes for Rainbow Trout warrant some caution (particularly downstream of the Project), this result would seem to imply that Rainbow Trout are less spatially extensive in their use of the mainstem Peace River.

In conclusion, our work to date has resulted in 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. In the coming months, assignments will continue for samples collected in 2022, and we will be continuing work we have started on: (i) developing more sensitive assays for Bull Trout and Rainbow Trout for the analysis of demographic characteristics (e.g., effective

population size, genetic variation, parentage), and (ii) population structure of three non-game species (Longnose Dace, Redside Shiner, and Slimy Sculpin).

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