Native Kokanee persistence in the Williston Reservoir

Fish & Wildlife Compensation Program – Peace Region Project
F21 Kokanee Genetics (PEA-F21-F-3361-DCA)
SUMMARY REPORT

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EXECUTIVE SUMMARY

The Fish & Wildlife Compensation Program Peace Basin Reservoirs Action Plan (FWCP 2014) identified a priority action to "*undertake a Kokanee assessment study to summarize status, trends, and aquatic and terrestrial ecosystem impacts and potential ecological risks associated with Kokanee introductions*" (Action ID: 2a-1). A direct contribution agreement was awarded to the University of Northern British Columbia (UNBC) by the Fish & Wildlife Compensation Program (FWCP) – Peace Region to examine whether native Kokanee (*Oncorhynchus nerka*) still persist in the Williston Reservoir or whether Columbia-origin Kokanee introduced in the 1990s have displaced the native population.

In August 2021, pelagic surveys were conducted in the Williston Reservoir by gill net and trawl to capture Kokanee for subsequent genotyping. To assess genetic relationships for the Kokanee, four reference populations were included in our analysis. The first population was Kokanee from the headwaters of the Parsnip River (Arctic and Tacheeda Lakes). The second reference population was Columbia-type Kokanee samples collected in 2006, 2018 and 2019 from Germansen River and Russel Creek. Our earlier work showed that all tributary spawning Kokanee did not differ from the Columbia River donor populations and there was no evidence of introgression with the native Williston Kokanee. DNA was extracted from scale samples archived by the FWCP – Peace Region from 1988 through 2000 which provided genetic signatures for native Kokanee within the reservoir and were our third reference population. The fourth population was Kokanee from the headwaters of the Finlay River sampled from Thutade Lake.

We used 14 microsatellite loci to genotype 165 Kokanee collected in the August 2021 pelagic surveys and compared them to 623 previously genotyped Kokanee from the four reference populations. Our analysis identified four genetic clusters that were associated with sample location. The four clusters were defined as: PARSNIP that included all fish sampled from Arctic and Tacheeda Lakes; COLUMBIA-TYPE that included fish sampled from Germansen River and Russel Creek and all Kokanee sampled from the reservoir in 2021; NATIVE that included Kokanee caught in the Williston Reservoir watershed before the introduction of Columbia River origin fish; and THUTADE that included Kokanee sampled from Thutade Lake.

Our finding that all Kokanee sampled from the reservoir in 2021 were of Columbia River origin indicates that native Williston Kokanee have not persisted in the reservoir. Our earlier work found that Kokanee collected from the GM Shrum intake towers in 2016 and 2019 were also only Columbia-type. Additionally, attempts in 2019 to collect spawners from the lower reaches of the Finlay River where native Kokanee had traditionally spawned were only Columbia-type Kokanee. Native Williston Kokanee, therefore, appear to have been extirpated from the reservoir.

Based on our findings from extensive spatial and temporal sampling in the Williston Reservoir Watershed, more information on the Kokanee of this system is required to effectively manage the various genetic populations. Genotyping Kokanee in the Dinosaur Reservoir would identify a potential refugium for native Williston Kokanee as tributary spawning by Kokanee does not appear to happen. Additional analyses of the timing of divergence between Thutade and native Williston Kokanee could inform the rate of potential "re-introduction" of native genotypes into the reservoir. A survey of pelagic piscivores that incorporates diet assessments could also help assess the conservation value of Columbia-type Kokanee, which appear to be the dominant population in the Williston Reservoir.

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INTRODUCTION

The Williston Reservoir is the largest body of water in British Columbia and was created by the impoundment of the Upper Peace River following the construction of the WAC Bennett Dam in 1968 (Langston & Murphy 2008) which reached the maximum permissible operating level for the first time in 1972 (Hirst 1991). The reservoir's high annual drawdown of approximately 17 m and rapid flushing rate of 2.2 years have prevented the formation of a functional littoral zone. These factors combined with high dissolved oxygen levels and erosion of banks around the reservoir leading to decreased water clarity and low productivity have resulted in the reservoir becoming ultra-oligotrophic (Blackman et al. 1990; Stockner et al. 2005). There has also been a change in abundance of fishes following flooding of the upper Peace River. Species that were adapted to the pre-impoundment lotic environments, notably Arctic Grayling (*Thymallus arcticus*) and Mountain Whitefish (*Prosopium williamsoni*), have been replaced with those more suited to reservoir conditions, such as Kokanee (*Oncorhynchus nerka*), Lake Whitefish (*Coregonus clupeaformis*), and Lake Trout (*Salvelinus namaycush*) (Sebastian et al. 2009; Langston 2012).

Increases in the number of Kokanee in the reservoir have been quite dramatic over the years due to changes in the environment, but also due to management decisions. Sampling surveys in the reservoir conducted in 1974 captured only 8 Kokanee which represented just 0.14% of the total number of pelagic fish (Barrett & Halsey 1985). Fourteen years later in 1988, the number of Kokanee within the pelagic fish community had increased with 79 fish which represented 2.28% of the fish caught (Blackman 1992) – suggesting that Kokanee were becoming more abundant within the lentic environment of the reservoir (Blackman et al. 1990). To facilitate the expansion of Kokanee into tributaries of the Williston Reservoir that were more accessible to anglers and also provide a prey source for large piscivorous fish species, a stocking program was initiated using fish from the Columbia River (Blackman et al. 1990; Langston 2012). Stream-spawning Kokanee from Arrow Reservoir (Hill Creek) and Kootenay Lake (Meadow Creek) were stocked extensively in tributaries of Williston Reservoir from 1990 to 1998. During this time, over 3 million juvenile Kokanee were stocked into five rivers that flow into the Williston Reservoir (Langston & Murphy 2008). The five systems were: Carbon Creek, Dunlevy Creek, Manson River, Nation River, and Davis River; three systems on the east side of the reservoir and two rivers that flow into the southwest portion of the reservoir.

An assessment of the Columbia-origin Kokanee stocking program was conducted in 1994. Adult Kokanee in spawning condition were captured by gill nets in the embayments of Carbon and Dunlevy Creeks in the Peace Arm of the reservoir (Langston & Zemlak 1998). Although gill netting was limited to only two locations in the Peace Arm, the proportion of Kokanee collected in the pelagic survey was 11.9%, higher than had been observed in the previous surveys. DNA extracted from archived scale samples from these fish were genotyped. Three out of 86 Kokanee gill netted in Carbon Creek and Dunlevy Creek embayments were genotyped as native Williston Kokanee; all the rest were Columbia-origin Kokanee (Wilson & Shrimpton 2021). The Columbia-origin Kokanee, therefore, appeared to be successfully colonizing the reservoir shortly after their introduction. Locations for gill netting, however, were targeted to be close to the stocking locations as it was expected that spawners would return to their natal streams and should have been more abundant near their natal streams at this time. Native Williston Kokanee were only known to spawn in side-channels and sloughs of the lower Finlay River (Fielden 1991; McLean & Blackman 1991; Fielden 1992) and may have been less abundant in the Peace Arm of the reservoir at this time of the year.

The Kokanee population showed substantial initial growth and was estimated to be 1 million fish in the Williston Reservoir following a hydroacoustic assessment in 2000 (Sebastian et al. 2003), 10 years after the initiation of the stocking program. These measurements were complemented with a gill net sampling program in the same year. At this time, Kokanee represented a greater proportion of the pelagic fish community in the reservoir. Although only 47 Kokanee were captured, this represented a higher percentage of pelagic fish than previous work: 12.7% (Pillipow & Langston 2002). DNA extracted from archived scale samples from the fish caught in 2000 were also genotyped; 25 were native Williston Kokanee and 20 were Columbia-origin Kokanee (Wilson & Shrimpton 2020)—suggesting similar abundance for the two Kokanee populations. Interestingly, there was regional variation in genotypes, as only native Kokanee were captured in the Finlay Reach and most of the Kokanee were Columbia-origin in the Peace Arm. The findings suggested that the two populations of Kokanee may have been exploiting different regions of the reservoir, although the spatial pattern is more likely due to the timing of sampling; Columbia-origin Kokanee peak spawning occurs in mid-September (Langston 2012), whereas native Kokanee were observed spawning in the Finlay River between November and January (Fielden 1991; McLean & Blackman 1991).

By 2008, it was estimated that the Kokanee population could be up to 9 million fish, overtaking Lake Whitefish as the most abundant species in the pelagic zone of Williston Reservoir (Sebastian et al. 2009) and a substantial increase from the 1 million estimate made in 2000 (Sebastian et al. 2003). Kokanee represented 45.2% of the pelagic fish captured, although the survey in 2008 was limited to the Peace Arm. Unfortunately, no archived tissue or scale samples were available for genotyping, and it is unclear if any of these fish were native Kokanee. More recent sampling in 2021 at sites throughout the reservoir found that the proportion of Kokanee was even higher, at 58.9% of the pelagic species captured (O'Connor 2022). Gill net surveys, therefore, have shown a pattern of Kokanee becoming an increasingly greater proportion of the pelagic fish assemblage since the formation of the reservoir (Table 1).

Aerial enumerations conducted from 2002 to 2006 focused on tributary streams where Kokanee were observed to spawn and found that the distribution and abundance of Kokanee in tributaries to the Williston Reservoir poorly reflected the stocking patterns from the 1990's. By 2006, Kokanee were reported to spawn in at least 68 rivers and streams from the Parsnip River watershed to the Finlay River watershed (Langston 2012). Samples from Kokanee caught in 2006, 2018, and 2019 from spawning tributaries, however, reveal genetic signatures that are exclusively Columbia-origin Kokanee (Wilson & Shrimpton 2020; Wilson & Shrimpton 2021).

By sampling tributaries in mid-September which was originally identified as the peak spawning time during aerial surveys, we may miss the native Kokanee which were not previously observed in tributary streams and were documented to spawn later in the fall (Fielden 1991; McLean & Blackman 1991). Native Kokanee also originated from Thutade Lake in the headwaters of the Finlay River (Wilson & Shrimpton 2021). Thutade Lake Kokanee are shore-spawning fish (Blackman et al. 1990; Fielden 1991; McLean & Blackman 1991; Langston & Zemlak 1998). Reproductive ecotypes that differ by spawning location ("shore-spawning" native Williston Kokanee vs "stream-spawning" Columbia-origin Kokanee) or spawning date (September for Columbia-origin Kokanee vs November for native Williston Kokanee) can undergo or maintain genetic differentiation while existing as sympatric populations (Withler et al. 2000; Lemay & Russello 2015). Temporal isolation alone has been shown to be a stronger driver for genetic divergence than site-specific isolation (Young et al. 2004; Whitlock et al. 2018).

Consequently, in our previous work we sought opportunities to obtain Kokanee samples from the reservoir and that would not be biased with stream-spawners. Kokanee and other pelagic species are entrained into the GM Shrum intake towers of the WAC Bennett dam (Algera et al. 2020). Samples from Kokanee entrained in August 2016 and August 2019 provided us with an opportunity to obtain genetic samples from non-spawning fish that were also not spatially biased by tributary spawners. The Kokanee samples from the intake towers, however, were found to be exclusively Columbia-origin (Wilson & Shrimpton 2021). There may be a spatial bias for Kokanee in the reservoir, as Columbia-origin Kokanee had a greater abundance in the Peace Arm and native Kokanee were more prevalent in the Finlay Reach of the Williston Reservoir from gill net surveys in 2000 (Wilson & Shrimpton 2020).

To understand the effects of Kokanee introductions (i.e., stocked Kokanee from the Columbia River system) on the Williston Reservoir ecosystem, the Peace Basin Reservoirs Action Plan (FWCP 2014) identified a priority action to "*undertake a Kokanee assessment study to summarize status, trends, and aquatic and terrestrial ecosystem impacts and potential ecological risks associated with Kokanee introductions. This study would also develop appropriate recommendations for actions, as needed*" (Action ID: 2a-1). Understanding the current status of Kokanee will provide information on where and to what extent Kokanee could be influencing the ecosystems in the region. A survey of pelagic Kokanee from locations throughout the reservoir is required to determine whether native Kokanee have persisted in the reservoir. Once this is known, then appropriate management efforts can be made, if it is possible, to preserve the native Williston population of Kokanee.

TABLE 1. Summary of gill net surveys conducted in the Williston Reservoir from 1974 to 2021 indicating the number of Kokanee (*Oncorhynchus nerka*) samples collected for each location. Columbia-origin Kokanee were first introduced into the Williston watershed in 1990; all Kokanee sampled in 1974 and 1988 were native Williston fish. Values in brackets are number of Kokanee from the sample location genotyped as native Williston fish (Wilson & Shrimpton 2020).

Location	1974	1988	1994	2000	2008	2021
Factor Ross (FR)	2	17	–	6 [6]	–	–
Teare Creek (TC)	0	21	–	7 [7]	–	34
Finlay Forks (FF)	–	–	–	11 [7]	–	38
Blackwater (BW)	0	1	–	3 [2]	–	41
Heather Point (HP)	1	1	–	–	–	7
Clearwater (CW)	0	27	–	5 [2]	29	36
Carbon (CA)	–	–	81[3]	–	–	–
Dunlevy (DN)	1	–	23	–	36	–
Forebay (FO)	4	12	–	13 [1]	15	–
Total	8	79	104	47	80	156
Percent	0.14	2.28	11.9	12.7	45.2	58.9

Locations shown on FIG. 1. Data for 1974 from Table 3 in Barrett & Halsey (1985). Data for 1988 from Table 3 in Blackman (1992). Data for 1994 from Appendix 2 in Langston & Zemlak (1998). Data for 2000 from Appendix A in Pillipow & Langston (2002). Data for 2008 from Appendix 10 in Sebastian et al. (2009). Data for 2021 from Table 2 in O'Connor (2022).

METHODS

FISH CAPTURE METHODS – Pelagic fish surveys were conducted on Williston Reservoir in August 2021 to replicate the earlier surveys conducted in 1988 (Blackman 1992), 2000 (Pillipow & Langston 2002) and only in the Peace Reach in 2008 (Sebastian et al. 2009). The 2021 survey followed standardized hydroacoustic-trawl methods used throughout the Province of BC, but modified for the size of the Williston Reservoir. Night transects for the acoustics and trawl components were conducted by the BC Ministry of Forests, Lands and Natural Resources Operations, and Rural Development. Overnight gill net calibration sets were conducted concurrently by Chu Cho Environmental. Locations where trawl and gill net surveys were conducted in 2021 are shown in FIG. 1. Number of fish caught at each location by method is presented in Table 2. For genetic variability and structure analyses, all Kokanee collected through trawl methods were combined into one group (76 fish) within the 2021 samples.

REFERENCE POPULATIONS – Previously genotyped Kokanee samples collected from the Williston Reservoir watershed from 1988 to 2019 were used as reference populations for comparison to the 2021 Kokanee samples (Wilson & Shrimpton 2020; Wilson & Shrimpton 2021; TABLE 3). Our earlier studies showed that differences did not exist among sample years at any given location using an analysis of molecular variance (AMOVA) in GenAlEx version 6.51b2 (Peakall & Smouse 2012). Consequently, reference populations were combined by sample location due to low values of F_{ST} and overall genetic similarity. The four reference populations were Parsnip, Columbia-type, Native Williston, and Thutade Lake.

Population 1 – Parsnip. Kokanee are found in two headwater lakes in the Parsnip River watershed: Arctic Lake and Tacheeda Lake (FIG. 1). There is some speculation that the source of Kokanee in Tacheeda Lake was from an unsanctioned transport of Arctic Lake Kokanee. Genetic samples support the stocking event due to the genetic similarity between the lakes, but also the smaller number of alleles in Tacheeda Lake (Wilson & Shrimpton 2021). The outflows from Arctic and Tacheeda Lakes are small and there are numerous obstructions, but it may be possible on high flow years for fish to move to the Parsnip River and potentially interact with fish spawning in its tributaries. Kokanee samples were genotyped from Arctic Lake captured in 2006 (50) and 2019 (18) and Tacheeda Lake in 2004 (50) and 2018 (30).

Population 2 – Columbia-type. Kokanee from Arrow Reservoir (Hill Creek) and Kootenay Lake (Meadow Creek) were stocked extensively in tributaries of Williston Reservoir from 1990 to 1998 (Langston & Murphy 2008). Columbia-origin Kokanee have since strayed to multiple rivers to spawn throughout the Williston Reservoir. There is no genetic difference among the tributaries where Kokanee now spawn in the Williston watershed (Wilson & Shrimpton 2021). We used spawners collected over multiple years from two locations where Kokanee spawn in September as the Columbia-type reference population: Germansen River (130) and Russel Creek (128).

Population 3 – Native Williston (Native Rsvr.). Kokanee were caught in the Williston Reservoir watershed before the introduction of Columbia River origin fish. Genotypes were obtained from scales collected from gill net surveys from 3 locations in the reservoir in 1988 (36), from gill netting in the Peace Arm and lower Finlay River in 1989 (34), and gill netting in the forebay to the WAC Bennett Dam in 1990 (15). Kokanee gill netted from the reservoir in 2000 also revealed that 25 of the fish were native Williston Kokanee. Within the reservoir, there is no physical barrier to prevent introgression between native Williston Kokanee and the Columbia origin Kokanee,

although temporal and spatial differences in spawning likely kept the two populations separate (Fielden 1991; Fielden 1992; Langston 2012).

Population 4 – Thutade Lake (Thutade). Kokanee are found in Thutade Lake in the headwaters of the Finlay River (FIG. 1). Due to the close genetic relationship with the Native Williston Kokanee (Wilson & Shrimpton 2021), Thutade Lake was likely the source population for natural colonization of the reservoir by Kokanee. There is no opportunity for fish to move from the reservoir or lower Finlay River back into Thutade Lake, however, as Cascadero Falls downstream of the outlet from Thutade Lake is impassable. Kokanee samples were genotyped from fish captured in Thutade Lake in 2003 (87) and 2017 (20).

GENETIC ANALYSIS – Tissue samples in 95% ethanol from Kokanee collected in 2021 from the Williston Reservoir were submitted directly to the Pacific Biological Station (PBS) Molecular Genetics Lab, Nanaimo, BC, for genotyping. Fish were genotyped for 14 microsatellite loci: *Ots2*, *Ots3* (Banks et al. 1999), *Ots100*, *Ots103*, *Ots107*, and *Ots108* (Beacham et al. 1998; Nelson & Beacham 1999), *Oki1a*, *Oki1b*, *Oki6*, *Oki10*, *Oki16*, and *Oki29* (Smith et al. 1998; Nelson et al. 2003), *One8* (Scribner et al. 1996), and *Omy77* (Morris et al. 1996). These loci are commonly used and have demonstrated to work well to characterize Sockeye Salmon and Kokanee populations (Beacham & Withler 2017).

Microsatellite genotypes were tested for duplicates using Microsatellite Toolkit (Park 2001). Duplicated genotypes in Russel Creek 2019 (n = 1) and Thutade Lake 2003 (n = 9) were excluded from all analyses as they were most likely the result of duplicated sampling. Duplicated genotypes in Tacheeda Lake 2004 (n = 17) were retained because of the overall low genetic diversity of the sample group; these were thought to be the result of family structure in the group.

Microsatellites are often prone to null alleles that result from base pair mutations at the PCR priming site (Banks et al. 1999; Holm et al. 2001). Both large allele dropout and the presence of null alleles may contribute to inaccurate amplification of certain loci between different populations (Banks et al. 1999). The presence of null alleles was tested using the program MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004).

Linkage disequilibrium between pairs of loci within each group was tested, and significant deviations from Hardy-Weinberg equilibrium in each group was examined using an exact test based on 1,000 Monte Carlo permutations of alleles in GENEPOP v1.1.7 package for R v4.0.3 (Guo & Thompson 1992; Rousset 2008). To evaluate genetic variation, basic population and genetic descriptive statistics were also performed in R. Expected heterozygosity (H_e), observed heterozygosity (H_o), and mean allelic richness (A_R) were calculated using HIERFSTAT v0.5–7 package (Goudet et al. 2020). The number of alleles (A) was determined with ADEGENET v2.1.3 package (Jombart 2008; Jombart & Ahmed 2011).

The Kokanee sampled in 2021 were analyzed by sample location for preliminary genetic structure and variability information. Pairwise population differentiation was evaluated as θ (Weir & Cockerham 1984) in HIERFSTAT v0.5–7 package for R. To determine significant genetic distances between groups sampled in 2021, 10,000 bootstraps were used with a lower and upper quantile for confidence intervals of 0.025 and 0.975 (function *boot.ppfst*). This analysis was also applied to the dataset of representative populations and 2021 samples. Global *F*-statistics were calculated with GENEPOP v1.1.7 package for R.

To assess how the 2021 samples aligned with the representative populations, a neighbor-joining tree (Saitou and Nei 1987) was constructed using Cavalli-Sforza & Edwards' (1967) chord distance (D_C) as a measure of genetic distance between populations. The analysis incorporated 10,000 bootstrap replications using TREEFIT v1.2 (Kalinowski 2009) and the tree was visualized in FIGTREE v1.4.4 (Rambaut 2018).

ASSESSING POPULATION OF ORIGIN – We used the software program STRUCTURE to analyze the microsatellite data for population structure (Pritchard et al. 2000). Sampling from multiple potential source populations allows for the assessment of the number of clusters (*K*) that most likely represents real population structure, reported with assigned values of likelihood (Pritchard et al. 2000). STRUCTURE sequentially imposes population structure and groups genotypes by clusters that maintain assumptions of Hardy-Weinberg equilibrium and unlinked loci, and allows for the assignment of unknown-origin individuals to a particular population (Pritchard et al. 2000). We used STRUCTURE to determine the number of clusters and inferred population structure of all genotypes. Based on our previous analyses (Wilson & Shrimpton 2020; Wilson & Shrimpton 2021), there is no evidence of hybridization among populations in the Williston watershed. The models used were therefore correlated allele frequencies and no admixture, with a burn-in period of 100,000 followed by 300,000 iterations. Values of *K* from 1 to 5 were tested and each *K*-value was replicated 10 times to survey all potential structure at the genetic population level. Population priors were used to assist with assigning *K*. The most effective value of *K* (ΔK) was identified *ad hoc* via the web-based program STRUCTURE HARVESTER (Earl & von Holdt 2011; Evanno et al. 2005). Cluster results and likelihoods were visualized with Cluster Markov Packager Across *K* (CLUMPAK; Kopelman et al. 2015).

A Discriminant Analysis of Principal Components (DAPC) was also used to represent the pattern of population partitioning, as it does not require *a priori* knowledge of the structure of biological populations nor assumptions of Hardy-Weinberg equilibrium or linkage disequilibrium (Jombart et al. 2010). We visualized the genetic differentiation of the representative dataset with a DAPC to assess structure patterns and identify clusters with maximized among-population variation. We used the ADEGENET v2.1.3 package for R, and the optimum number of principal components (PCs) was cross-validated with the lowest root mean squared error (function *xval*) as recommended by Jombart & Collins (2015). The Kokanee collected in 2021 were initially assessed apart from the reference populations of the Williston watershed and with the addition of previously-genotyped donor populations collected from the Columbia River system (Hill Creek and Meadow Creek). DAPCs were conducted with the 2021 samples and Williston reference populations (TABLE 3) with a series of optimal number of clusters (*K*).

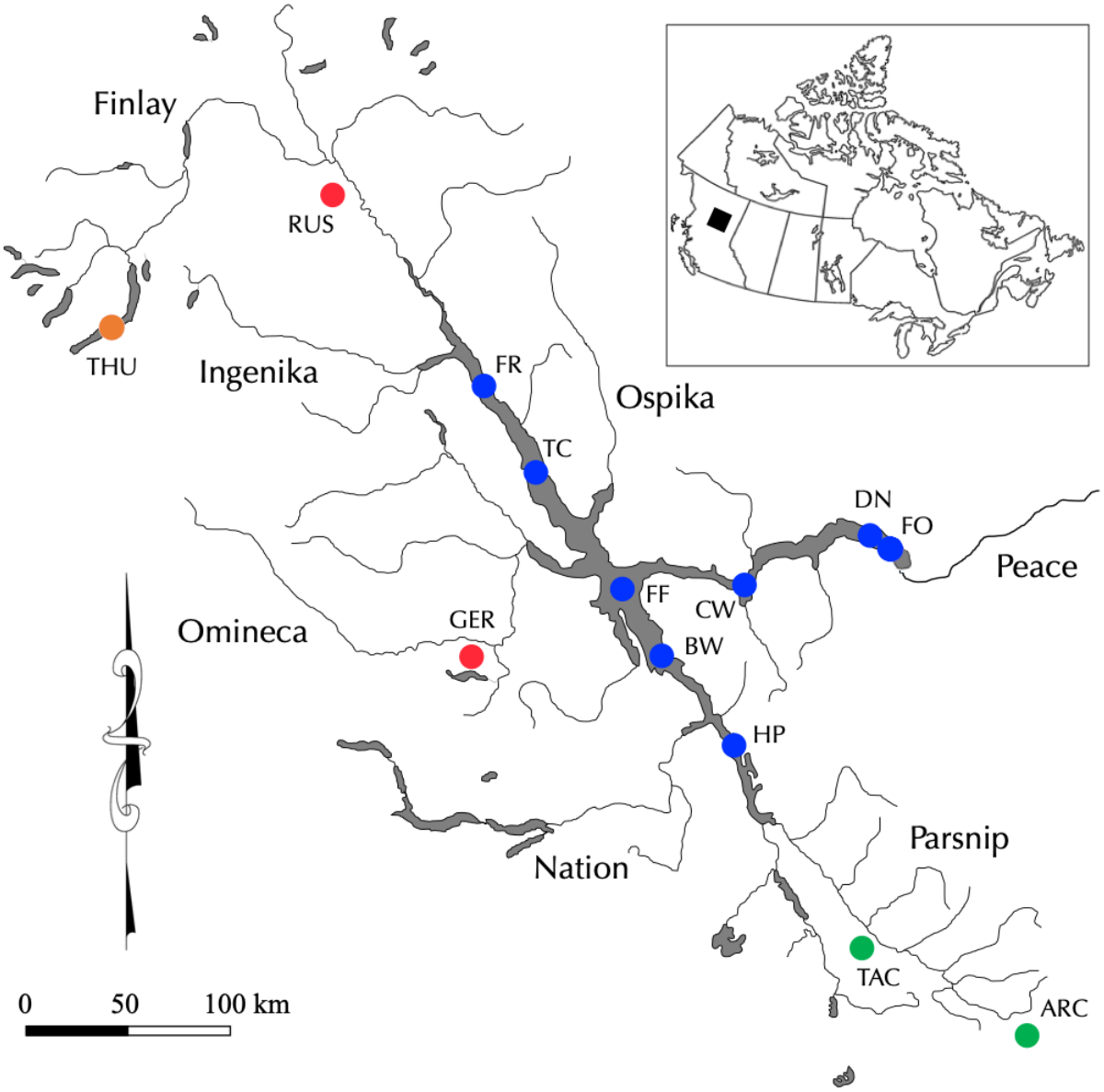

FIG. 1. The Williston Reservoir watershed and tributaries where Kokanee (*Oncorhynchus nerka*) were collected. **RED** symbols are locations of sample sites for Columbia-type Kokanee that spawn in tributary streams to the reservoir: Germansen River (GER) and Russel Creek (RUS). **GREEN** symbols are locations where native Kokanee were sampled from lakes in the headwaters of the Parsnip River: Arctic Lake (ARC) and Tacheeda Lake (TAC). The **ORANGE** symbol is the location where samples were collected from Thutade Lake (THU). **BLUE** symbols indicate locations where Kokanee were collected in the reservoir between 1988–2000 and in 2021, near Factor Ross Creek (FR), Teare Creek (TC), Finlay Forks (FF), Blackwater Creek (BW), Clearwater Creek (CW), Dunlevy Creek (DN), and the Forebay (FO).

TABLE 2. Number of Kokanee (*Oncorhynchus nerka*) samples that were collected in 2021 by location, method, and region. Genotypes indicate the number of samples from Kokanee that were successfully amplified for at least 10 microsatellites. For genetic variability and structure analyses, all Kokanee collected through trawl methods were combined into one group (Williston Rsvr.) within the 2021 samples.

Location	Method	Caught	Submit	Genotypes	Region	Genotypes
Teare Creek (TC)	Gill net	34	29	25	Finlay Reach	42
Teare Creek (TC)	Trawl	18	18	17		
Finlay Forks (FF)	Gill net	38	30	30	Finlay Forks	53
Finlay Forks (FF)	Trawl	25	23	23		
Blackwater (BW)	Gill net	41	5	5	Parsnip Reach	8
Heather Point (HP)	Gill net	7	7	3		
Clearwater (CW)	Gill net	36	27	26	Peace Arm	62
Dunlevy (DN)	Trawl	45	40	36		
Total		244	179	165		165

Gill net samples were collected by Bryce O'Connor (Chu Cho Environmental LLP) and trawl samples were collected by Tyler Weir and David Johner (BC Ministry of Forests, Lands, Natural Resource Operations, and Rural Development).

TABLE 3. Number of Kokanee (*Oncorhynchus nerka*) samples that were successfully genotyped by location, year, and sample type.

Location	1988	1989	1990	2000	2003	2004	2006	2017	2018	2019	2021
PARSNIP											
Arctic							**50**			**18**	
Tacheeda						**50**			**30**		
COLUMBIA-TYPE											
Russell							**49**		**40**	**39**	
Germansen							**50**		**40**	**40**	
NATIVE RSVR.											
Reservoir	**36**	**34**	**15**	**25**							
THUTADE											
Thutade					**87**			**20**			
RSVR. 2021											
Reservoir											**165**
TOTAL	**36**	**34**	**15**	**25**	**87**	**50**	**149**	**20**	**140**	**97**	**165**

Samples from 2003 to 2006 in **RED** were genotyped for the Peace / Williston Fish & Wildlife Compensation Program by Ruth Withler at the Pacific Biological Station (PBS) and data provided to UNBC. Samples in **BLUE** were genotyped at PBS for PEA-F19-F-2870-DCA; 1988 to 2000 were archived scale samples provided by C. Coady (FWCP – Peace Region), 2018 samples from Williston tributaries were collected by DWB for PEA-F19-F-2895-DCA. Samples in **GREEN** were genotyped at PBS for PEA-F20-F-3143; 2016 and 2017 samples were collected by Chu Cho Environmental for PEA-F17-F-1471 and PEA-F18-F-2296, respectively; 2019 samples were collected by DWB for PEA-F20-F-3359-DCA. Samples in **BLACK** were genotyped for the present FWCP – Peace Region funded project PEA-F21-F-3361-DCA and collected as described in TABLE 2.

RESULTS

MICROSATELLITE MARKERS – Across all loci and sample groups from 2021 and representative populations, there was evidence of 2 significant null alleles present due to homozygote excess: locus *Oki10* (0.057, $P < 0.01$) and locus *Ots3* (0.080, $P < 0.001$) in fish from Thutade Lake. Both null alleles maintained frequencies below 0.10. Null allele frequencies equal to or less than 0.10 may be acceptable to use without applied corrections (Huang et al. 2016) and were maintained in subsequent analysis. There was no evidence for scoring error (stuttering) or allele dropout at any locus.

Tests for linkage disequilibrium between pairs of loci within each group and corrected for Benjamini-Hochberg FDR resulted in statistically significant departures in 16 out of 455 tests (FDR $\alpha = 0.00148$), but departures were not concentrated on specific locus pairs (Kruskal-Wallis test, $P = 0.99$). Therefore, each locus represented an independent measure of genetic variation and divergence. Multiple tests (294) were conducted to identify deviations from Hardy-Weinberg, and after corrections 11 tests deviated from Hardy-Weinberg equilibrium by way of heterozygote deficiency (FDR $\alpha = 0.0012$). However, these deviations were not concentrated on specific loci or groups (ANOVA, $P = 0.95$). All loci were polymorphic and retained in analyses.

GENETIC VARIABILITY – Examining the genetic distances among the 2021 sample locations with Weir & Cockerham's (1984) θ estimates, all distances were significantly different from zero (TABLE 4). The distance values for these groups were extremely small, ranging from 0.000 to 0.043—indicating a lack of genetic differentiation among 2021 sample locations. Therefore, the 2021 samples were combined and considered one single sample group for the remainder of the analyses.

The mean ± SE H_e averaged across all 14 loci and 5 groups (Parsnip, Columbia-type, Native Rsvr., Thutade, and Rsvr. 2021) was 0.60 ± 0.027 (TABLE 5), and ranged from 0.40 ± 0.062 (Parsnip) to 0.71 ± 0.044 (Rsvr. 2021) and 0.71 ± 0.043 (Columbia-type). The mean ± SE H_o for all loci and groups was 0.59 ± 0.028. Parsnip once again reported the lowest H_o of 0.39 ± 0.064, with 0.71 ± 0.044 (Columbia-type) and 0.70 ± 0.044 (Rsvr. 2021) on the higher end. The mean ± SE allelic richness (A_R) across loci and groups was 8.71 ± 0.705, and ranged within groups from 3.87 ± 0.791 (Parsnip) to 11.69 ± 0.1.560 (Rsvr. 2021). The A_R for Columbia-type, Rsvr. 2021, and Thutade all exceeded 10.00, and the Native Rsvr. group averaged 6.22 ± 1.194. The greatest number of alleles for a group was sampled from the Columbia-type group (199), followed by Rsvr. 2021 (187). The measurements of genetic variation of this subset of representative populations and the 2021 genotypes are consistent with our previous findings: the native groups (Parsnip, Native Rsvr.) consistently reported the lowest genetic diversity among all the groups, and the Columbia-origin lineage exhibited some of the highest genetic diversity and allelic richness. Additionally, Thutade maintained a higher mean allelic richness and expected heterozygosity ($A_R = 10.34 \pm 1.667$; $H_e = 0.64 \pm 0.059$) than other native groups, as values were closer to the mean allelic richness and expected heterozygosity values of Columbia-type group.

The global F_{ST} across all loci and groups in this subsampled dataset was 0.2011 (TABLE 6). The genetic distance values (Weir and Cockerham's 1984 θ estimates) among the representative populations and the 2021 sample group were all significantly different from zero (TABLE 7). Parsnip individuals exhibited the greatest genetic distance with all other groups, ranging from 0.328 (Parsnip–Columbia-type) to 0.395 (Parsnip–Native Rsvr.). Thutade and Native Rsvr. showed a

genetic distance of 0.079, and both of these groups showed greater similarity to Columbia-type fish than those from the Parsnip group. The smallest genetic distance was between Columbia-type and Rsvr. 2021 (0.001), indicating that these two groups share a high degree of genetic similarity.

The neighbor-joining tree of Cavalli-Sforza and Edwards' chord distances (D_C) clustered the Columbia-type and Rsvr. 2021 groups (100%; FIG. 2). In this case, Thutade remained fairly separate from the Native Rsvr., which clustered more closely with the Parsnip group (52%). This is likely due to the high A_R of Thutade as compared to the latter groups.

POPULATION STRUCTURE – We ran the program STRUCTURE with *a priori* knowledge of population assignment and sampling location for each fish. STRUCTURE plots showing a range of *K* from 1 to 5 demonstrate the emergence of informative patterns with increasing *K* (FIG. 3). The *K* values of 1, 2, and 3 clusters were all given the highest probability of reflecting real-world population structure of this dataset, with each assigned a Markov clustering (MCL) similarity of 1.000. A *K* of 2 was rejected as an oversimplification of the Williston watershed Kokanee populations, as it grouped all Columbia-type, Thutade, Native Rsvr., and Rsvr. 2021 fish into one genetic population. A *K* of 3 was more parsimonious, as it grouped the genetically-similar Thutade and native Williston fish—with the latter known to have diverged from the former in recent (i.e., post-glacial) history (Wilson & Shrimpton 2021). Nevertheless, a *K* of 4 clusters (MCL = 0.998) is the most likely to accurately reflect the Williston watershed system as it clearly differentiated the Thutade and native Williston populations. Further extending the number of clusters (*K* = 5; MCL = 0.985) did not reveal informative subpopulation structure in any of the representative populations or the 2021 sample group. The Kokanee collected from the reservoir in 2021 were entirely assigned to the Columbia-type cluster, and did not show evidence of any native Williston genotype.

For the DAPC, the 2021 sample groups were assessed independently in a preliminary investigation of potential population structure among sample locations. The value of *K* associated with the lowest Bayesian Information Criterion (BIC) value was 2. Genotypes of fish sampled from donor populations in the Columbia River system, Hill Creek (100) and Meadow Creek (345), were added to assist in differentiating the proposed two clusters. With these additions, the 2021 sample groups were confirmed to cluster by putative source lineages, with the majority aligning with Hill-type signatures (FIG. 4).

For the combined dataset of representative populations and the 2021 sample group, a number of *K* values (*K* = 2 to 6) were systematically assessed and a *K* = 4 was ultimately selected (FIG. 5). Variance was assessed using 120 PCs and 3 discriminant functions that explained approximately 90% of the dataset. A four-cluster system supported the STRUCTURE analysis: each of the four representative populations were well differentiated, and the 2021 sample group clustered entirely with the Columbia-type group. With trials of increasing *K*, both Columbia-type and Rsvr. 2021 groups were further split into multiple overlapping clusters that did not align by sample location (FIG. 6). It should also be noted that in this DAPC, as in previous DAPCs of the comprehensive dataset of Williston watershed Kokanee (Wilson & Shrimpton 2021), individuals assigned to Thutade were found in both Columbia-type and Native Rsvr. groups, while individuals with proportions of Native Rsvr. genotype were found in Thutade. These cluster assignments are not supported by STRUCTURE and are likely an artifact of this method of genetic population analysis, past gene flow between these lineages, and/or the type of molecular marker (i.e., microsatellite loci) used herein.

TABLE 4. Pairwise Weir & Cockerham (1984) θ genetic distance estimates among Kokanee sampled from the Williston Reservoir in 2021. θ values are represented below the diagonal, and HIERFSTAT boostrapping over loci confidence intervals are presented in brackets above the diagonal. All Kokanee collected through trawl methods were combined into the Williston Rsvr. Group (see TABLE 2).

	Finlay (FF)	Williston Rsvr.	Teare Creek (TC)	Clearwater (CW)	Blackwater (BW)	Heather Point (HP)
Finlay Forks (FF)		(0.000—0.000)	(0.000—0.008)	(0.000—0.016)	(0.000—0.032)	(0.000—0.050)
Williston Rsvr.	0.000		(0.000—0.004)	(0.000—0.006)	(0.000—0.022)	(0.000—0.058)
Teare Creek (TC)	0.000	0.000		(0.000—0.004)	(0.004—0.034)	(0.000—0.053)
Clearwater (CW)	0.006	0.000	0.000		(0.002—0.046)	(0.000—0.048)
Blackwater (BW)	0.017	0.010	0.019	0.024		(0.000—0.091)
Heather Point (HP)	0.016	0.018	0.015	0.008	0.043	

TABLE 5. Measurements of genetic variation of Williston watershed Kokanee by sample group. N is the sample size, A represents the total number of alleles across all loci per group, and A_R is the rarefied allelic counts across all loci per sample group. Expected heterozygosity and observed heterozygosity are represented by H_e and H_o, respectively. H_o did not depart from H_e for any particular group across all loci. For each parameter, standard errors are given in parentheses.

Group	N	A	A_R	H_e	H_o
Parsnip	148	57	3.87 (0.791)	0.40 (0.062)	0.39 (0.064)
Columbia-type	258	199	11.45 (1.475)	0.71 (0.043)	0.71 (0.044)
Native Rsvr.	110	92	6.22 (1.194)	0.53 (0.064)	0.52 (0.063)
Thutade	107	152	10.34 (1.667)	0.64 (0.059)	0.64 (0.056)
Rsvr. 2021	165	187	11.69 (1.560)	0.71 (0.044)	0.70 (0.044)

TABLE 6. *F*-statistics (Weir & Cockerham 1984) of five groups of Kokanee sampled from the Williston watershed.

F_{ST}	0.2011
F_{IT}	0.2088
F_{IS}	0.0096

TABLE 7. Pairwise Weir & Cockerham (1984) θ genetic distance estimates among Kokanee sampled from the Williston Reservoir between 1988 and 2021. θ values are represented below the diagonal, and HIERFSTAT boostrapping over loci confidence intervals are presented in brackets above the diagonal.

	Parsnip	Columbia-type	Native Rsvr.	Thutade	Rsvr. 2021
Parsnip		(0.240–0.410)	(0.280–0.503)	(0.235–0.421)	(0.245–0.423)
Columbia-type	0.328		(0.102–0.218)	(0.056–0.162)	(0.000–0.003)
Native Rsvr.	0.395	0.157		(0.049–0.114)	(0.108–0.228)
Thutade	0.333	0.105	0.079		(0.059–0.175)
Rsvr. 2021	0.339	0.001	0.165	0.113	

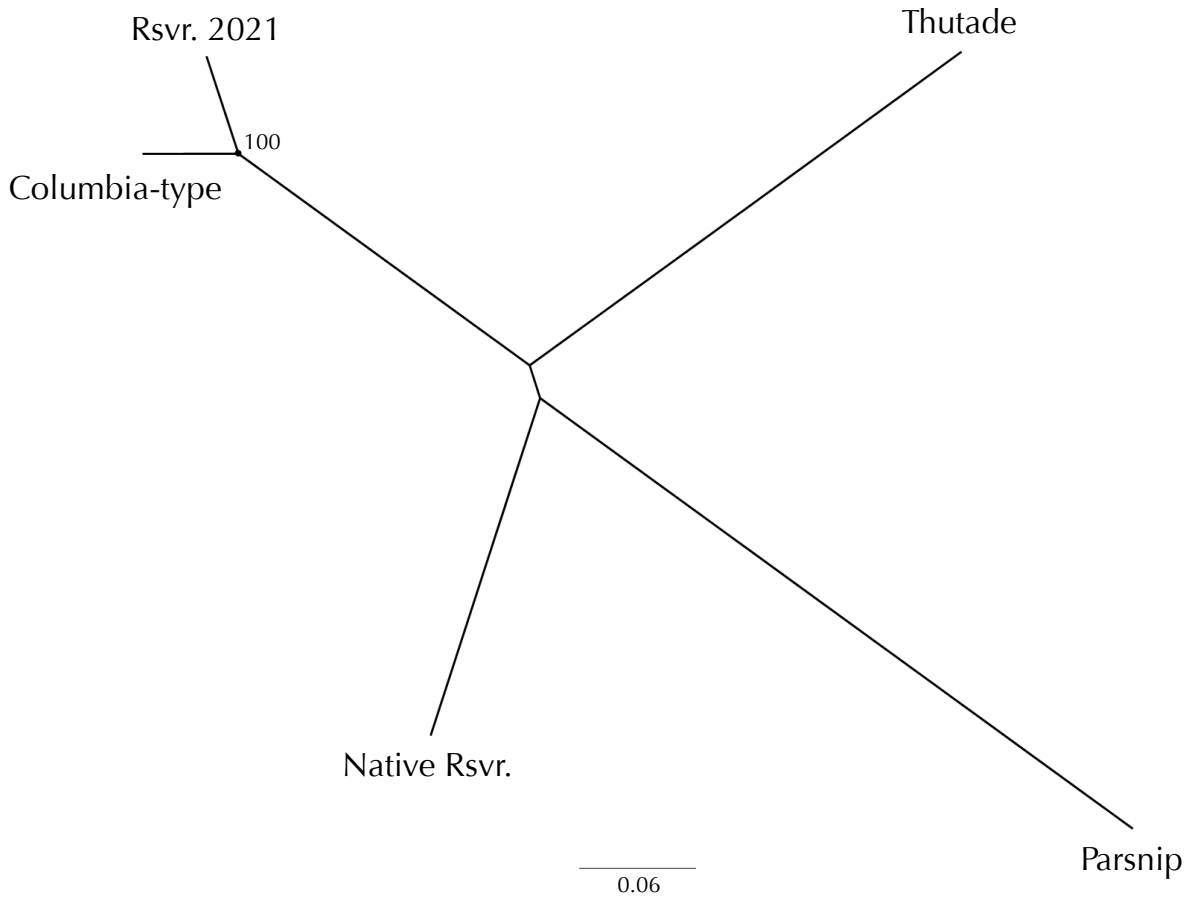

FIG. 2. Neighbor-joining tree constructed using Cavalli-Sforza & Edwards (1967) chord distance (D_C) inferred from variation at fourteen microsatellite loci in 21 groups of Williston watershed Kokanee. Numbers represent percentage of 10,000 bootstrap replicates. Percentages below 50% are not reported.

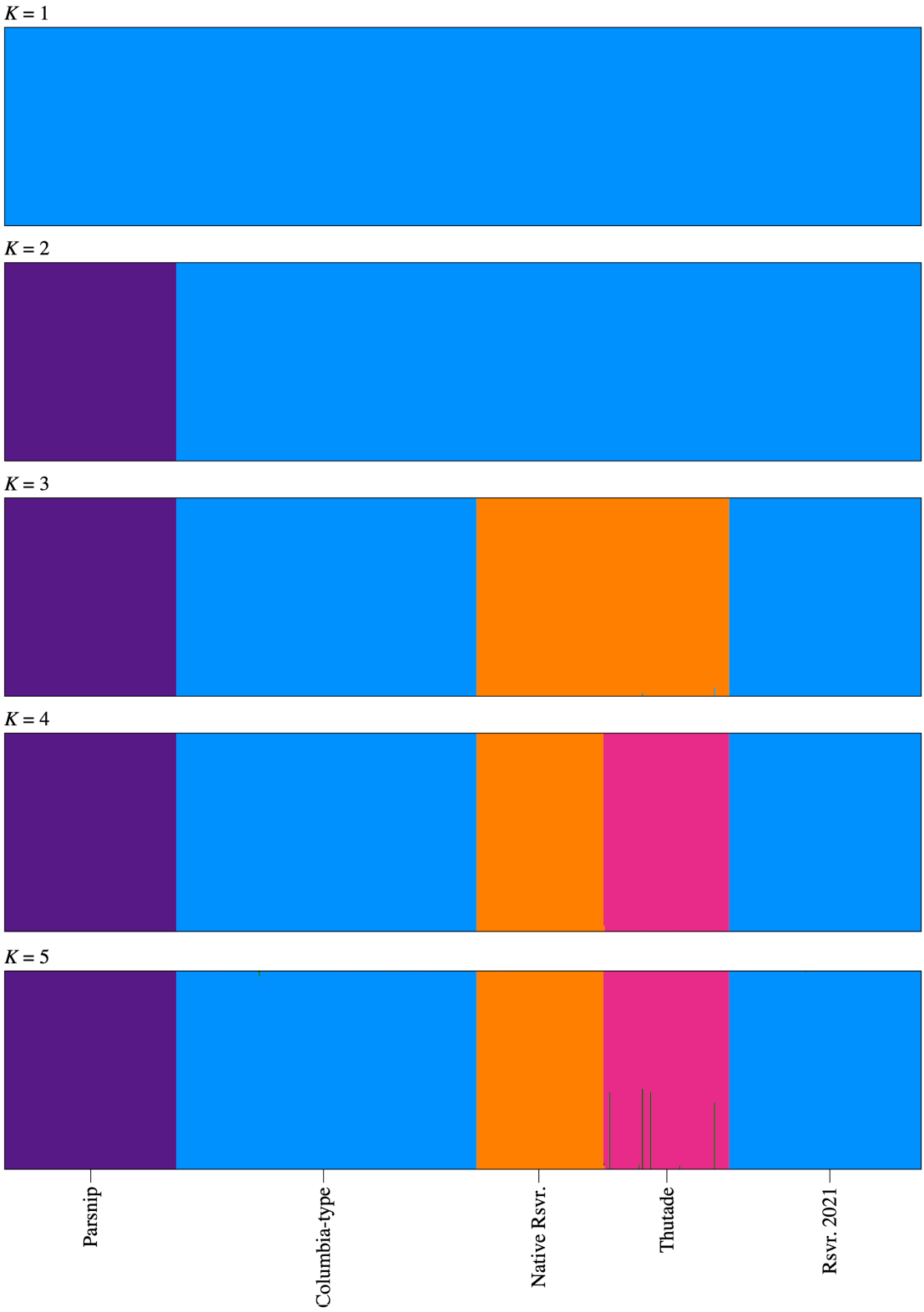

FIG. 3. Structure analysis for the combined representative populations and the Rsvr. 2021 sample group assayed at fourteen microsatellite loci. Each fish is represented by a vertical line which exhibits the proportional composition of each fish's genome across genetic clusters for $K = 1$ to 5. Sampling locations are shown in FIG. 1. Sample sizes are shown in TABLE 3.

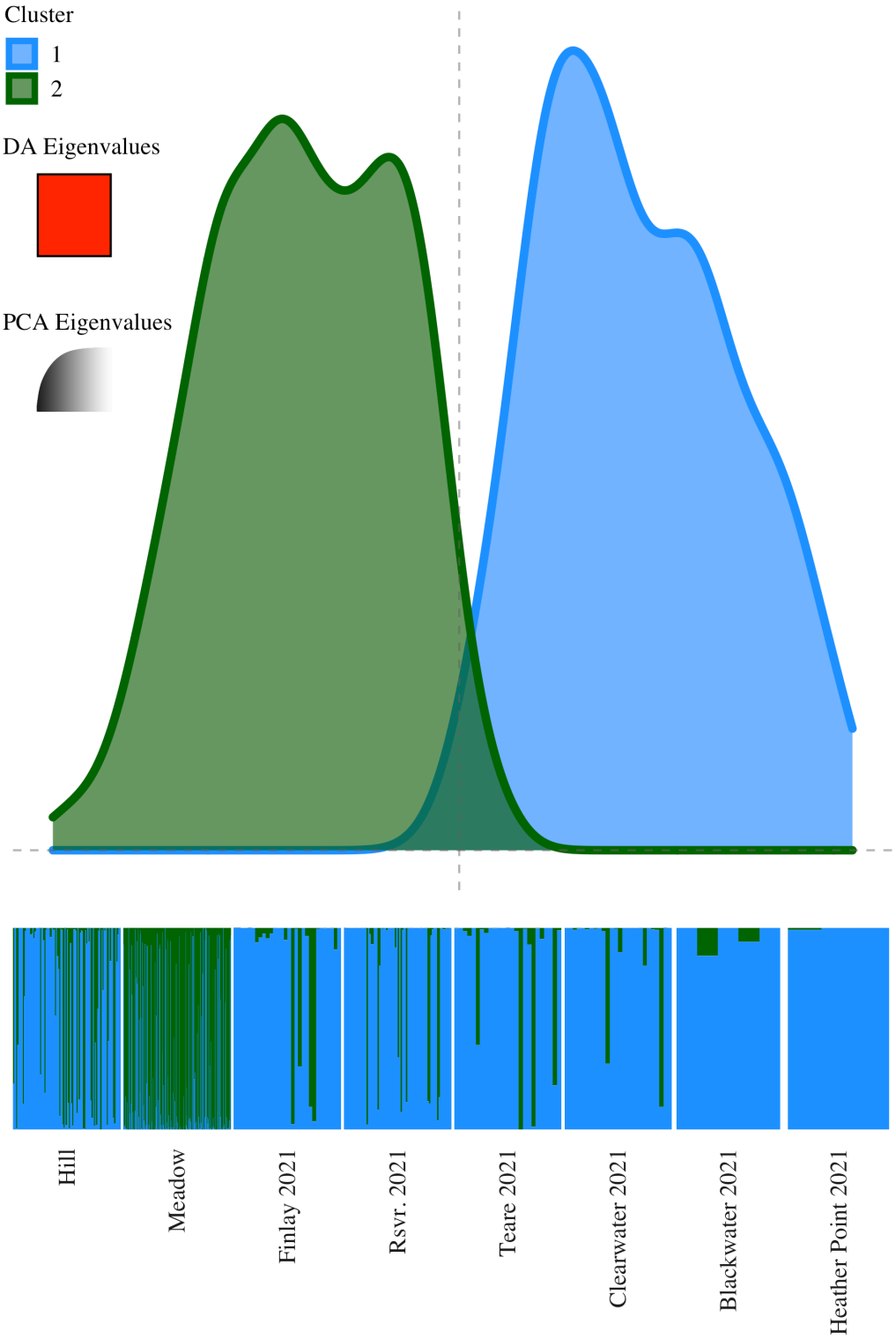

FIG. 4. A Discriminant Analysis of Principal Components (DAPC) for the Kokanee sampled in 2021 from the Williston Reservoir by gill net at five locations and trawl surveys at three locations (grouped as Rsvr. 2021). Genotypes of fish sampled from donor populations in the Columbia River system, Hill Creek (100) and Meadow Creek (345), were included in the analysis. A *K* of 2 appropriately described the genetic differentiation observed among these sample groups.

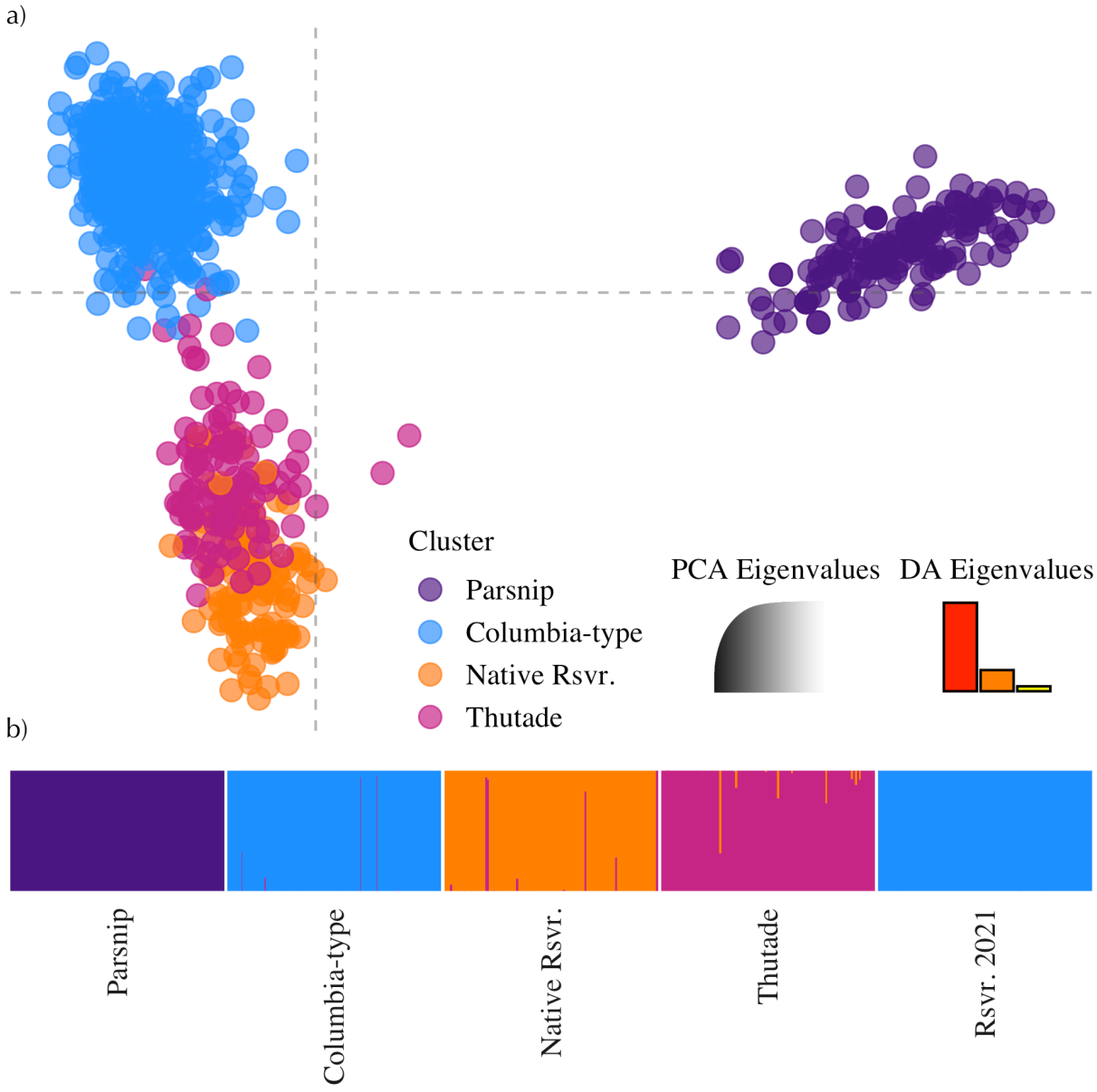

FIG. 5. Plots of a Discriminant Analysis of Principal Components (DAPC) for fourteen microsatellites across 4 reference populations (Parsnip, Columbia-type, Native Rsvr., and Thutade) and Kokanee sampled in 2021 (Rsvr. 2021) from the Williston Reservoir. Individual genotypes in the scatterplot (a) are represented by dots, and individual membership probabilities (b) are presented as vertical bars; genetic clusters are color-coded. A *K* of 4 was the most parsimonious representation of the population structure of Williston watershed Kokanee, as the Columbia-type and Rsvr. 2021 were clustered together into one genetic population.

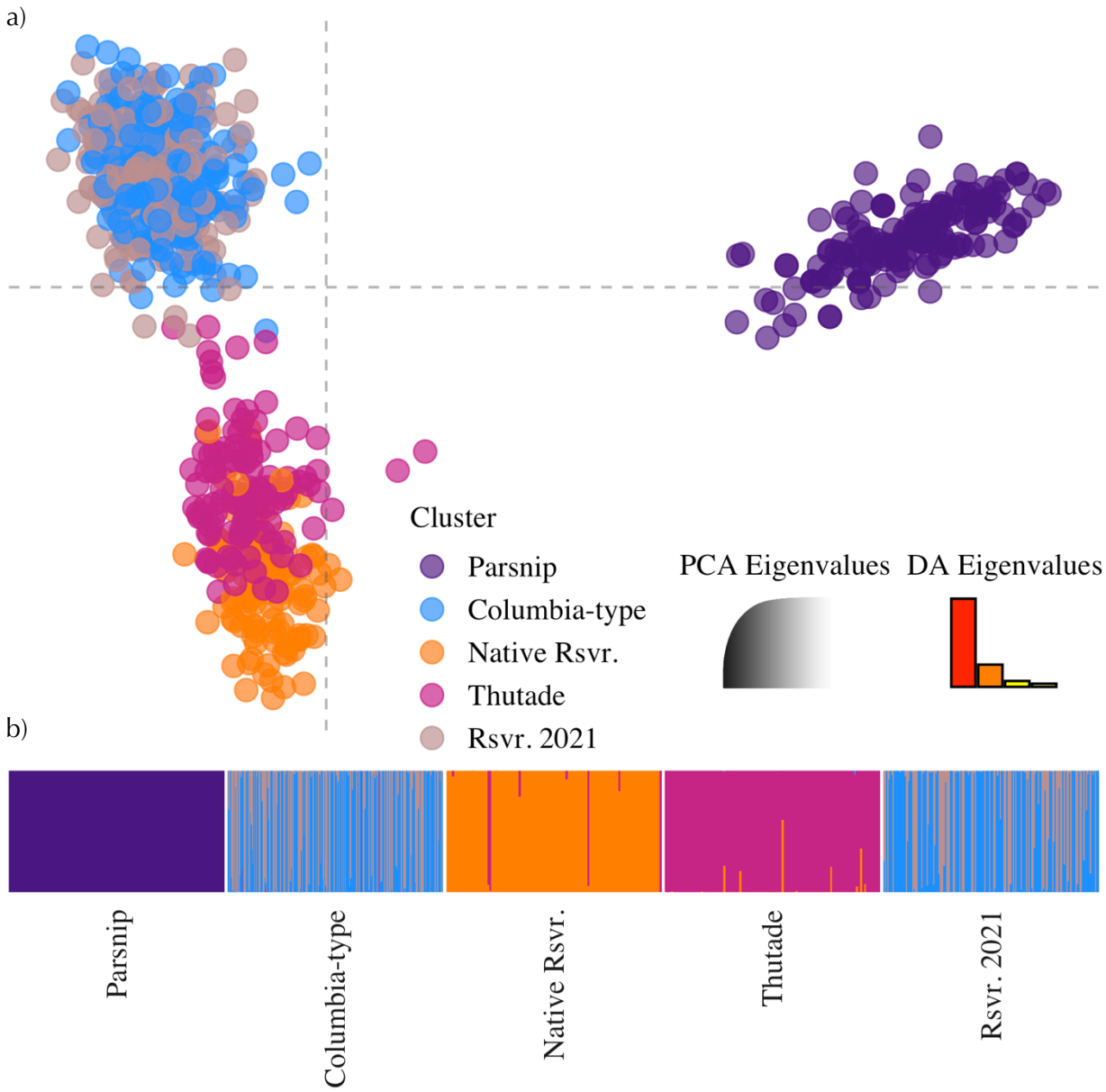

FIG. 6. A Discriminant Analysis of Principal Components (DAPC) for 4 reference populations (Parsnip, Columbia-type, Native Rsvr., and Thutade) and Kokanee sampled in 2021 (Rsvr. 2021) from the Williston Reservoir. Individual genotypes in the scatterplot (a) are represented by dots, and individual membership probabilities (b) are presented as vertical bars; genetic clusters are color-coded. A *K* of 5 identified the Rsvr. 2021 group, but it remained closely clustered to the Columbia-type Kokanee sampled in tributaries to the reservoir. Increasing the value of *K* resulted in both Columbia-type and Rsvr. 2021 sample groups further splitting into multiple overlapping clusters, and was not informative of true population structure.

DISCUSSION

Our finding that all Kokanee sampled from the reservoir in 2021 were of Columbia River origin indicates that native Williston Kokanee have not persisted in the reservoir. In contrast to our previous population genetic analysis of Kokanee within the Williston watershed, fish were not sampled from spawning locations in tributaries to the reservoir in 2021—rather, they were collected from the pelagic environment of the reservoir via gill netting and trawl surveys. This was in an effort to decrease the inherent bias of sampling in the natal streams where Columbia-origin fish were originally stocked and have subsequently strayed, and where native Kokanee were not known to frequent prior to stocking events. Additionally, our goal was to conduct gill netting following the timing (August and September) and procedures put forth by Pillipow & Langston (2002), when native Kokanee were last sampled from the Williston system. With the addition of trawl methods and the elimination of age class bias, the chances of intercepting any native Kokanee persisting in the body of the reservoir should have been attainable.

Our genetic variability and population structure results were verified through multiple statistical methods. Both Cavalli-Sforza & Edwards' (1967) D_C and Weir and Cockerham's (1984) θ genetic distance measurements are based on genetic drift as the primary driving factor of population differentiation (vs the assumptions about evolutionary models or rates of mutation inherent with other distance measurements) and were consistent in their placement of the Columbia-type and 2021 reservoir samples in close genetic proximity. The use of the Bayesian method STRUCTURE, which maintains assumptions of Hardy-Weinberg equilibrium and unlinked loci, in conjunction with a Principal Component Analysis (PCA) and other multivariate models (Discriminant Analysis) without assumptions or *a priori* population information, may be the most effective combined method for accurately recreating real-world population structure (Porras-Hurtado et al. 2013). For this subset of the sampled genotypes, a no admixture model of STRUCTURE was employed that further specified that admixture among populations was not a distinct possibility—that is, the genetic population assigned to an individual would be limited to only one of the present populations. While this would not be ideal for preliminary investigative purposes, the no admixture model is considered to be an appropriate method by which to assess highly-distinct populations (Porras-Hurtado et al. 2013), as was previously found to be the case for Williston watershed Kokanee. Through both of these methods, it was confirmed that the Kokanee sampled from the reservoir in 2021 did not include any native Williston genotypes. The cluster patterns and genetic relationships among all representative populations remained consistent with the full dataset analysis conducted earlier (Wilson & Shrimpton 2020; Wilson & Shrimpton 2021), and no further subpopulation structure was revealed in this examination.

There are a few intrinsic limitations when regarding a system of the scale of the Williston watershed, largely those concerning temporal and spatial coverage of Kokanee sampling. It is important to note that unlike the sampling undertaken by Pillipow & Langston (2002), no fish were collected from Factor Ross in 2021—the most northerly region of the reservoir and the region where the number of native Kokanee was typically higher. Factor Ross is one of the two locations sampled in 2000 from which only native Williston Kokanee were collected. Since that time, the distribution of Columbia-origin Kokanee spawners has shifted from the Peace and Parsnip reaches, and the runs with the greatest number of fish and the most consistent number of spawners are now found in tributaries to the Finlay River (McDermot-Fouts 2019; Robinson 2020). This suggests that Columbia-origin Kokanee may have displaced the native Kokanee in the area they historically occupied. Columbia-type Kokanee are not only abundant in the Finlay Reach—lower catch per

unit effort (CPUE) was reported for Kokanee in the Parsnip Reach at Heather Point than other sites, so Columbia-type Kokanee appear to be abundant throughout both the Finlay Reach and the Peace Arm of the reservoir (TABLE 2).

In 2000, over half of the Kokanee sampled from the Williston Reservoir (25/45) were native fish. Including this dataset, over 1,900 Kokanee have been collected, genotyped, and analyzed since 2000, and none of them have been found to be native Williston genotypes. Given their low (but increasing) population in the years before Columbia-type Kokanee were introduced, compounded with the expansion and extensive straying of these stocked fish (Shrimpton et al. 2022), this study reinforces that native Williston Kokanee have been extirpated from the reservoir.

Native Kokanee within the reservoir represented a distinct genetic cluster due to divergence from their population of origin—the Thutade Lake population. If this divergence occurred after flooding of the Williston Reservoir in 1968, development of a native Williston Kokanee genetic signature would have been extremely rapid (in just 5 generations; Wilson & Shrimpton 2021), as the fish sampled in 1988 clustered with all native Kokanee sampled in subsequent years. Evolution of reproductive isolation in Sockeye Salmon following colonization to a novel habitat in 1937 was found to have occurred by 1992, just 56 years or less than 13 generations (Hendry et al. 2000)—suggesting divergence due to segregation can occur in relatively few generations. Cascadero Falls ensured that the native Williston Kokanee could not be re-introduced to Thutade Lake. No migration barrier, however, exists for downstream movement of Thutade Kokanee to the reservoir. Given the short period of time from flooding of the reservoir to development of a distinct genetic native Williston Kokanee signature, it would be unlikely for these fish to have colonized the lower Finlay after 1968.

It is probable, therefore, that the native Kokanee existed in the lower parts of the Finlay River before flooding. There is evidence that Kokanee can survive and grow in the largely fluvial environment below the lakes in the headwaters of the Finlay and Parsnip Rivers. Babaluk et al. (2000) reported on a single Kokanee captured in Great Slave Lake in 1991 and posited that the only source populations were from the upper Peace River: Thutade Lake or Arctic Lake. The Kokanee collected from Great Slave Lake in 1991 was too old (6 years) and too large (40.3 cm) to have originated from the program that stocked Columbia-origin fish into the Williston watershed as it was initiated in 1990 (Langston & Murphy 2008). Based on our earlier work (Wilson & Shrimpton 2020; 2021), this fish was likely a native Williston Kokanee derived from the Thutade Lake population. The finding by Babaluk et al. (2000), therefore, indicates that native Williston Kokanee could survive extensive downstream migrations in a fluvial habitat.

It is not clear, however, when Kokanee might have established a population in the Upper Peace River. Reports from FC Swannell's survey of the Finlay River and tributaries indicated *silver trout* in a game record from 1914 (Patterson 1968)—long before the formation of the Williston Reservoir. The record also included Rainbow Trout and Dolly Varden [which would have been Bull Trout], suggesting the survey team were familiar with salmonids in the system and the *silver trout* may have been Kokanee. Where the fish that eventually colonized the reservoir might have resided below Cascadero Falls is also unknown. The game record from FC Swannell's survey covered territory from Fort Graham north to Kwadacha and included a trip up the Ingenika as far as the canyon at river km 117 containing a waterfall (Patterson 1968). It is not clear where fish were caught, but suitable habitat for rearing was likely limited. The number of *silver trout* was low, only five fish compared to 27 Rainbow Trout and 47 Bull Trout—consistent with little available

lacustrine habitat in the region at the time. Kokanee in the Williston Reservoir were not abundant in the 1974 survey (0.14%; Barrett & Halsey 1985), but numbers increased in a relatively short period of time once there was abundant lacustrine habitat following completion of the WAC Bennett Dam (Blackman 1992). Appropriate spawning habitat is often a bottleneck for life history completion in salmonids (Quinn 2005). Locations where native Kokanee were documented to spawn in the 1990s were in the lower Finlay River upstream of the regions affected by the flooded waters of the reservoir (Fielden 1991; Fielden 1992). Consequently, Kokanee may have been able to persist in the Upper Peace River before formation of the Williston Reservoir.

There remains potential for the "re-introduction" of native Williston Kokanee to the reservoir through Thutade Kokanee that pass over Cascadero Falls in the Finlay River. Founder effects and genetic drift, however, would mean that the subsequent population would not be identical to the unique genotype of the original native Williston population. Additionally, it is unknown how long the Thutade and Williston populations had been reproductively isolated. If migrants from Thutade Lake did establish a functional independent population as defined by McElhany et al. (2000), it remains questionable whether they could compete with large numbers of Columbia-type Kokanee in the highly oligotrophic system.

This study also reinforces the lack of introgression of Columbia-type genotypes with Arctic and Tacheeda populations and, given the most recent aerial surveys (McDermot-Fouts 2019; Robinson 2020), the future likelihood of Columbia-origin Kokanee invading these lakes remains low. It is possible for Kokanee to migrate from the reservoir upstream in the Parsnip and into Arctic Lake. Few Kokanee, however, spawn in streams flowing into the Parsnip Reach and none have been documented to move far up the Parsnip River (McDermot-Fouts 2019; Robinson 2020). There is also no opportunity for Kokanee to move from the reservoir or lower Finlay River into Thutade Lake as Cascadero Falls located downstream of the outlet from Thutade Lake is impassable. Consequently, introgression of Columbia Kokanee with Thutade Lake Kokanee in the headwaters of the Finlay River will not occur and introgression with Arctic Lake Kokanee appears unlikely.

Our study demonstrates not only a pattern of increasing proportion of Kokanee in the pelagic fish community in the reservoir, but also an increasing proportion of Columbia-origin Kokanee (TABLE 1). The Williston Reservoir is highly oligotrophic and may be exerting a tremendous selective pressure for the most effective filter feeders in the pelagic environment. Early surveys suggest that native Kokanee competed well with other pelagic species when the assemblage shifted to Lake Whitefish, Peamouth (*Mylocheilus caurinus*), and Kokanee after the impoundment of the Peace River (Blackman 1992). The marked increase in Kokanee numbers that accompanied the introduction of Columbia-origin Kokanee, however, seems to suggest that this population has the ability to outcompete not only other species, but also the native population of Kokanee that existed in the Williston Reservoir.

RECOMMENDATIONS

This report is based on findings from Kokanee samples collected over three decades following the formation of the Williston Reservoir and over a wide range of locations within the watershed. The 2021 samples were collected from multiple locations throughout the reservoir as earlier work suggested spatial differences between the native and Columbia-type Kokanee. Despite the wider spatial sampling, all Kokanee were genotyped as Columbia-type, indicating that the genetically distinct native Kokanee population has been extirpated from the Williston Reservoir. More

information on Kokanee within the Williston Reservoir watershed is still required to effectively manage this species and understand the origin of the native Williston Kokanee. Based on our findings, we have the following recommendations for future work.

RECOMMENDATION 1. ASSESS THE CONSERVATION VALUE OF INTRODUCED COLUMBIA ORIGIN KOKANEE – Columbia-origin Kokanee may be providing conservation benefits within the Williston Reservoir watershed. Non-native species may provide food resources for species considered important or desirable, but also provide desirable ecosystem functions (Schlaepfer et al. 2011). Predator-prey dynamics within the pelagic environment, therefore, should be examined. Bull Trout (*Salvelinus confluentus*) are known predators of Kokanee in the Williston Reservoir watershed (L. Gleeson, *personal communication*), but other piscivores such as Lake Trout (*S. namaycush*) may also rely on Kokanee as a prey source. Such work would align with Sub-objective 2 from the Rivers, Lakes, and Reservoirs Action Plan: *understand the relationships between reservoir productivity, kokanee, lake trout and other fish populations, and aquatic and terrestrial food webs* specifically *Action #4: assess Williston Reservoir productivity, including relationships to historical reservoir productivity and kokanee, lake trout, and/or other fish species productivity* (FWCP 2020). A survey of pelagic fish species should target top level predators in the reservoir and incorporate methods to assess diet. An approach that has been successful in determining stomach content analysis and testing for prey species is the use of molecular genetic dietary tools (Carreon-Martinez et al. 2011; O'Dell et al. 2020).

RECOMMENDATION 2. DETERMINE THE POPULATION ORIGIN FOR KOKANEE IN THE DINOSAUR RESERVOIR – Although Kokanee are not abundant in the Dinosaur Reservoir, they are present—5% of fish caught in an earlier survey were Kokanee (Murphy et al. 2004). Entrainment of Kokanee at the GM Shrum intake towers of the WAC Bennett Dam occurs (Algera et al. 2020). Fish sampled from the intake towers in 2016 and 2019 were all genotyped as Columbia-type Kokanee. Consequently, the source of Kokanee in Dinosaur Reservoir is most likely Columbia-type from the Williston Reservoir, however, little habitat is available for tributary spawners (Murphy et al. 2004). Additionally, a 2018 aerial survey found no Kokanee spawners in Gething Creek (McDermot-Fouts 2019), one of the few tributaries to the Dinosaur Reservoir where stream spawning could occur. Habitat at the tailrace of the WAC Bennett Dam (Pattenden & Ash 1993) may be suitable for shore spawners, such as Kokanee that originated from Thutade Lake, and may support a population of native Kokanee in a reservoir where spawning habitat is not suitable for Columbia-type Kokanee. Genotyping Kokanee from Dinosaur Reservoir, therefore, will confirm origin of these fish. This work aligns with Sub-objective 5 from the Rivers, Lakes, and Reservoirs Action Plan: *maximize the population viability of priority aquatic species* specifically *Action #16: conduct research on priority, but lesser-known, fish populations related to conservation status, critical habitats, and key limiting factors* (FWCP 2020). It is unlikely, but if Dinosaur Reservoir Kokanee differ from those in the Williston Reservoir, management decisions should be made to conserve them.

RECOMMENDATION 3. DETERMINE THE EFFECTIVE POPULATION SIZE FOR NATIVE AND COLUMBIA-ORIGIN KOKANEE IN THE WILLISTON RESERVOIR – Small population size is often linked to loss of genetic variation and periodic population bottlenecks accelerate the erosion of genetic diversity. Loss of genetic diversity, therefore, is expected to have a negative effect on population fitness and viability (Heath et al. 2002). The rate of loss in genetic diversity, however, is dependent on the effective population size, N_e, rather than the actual number of animals in a population, N (Kalinowski & Waples 2002). N_e is a fundamental parameter, therefore, for conservation and management when assessing population fitness. N_e can be calculated from the temporal change in allele frequencies

(Waples 1990). We have data from genotyping the native and Columbia-origin Kokanee in the Williston watershed: native Williston from 1988, 1989, 1990 to compare with 2000; Thutade from 2003 & 2017; Arctic from 2006 & 2019; Tacheeda from 2004 & 2018; Germansen, Pelly & Russel where Columbia-type Kokanee spawned in 2006, 2018 & 2019; and also the donor populations, Hill & Meadow Creeks. The calculation of N_e is relatively insensitive to variation in generation time and overlapping generations. Inaccuracies in the estimation of N when the population size is greater than 1000 spawning fish also has little effect on estimating N_e which is beneficial for our application as population size is not accurately known for the native populations of Kokanee. This work aligns with Sub-objective 5 from the Rivers, Lakes, and Reservoirs Action Plan: *maximize the population viability of priority aquatic species* specifically *Action #16: conduct research on priority, but lesser-known, fish populations related to conservation status, critical habitats, and key limiting factors* (FWCP 2020). The small number of alleles (A), low rarefied allelic counts (A_R), and low levels of heterozygosity for the Parsnip River populations of Arctic Lake and Tacheeda Lake are of concern for their persistence. Determining N_e will provide us with an estimate of resilience to stochasticity for Kokanee populations in these two lakes and whether management practices need to be revised. Calculation of N_e for the other populations within the watershed will be of value for comparison.

RECOMMENDATION 4. GENOTYPE KOKANEE SAMPLES FROM FISH GILL NETTED FROM THE RESERVOIR IN 2008 – Our data shows a gradual reduction and ultimately extirpation of native Williston Kokanee from the reservoir after the introduction of the Columbia-type Kokanee (TABLE 1). How long native Kokanee persisted after the introduction is not clear. Samples from Kokanee caught in the reservoir approximately every decade have been genotyped (gill netted in 1990 and before, 2000, and 2021), except for the fish sampled in 2008. The 2008 gill net sampling, however, was only conducted in the Peace Arm (Sebastian et al. 2009). Although spatial separation in the reservoir was found for the different Kokanee genotypes based on the 2000 gill net surveys (Wilson & Shrimpton 2020), native Kokanee were genotyped from fish sampled in the Peace Arm in the 1994 samples (Wilson & Shrimpton 2021), and information on scale envelopes indicated capture location as "*Clearwater*" for Kokanee caught in 1988, "*Dunlevy Yacht Club*" for Kokanee caught in 1989, and "*Bennett Dam draft tube*" for Kokanee caught in 1990 – all locations in the Peace Arm of the reservoir. If scale samples from the 80 Kokanee gill netted in 2008 have been archived by the FWCP – Peace Region, genotyping will reveal if native Kokanee were still present in the reservoir at that time. Such findings will elucidate the dynamics of competition between the two populations of Kokanee and the rate at which the native Williston Kokanee were extirpated. This information is required if there is a management desire to re-introduce Kokanee from Thutade Lake into the reservoir and *recreate* native Kokanee in the Williston Reservoir. Findings from this work do not directly align with the current Rivers, Lakes, and Reservoirs Action Plan (FWCP 2020), but understanding the dynamics of interactions between the Columbia-origin Kokanee and other fish in the reservoir may inform directions for future FWCP – Peace Region Actions Plans.

RECOMMENDATION 5. DETERMINE TIMING OF KOKANEE COLONIZATION OF THE UPPER PEACE RIVER WATERSHED BELOW CASCADERO FALLS – Integration of both genetic and population data is important to understand patterns and timing of historic changes in abundance within a population (Wommach et al. 2015). Such an approach, therefore, could be used to determine timing of colonization by looking for genetic signatures that are indicative of when bottlenecks and expansion occurred within a population. There are a number of analyses that can be used with our genotype data. Different tests have power to detect changes in population size at different temporal and spatial scales (Girod et al. 2011), which can aid in the development of a more

complete comparison between historic and contemporary population sizes (Palsbøll et al. 2013). Tests examining changes in heterozygosity can be used to detect bottlenecks in population size if recent, severe, or short in duration (Luikart & Cornuet 1998). The sign test for heterozygosity excess (Cornuet & Luikart 1996) is based on the expectation that when a population goes through a drastic decrease in size, the number of rare alleles in the population will decrease rapidly. To detect signatures of older bottlenecks that would indicate a Kokanee population existed prior to impoundment of the Upper Peace River, alternative analyzes can be performed. The *M* ratio test is based on estimation of the change in allele numbers observed in a recently reduced population (Garza & Williamson 2001) and focuses on the discrepancy expected between the number of alleles (*k*) and the range of allele sizes (*r*). For a population that has gone through a reduction in size, it is predicted that *k* will be reduced at a faster rate than *r*, thus producing a smaller than expected ratio (*M*) for the population. For detecting such patterns that are more ancient, the Bayesian method developed by Storz & Beaumont (2002) estimates posterior probabilities of ancestral (N_1) and current (N_0) effective population size, the time since the change in population size (x_a), and mutation rate (μ) using Markov Chain Monte Carlo simulations based on variation in microsatellite loci.

Analysis of historic bottlenecks would be valuable for all three native Kokanee populations in the Williston Reservoir Watershed: the native Williston Kokanee, Thutade Lake Kokanee, and Kokanee from the headwaters of the Parsnip River found in Arctic and Tacheeda Lakes. Sample size may limit the power of the analysis and may require additional samples—something relatively easily done for Thutade Lake, and Arctic and Tacheeda Lakes. The archived scale samples were provided by the FWCP – Peace Region for our earlier work and more samples may not exist. The scale samples from the reports that defined spawning locations for native Williston Kokanee in the Finlay River (Fielden 1991; 1992) were not included in our earlier work and it may be possible to augment our data set if these samples still exist; 104 & 57 samples in 1990 & 1991, respectively. The information developed from this work does not align with the current Rivers, Lakes, and Reservoirs Action Plan (FWCP 2020), but understanding the phylogenetic relationships of the native Kokanee in the watershed is important to properly conserve and manage these populations and may inform the direction of future FWCP – Peace Region Actions Plans.

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