Genetic Population Structure and Demographics of Kokanee Introduced to the Williston Reservoir

Fish & Wildlife Compensation Program – Peace Region Project
F20 Kokanee Genetics and Demographics (PEA-F20-F-3143-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 four research questions that are important to adequately manage Kokanee (*Oncorhynchus nerka*) introduced into the Williston Reservoir watershed: 1. Determine Kokanee age at maturity which will inform our population trend analysis; 2. Assess Kokanee fecundity (reproductive status) to determine population structure and to what extent the current population could expand; 3. Assess potential introgression of Columbia-origin Kokanee with native Kokanee populations; and 4. Assess the degree of homing and fidelity of Kokanee to the originally stocked streams.

Ages of Kokanee spawners was determined from otolith annuli. The grand mean of ages for Kokanee examined in our study included fish collected in 1989, 1994, 2006, 2018, and 2019 and was 2.85 ± 0.13 (SD) years across the different groups and sample years. This indicates that most Kokanee spawn in their 4th year and generation time from fertilization to sexual maturation and spawning is 4 years in the Williston Reservoir watershed. Age estimates between groups of Williston Reservoir Kokanee did not differ significantly by spawning location or by sample year.

Sizes of Kokanee at maturity have changed over time and are getting smaller. Native Kokanee caught between 1988 and 1991 were the largest fish caught in the Williston Reservoir watershed over the years that Kokanee have been sampled. The first cohort of Columbia origin Kokanee that matured in the Williston Reservoir watershed were smaller than the native Kokanee, but significantly larger than Columbia origin Kokanee caught in 2006, 2018, 2019, and 2020. Spawners sampled in 2018, 2019, and 2020 were the smallest cohorts.

Although mature Kokanee were collected in September in 2018 and 2019, which was near the peak of the spawning period based on earlier observations, many of the females had few eggs indicating that they had spawned prior to collection. Spawners were collected earlier in September of 2020 and a greater proportion of the females had eggs that were still tightly retained within the skeins indicating that they had not spawned. Mean fecundity was 212 ± 53 (SD) which is substantially less than measurements for mature Kokanee from 1994. The lower fecundity was directly related to the smaller size for the cohort that matured in 2020 – which did not differ in size from spawners collected since 2017.

To understand genetic relationships among populations of Kokanee in the Williston Reservoir watershed, samples were collected to identify spatial and temporal patterns. Scale samples archived by the FWCP – Peace Region from 1988 through 2000 were donated to provide genetic signatures for native Kokanee within the reservoir and changes that occurred following the introduction of Kokanee from the Columbia River watershed. Kokanee samples collected in 2006, 2018 and 2019 from multiple tributary streams to the reservoir were used to assess spatial variation and homing potential to natal streams. Kokanee collected from Hill Creek and Meadow Creek in the Columbia River watershed that were the donor populations for the fish stocked into the Williston Reservoir were also included in the analysis. We used 14 microsatellite loci to genotype 1870 Kokanee. Our analysis identified five genetic clusters that were associated with sample location, but within sample locations there was no difference among years. The five clusters were defined by location: Cluster 1 included all fish sampled from Arctic and Tacheeda Lakes; Cluster 2 included fish sampled from the Meadow Creek, but also a small number of fish (<10) sampled from the reservoir in 1994 and 2000; Cluster 3 included all of the Kokanee sampled from spawning tributaries to the reservoir in 2006, 2018 and 2019, most of fish sampled from the reservoir embayments in 1994, and aligned most closely with the Columbia River watershed Hill Creek fish; Cluster 4 included Kokanee caught in the Williston Reservoir watershed before the introduction of Columbia River origin fish and more than half of the fish caught in the reservoir in August of 2000; Cluster 5 included Kokanee sampled from Thutade Lake.

Kokanee sampled from the reservoir in 2000 revealed that native Kokanee persisted in the reservoir after the introduction of Columbia origin fish. Kokanee sampled from the tributaries in 2006, 2018 and 2019, however, were all assigned to a single cluster (Hill Creek) and did not include any native Williston Kokanee fish. Genotyping of Kokanee collected from the Peace Arm in 2016 and 2019 revealed only fish that were part of the Hill Creek cluster. Although we found no evidence of introgression of the Columbia origin Kokanee stocked into the Williston Reservoir watershed with native Kokanee populations, we also found no evidence that native Williston Kokanee still persist in the reservoir. A lack of genetic differences among Columbia origin Kokanee spawning in multiple tributaries in the watershed suggests that spawners are not exhibiting fidelity to their natal streams and that reproductive isolation has not occurred. Alternately, insufficient time has not allowed for differentiation of genotypes if Kokanee are showing fidelity to their natal streams. Additional research initiatives are recommended to better understand the distribution, homing ability, physical characteristics, and conservation value of Kokanee in the Williston reservoir. These include determine native Kokanee persistence in the reservoir, characterize locations where Kokanee spawn, determine homing of Columbia origin Kokanee to tributary streams, assess the mechanism for rapid decrease in size of Kokanee following stocking, assess intra-population competition through morphometric analysis of Columbia origin and native Kokanee, and estimate the conservation value of introduced Columbia origin Kokanee.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The Williston Reservoir was created in 1968 following the construction of the WAC Bennett Dam and impounding the Peace River in the canyon near Hudson's Hope, BC, for hydroelectric generation. With a surface area of 1,779 km^2, the Williston Reservoir is the largest lentic freshwater system in British Columbia. A native population of Kokanee (*Oncorhynchus nerka*) was established in the Williston Reservoir when it formed after the completion of the WAC Bennett Dam (Blackman et al. 1990). Changes in relative abundance of Kokanee in Williston Reservoir increased from 0.1% of fish gill netted in 1974 to 2.3% of captures based on data from the same locations sampled in 1988. The origin of the native Kokanee in the reservoir may have been from two sources as populations of Kokanee exist at the southern and northern regions of the watershed. In the south, Arctic and Tacheeda Lakes in the headwaters of the Parsnip River have shore spawning Kokanee. In the north, Thutade Lake in the headwaters of the Finlay River also has Kokanee that appear to be shore spawners.

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 and Murphy 2008). The five systems were: Carbon Creek, Davis River, Dunlevy Creek, Manson River, and Nation River; three systems on the east side of the reservoir and two rivers that flow into the southwest portion of the reservoir. An aerial enumeration study conducted from 2002 to 2006 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). Systems with the greatest abundance of Kokanee were found to be Russell Creek (Finlay River tributary), Ingenika River, Omineca River, Osilinka River, and Germansen River – some years with up to 250 000 spawners within a single river. Spawning Kokanee, therefore, appear to have selected tributaries in the Williston watershed that flow into the northwestern portion of the reservoir – not the regions originally stocked. These patterns, however, may have changed even further in the past decade –in response to a survey conducted in 2016 of individuals living in communities in the northern portion of the watershed and anglers fishing in the region, Kokanee were described as "everywhere" (Coxson et al. 2017).

The ecosystem effects of increased Kokanee abundance on the Williston Reservoir and its tributaries are not well understood. 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). This is also a substantial increase in the estimated Kokanee population from 1 million fish in the Williston Reservoir assessed by hydroacoustic measurements made in 2000 (Sebastian et al. 2003). It is also possible that Kokanee may have expanded into lakes connected to Williston Reservoir via connecting tributaries.

Sockeye Salmon, anadromous *O. nerka*, have a fairly consistent generation time of 4 years, although some variation in age at maturity does exist and this seems to be related to latitude; growth is slower in colder more northerly lakes and fish usually leave their natal lake and migrate to the ocean at an older age (Burgner 1991). The generation time for Kokanee in the Williston

Reservoir, however, is not known. Kokanee age estimates from the donor populations in Hill Creek and Meadow Creek indicates that age at maturity is more variable, but most of the fish are 3 years of age which would indicate a generation time of 4 years (M. Neufeld, unpublished data). Results from a gill net survey of Kokanee from the Williston Reservoir appear to show three distinct age classes for Kokanee caught in the reservoir in mid-July (Sebastian et al. 2009). The overlap in size between the oldest age class (3) and the size of Kokanee spawners supports the age assessment, however, some much larger fish were also found on the spawning grounds and could indicate the fish are more than 4 years of age. Generation time is an important parameter to know when managing a species to assess life history information such as recruitment and age class survival.

Reproductive potential is also an important factor for stewards to understand to effectively manage a species. Semelparous species, such as Kokanee, are capital breeders and their total reproductive potential is invested in a single spawning event. Number of offspring produced by a population can then be used to assess life history characteristics and to forecast population growth and egg to adult survival. Fecundity, however, is positively correlated with female size (Elgar 1990); fecundity is related directly to mass and to length cubed. Historic estimates of fecundity for Kokanee sampled from the embayments to Carbon and Dunlevy Creeks in the reservoir and Philip Creek in 1994 were 470 ± 57 for females that had an average fork length of 26.7 ± 0.9 cm (n = 32; Langston and Zemlak 1998).

It is unknown whether the original colonization of the reservoir was from Kokanee that moved downstream from Thutade Lake or Arctic and Tacheeda Lakes. The effect of introduction of an exotic race of Kokanee on the native Kokanee populations, however, presents the potential for genetic introgression between the populations. 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 up from the Parsnip River into these lakes. The potential for genetic introgression in the Parsnip headwaters, therefore, is high – except Kokanee escapement to the Parsnip Reach has been very low in recent years (McDermot-Fouts 2019; Robinson 2020). There is no opportunity for fish to move from the lower Finlay River into Thutade Lake, however, as Cascadero Falls downstream of the outlet from Thutade Lake is impassable. 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 may keep the two populations separate (Fielden 1991; Fielden 1992; Langston 2012).

Extensive straying of Columbia origin Kokanee has occurred in the Williston Reservoir following stocking. Lack of spawners returning to streams where they were introduced in the 1990s suggests Kokanee are selecting specific habitat features that differ from the locations where they were originally stocked. Stream depth, velocity and physical properties of the channel, such as substrate size are important features of spawning habitat for salmonids, but intragravel factors are the main variables influencing spawning site selection at the microhabitat scale for other stream spawning salmonids in BC such as Coho Salmon *O. kisutch* (McRae et al. 2012) – particularly temperature where variability in intragravel water temperature is more stable than surface water (Tuor and Shrimpton et al. 2019). Selection of a spawning site is crucial because the highest rates of mortality in salmonids generally occur during the incubation period (Quinn 2005), and this mortality is closely related to the features of the spawning / incubation site. Consequently, salmonids often show fidelity to specific spawning locations within a watershed – even at the microhabitat scale (Turcotte and Shrimpton 2020). Fidelity to spawning locations leads to reproductive isolation and genetic differentiation among populations. Evolution of reproductive isolation in Sockeye Salmon following colonization to a novel habitat was found to occur in under 13 generations (Hendry et al. 2000). Since the introduction of Columbia origin Kokanee to the reservoir between 1990 and 1998, five to seven generations have passed – fewer than the number observed by Hendry et al. (2000) to result in reproductive isolation. Colonization of spawning streams by few individuals, however, may have resulted in founder effects (Templeton 1980) resulting in substantial genetic differences among tributary streams.

In an effort to understand the effects of Kokanee introductions (i.e. stocked Kokanee) on the aquatic food web, 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. There are four research questions that need to be answered to begin to adequately manage Kokanee introduced into the Williston Reservoir watershed:

1. Determine age at maturity for Kokanee in the watershed to inform population trend analysis;
2. Assess Kokanee fecundity and reproductive investment to determine population structure and to what extent the current population could expand;
3. Assess potential introgression of Columbia-origin Kokanee with native Kokanee populations in the watershed; and,
4. Assess the degree of homing and fidelity of Kokanee to their natal spawning streams.

METHODS

AGE AND SIZE AT MATURITY – Mature Kokanee spawners sampled from streams in 2006, 2018, and 2019 (FIG. 1) were frozen prior to otolith extraction. Otoliths were removed from Kokanee carcasses after they were partially thawed. Additional otoliths archived by the FWCP – Peace Region from mature Kokanee captured in the lower Finlay River in 1989 and captured from Philip Creek in 1994 were included in the analysis. Morphometric data from the otolith storage envelopes for the 1989 and 1994 included length and mass data.

Each otolith was viewed under a dissecting microscope (Leica S9i Stereomicroscope; Opti·Tech, Toronto, ON) and image captured with integrated 10 MP digital color camera with CMOS sensor to visualize individual annuli. Three individuals viewed digital images independently and assigned an age based on the number of visible annuli. Otoliths from spawning Kokanee sampled in 2006 and 2018 were also submitted to North South Consultants Inc. (Winnipeg, MB) for age verification. Otoliths from each fish, therefore, were assessed by up to four estimators: North-South Consultants (n = 100), J. Bottoms – UNBC (n = 226), M. Shrimpton – UNBC (n = 450), and P. Wilson – UNBC (n = 464). A consensus age for each individual was determined with the mode value across all estimations. One-way Analysis of Variance (ANOVA) was used to compare age estimates across all groups. To test for differences across time, a two-way ANOVA with replication was used to compare age estimates for Germansen River, Pelly Creek, and Russell Creek sampled in 2006 and 2018.

To assess changes in size over time without potential bias introduced by the effects of freezing (Armstrong and Stewart 1997; Leonard et al. 2021), we compared field values from scale envelopes for 1989 and from FWCP reports for 1990, 1991, 1994 and 2000 to the field measurements from fish collected in 2006, 2016 (age only), 2017 (age only), 2018, and 2019. A summary of samples used in our analysis is provided below.

1989 – Data included length and mass measurements recorded on scale envelopes for fish caught in the lower Finlay River on 11Oct1989 (n = 20) were native Williston Kokanee.

1990 – Data for mature Kokanee from the Finlay River caught between 15Oct1990 and 3Nov1990 (n = 104) (Fielden 1991) were native Williston Kokanee.

1991 – Data for mature Kokanee from the Finlay River caught between 20Sep1991 and 26Sep1991 (n = 56) (Fielden 1992) were native Williston Kokanee.

1994 – Data for mature Kokanee caught in 1994 in the Carbon Creek embayment (26Aug1994; n = 66), Dunlevy Creek embayment (24Aug1994; n = 23), and from Philip Creek (1Oct1994; n = 31) (Langston and Zemlak 1998) were among the first cohort of maturing Columbia River origin fish.

2000 – Data from 2000 were Kokanee from six locations in the Williston Reservoir caught between 24Aug2000 and 3Sep2000. Only mature or maturing fish were included (n = 34) (Pillipow & Langston 2002). Genotyping indicated that of the 34 fish that were mature, 21 were native Williston Kokanee and 13 were Columbia origin Kokanee (see below).

2006 – Unpublished data for mature Kokanee caught in 2006 from tributaries to the Williston Reservoir (n = 350) from seven locations were provided by Randy Zemlak (BC Hydro); Germansen River (n = 50), Pelly Creek (n = 50), Tsaydiz Creek (n = 50), Russell Creek (n = 50), Bower Creek (n = 50), Finlay River (n = 50), and Finlay River side channel (n = 50).

2016 – Otoliths were extracted from mature Kokanee that were collected by Chu Cho Environmental under FWCP Project No. PEA-F17-F-1471 contract to UNBC (n = 39) in

September 2016 from three locations; Osilinka River (n = 13), Aley Creek (n = 16), and Stevenson Creek (n = 10).

2017 – Otoliths were extracted from mature Kokanee that were also collected by Chu Cho Environmental under FWCP Project No. PEA-F18-F-2296 contract to UNBC (n = 20) in September 2017 from two locations; Aley Creek (n = 10) and Stevenson Creek (n = 10).

2018 – Mature Kokanee were collected by DWB Consulting Services Ltd. (DWB) under FWCP Project No. PEA-F19-F-2895-DCA (n = 284) in September 2018 from seven locations; Germansen River (n = 40), Manson River (n = 41), Dunlevy Creek (n = 41), Aley Creek (n = 41), Pelly Creek (n = 41), Russell Creek (n = 40), and Cuttoff Creek (n = 40).

2019 – Mature Kokanee were also collected by DWB under FWCP Project No. PEA-F20-F-3359-DCA (n = 123) in September and October 2019 from four locations; Germansen River (n = 40), Aley Creek (n = 43), and Russell Creek (n = 40). Fish collected in the lower Finlay / Akie Rivers (n = 20) were excluded from analyses as fork length was not recorded.

Fork length (L_F; cm) and mass (g) of spawners were used to calculate condition factor (K) with the equation $100 \times M \cdot L_F^{-3}$. Length measurements and condition factor (K) among the different sampling locations were grouped by collection year, except for 2000 where we separated the Native and Columbia origin fish based on genotype (see below). Data were tested for normality and homoscedasticity with Levene's tests and Shapiro-Wilk tests. Differences among years was assessed using a Kruskal-Wallis one-way Analysis of Variance (ANOVA) and *post hoc* Dunn's test to compare means between years. Data are presented ± 1 standard deviation (SD).

FECUNDITY & REPRODUCTIVE INVESTMENT – Mature Kokanee spawners were collected on 18Sep2018 from Pelly Creek, 18Sep2018 from Aley Creek, 19Sep2018 from Russel Creek, 19Sep2018 from Cuttoff Creek, 20Sep2018 from Germansen River, 21Sep2018 from Manson River, and 5Oct2018 from Dunlevy Creek (McDermot-Fouts 2019) and frozen for shipment to the University of Northern British Columbia. Fish were partially thawed to remove gonads. There were 146 females collected from the seven spawning tributary locations. Most of the females collected in 2018 had few or no eggs (n = 95) or had eggs that were loose within the skeins and evidence that they had spawned (n = 24). Of the 24 females that had not appeared to have spawned or who had eggs that were still tight in the skeins, 14 were from Aley Creek, 7 were from Cutoff Creek, 3 were from Manson River, 2 were from Germansen River, and 1 was from Pelly Creek. Mature Kokanee spawners were also collected on 14Sep2019 from Russel Creek, 15Sep2019 from Aley Creek, 16Sep2019 from Germansen River, and 22Oct2019 from Finlay River / Akie River (Robinson 2020) and processed as in 2018. Although sampling was conducted slightly earlier in 2019, few females still retained eggs. There were 55 females collected from the four spawning tributary locations. Most of the females collected in 2019 had few or no eggs (n = 42) or had eggs that were loose within the skeins and evidence that they had spawned (n = 12). Only 1 female from Aley Creek had eggs that were still tight within the skeins. Consequently, spawner collection was targeted earlier in September for 2020 to increase the probability of collecting pre-spawned female Kokanee and determine fecundity. Kokanee were collected on 9&12Sep2020 from Aley Creek, 11Sep2020 from Russel Creek, and 13&14Sep2020 from Germansen River (M. Husereau, Chu Cho Environmental). A greater proportion of female spawners still had eggs and for many the eggs were still tight within the skeins.

To estimate fecundity and reproductive investment, frozen Kokanee were partially thawed. Each animal was measured for fork length (L_F; cm) and mass (M; g). An incision was made from the vent to the isthmus and gonads removed while still partially frozen and firm. Ovaries (or testes) were placed on an electronic scale to measure gonad mass (M_G). Eggs were then placed in a 50%

solution of isopropanol for at least one week to preserve the eggs. Isopropanol hardened the eggs so that they would not rupture when handled. Following preservation, eggs were stripped from the skeins and individually counted. Due to the small size of the spawning Kokanee, fecundity was low and counting the eggs was faster than subsampling to estimate egg number. Because the ovaries were removed frozen from the female Kokanee, gastrointestinal tissue was often included in the ovary mass – which was subtracted from the egg mass after preservation. Physical characteristics were compared among the three spawning locations for pre-spawned females only (fork length [L_F; cm], mass [M; g], condition factor [K; $100 \times M \cdot L_F^{-3}$], gonadal somatic index [GSI; $100 \times M_G \cdot M^{-1}$], fecundity [n], and egg size [mg]) using a one-way ANOVA followed by Tukey's HSD test if significant differences were found among the populations. Although, both L_F and M decrease significantly following death and frozen storage (McQueen et al. 2019), we did not incorporate a correction factor as comparisons were only made among the 2020 spawners.

Our measures of fecundity were compared to published values from 25 Kokanee populations and 48 Sockeye Salmon populations (McGurk 2000), and also data from the Columbia River populations from which the stocked populations were derived. Meadow Creek as yearly means from 1969 to 2017 (Steve Arndt, BC MoFLNRORD, unpublished data) and Hill Creek as yearly means from 1983 to 2017 (Matt Neufeld, BC MoFLNRORD, unpublished data) were compared to the 2020 Williston Reservoir spawners. Additionally, fecundity data reported for Kokanee gill netted in Carbon Embayment, Dunlevy Embayment, and Philip Creek were included in our analysis (Langston and Zemlak, 1998). For comparison with these data, we corrected for the decrease in L_F measurements for the frozen 2020 spawners by multiplying by 2.25% – the average estimate for shrinkage associated with freezing from published values (Armstrong and Stewart 1997; Buchheister and Wilson 2005; Florin and Lingman 2008; McQueen et al. 2019).

GENETIC ANALYSIS – MICROSATELLITE MARKERS – Scale samples from Kokanee gill netted in the reservoir in the late 1980s before the stocking program, in 1994 shortly after the stocking program was initiated, and in 2000 a decade after stocking, were donated to UNBC by the FWCP – Peace Region. For the sample collection that occurred in 2018 and 2019, 10 regions were selected to represent the geographic range of Kokanee in the Williston watershed, including areas where genetic introgression with native populations may occur (FIG. 1; TABLE 1). Mature spawners were collected in 2018 by DWB Consulting Services Ltd. (DWB) at the peak of spawning for Columbia River origin Kokanee (mid-September). In 2019, mature spawners were collected in mid-September, but also late October in 2019 in the lower Finlay River in an attempt to collect native Kokanee when and where they were historically known to spawn: side channels of the lower Finlay in late October until late November (Fielden 1990; Fielden 1991). Additional Kokanee samples for a UNBC nutrient enrichment study collected in 2016 and 2017 from the Osilinka River, Aley Creek, Stevenson Creek and Thutade Lake were included. Adipose fin clips from Kokanee collected from the GM Shrum intake towers of the WAC Bennett Dam in 2016 and 2019 were donated by BC Hydro and Carleton University for inclusion in this study (TABLE 1).

Capture methods varied depending on the water body type; stream sampling was accomplished predominately through the use of a backpack electrofishing unit (Model S-R 12-B, Smith-Root Inc., Vancouver, WA, USA) and stunned fish were collected with a seine or dip net. The shore-spawning Kokanee of Tacheeda and Arctic Lakes were sampled through the use of sinking and floating gill nets (McDermot-Fouts 2019; Robinson 2020). Fish were anesthetized in a solution of $100 \text{ mg} \cdot L^{-1}$ tricaine methane sulfonate (MS-222) buffered with sodium bicarbonate, euthanized by concussion and exsanguination, and frozen at -20 ºC until further processing (McDermot-Fouts

2019; Robinson 2020). Muscle tissue or adipose fin samples were collected from Kokanee individuals and stored in 95% ethanol at -20 ºC.

Kokanee collected from various sampling locations throughout the Williston Reservoir watershed in 2003, 2004, 2006 and 2010 (TABLE 1) were previously genotyped for the FWCP – Peace Region and data provided to us by Ruth Withler of the Pacific Biological Station (PBS) Molecular Genetics Lab, Nanaimo, BC. Tissue samples in 95% ethanol from Kokanee collected in 2016, 2017, 2018, and 2019 were submitted directly to PBS for genotyping. Scale samples provided by FWCP – Peace Region were placed in scale envelopes and stored at room temperature for an extended time and quality of DNA was uncertain. Fin clips from Kokanee sampled from the GMS intake towers and donated by U Carleton were dried and DNA quality was also uncertain. Consequently, DNA extractions from archived scales and dried fin clips were performed before submission to PBS to ensure quality of DNA. We used the QIAamp DNA Micro® kits (Qiagen, Toronto, ON) following the manufacturers protocol, with the modification of overnight incubation at 37 ºC. Two elutions of 50 µl per sample were collected; the first elution was submitted to PBS for genotyping and the second reserved at UNBC at -20 ºC for any future analyses.

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) that 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.

Many locations were sampled multiple or consecutive years. To determine whether there were significant differences between sample years, an analysis of molecular variance (AMOVA) in GenAlEx version 6.51b2 (Peakall and Smouse 2012) was performed. No differences were found for locations sampled from different years, consequently groups were combined by sample location due to low values of F_{ST}. The total number of sample groups across time and year, therefore, was reduced from 42 to 21. 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 and 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 and Ahmed 2011).

ASSESSMENT OF GENETIC INTROGRESSION – Potential introgression of Columbia River origin Kokanee with native Kokanee populations in the Williston Reservoir watershed was assessed using two approaches. First, we assessed population subdivision of the sample groups by constructing a neighbor-joining tree (Saitou and Nei 1987) using Cavalli-Sforza and 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). The use of D_C requires no assumptions about evolutionary models or rates of mutation, and has been shown to consistently perform well in obtaining phylogenies (Takesake and Nei 1996).

Second, 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 admixed 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. The models used were correlated allele frequencies and admixture, with a burn-in period of 100,000 followed by 300,000 iterations. Values of K from 1 to 21 were tested and each K-value was replicated 10 times to survey all potential structure at the genetic population level. Sample location priors were used in an attempt to analyze population structure with slight geographic bias to assist with assigning K. The most effective value of K (ΔK) was identified *ad hoc* via the web-based program STRUCTURE HARVESTER (Earl and von Holdt 2011; Evanno et al. 2005). Cluster results and likelihoods were visualized with Cluster Markov Packager Across K (CLUMPAK; Kopelman et al. 2015). We evaluated genotypes of Kokanee caught in the Williston Reservoir before and after stocking with Columbia River Kokanee with the two native populations in the watershed to evaluate potential subpopulation structure, examine evidence of introgression with native groups, and genetic drift between different spawning locations.

ASSESSMENT OF HOMING TO NATAL SPAWNING LOCATIONS – Model-based genetic clustering methods have been shown to provide resolution of populations at different scales. Warnock et al. (2010) found a hierarchical model of population structure, in which local populations were nested within genetic basin-wide populations. The results from STRUCTURE contrasted with using simple F_{ST} based approaches among *a priori* designated populations. To examine potential genetic differences due to reproductive isolation that results from homing and fidelity to spawning locations, we compared genotypes of Kokanee from only the different tributary streams using STRUCTURE, but also with Discriminant Analysis of Principal Components (DAPC). Analyzing genetic information with a Principle Component Analysis (PCA) and other multivariate models (Discriminant Analysis) that do not require assumptions of Hardy-Weinberg Equilibrium or linkage disequilibrium has shown to be an effective method for recreating real-world population structure (Porras-Hurtado et al. 2013). DAPC replicates Bayesian clustering method of STRUCTURE by assigning populations to particular discriminated clusters, and provides a visual representation of the pattern of population partitioning (Jombart et al. 2010). We visualized the genetic differentiation among Kokanee sampled from tributary spawning locations determined with DAPC using the ADEGENET v2.1.3 package for R.

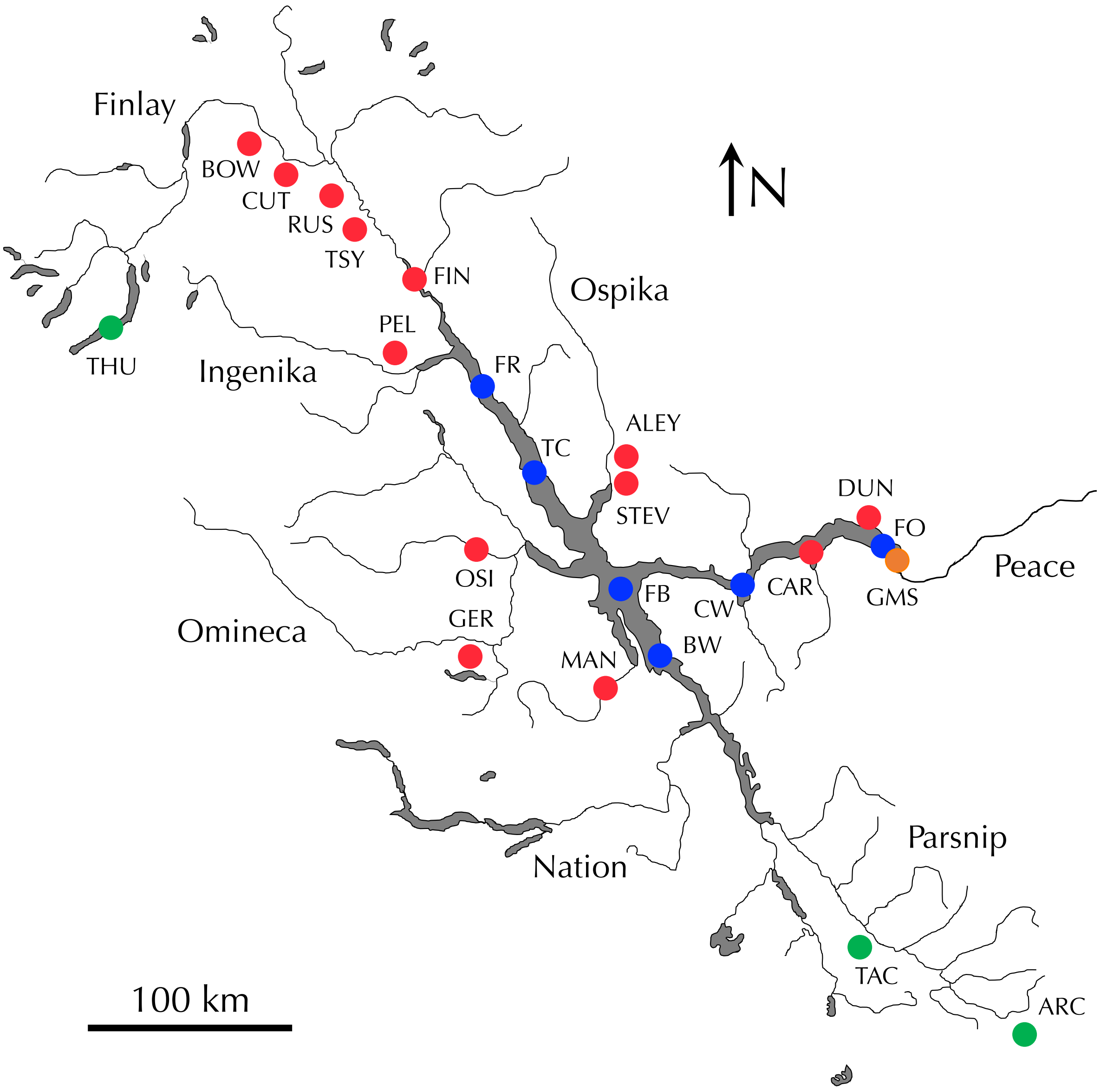

FIG. 1. The Williston Reservoir watershed and tributaries where Kokanee (*Oncorhynchus nerka*) were collected. **RED** symbols are locations of sample sites for Kokanee that spawn in tributary streams to the reservoir. **GREEN** symbols are locations where native Kokanee were sampled from lakes in the headwaters of the Parsnip and Finlay Rivers. **BLUE** symbols indicate locations where Kokanee were collected in the reservoir. **ORANGE** symbol is the location where samples were collected from the GM Shrum intake towers to the WAC Bennett Dam. Abbreviations for sample locations are Arctic Lake (ARC), Tacheeda Lake (TAC), Manson River (MAN), Germansen River (GER), Osilinka River (OSI), Pelly Creek (PEL), Finlay River (FIN), Tsaydiz Creek (TSY), Russel Creek (RUS), Cuttoff Creek (CUT), Bower Creek (BOW), Thutade Lake (THU), Aley Creek (ALEY), Stevenson Creek (STEV), Carbon Embayment (CAR), Dunlevy Embayment & Creek (DUN), GM Shrum intake towers (GMS). Samples collected in the Williston Reservoir in 2000 were collected at locations near Factor Ross Creek (FR), Teare Creek (TC), Finlay Bay (FB), Blackwater Creek (BW), Clearwater Creek (CW), and the Forebay (FO).

TABLE 1. Number of Kokanee (*Oncorhynchus nerka*) samples that were successfully genotyped by location, year and sample type.

Location	1988	1989	1990	1994	2000	2003	2004	2006	2010	2016	2017	2018	2019
Williston	**36**	**34**	**15**		**45**								
GMS										**95**			**40**
Russell								**49**				**40**	**39**
Bower								**50**					
Cuttoff												**40**	
Finlay								**92**					**20**
Tsaydiz								**49**					
Pelly								**49**				**40**	
Osilinka										**13**			
Germansen								**50**				**40**	**40**
Manson												**41**	
Aley										**16**	**10**	**41**	**42**
Stevenson										**10**	**7**		
Dunlevy				**23**								**41**	
Carbon				**63**									
Thutade						**87**					**20**		
Arctic								**50**					**18**
Tacheeda							**50**					**30**	
Meadow						**196**	**100**					**49**	
Hill									**60**			**40**	
TOTAL	**36**	**34**	**15**	**86**	**45**	**283**	**150**	**389**	**60**	**134**	**37**	**402**	**199**

Samples from 2003 to 2010 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 **BLACK** 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, 2018 samples from Meadow Creek and Hill Creek were collected by Matt Neufeld and Steve Arndt (BC MoFLNRORD). 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 **BLUE** were collected by R. Zemlak (BC Hydro), Taylor Ward and Dirk Algera (U Carleton) from the intake towers of the GM Shrum generating station for the WAC Bennett DAM, donated to UNBC, and genotyped at PBS for a UNBC Research Project Award to P. Wilson.

RESULTS

AGE & SIZE AT MATURITY – Annuli were clearly visible for many, but not all, of the whole mount images of otoliths for Kokanee sampled from the Williston watershed (FIG. 2). The grand mean of ages for Kokanee examined in the study was 2.85 ± 0.13 (SD) years across the different groups and sample years. This indicates a generation time of 4 years, from spawning event to 4 subsequent years of growth (3 otolith annuli + 1 more year of growth) before sexual maturation, for fish in the Williston Reservoir watershed. Age estimates between groups of Williston Reservoir Kokanee did not differ significantly (ANOVA, $P = 0.079$) (TABLE 2). Additionally, a two-factor ANOVA with replication examining Germansen, Pelly, and Russel in 2006 and 2018 did not find significant differences between locations ($P = 0.18$) or years ($P = 0.89$).

Matt Neufeld and Steve Arndt (BC MoFLNRORD) provided us with historic data on age and size at maturity for Hill Creek and Meadow Creek, the original populations that were used to stock Kokanee into the Williston Reservoir watershed. Most of the Kokanee in the reservoir from native populations and of Columbia River origin historically have matured at age 3 consistent with our otolith annuli estimates of age at maturity.

Sizes of Kokanee at maturity have changed over time and are getting smaller (FIG. 3; TABLE 3). Kokanee caught in the lower Finlay River in 1989, 1990 and 1991 were native Kokanee from the Upper Peace River system. Native Kokanee were the largest fish caught in the Williston Reservoir watershed over the years that Kokanee have been sampled and were significantly larger than the first cohort of mature stocked Kokanee (29.7 ± 1.78 cm for native Kokanee verses 26.8 ± 1.03 cm for Columbia Kokanee) and larger than all the other cohorts (TABLE 4). Of the native Kokanee caught over the 3 years, fish caught in 1990 were on average the largest, but did not differ in length from spawners caught in 1989 or 1991 (TABLES 3 & 4).

Kokanee sampled in 1994 were some of the first Columbia River origin Kokanee to mature; STRUCTURE assigned these fish to the Hill Creek cluster (see below). Fish sampled in 1994 were caught in the Peace Arm of the Williston Reservoir in embayments of creeks where Columbia Kokanee were stocked and in Philip Creek, a tributary to the Nation River where Kokanee were also stocked (FIG. 3). The Columbia origin fish were similar in size among the three locations, but significantly smaller than the native Kokanee. The Kokanee caught in 1994 differed in colouration from the Kokanee caught in the reservoir before the stocking program was started; Kokanee caught in the Williston Reservoir exhibited bright red bodies with emerald green heads, whereas Kokanee from the Finlay River had rusty reddish brown bodies and heads (Langston and Zemlak 1998).

Of the 45 Kokanee gill-netted from the reservoir in 2000, 34 were considered mature or maturing (Pillipow and Langston 2002). Although the number of samples was small, the fish appeared to show a bimodal length distribution; the lower mode with a mean of approximately 22 cm and the upper mode with a mean greater than 26 cm (FIG. 3). Genotyping revealed that the fish were a mix of native Williston Kokanee and Columbia origin Kokanee (see below). The majority of fish in the lower mode were native Kokanee (13 of 15) and the majority of fish in the upper mode were Columbia origin Kokanee (11 of 19). Although, the two populations of Kokanee from the 2000 samples did not differ in length, the larger Columbia origin Kokanee did not differ in size from the 1994 cohort, whereas the native Williston Kokanee were significantly smaller than the 1994 Columbia fish (TABLES 3 & 4).

Kokanee caught in tributaries to the Williston Reservoir in 2006 were on average smaller than the fish sampled in 1994, but formed a bimodal distribution. Kokanee sampled in 2006 also morphologically resembled the Columbia River origin fish and formed part of the genetic cluster that was characteristic of the Columbia River origin Kokanee – particularly Hill Creek (one of the donor populations introduced into the watershed; see below) rather than to the native Kokanee populations in the watershed, Thutade Lake, Arctic Lake or North Tacheeda Lake. Kokanee sampled from Germansen River were predominantly from the upper size mode and were distinctly larger than fish caught in the other tributary streams. The larger mode was similar in size to the fish sampled in 1994 (FIG. 3). Fish from more northerly tributaries that are part of the Finlay River watershed were smaller; the largest number of fish were part of the 24-cm size class and there were few larger fish. Fish caught in Pelly Creek exhibited a range of size, although most were part of the smaller modal group. As there were no genetic differences among the 2006 cohort, all fish were combined for statistical comparison. The 2006 fish were significantly smaller than the native Williston Kokanee prior to stocking and the 1994 Columbia origin Kokanee and significantly larger than the 2018 to 2019 cohorts (TABLES 3 & 4). The 2006 fish did not differ in size from native or stocked Kokanee sampled in 2000.

Kokanee spawners from the 2018 to 2019 cohorts were significantly smaller than fish caught in earlier years (TABLES 3&4). The most abundant size class was 22 cm for 2018 and 2019 (FIG. 3), although the range of sizes of fish varied among the rivers and by year. For 2018, fish caught in the more southerly tributaries tended to be larger than fish caught in the tributaries to the Finlay River. In 2019, however, the most abundant size class for the Russel Creek spawners was 23 cm, whereas the most abundant size class for Germansen River and Aley Creek was 22 cm.

We also found differences in condition factor (K; $100 \times M \cdot L_F^{-3}$) for fish sampled at different times from spawning locations in the reservoir. Native Williston Kokanee sampled from 1989 to 1991 and in 2000 had the highest average values for K (TABLE 3). Stocked Columbia River origin Kokanee that were sampled in 2006 and earlier had intermediate values of K that did not differ from the Columbia origin Kokanee sampled in 2000 (TABLES 3 & 4). In contrast, cohorts sampled from 2018 and 2019 had values of K that were significantly less than cohorts sampled in 2006 and earlier (TABLE 4).

FECUNDITY & REPRODUCTIVE INVESTMENT – Most of the Kokanee caught between 18Sep2018 and 20Sep2018 had already spawned. Only 19% of the mature females collected from tributary spawning locations in 2018 retained large numbers of eggs and appeared not to have spawned. Similarly, most of the Kokanee caught between 14Sep2019 and 16Sep2019 were also spent; 4% of females still retained large numbers of eggs. In 2020, Kokanee in the spawning tributaries were caught earlier than the previous years; 9Sep2020 to 14Sep2020 and the proportion of female spawners that were still gravid was higher. Additionally, female spawners were specifically targeted for collection from Germansen River, Russel Creek, and Aley Creek in 2020 to investigate fecundity. Although eggs were free of the skeins for some fish and there were indications that some of the fish had spawned or partially spawned, eggs were still tightly attached to the skeins in many of the spawners (FIG. 4). A summary of mean reproductive traits for female and male Kokanee caught in Williston Reservoir tributaries is provided in TABLE 5. Comparison of female pre-spawners collected from Germansen River, Russel Creek and Aley Creek indicated that fork length ($F_{2,61} = 2.06$; $P = 0.137$) and mass ($F_{2,61} = 1.02$; $P = 0.366$) did not differ, but the ratio of mass to length (condition factor; K) differed significantly ($F_{2,61} = 5.97$; $P = 0.0043$) and was greater for female spawners that were still gravid that were collected from Russel Creek and Aley Creek than females from Germansen River. None of the other values for reproductive investment, GSI ($F_{2,61}$ = 1.48; $P = 0.234$), F ($F_{2,61}$ = 1.04; $P = 0.362$), and egg size ($F_{2,61}$ = 0.118; $P = 0.889$), differed among the three spawning locations.

There was a slight but significant relationship between fecundity and length ($F_{1,62}$ = 9.16; P = 0.0036), but no relationship existed between egg size and length ($F_{1,62}$ = 2.673; $P = 0.107$). Additionally, Kokanee sampled in 2020 were smaller than Kokanee from the Williston Reservoir watershed before (1990) and after Columbia River Kokanee were stocked (1994) (TABLE 5; FIG. 3). The smaller body size limits fecundity as number of eggs females produce is directly proportional to fish size for both non-anadromous, Kokanee, and anadromous, Sockeye Salmon, populations of *O. nerka* (FIG. 5A; McGurk 2000). Kokanee from the Columbia River donor populations have shown considerable variation in size among years with corresponding variation in fecundity. Considerable variation also exists among different populations of Kokanee and Sockeye Salmon, with the larger anadromous life history form exhibiting much greater fecundity (FIG. 5A; McGurk 2000). The decrease in size between fish caught in 1994 and 2020 resulted in a considerable decrease in fecundity – conforming to the length-fecundity relationship determined for Kokanee by McGurk (2000); $F = 0.0159L^{3.131}$, where F is fecundity and L is fork length (FIG. 5B).

Spawners were generally less fecund than the regression line determined for the data from McGurk (2020) – of the 64 mature female Kokanee sampled in 2020 that had not spawned, fecundity was higher than the model reported by McGurk (2000) for only 17; 47 fish had lower fecundity than the model. Reproductive investment for the Williston Reservoir Kokanee, therefore, is proportionately less for a given length than the model derived from 25 Kokanee populations reported in McGurk (2000). In contrast to the findings for 2020, female spawners sampled in 1994 that were some of the first Columbia River origin fish to mature in the reservoir (see below) were generally more fecund than the predicted values from the McGurk (2000) model; 19 were more fecund and 13 were less fecund (FIG. 5B).

POPULATIONS GENETICS – MICROSATELLITE MARKERS – Genetic signatures did not differ significantly for locations where Kokanee were collected over multiple years; all F_{ST} values were close to zero or negative ($F_{ST} \leq 0.011$), indicating random mating under Hardy-Weinberg equilibrium. Kokanee collected in 2000 from the Reservoir were maintained as a separate group from other fish caught in the reservoir ($F_{ST} = 0.082$), while those from before (1988 to 1990; Native) and after (2016, 2019; GMS) were respectively combined. Sampling locations were combined for all subsequent analysis and group sample sizes by location ranged from 13 (Osilinka) to 345 (Meadow) individuals (TABLE 6).

Across all loci and sample groups, there was evidence of 3 significant null alleles present due to homozygote excess: locus Ots3 in fish from Aley Creek (0.086, $P < 0.001$), and locus Oki10 (0.057, $P < 0.01$) and locus Ots3 (0.080, $P < 0.001$) in fish from Thutade Lake. All 3 null alleles maintained frequencies below 0.10. There was no evidence for scoring error (stuttering) or allele dropout at any locus.

The mean H_e averaged across all 14 loci and 21 groups was 0.66 ± 0.022 (TABLE 6), and ranged from 0.38 ± 0.064 (Tacheeda) to 0.72 ± 0.049 (Hill) and 0.72 ± 0.047 (Osilinka). Of the tributary groups, mean H_e was 0.70 ± 0.002. The mean H_o for all loci and groups was 0.65 ± 0.021. Tacheeda once again reported the lowest H_o of 0.38 ± 0.066, with 0.71 ± 0.049 (Hill) and 0.71 ± 0.043 (Germansen) on the higher end. The mean allelic richness (A_R) across loci and groups was

5.99 ± 0.258, and ranged within groups from 2.82 ± 0.454 (Tacheeda) to 6.97 ± 0.944 (Meadow). The A_R for tributary groups all exceeded 6.00, while the native groups averaged 3.93 ± 0.701. The greatest number of alleles for a group was sampled from Meadow (215), with Germansen (178) and Thutade (152) reporting the greatest number of alleles for the tributary groups and native groups, respectively. The native groups consistently reported the lowest genetic diversity among all the groups, and the putative source populations of Hill and Meadow exhibited the some of the highest genetic diversity and allelic richness. Thutade appeared to have higher mean allelic richness and expected heterozygosity (A_R = 5.93 ± 0.793; H_e = 0.64 ± 0.059) than other native groups, as they were more equivalent to the mean allelic richness and expected heterozygosity values of tributary groups. The genetic diversity of the tributary groups fell close to but lower than the values of the Columbia River source populations.

POPULATIONS GENETICS – ASSESSMENT OF POTENTIAL INTROGRESSION – The neighbor-joining tree of Cavalli-Sforza and Edwards' chord distances (D_C) clustered the tributary groups largely together and with Hill and Meadow (84%; FIG. 6). The Osilinka and Stevenson groups appeared somewhat apart from the rest of the tributary groups (73%), but this could have been due to their low sample sizes (Osilinka = 13 individuals; Stevenson = 17 individuals) rather than a true reflection of genetic distance. The Forebay group, comprised of Kokanee that were collected from the GM Shrum intake towers near the WAC Bennett Dam in 2016 and 2019, also clustered with the tributary groups. Native Williston Kokanee were closest to the Thutade Lake fish, suggesting a common ancestry. Notably, Kokanee sampled from the reservoir in 2000 separated from the tributary spawner groups (100%), but were positioned between the Thutade Lake and Williston Tributary groups – suggesting introgression between the native Williston and Columbia origin Kokanee. Arctic and Tacheeda clustered together, but distinctly apart from the rest of the samples (100%).

We ran the program STRUCTURE with *a priori* knowledge of geographic location for each fish. STRUCTURE plots showing a range of K from 2 to 6 demonstrate the emergence of informative patterns with increasing K (FIG. 7). A K value of 2 clusters with Markov clustering (MCL) similarity of 0.994 was given the highest probability. For K of 2, samples from native Kokanee populations in the Williston Watershed (Arctic, Tacheeda, Thutade, and the fish sampled in the 1990s from the reservoir) clustered together as one genetic population. All other samples comprised the second genetic population, except for the fish sampled in 2000 which were assigned to either cluster with no indication of admixture. A K of 2 clusters was not considered to be the best fit as samples collected from Thutade Lake in 2003 and 2017 showed admixture between the two clusters which is highly unlikely given that Cascadero Falls is impassable to upstream migration. Additionally, limiting the analysis to two clusters does not reflect the geographic distribution of Kokanee in the watershed and was not consistent with F_{ST} estimations. A K value of 2, therefore, was considered an oversimplification.

A K of 3 clusters separated the Williston Watershed Kokanee into two groups: fish from the Parsnip River watershed, which were distinct from the cluster that contained native Williston Kokanee caught before stocking (1989, 1990, and 1991), and the Thutade Lake fish. The Columbia River samples, Hill Creek and Meadow Creek, and all the Kokanee sampled from tributaries and the reservoir forebay formed cluster 3.

A K of 4 was the second most supported cluster probability (MCL = 0.969) and was similar to K of 3 as it segregated Arctic and Tacheeda to one cluster, Thutade and the native Williston fish to a separate cluster, but also differentiated between the two source populations of Columbia-origin

Kokanee; Hill Creek and Meadow Creek. The samples from Hill Creek also showed signs of admixture with Meadow Creek genotypes (FIG. 7) – consistent with the stocking of Hill Creek with kokanee from Meadow Creek (Langston and Murphy 2008). Since initial stocking in the Williston reservoir, Meadow Creek signatures do not appear to have persisted as a distinct genetic population in the watershed. The tributary spawning Kokanee and the Kokanee sampled from the dam intake towers in 2016 and 2019 were dominated by Hill-type genotypes. The Carbon group, containing Kokanee collected in 1994 from Carbon embayment, shortly after the stocking program began, also contained 3 individuals with Native Williston signatures (FIG. 8).

The third most-supported cluster probability, K = 5 (MCL = 0.959), was considered the most parsimonious. For a K of 5, the assignment was similar to K of 4, except the native Williston fish sampled in 1989 to 1991 were differentiated from Thutade Lake. Separation of Kokanee caught in the reservoir from Thutade Lake did not occur until a K of 5 further supports that Thutade Lake was the source population for Kokanee in the reservoir prior to stocking Columbia River Kokanee. A K of 6 offered no further information, but forced a split in the Hill Creek cluster (FIG. 7).

We found no evidence of introgression of Columbia origin Kokanee with native Williston Kokanee populations in our analysis – both for the two lakes in the headwaters of the Parsnip River watershed (FIG. 7) or for Kokanee sampled in the reservoir (FIG. 8). Kokanee gill-netted in Carbon embayment in 1994 were mainly Hill type, with some fish showing admixture between Hill and Meadow types, but three fish were clearly assigned to the Thutade / native Williston cluster (K = 4) or just the Williston cluster (K = 5). Additionally, Kokanee gill-netted from six locations throughout the reservoir in 2000 were assigned to one of three clusters, Hill (n = 15), Meadow (n = 5), or Williston (n = 25), with no indication of admixture (FIG. 8). Further, fish collected in 2016 and 2019 at the dam intake towers (GMS) exclusively belonged to the Hill cluster and we found no indication of native Williston genotypes – or admixture between the two clusters.

POPULATIONS GENETICS – ASSESSMENT OF HOMING TO NATAL SPAWNING LOCATIONS – The neighbor-joining tree (FIG. 6) and the STRUCTURE analysis (FIG. 7) on all Kokanee genotyped indicated no differences among the stocked Columbia origin Kokanee that had colonized multiple tributary streams in the Williston Reservoir watershed. Reducing the data to only genotypes from Kokanee sampled from spawning locations after stocking Columbia origin fish did not indicate a hierarchical model of population structure within the watershed independent of the modelling approach used (data not shown). For the DAPC analysis, genotypes of fish from all the tributary streams clumped together and the 95% confidence intervals for each spawning location showed considerable overlap (FIG. 9). Additionally, few DAPC scores were scattered outside the 95% confidence intervals; a notable exception is for 3 fish sampled from the Carbon Embayment in 1994 with low values for the second discriminant function (negative values on the Y-axis). These three fish were the same samples assigned by STRUCTURE to be native Williston Reservoir Kokanee.

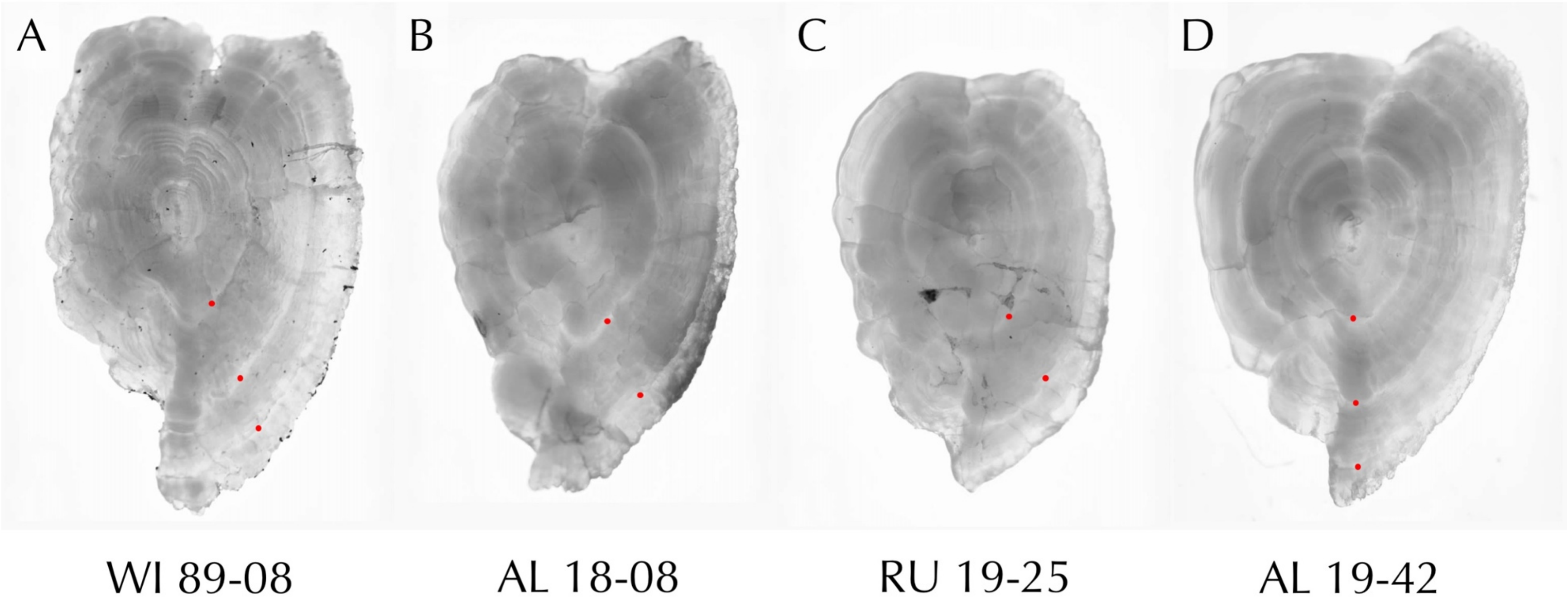

FIG. 2. Light microscope images from mature Kokanee (*Oncorhynchus nerka*) spawners caught in the Williston Reservoir watershed. A – native Williston Reservoir Kokanee (WI 89-08; 29.2 cm, 340 g) caught in the lower Finlay River in 1989. B – Columbia River origin Kokanee (AL 18-08; 22.1 cm, 125.6 g) caught in Aley Creek in 2018. C – Columbia origin Kokanee (RUS 19-25; 21.9 cm, 99.1 g) caught in Russel Creek in 2019. D – Columbia origin Kokanee (ALEY 19-42; 22.2 cm, 128.4 g) caught in Aley Creek in 2019. Red dots indicate annuli.

TABLE 2. Number of samples, fork length, mass, and age for Williston Reservoir Kokanee. Data are means ± standard deviation (range in parentheses). Significant differences in age estimates between groups were not identified (ANOVA, $P = 0.079$). Sample size (n) was the same for size and age measurements for all groups except fish caught in 2006 where sample size for length and mass is provided followed by sample size for age determination in brackets.

YEAR	LOCATION	n	LENGTH	MASS	AGE
1989	Williston	13	30.4 ± 2.7 (28.6 – 38.7)	352.9 ± 29.9 (300 – 400)	3.08 ± 0.49 (2 – 4)
1994	Philip	27	27.3 ± 1.0 (25.9 – 29.0)	205.9 ± 46.8 (142 – 312)	3.07 ± 0.47 (2 – 5)
2006	Germansen	50 (35)	26.1 ± 1.7 (23.2 – 30.5)	225.2 ± 48.3 (164 – 415)	2.94 ± 0.42 (2 – 4)
2006	Pelly	50 (39)	24.1 ± 1.8 (21.5 – 28.5)	187.8 ± 36.9 (125 – 290)	2.74 ± 0.59 (2 – 4)
2006	Russel	50 (39)	24.7 ± 1.6 (22.0 – 28.0)	178.5 ± 36.2 (108 – 271)	2.69 ± 0.52 (2 – 4)
2016	Osilinka	13	22.6 ± 1.8 (19.4 – 24.9)	100.9 ± 34.5 (50 – 185)	2.69 ± 0.75 (2 – 4)
2016	Stevenson	10	22.2 ± 1.4 (19.8 – 24.3)	108.5 ± 31.9 (51 – 150)	2.80 ± 0.42 (2 – 3)
2017	Stevenson	10	21.4 ± 0.7 (20.5 – 22.7)	103.2 ± 21.5 (76 – 146)	2.80 ± 0.42 (2 – 3)
2018	Germansen	39	21.9 ± 1.0 (20.0 – 23.7)	117.0 ± 22.4 (85 – 159)	2.79 ± 0.52 (2 – 4)
2018	Pelly	40	21.4 ± 1.1 (18.7 – 23.8)	103.6 ± 15.3 (67 – 142)	2.83 ± 0.50 (2 – 3)
2018	Russell	40	21.3 ± 1.2 (19.4 – 23.8)	104.0 ± 22.8 (69 – 152)	2.75 ± 0.49 (2 – 4)
2018	Aley	41	22.1 ± 0.9 (20.0 – 23.8)	127.2 ± 17.7 (88 – 167)	2.71 ± 0.64 (1 – 4)
2019	Germansen	40	21.4 ± 0.9 (19.9 – 24.0)	105.1 ± 15.1 (77 – 138)	2.78 ± 0.53 (2 – 4)
2019	Russel	37	21.5 ± 0.9 (19.0 – 23.0)	106.6 ± 17.3 (74 – 146)	2.95 ± 0.47 (2 – 4)
2019	Aley	41	21.3 ± 0.9 (19.4 – 23.5)	123.1 ± 24.1 (83 – 183)	2.90 ± 0.37 (2 – 4)

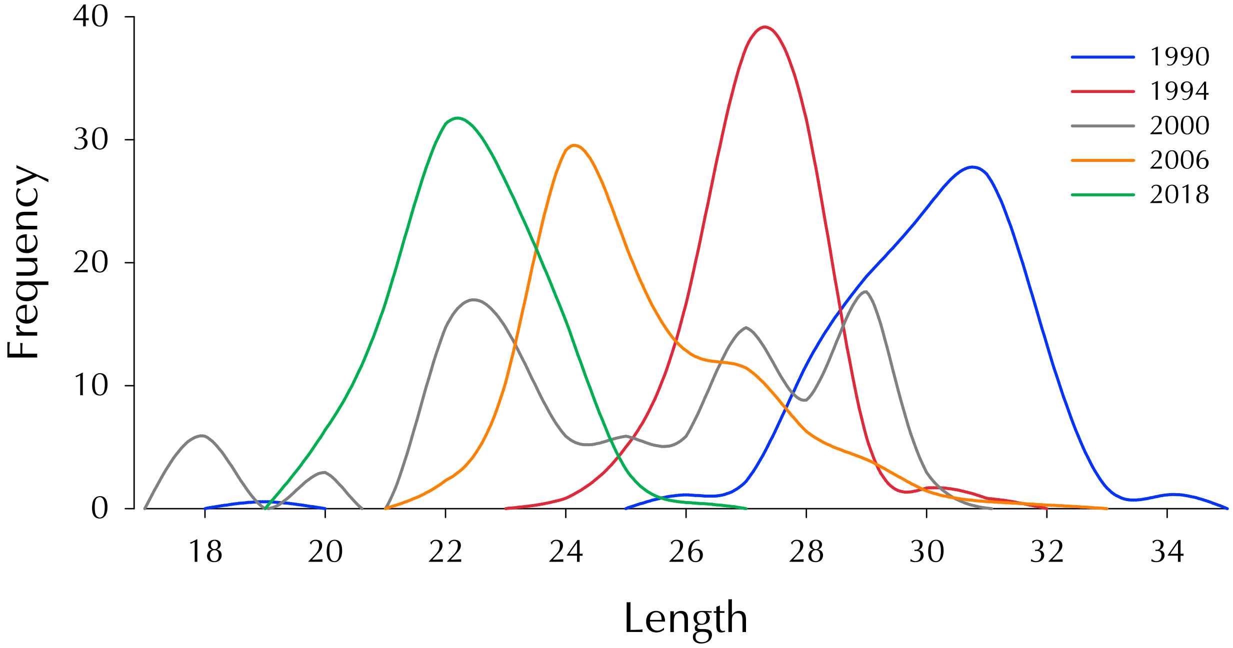

FIG. 3. Frequency of length classes (cm) for Kokanee (*Oncorhynchus nerka*) caught in the Williston Reservoir and tributaries to the reservoir.

"1990" were mature Kokanee from the Finlay River collected in 1989, 1990 and 1991 (n = 181). Kokanee were caught on 11Oct1989 (n = 21) (data on scale envelopes provided by FWCP-Peace Region), between 15Oct1990 and 3Nov1990 (n = 104) (Fielden 1991), and between 20Sep1991 and 26Sep1991 (n = 56) (Fielden 1992).

"1994" were mature Kokanee caught in 1994 in the Carbon Creek embayment (26Aug1994; n = 66), Dunlevy Creek embayment (24Aug1994; n = 23), and from Philip Creek (1Oct1994; n = 31) (Langston and Zemlak 1998). All fish were of Columbia River origin, except 3 that were native Williston Kokanee determined by genotyping.

"2000" were Kokanee gill-netted from six locations in the Williston Reservoir caught between 24Aug2000 and 3Sep2000. Only mature or maturing fish were included (n = 34) (Pillipow & Langston 2002).

"2006" were mature Kokanee caught in 2006 from tributaries to the Williston Reservoir (n = 350) from six locations; Germansen River (n = 50), Pelly Creek (n = 50), Russell Creek (n = 50), Bower Creek (n = 50), Tsaydiz Creek (n = 50), Finlay River (n = 50), and Finlay River side channel (n = 50). Unpublished data provided by Randy Zemlak (BC Hydro).

"2018" were mature Kokanee collected in 2018 and 2019 (n = 407). In 2018 Kokanee were collected by DWB Consulting Services Ltd. (DWB) under FWCP Project No. PEA-F19-F-2895-DCA (n = 284) in September 2018 from seven locations; Germansen River (n = 40), Manson River (n = 41), Dunlevy Creek (n = 41), Aley Creek (n = 41), Pelly Creek (n = 41), Russell Creek (n = 40), and Cuttoff Creek (n = 40). In 2019 mature Kokanee were collected by DWB Consulting Services Ltd. under FWCP Project No. PEA-F20-F-3359-DCA (n = 123) in September and October 2019 from three locations; Germansen River (n = 40), Aley Creek (n = 43), and Russell Creek (n = 40).

TABLE 3. Average fork length (cm) and condition factor (K; g · cm^{-3}) for spawning Kokanee sampled in the Williston Reservoir watershed by year. Number of samples (n) varied among the years. Data are means ± SD (range in parentheses). Samples collected in 1991 and earlier (1989, 1990 & 1991) were native Williston Reservoir Kokanee, 1994 were Columbia origin Kokanee (3 Williston Kokanee were removed based on genotyping – see below), in 2000 data was separated based on genotyping for Williston (2000^{W}) and Columbia (2000^{C}) fish, and after 2000 (2006 to 2019) genotyping revealed that all fish were Columbia origin. Values with a common letter do not differ (TABLE 4).

Group	n	Length	K_{FL}
1989	35	29.04 ± 1.57 (23.9–31.9)^{w}	1.37 ± 0.12 (1.09–1.54)^{a}
1990	119	29.72 ± 1.84 (21.7–33.8)^{w}	1.31 ± 0.11 (1.02–1.55)^{ab}
1991	56	28.62 ± 1.93 (19.0–31.7)^{w}	1.28 ± 0.15 (1.03–1.69)^{bc}
1994	117	26.79 ± 0.97 (23.7–29.9)^{x}	1.23 ± 0.22 (0.77–1.53)^{c}
2000^{W}	21	24.16 ± 3.31 (16.1–29.2)^{y}	1.29 ± 0.13 (0.75–1.47)^{c}
2000^{C}	13	25.87 ± 3.02 (17.3–29.0)^{xy}	1.19 ± 0.07 (1.07–1.35)^{cd}
2006	350	24.85 ± 1.87 (21.6–32.4)^{y}	1.15 ± 0.19 (0.69–1.52)^{d}
2018	284	22.08 ± 1.35 (19.1–28.4)^{z}	1.05 ± 0.17 (0.52–1.44)^{f}
2019	123	21.81 ± 0.99 (19.3–24.2)^{z}	1.08 ± 0.11 (0.76–1.40)^{e}

TABLE 4. Pairwise comparison table of the *P* values of Kruskal-Wallis tests and *post hoc* Dunn's tests comparing fork length (cm; above diagonal) and condition factor (K; below diagonal) of Kokanee collected across years. Groups as defined in TABLE 3.

	1989	1990	1991	1994	2000^{W}	2000^{C}	2006	2018	2019
1989		0.616	0.643	0.009	<0.001	0.020	<0.001	<0.001	<0.001
1990	0.152		0.249	<0.001	<0.001	0.003	<0.001	<0.001	<0.001
1991	0.023	0.170		0.012	<0.001	0.033	<0.001	<0.001	<0.001
1994	<0.001	<0.001	0.142		<0.001	0.389	<0.001	<0.001	<0.001
2000^{W}	0.188	0.725	0.568	0.113		0.085	0.219	<0.001	<0.001
2000^{C}	0.002	0.017	0.122	0.414	0.082		0.252	<0.001	<0.001
2006	<0.001	<0.001	<0.001	<0.001	<0.001	0.522		<0.001	<0.001
2018	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001		0.308
2019	<0.001	<0.001	<0.001	<0.001	<0.001	0.045	<0.001	0.044	

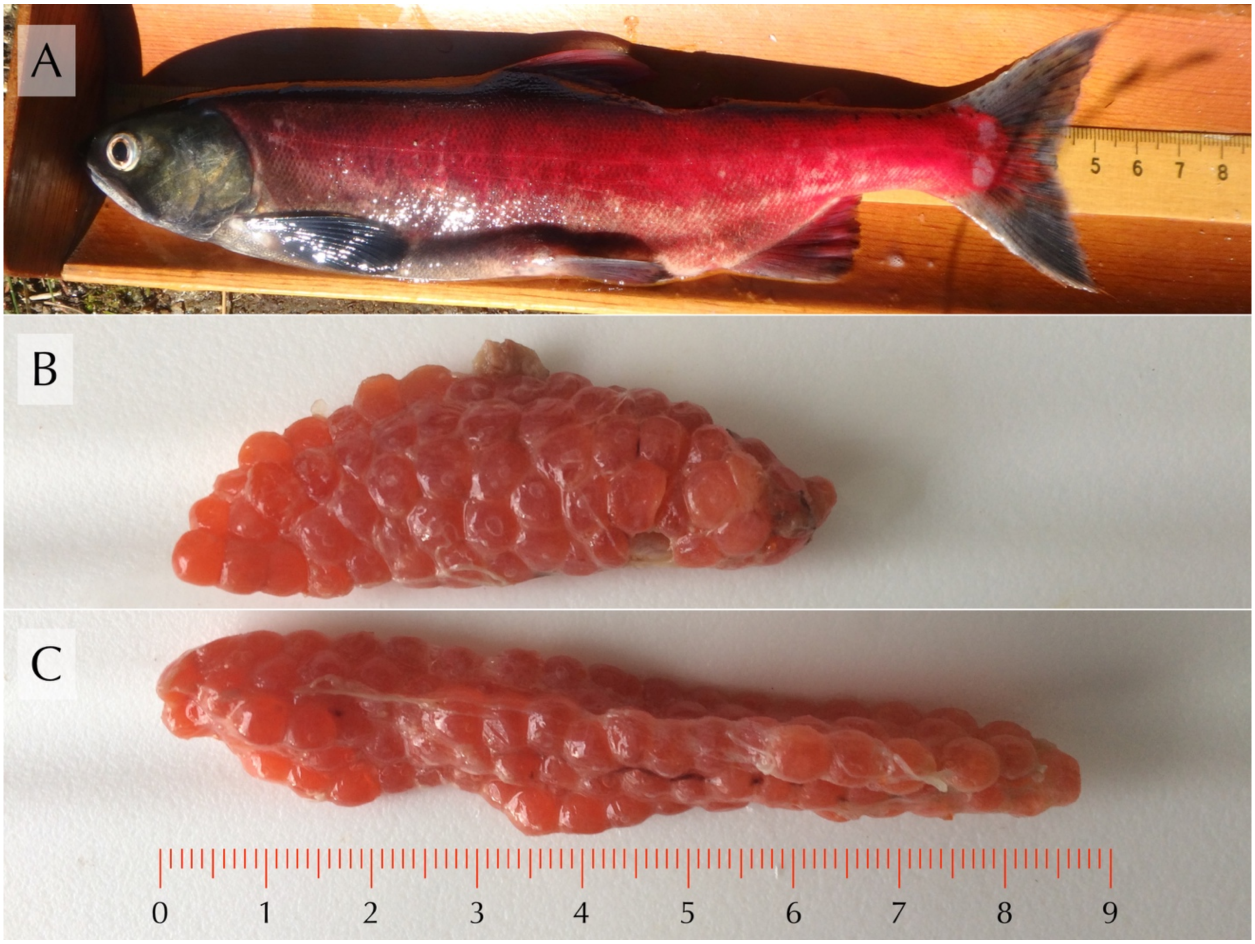

FIG. 4. A. Mature female Kokanee (*Oncorhynchus nerka*) spawner collected from the Osilinka River on 13Sep2016, 23.9 cm in length (photograph by Lindi Anderson, Chu Cho Environmental). B & C. Eggs still within skeins from a mature female Kokanee collected from the Germansen River (GER 20-03) on 13Sep2020, 22.4 cm, 121.7 g. Gonad mass was 11.84 g, gonadal somatic index was 9.73%, fecundity was 204 eggs, and egg size was 58.0 mg.

TABLE 5. Mean fork length, mass, and measures of reproductive investment for Kokanee (*Oncorhynchus nerka*) from three locations in the Williston Reservoir watershed where Columbia origin fish spawn. Data is separated into females and males. Sample size is given as n and variation reported as standard deviation (SD). Some of the females had already spawned; for the females sampled that had not spawned, gonad mass, gonadal somatic index (GSI), fecundity, and egg size are listed. Females were selectively caught for the analysis to ensure sufficient number of fish would be sampled to determine reproductive investment. Fish were collected from Germansen River (13&14Sep2020), Russel Creek (11Sep2020), and Aley Creek (9&12Sep2020). Mature Kokanee were collected by Chu Cho Environmental under FWCP Project No. PEA-F21-F-3363-DCA to DWB Consulting Services Ltd.

	Germansen River	Russel Creek	Aley Creek
ALL FEMALES			
n	36	28	34
Length (cm)	21.6 ± 0.9	21.0 ± 1.1	21.4 ± 0.9
Mass (g)	101.7 ± 15.0	103.3 ± 17.6	109.6 ± 14.1
PRE-SPAWNED FEMALES			
n	15	23	26
Length (cm)	21.8 ± 0.7	21.1 ± 1.2	21.4 ± 1.0
Mass (g)	110.9 ± 14.7	106.8 ± 16.2	112.6 ± 12.8
K ($g \cdot cm^{-3}$)	1.06 ± 0.06[a]	1.13 ± 0.07[b]	1.15 ± 0.09[b]
Gonad mass (g)	12.7 ± 3.5	13.2 ± 2.7	13.9 ± 3.1
GSI (%)	11.3 ± 2.0	12.4 ± 2.0	12.3 ± 2.1
Fecundity	200 ± 48	207 ± 42	223 ± 64
Egg size (mg)	63.0 ± 6.7	64.5 ± 10.1	63.7 ± 9.0
POST SPAWNED FEMALES			
n	21	5	8
Length (cm)	21.5 ± 0.9	20.5 ± 0.3	21.4 ± 0.8
Mass (g)	95.1 ± 11.5	87.2 ± 16.1	99.9 ± 14.8
K ($g \cdot cm^{-3}$)	0.96 ± 0.06	1.01 ± 0.15	1.02 ± 0.10
MALES			
n	4	3	6
Length (cm)	20.8 ± 0.5	19.8 ± 0.9	21.1 ± 0.7
Mass (g)	97.4 ± 13.6	91.8 ± 16.0	116.7 ± 11.7
K ($g \cdot cm^{-3}$)	1.08 ± 0.19	1.17 ± 0.07	1.24 ± 0.12
Gonad mass (g)	1.68 ± 0.42	1.75 ± 0.37	1.24 ± 0.85
GSI (%)	1.71 ± 0.21	1.95 ± 0.53	1.04 ± 0.67

Values with a common letter do not differ significantly ($P < 0.05$) for the pre-spawned females. No letter indicates that no differences were found among the three spawning locations.

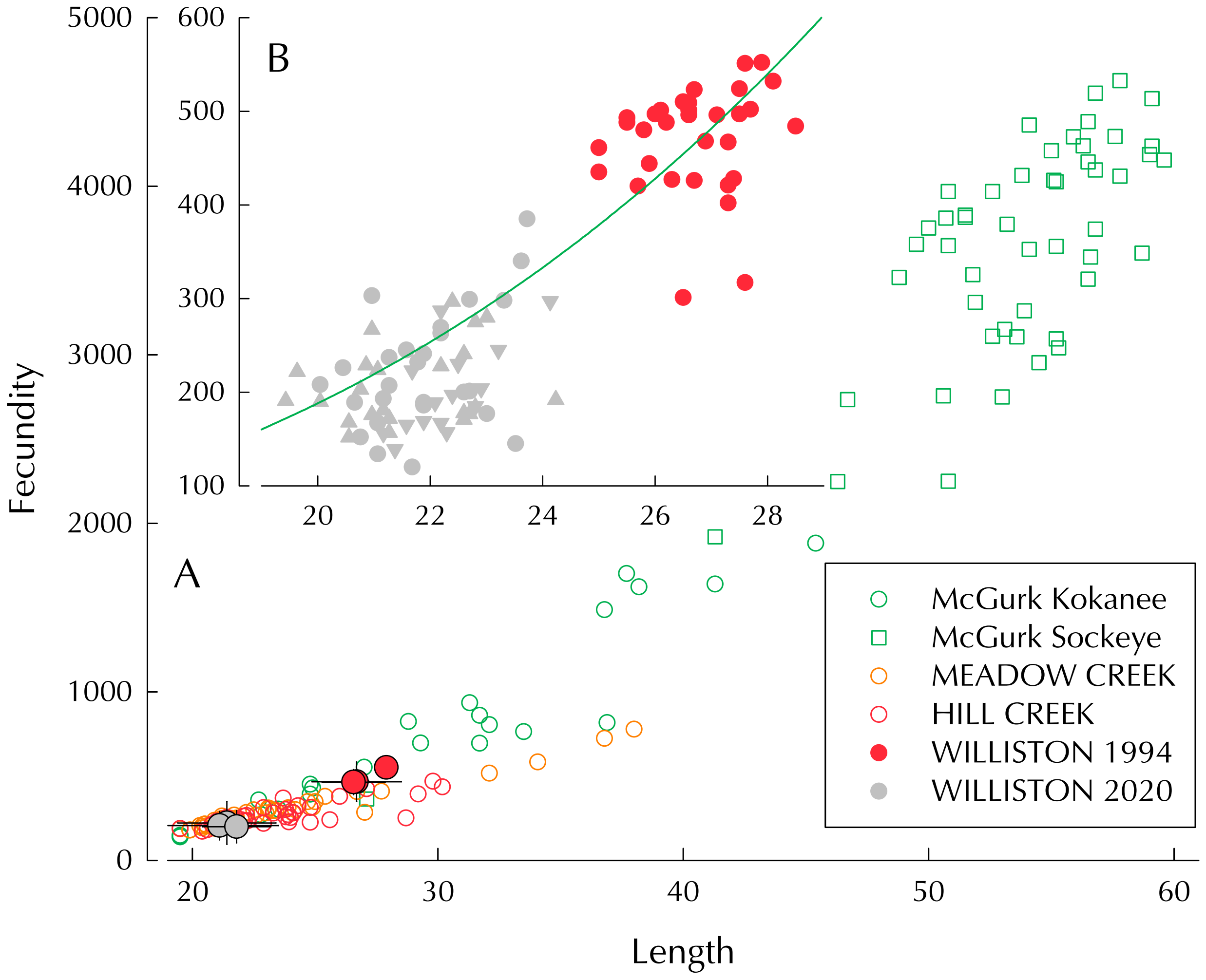

FIG. 5. A. Fecundity measurements plotted verses fork length (L_F; cm) for female Kokanee and Sockeye Salmon (*Oncorhynchus nerka*) for multiple populations. McGurk (2000) data are means from 25 Kokanee populations and 48 Sockeye Salmon populations. Meadow Creek are yearly means from 1969 to 2017 (Steve Arndt, BC MoFLNRORD, unpublished data). Hill Creek are yearly means from 1983 to 2017 (Matt Neufeld, BC MoFLNRORD, unpublished data). Williston 1994 are means ± 2 SD for Kokanee gill netted in Carbon Embayment, Dunlevy Embayment, and Philip Creek (Langston and Zemlak, 1998). Williston 2020 are means ± 2 SD for Kokanee sampled from two tributary streams; Germansen River. Aley Creek and Russel Creek (Jade Reynolds, unpublished data). B. Fecundity measurements for individual female Kokanee from the Williston Reservoir watershed collected in 1994 and 2020 at the locations indicated above. For the 2020 fecundity measurements, ▲ are fish from Russel Creek, ● are fish from Aley Creek, and ▼ are fish from Germansen River (Jade Reynolds, unpublished data). Regression line was derived from Kokanee data for 25 populations from McGurk (2000); $F = 0.0159L^{3.131}$.

TABLE 6. Sample groups and measurements of genetic variation for Kokanee (*Oncorhynchus nerka*) used to access potential introgression and homing to natal streams in the Williston Reservoir watershed.

Group	n	A	A_R	H_e	H_o
Arctic Lake (ARC)	68	56	3.12 ± 0.526	0.40 ± 0.064	0.40 ± 0.067
Tacheeda Lake (TAC)	80	49	2.82 ± 0.454	0.38 ± 0.064	0.38 ± 0.066
Hill Creek (HIL)	100	168	6.83 ± 0.903	0.72 ± 0.049	0.71 ± 0.049
Meadow Creek (MED)	345	215	6.97 ± 0.944	0.70 ± 0.050	0.70 ± 0.047
Carbon Embayment (CAR)	63	150	6.62 ± 0.890	0.71 ± 0.044	0.69 ± 0.051
Dunlevy Creek (DUN)	64	143	6.27 ± 0.827	0.69 ± 0.050	0.68 ± 0.057
Manson Creek (MAN)	41	138	6.50 ± 0.951	0.70 ± 0.047	0.68 ± 0.050
Germansen River (GER)	130	178	6.48 ± 0.827	0.71 ± 0.044	0.71 ± 0.043
Osilinka River (OSI)	13	103	6.92 ± 0.897	0.72 ± 0.047	0.69 ± 0.049
Pelly Creek (PEL)	89	173	6.54 ± 0.826	0.71 ± 0.043	0.70 ± 0.039
Finlay River (FIN)	112	169	6.51 ± 0.823	0.71 ± 0.045	0.70 ± 0.046
Tsaydiz Creek (TSY)	49	136	6.18 ± 0.789	0.70 ± 0.042	0.70 ± 0.044
Russel Creek (RUS)	128	170	6.44 ± 0.793	0.71 ± 0.043	0.70 ± 0.045
Cutoff Creek (CUT)	40	131	6.32 ± 0.840	0.70 ± 0.046	0.68 ± 0.049
Bower Creek (BOW)	50	136	6.27 ± 0.814	0.70 ± 0.044	0.69 ± 0.045
Aley Creek (ALEY)	109	169	6.38 ± 0.828	0.70 ± 0.045	0.69 ± 0.046
Stevenson Creek (STEV)	17	97	6.10 ± 0.864	0.69 ± 0.044	0.66 ± 0.045
Williston_Forebay (GMS)	135	184	6.56 ± 0.832	0.71 ± 0.043	0.68 ± 0.043
Williston_2000 (2000)	45	133	6.22 ± 0.795	0.69 ± 0.053	0.61 ± 0.054
Williston_Native (1990)	85	89	3.84 ± 0.577	0.52 ± 0.064	0.53 ± 0.064
Thutade Lake (THU)	107	152	5.93 ± 0.793	0.64 ± 0.059	0.64 ± 0.056

Genotypes (n) were combined by sample location over multiple years (AMOVA; $F_{ST} < 0.01$; $P > 0.05$). Williston_2000 was retained as a separate group because of an excess of null alleles that indicates a Wahlund effect (Khrustaleva et al. 2017). Measurements of genetic variation of Kokanee by sample group. 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. Estimates of variation are provided as standard error.

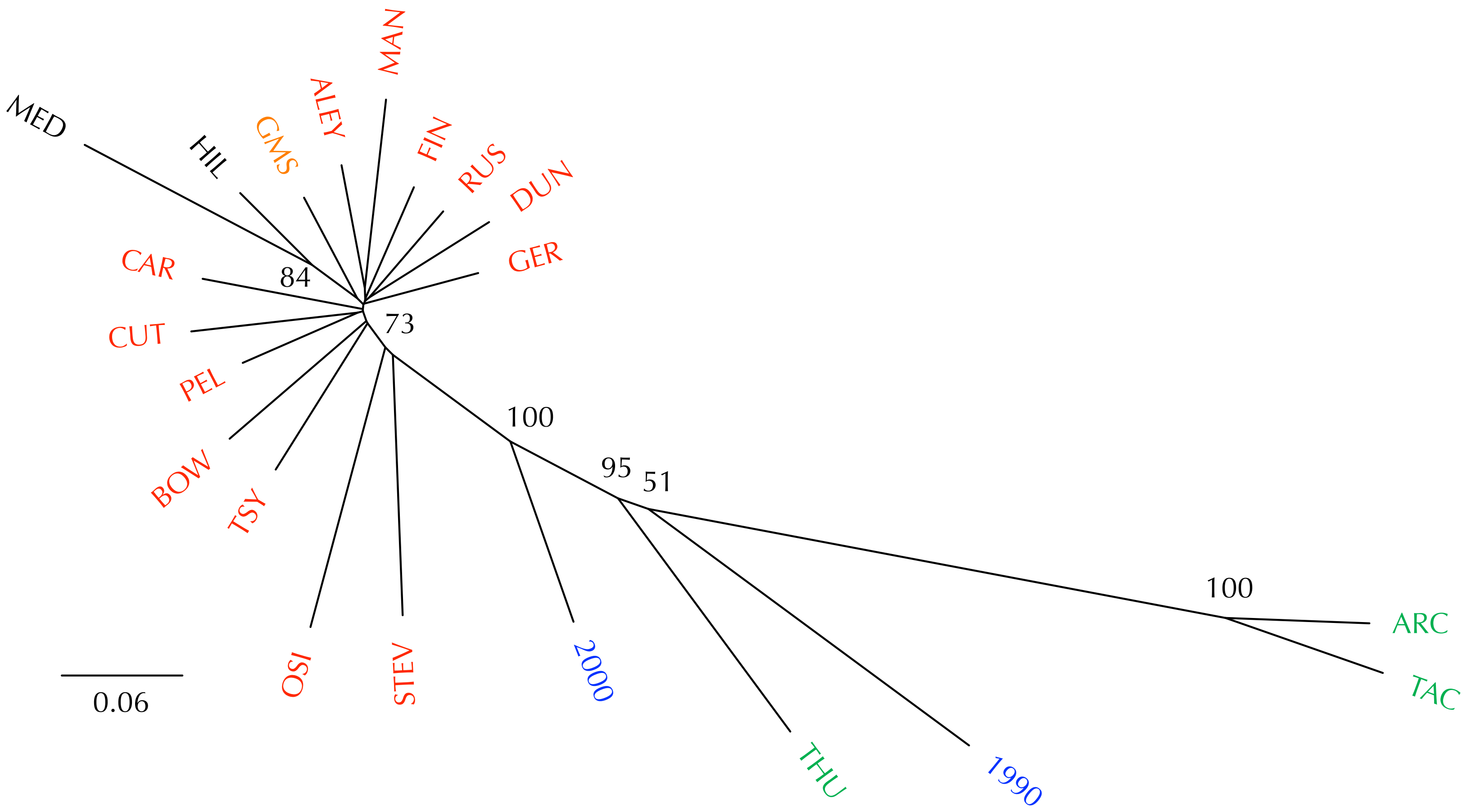

FIG. 6. Neighbor-joining tree constructed using Cavalli-Sforza and Edwards (1967) chord distance (D_C) inferred from variation at fourteen microsatellite loci in 21 groups of Williston watershed Kokanee (*Oncorhynchus nerka*). Numbers represent percentage of 10,000 bootstrap replicates. Percentages below 50% are not reported. **RED** labels are Kokanee sampled from tributary streams to the reservoir. **GREEN** labels are native Kokanee sampled from lakes in the headwaters of the Parsnip and Finlay Rivers. **BLUE** labels are Kokanee collected in the reservoir. **ORANGE** are samples collected from the GM Shrum intake towers to the WAC Bennett Dam. **BLACK** are Kokanee sampled from the original donor populations in the Columbia River watershed.

FIG. 7. STRUCTURE analysis for 19 locations where Kokanee (*Oncorhynchus nerka*) were sampled in the Williston Reservoir watershed and for the two locations where Columbia River origin Kokanee were obtained to stock into Williston Reservoir tributaries between 1990 and 1998. Analysis was conducted for fourteen microsatellite loci. Each fish is represented by a vertical line which exhibits the proportional composition of each fish's genome across K genetic clusters from K of 2 to 6. Abbreviations for sample locations in the Williston Reservoir Watershed are: Arctic Lake (ARC), Tacheeda Lake (TAC), Carbon Embayment (CAR), Dunlevy embayment & Creek (DUN), Manson River (MAN), Germansen River (GER), Osilinka River (OSI), Pelly Creek (PEL), Finlay River (FIN), Tsaydiz Creek (TSY), Russel Creek (RUS), Cuttoff Creek (CUT), Bower Creek (BOW), Aley Creek (ALEY), Stevenson Creek (STEV), GM Shrum intake towers (GMS), Williston Reservoir (2000), Williston Reservoir & lower Finlay River (1990), and Thutade Lake (THU). Sampling locations are shown in FIG. 1. Columbia River Kokanee populations are Hill Creek (HIL) and Meadow Creek (MED). Sample sizes are shown in TABLE 1.

FIG. 8. STRUCTURE analysis indicating the origin and change in abundance of native Williston Reservoir Kokanee (*Oncorhynchus nerka*). Analysis was conducted for fish sampled at 21 locations as shown in Figure 7, but only showing Kokanee that were obtained from sampling in the embayments, the reservoir, the lower Finlay River, and Thutade Lake. Analysis was conducted for 14 microsatellite loci. Each fish is represented by a vertical line which exhibits the proportional composition of each fish's genome across K genetic clusters for K of 4 and 5. Abbreviations are as follows: Carbon Creek (CAR) where Kokanee were sampled in 1994 from the embayment (n = 63); Dunlevy Creek (DUN) where Kokanee were sampled in 1994 from the embayment (n = 23) and from the creek in 2018 (n = 41); GM Shrum intake towers (GMS) to the WAC Bennett Dam where Kokanee were sampled in 2016 (n = 123) and 2019 (n = 40); Kokanee gill-netted from six locations in the Williston Reservoir (2000) caught between 24Aug2000 and 3Sep2000 (n = 45) (Pillipow & Langston 2002); archived scale samples (1990) collected at various locations in the reservoir in 1988 (n = 36), 1989 (n = 34), and 1990 (n = 15); and Thutade Lake (THU) were Kokanee sampled in 2003 (n = 87) and 2017 (n = 20).

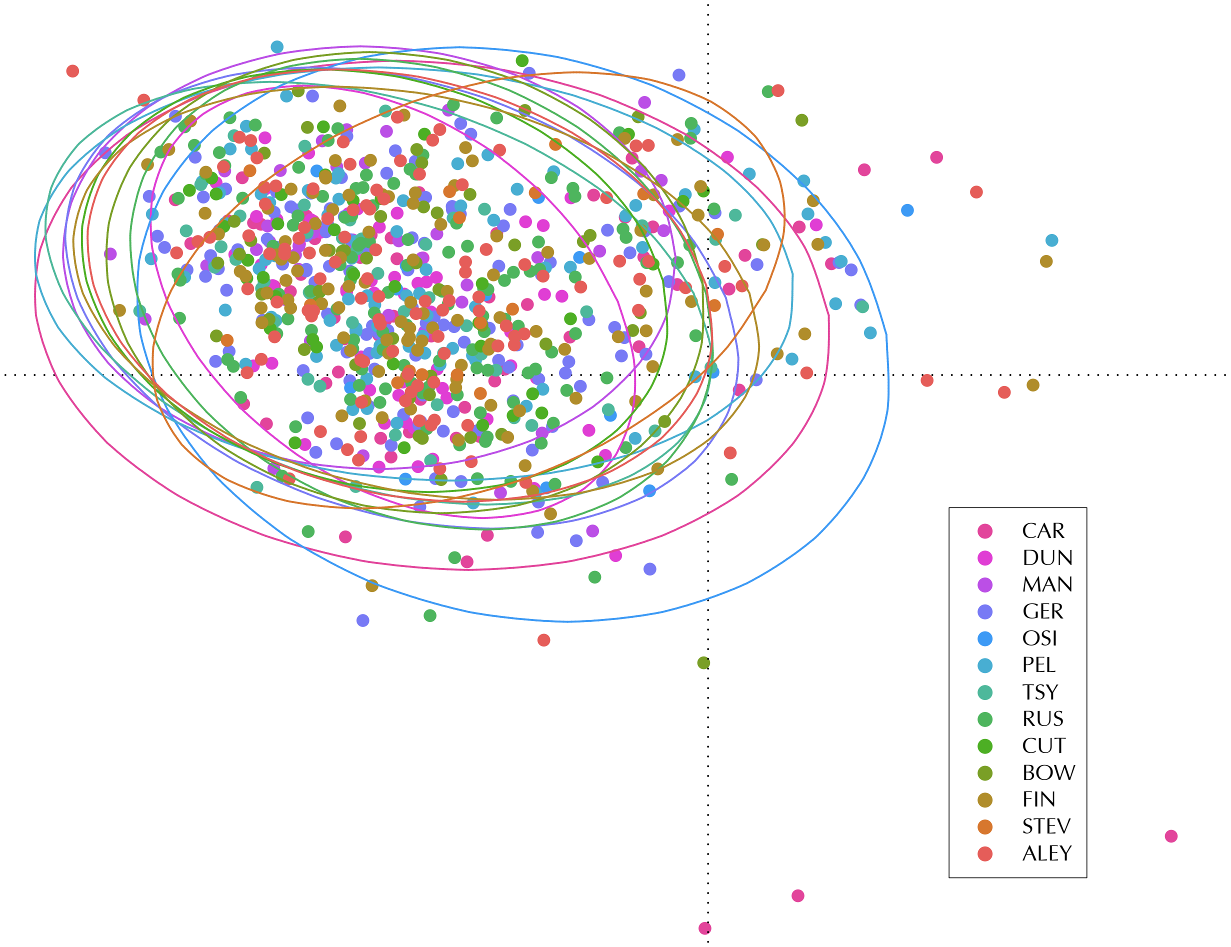

FIG. 9. A. Discriminant Analysis of Principal Components (DAPC) for 13 locations in the Williston Reservoir where Kokanee (*Oncorhynchus nerka*) were sampled. DAPC scores are plotted for Kokanee collected from Carbon Embayment (CAR), Dunlevy embayment & Creek (DUN), Manson River (MAN), Germansen River (GER), Osilinka River (OSI), Pelly Creek (PEL), Finlay River (FIN), Tsaydiz Creek (TSY), Russel Creek (RUS), Cuttoff Creek (CUT), Bower Creek (BOW), Stevenson Creek (STEV), and Aley Creek (ALEY). Ellipses represent 95% confidence intervals for each sampling location.

DISCUSSION

AGE & SIZE AT MATURITY – Determination of age at maturity for the spawners collected in 2006, 2018 and 2019 indicated that Kokanee were predominantly 3 years old and would have a generation time of 4 years. The cohorts that matured in 2006, 2018 and 2019 also did not differ significantly in age from Columbia origin Kokanee sampled in 1994 or native Williston Kokanee sampled in 1989 (TABLE 2). Although the average age for spawning Kokanee was 3 years, there was considerable range in age of spawners from 1 to 5 years. A range in spawner ages has also been found for the Columbia River populations where the donor stock originated: Hill Creek and Meadow Creek. Hill Creek spawners from 1990 to 2018 ranged from 2 to 4 years, indicating generation times from 3 to 5 years. The proportion of each age class varied among years; age 2 spawners averaged 30% (ranged from 0 to 94%), age 3 spawners averaged 59% (ranged from 6 to 93%), age 4 spawners averaged 11% (ranged from 0 to 92%) (M. Neufeld, unpublished data). Meadow Creek spawners from 1989 to 2017 ranged from 1 to 5 – indicating generation times from 2 to 6 years. The proportion of each age class also varied among years; age 1 spawners were rare and averaged 0.2% (ranged from 0 to 4%), age 2 spawners averaged 12% (ranged from 0 to 42%), age 3 spawners averaged 73% (ranged from 7 to 96%), age 4 spawners averaged 13% (ranged from 0 to 87%), and age 5 spawners averaged 0.7% (ranged from 0 to 7%) (S. Arndt, unpublished data). Consequently, there is considerable temporal variation in spawner age for the original Columbia River donor populations that were stocked in the Williston watershed. It is possible that such variation also exists for Kokanee that have now colonized the different tributaries to the reservoir, but we lack the temporal data necessary to assess any changes over time.

Spatial differences in age at maturity also exist among populations of Kokanee from different tributary streams to Kootenay Lake, BC, which include Meadow Creek (Vernon 1957). Most of the fish were aged 3 years that spawned in the northern tributaries to Kootenay Lake, whereas spawners in tributaries to the west arm and south end of Kootenay Lake were predominantly 2-year-old fish. Although the spatial differences in spawning locations are much greater in the Williston Reservoir than Kootenay Lake, fish spawning in the different tributaries may not have diverged and adapted to local conditions that may lead to differences in age at maturity.

A striking finding from our study was the change in size at maturity for Kokanee over time. Size at maturity differs among populations (McGurk 2000), but variation in size has also been found within populations over time (M. Neufeld & S. Arndt, unpublished data). Native Kokanee from the late 1980s and early 1990s were the largest and our genetic data indicates that native Kokanee from 2000 were significantly smaller. Columbia origin Kokanee were also the largest in 1994 and fish sampled more recently were significantly smaller. Smaller size of spawners from 2018 to 2019, however, is not due to a shift in age at maturation as age of spawners did not differ significantly among cohorts from different years. The shift in size of spawners was more likely linked to density dependent factors and increased competition that are limiting growth of Kokanee due to the highly oligotrophic status of the reservoir (Sebastian et al. 2003; Sebastian et al. 2009). Significantly lower K for the 2018 and 2019 cohorts support density dependent effects (TABLE 3).

FECUNDITY & REPRODUCTIVE INVESTMENT – The decrease in size at maturity for Kokanee from the Williston Reservoir watershed has resulted in a marked decline in fecundity. Reproductive potential, particularly number of eggs, is proportional to female size across a wide range of fishes (Elgar 1990). Native Williston Kokanee sampled in 1990 and 1991 were the largest at maturity (FIG. 3) and also had the highest average number of eggs: 500 ± 75 (SD) (Fielden 1991; Fielden

1992). Columbia River origin Kokanee sampled in 1994 were also larger than the fish sampled in 2020; the average number of eggs was 472 ± 52 (SD) (Langston and Zemlak 1998). We found an average fecundity for females collected in the samples from 2020 of 212 ± 53 (SD). The decline in size at maturity has resulted in lower numbers of eggs produced per female. In combination with the decreased estimates of escapement for 2018 (McDermot-Fouts 2019) and 2019 (Robinson 2020) for Kokanee throughout the Williston Reservoir watershed, our data indicates that future population growth for Kokanee may be less than it was historically within the watershed.

Kokanee sampled from Williston Reservoir spawning streams in 2020 were also less fecund per given length than values predicted by the regression model from McGurk (2020), whereas this was not the case for female spawners sampled in 1994. The lower fecundity may be due to egg number vs egg size trade-offs. How gonadal mass may be partitioned is related to the relative importance of number of future offspring compared to factors that affect local optima for egg size (Tuor et al. 2020). Egg size did not differ among the locations where Kokanee were sampled and a minimum egg size might be necessary for embryo survival due to the cold hyporheic temperatures fish experience within the redd environment in the tributaries to the Williston Reservoir (Williamson CJ, Zimmerman JT, Shrimpton JM, unpublished data; also see Tuor and Shrimpton 2019). We cannot directly compare egg size to that of fish collected in 1994 by Langston and Zemlak (1998) as the fish were not sampled from spawning tributaries and were not fully mature. Consequently, we do not know if egg size has decreased with size of female spawners. McGurk (2000), however, found that kokanee maintain a constant egg size while increasing egg number with increasing body size – which would suggest that egg size at maturity may have been similar for the fish sampled in 1994 to that for fish sampled in 2020. The decline in size of Kokanee over time, therefore, will limit numbers of potential offspring per female, but changes in egg size may be constrained by a minimum amount of energy required for embryo survival (Tuor et al. 2020).

POTENTIAL FOR INTROGRESSION –The most parsimonious number of clusters determined by STRUCTURE yielded a K value of five when the program was run with *a priori* knowledge of geographic location for each fish. Kokanee from the upper Peace separated into three clades; Thutade Lake sampled in 2003 and 2017, Arctic / Tacheeda Lakes sampled in 2004, 2006, 2018 and 2019, and native Williston Reservoir fish sampled in 1988, 1989, and 1990 before the introduction of Columbia River origin fish. The other two clades were the two Columbia River origin populations: Meadow Creek and Hill Creek (FIG. 7). STRUCTURE assigned Kokanee captured in the reservoir in 2000 to one of three clades: the native Williston clade, and the Hill Creek and Meadow Creek clades. Fish sampled from the tributaries in 2006, 2016, 2017, 2018 and 2019 (FIG. 7) were all assigned to a single clade, Hill Creek, with genetic signatures that did not differ among the spawning tributaries (FIG. 9).

Clearly no introgression of Columbia origin Kokanee has occurred with fish from Arctic and Tacheeda Lakes. The almost complete absence of Kokanee from spawning streams in the Parsnip Reach and Parsnip River tributaries in 2018 (McDermot-Fouts 2019) and 2019 (Robinson 2020) based on aerial surveys indicates that the future likelihood of Columbia origin Kokanee invading these lakes is low. A ground survey of tributaries in the Parsnip Reach on 18Sep2017 indicated the presence of Kokanee. Small numbers of Kokanee were observed in Cutthumb Creek, but not other eastern Parsnip Reach tributaries and not the week following (Coxson et al. 2018). The fish were bright red with colouration characteristic of Columbia origin Kokanee.

There is 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 the headwaters of the Finlay River will not occur. Within the reservoir, there is no physical barrier to prevent introgression between native Williston Kokanee and the Columbia origin Kokanee – making this population of Kokanee vulnerable to introgression. Clustering analyses with either STRUCTURE and DAPC indicated that Kokanee with genotypes characteristic of native Williston fish were present in samples collected from the reservoir in 1994 and 2000. Three Kokanee gill-netted in Carbon Creek embayment were assigned to the Williston cluster. Most (56%) of the Kokanee gill-netted from six location in the reservoir in 2000 were assigned to the Williston cluster and there was no indication of admixture between the Kokanee genotyped as Williston fish and either the Hill Creek or Meadow Creek clusters – indicating no introgression between native Williston and Columbia origin Kokanee. Rapid introgression between populations of *O. nerka* has been observed (Veale and Russello 2016). Sockeye Salmon were reintroduced to Skaha Lake, British Columia, as juveniles and upstream fish passage constructed in 2004; Sockeye Salmon and hybrids with Kokanee were first detected in 2008 at low levels, but the number of hybrids among age 0 juveniles had increased to over 15% by 2014 – only a decade after fish passage to the lake had been restored. Lack of introgression in the Williston Reservoir, therefore, appears remarkable.

The mechanism for preventing introgression may be due to spatial and temporal separation in spawning patterns. Native Williston Reservoir Kokanee were only confirmed to spawn in the lower reaches of the Finlay River in side channels and sloughs (Fielden 1991; Fielden 1992) – likely some of the limited habitat in the watershed suitable for fish that originated from the shore spawning population in Thutade Lake. Both Hill Creek and Meadow Creek populations are tributary spawners and represent a different ecotype from shore spawners. Temporal differences in spawning date, rather than spawning location for different ecotypes maintained reproductive isolation for Kokanee populations in Lake Pend Oreille, Idaho (Whitlock et al. 2018). The native Williston Kokanee were recorded to spawn in late October to early December (Fielden 1992), whereas the peak of spawning for the Columbia River Kokanee occurs in mid-September (Langston 2012). It is possible that spatial and temporal limitations of sampling in 2006 and 2018 may not have captured native fish. Consequently, in 2019 DWB Consulting Services Ltd. targeted sampling of Kokanee spawners in the lower Finlay River in late October. Kokanee were collected on 22Oct2019 from two locations: a side channel of the Finlay River approximately 8 km north of the Akie River confluence and a site on the lower Akie River. All of the fish sampled were genotyped as Columbia Kokanee and exhibited the bright red pigmentation characteristic of the Hill Creek stock, but all the females (14 of 20 Kokanee sampled) had previously spawned with no eggs remaining (n = 12) or few eggs (13 and 61 eggs) – further supporting temporal separation.

Although the 2000 sampling revealed that native Kokanee persisted in the reservoir after the introduction of Columbia origin fish, Kokanee sampled from the intake towers at the WAC Bennett Dam in 2016 and 2019 were all Columbia origin fish. The proportion of native Williston Kokanee sampled in 2000 differed by location where gill nets were set in the reservoir (Wilson and Shrimpton 2020). Columbia origin Kokanee, both Hill Creek and Meadow Creek, were more abundant in the Peace Reach and only native Williston Kokanee were caught in the Finlay Reach. The spatial separation by population in 2000 suggests segregation in the reservoir by ecotype, but it is more likely that the spatial differences were due to timing of sampling. Gill net sampling was conducted at the end of August and early September 2000. Temporal differences in spawning date between native Williston Kokanee and Columbia origin Kokanee may have contributed to the greater abundance of native fish in the samples as Columbia Kokanee may have migrated out of the reservoir and into spawning tributaries. This may also account for the spatial difference in genotypes for Kokanee sampled in 2000. Many of the spawning locations in the northern part of the watershed are tributaries to the major rivers flowing into the reservoir: the Finlay, Ingenika, Omineca, and Ospika Rivers – requiring more extensive migrations than streams flowing into the Peace Reach. Initiation of migration to spawning sites has been shown to occur earlier for Bull Trout (*Salvelinus confluentus*) that have to migrate further (Bahr and Shrimpton 2004). If this pattern also occurs in Kokanee in the Williston Reservoir, it may indicate that migration strategies differ by spawning location and that the introduced Kokanee are homing to specific natal locations.

HOMING TO NATAL STREAMS – Salmon are famous for their ability to return "home" to the natal stream where they were spawned. The rationale for selecting streams and rivers within the Williston watershed where Kokanee were stocked appears to have relied on this innate ability to home and the assumption that recreational opportunities for anglers would be provided where fish were stocked (Blackman et al. 1990). The extensive colonization of new rivers and streams in the Williston Reservoir watershed indicates that straying occurred shortly after the fish were stocked. Straying is an important life history trait for salmon to colonize new environments. Work on two species found in the Williston Reservoir watershed, Pygmy Whitefish (*Prosopium coulterii*) and Arctic Grayling (*Thymallus arcticus*), reveal that dispersal occurred from at least two major Pleistocene glacial refugia; Beringia and Cascadia (Stamford and Taylor 2004; Witt et al. 2011). Our work has not assessed potential natural dispersal of Kokanee into the Williston Reservoir watershed, but the substantial genetic differences between the Thutade Lake Kokanee and Arctic / Tacheeda Lakes Kokanee suggest dispersal into the watershed may have occurred from two different refugia.

Straying of Columbia origin Kokanee throughout the Williston watershed was rapid – colonization of more than 68 new watersheds occurred within a decade to streams that were greater than 200 km from the locations where they were stocked (Langston 2012). Such rapid rates of straying over long distances are not without precedence. Tagging studies have revealed rates and distances of straying by salmonids. The majority of strays are often recovered within 25 km of natal locations (Hard and Heard 1999), but straying has been recorded over distances up to 480 km for Chinook Salmon *O. tshawytscha* (Candy and Beacham 2000). Chinook Salmon that were transplanted from the Sacramento River, California, to the South Island of New Zealand between 1901 and 1907 (McDowall 1994) have shown extensive dispersal. By 1915, salmon had colonized rivers 200 km north of the initial transplant site (Quinn et al. 2001). Rates of invasion by Chinook Salmon in South America have also been rapid, reaching their maximum extent in under 25 years (Correa and Gross 2008).

A number of factors contribute to dispersal of fish to new areas and include habitat availability, disturbance, and populations size. There are numerous tributaries to the reservoir that appear well suited for salmonids to spawn – consequently habitat was available. Higher straying rates have been reported for altered river systems. Quinn et al. (1991) found high rates of straying (average of 17%) in fall Chinook Salmon from the Lower Columbia River using coded wire tags; straying rates were higher in response to a disturbance and into larger river systems with more spawners. Creation of the Williston Reservoir was clearly a disturbance to the watershed, but at the time of the Kokanee stocking program, productivity was high (Sebastian et al. 2003) – the changes associated with flooding the watershed would also favour dispersal. There is also evidence that larger populations function as sources of dispersal (Palstra et al. 2007). The large number of Kokanee stocked into the watershed from Hill Creek and then Meadow Creek would also have functioned as a source for dispersal.

Although rates of straying in the first few generations after transplanting salmon to a novel environment are high, lower rates of straying appear to occur after the populations have been established (Quinn et al. 2001). Reduced straying and fidelity to spawning locations would create reproductively isolated populations and lead to genetic differentiation. Significant levels of genetic differentiation and strong isolation by distance have been found in another stream spawning salmonid, Arctic Grayling, within the Williston watershed (Stamford and Taylor 2005; Shrimpton et al. 2012). The size of the watershed is clearly large enough for population level differences to exist. Columbia Kokanee, however, have only been in the watershed since 1990 at the earliest, which may not be enough time to establish differences.

The length of time required to establish population genetic structure will vary depending on selective pressures at the local habitat scale, but also the potential for founder effects if few individuals establish the new population. When population densities are low, strays may experience high productivity due to negligible competition and exhibit rapid population growth (Quinn et al. 2001). Reproductive isolation and genetic differentiation was documented in Sockeye Salmon following colonization to a novel habitat in under 13 generations (Hendry et al. 2000). From the time of stocking (1990 to 1998) to when the Kokanee samples were collected for genotyping in our study (2018 and 2019), potentially five to seven generations had existed. Phenotypic differences, such as the larger size of 2006 Germansen River spawners compared to mature Kokanee from other spawning locations in 2006 (Wilson and Shrimpton 2020) suggest selection – but no genetic differences were observed. The number of generations, therefore, may be insufficient to establish genetic differences among the spawning tributaries or gene flow due to straying and lack of fidelity to spawning streams may limit population differentiation. Native Williston Kokanee were likely separated from Thutade Lake over a similar period of time, yet our analysis using STRUCTURE indicated two distinct genetic clusters. Consequently, genetic differentiation appears possible within this time frame if gene flow is restricted.

Our work has shown that the native Williston Kokanee were derived from the Thutade Lake population. Kokanee caught from 1988 to 2000 were similar to the Thutade Lake fish, but could be differentiated as a separate cluster by STRUCTURE. Fish caught in 1988 may have been separated from the Thutade Lake population for as little as 20 years or potentially 5 generations. If Kokanee had colonized the lower portions of the Finlay River prior to 1968 it is not clear where fish might have established a lacustrine population – although we cannot rule out the possibility that Kokanee "migrated" down from Thutade Lake prior to the completion of the WAC Bennett Dam. Once fish colonized the lower Finlay, they were unable to migrate back to Thutade Lake due Cascadero Falls which are impassable to upstream migration. Relatively few generations make it unlikely that natural selection would have resulted in genetic differentiation. It is more likely the differences were due to founder effects (Templeton 1980).

We found absolutely no indication of genetic differences among the tributary spawning Kokanee in our study. Although there have been few generations to establish genetic structure, the large scale of the watershed make this somewhat surprising. The large number of Kokanee stocked (over 3.3 million; Langston and Murphy 2008) and the dynamic nature of the reservoir may continue to favour dispersal over homing to natal streams.

RECOMMENDATIONS

This report is based on findings from Kokanee samples collected over 3 decades following the formation of the Williston Reservoir and over a wide range of locations within the watershed. We found Kokanee within the Williston Reservoir watershed have a 4-year generation time. A significant decrease in size at maturation was also found that has resulted in lower fecundity and reproductive potential. Genetic analysis did not find evidence for introgression between native and Columbia origin stocks, but also failed to reveal fidelity to spawning locations. More information on Kokanee within the Williston Reservoir watershed is still required to effectively manage this species. Based on our findings, we have the following recommendations for future work.

RECOMMENDATION 1. DETERMINE NATIVE KOKANEE PERSISTENCE IN THE RESERVOIR – Results from our genetic analysis are a concern for native Kokanee. Native Kokanee within the reservoir represented a separate genetic cluster due to divergence from the Thutade Lake population – and appear to be extirpated from the watershed. The lack of any genotypes indicating native Williston Kokanee from samples collected in 2006, 2016, 2017, 2018 and 2019 may be due to a bias in sampling for locations where Columbia origin Kokanee are known to spawn. Consequently, further sampling is required to assess whether native Kokanee are still found within the reservoir. By sampling tributaries in mid-September, we may miss the native Kokanee which were not previously observed in tributary streams and appear to spawn later in the fall based on stage of maturation. A survey similar to that conducted in 2000 should be undertaken to determine whether native Kokanee have persisted in the reservoir. Once this is known, then appropriate management efforts can be made to preserve the native population of Kokanee. Funding was awarded to UNBC for a project titled "Native Kokanee persistence in the Williston Reservoir (PEA-F21-F-3361-DCA)". The work is planned for summer 2021.

RECOMMENDATION 2. DETERMINE PHYSICAL CHARACTERISTICS OF LOCATIONS WHERE KOKANEE SPAWN - There is considerable variability in the number of spawning Kokanee within tributaries across years, but also among tributary streams within years (Langston 2012; McDermot-Fouts 2019; Robinson 2020). We do not know what factors are important for Columbia River-origin Kokanee when selecting spawning locations and whether these are changing over time. Knowledge of physical characteristics that these fish are selecting for, or avoiding, in the locations where they spawn is important for protecting or limiting a species ability to complete their life cycle. Physical characteristics of spawning sites and the redd environment should be characterized for locations where Kokanee are known to spawn in high density and compared to areas where they were introduced and no longer spawn, and also where they have not been documented to spawn – but are not precluded from spawning. The Columbia River-origin Kokanee have strayed throughout large portions of the reservoir and it is not known whether they will continue to stray or have now selected specific locations where they show fidelity to specific spawning locations.

RECOMMENDATION 3. DETERMINE HOMING OF COLUMBIA ORIGIN KOKANEE TO TRIBUTARY STREAMS – We know little about the secondary impacts of the introduction of Kokanee to the Williston Reservoir watershed, including on other recreational and traditional use fish species – but also on the native Kokanee that were found in the reservoir before the introduction of Columbia origin Kokanee. Spawning Kokanee of Columbia origin are abundant in tributaries to the Finlay River and tributaries to the Finlay Reach. Historically, native Kokanee were observed to spawn in side channels of the Lower Finlay River. Determining whether introduced Kokanee show fidelity to specific locations for spawning will help us understand potential spatial overlap with native fish at a critical life history stage. This lack of knowledge is a key limitation on any planning for species specific management. Earlier work funded by the Peace / Williston Fish & Wildlife Compensation Program on differences in elemental signature for otoliths from Arctic Grayling (Clarke et al. 2007) and Slimy Sculpin *Cottus cognatus* (Clarke et al. 2015) captured among tributaries to the Williston Reservoir indicate large differences in elemental chemistry within the watershed. Recent work in our lab demonstrated that natal stream elemental signatures in otoliths of Coho Salmon were specific to spawning locations (Turcotte and Shrimpton 2020). These findings suggest that otolith microchemistry has strong potential to be successful for defining homing in Kokanee. Funding was awarded to UNBC for a project titled "Spawning site fidelity of Columbia Kokanee in the Williston (PEA-F21-F-3176)". Water samples collected from tributary streams where Kokanee spawn show considerable differences in ratios of Strontium to Calcium and Barium to Calcium, two elements that are incorporated in the otoliths of fish proportionate to the water where the fish reside. Laser ablation-inductively coupled plasma-mass spectrometry has been used to identify elemental signatures of otoliths from spawning Kokanee collected from Osilinka River, Pelly Creek, Russel Creek and Stevenson Creek. Data reduction is ongoing and will be reported in 2021.

RECOMMENDATION 4. ASSESS MECHANISM FOR RAPIDLY CHANGING LIFE HISTORY CHARACTERISTICS OF KOKANEE FOLLOWING STOCKING – Although the age at maturity has shown little change over time, there have been significant declines in the sizes of the fish resulting in much lower fecundity for female spawners – a finding that will significantly affect reproductive potential and population growth. Rapid changes in life history have been documented for non-native fish species entering a new environment (Bøhn et al. 2004). Reasons for such rapid changes may be associated with density dependent responses or phenotypic plasticity within a species. The decline in number of spawning Kokanee indicated by the recent Kokanee spawning aerial flight counts (McDermot-Fouts 2019; Robinson 2020 – compared to Langston 2012) suggest that the number of Kokanee may have declined in the reservoir – supporting a density dependent response. Such a decline could be linked to a change or decline in productivity and consequently carrying capacity. This could result in greater competition among other pelagic species, such as Lake Whitefish, for limited resources. It could also mean greater intra-specific competition between Columbia origin Kokanee and native Willliston Kokanee if they still persist in the watershed. Pelagic fish surveys similar to those conducted in the reservoir by Sebastian et al. (2003; 2009), therefore, are recommended.

RECOMMENDATION 5. ASSESS INTRA-POPULATION COMPETITION THROUGH MORPHOMETRIC ANALYSIS OF COLUMBIA ORIGIN AND NATIVE KOKANEE – Lack of native Williston Kokanee genotypes among any of the fish sampled in 2006, 2016, 2017, 2018 or 2019 indicates native fish may have been extirpated from the reservoir – potentially due to competition with the introduced Columbia origin Kokanee. Spatial and temporal differences in spawning likely precluded genetic introgression – consistent with our genetic findings – but would also have avoided competition among spawners with no overlap in redds for developing embryos and alevin. Following emergence from the gravel, native and introduced Kokanee fry likely migrate downstream to the reservoir where they will directly compete for resources in a highly oligotrophic environment until maturation. Considerable phenotypic differences existed between the native Williston Kokanee and the introduced Columbia origin Kokanee beyond pigmentation at maturity (Langston and Zemlak 1998). We found condition factor, a mass to length measurement and index of shape, differed significantly (TABLE 3). Other morphological features such as gill raker count may also have differed. Feeding efficiency on small zooplankton is positively correlated with numbers of gill rakers in Whitefish *Coregonus* sp from Lake Thun, Switzerland (Roesch et al. 2013). Mean gill raker numbers are also significantly higher in Kokanee than Sockeye Salmon (Wood and Foote 1996) – a heritable trait that is stable over time (Foote et al. 1999) – but also suggests that Kokanee may be more efficient at eating small plankton. Beacham and Withler (2017) provide evidence for a monophyletic origin for Kokanee from the Columbia River that reflects radiation of Kokanee from a glaciation refuge rather than independent evolution of the life history form from Sockeye Salmon. The lack of bright red colouration at maturity for the native Williston Kokanee (Langston and Zemlak 1998) indicates that these fish were derived from landlocked Sockeye Salmon following deglaciation (Craig and Foote 2001; Ricker 1938). Consequently, the native Kokanee may be phenotypically more similar to Sockeye Salmon than the stocked Columbia origin fish and may have fewer gill rakers. Competition may lead to extirpation or rapid evolution of phenotypes. The invasion of alewife (*Alosa pseudoharengus*) in Lake Michigan resulted in not only a decline in abundance of a native planktivorous cisco (*Coregonus hoyi*), but also a change in phenotype – gill rakers were reduced in number with a shift to bottom habitats and benthic prey (Crowder 1984). Whether differences in feeding efficiency existed between native and introduced Kokanee is not known. Morphometric analysis of pelagic species within the watershed that feed on zooplankton is recommended. Analysis should include native populations from Thutade Lake and Arctic Lake, Columbia origin fish captured in the reservoir and also from the donor Hill Creek and Meadow Creek populations, but also Lake Whitefish which historically were the most abundant pelagic species before the introduction of Kokanee (Blackman et al. 1990; Sebastian et al. 2009). Gill raker count of a historically-successful pelagic species such as Lake Whitefish may be informative as to why one lineage of Kokanee were more successful than the other, if morphometric differences between the two lineages are found. Fish collection could be linked to work conducted for Recommendation 4. Zooplankton surveys throughout the year to assess size distribution of potential prey items for these pelagic species will inform the potential size of prey for planktivorous species in the reservoir.

RECOMMENDATION 6. 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). If Kokanee numbers are at carrying capacity and declining, potential effects on other species within the reservoir system that rely on Kokanee as a prey species may be profound. Predator-prey dynamics within the pelagic environment, therefore, should be examined. Bull Trout are known predators on Kokanee in the Williston Reservoir watershed (L. Gleeson, personal communication), but other piscivores such as Lake Trout (*S. namaycush*) will also rely on Kokanee as a prey source. A survey of pelagic fish species should also 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).

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REFERENCES

Armstrong JD, Stewart DC (1997) The effects of initial length and body curvature on shrinkage of juvenile Atlantic salmon during freezing. *Journal of Fish Biology* **50**, 903–905.

Bahr MA, Shrimpton JM (2004) Spatial and quantitative patterns of movement in large Bull Trout (*Salvelinus confluentus*) from a watershed in north-western British Columbia, Canada, are due to habitat selection and not differences in life history. *Ecology of Freshwater Fish* **13**, 294–304.

Banks MA, Blouin MS, Baldwin BA, Rashbrook VK, Fitzgerald HA, Blankenship SM, Hedgecock D (1999) Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* **90**, 281–288.

Beacham TD, Margolis L, Nelson RJ (1998) A comparison of methods of stock identification for sockeye salmon (*Oncorhynchus nerka*) in Barkley Sound, British Columbia. *North Pacific Anadromous Fish Commission Bulletin* **1**, 227–239.

Beacham TD, Withler RE (2017) Population structure of sea-type and lake-type sockeye salmon and kokanee in the Fraser River and Columbia River drainages. *PLoS ONE* **12**, e0183713. https://doi.org/10.1371/journal.pone.0183713

Blackman BG, Jesson DA, Ableson D, Down T (1990) Williston Lake Fisheries Compensation Program Management Plan. Peace / Williston Fish & Wildlife Compensation Program Report No. 58 38pp.

Bøhn T, Sandlund OT, Amundsen P-A, Primicerio R (2004) Rapidly changing life history during invasion. *Oikos* **106**, 138–150.

Buchheister A, Wilson MT (2005) Shrinkage correction and length conversion equations for *Theragra chalcogramma*, *Mallotus villosus* and *Thaleichthys pacificus*. *Journal of Fish Biology* **67**, 541–548.

Burgner RL (1991) Life history of sockeye salmon (*Oncorhynchus nerka).* In Pacific Salmon Life Histories. Edited by C. Groot and L Margolis. UBC Press: Vancouver. Pp 3-101.

Candy JR, Beacham TD (2000) Patterns of homing and straying in southern British Columbia coded-wire tagged chinook salmon (*Oncorhynchus tshawytscha*) populations. *Fisheries Research* **47**, 41–56.

Carreon-Martinez L, Johnson TB, Ludsin SA, Heath DD (2011) Utilization of stomach content DNA to determine diet diversity in piscivorous fishes. *Journal of Fish Biology* **78**, 1170–1182.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic Analysis: Models and Estimation Procedures. *American Journal of Human Genetics* **19**, 233-257.

Clarke AD, Telmer KH, Shrimpton JM (2007) Using natural elemental signatures to determine habitat use and population structure for a fluvial species, the Arctic grayling, in a watershed impacted by a large reservoir. *Journal of Applied Ecology* **44**, 1156-1165.

Clarke AD, Telmer KH, Shrimpton JM (2015) Movement patterns of fish revealed by otolith microchemistry: a comparison of putative migratory and resident species. *Environmental Biology of Fishes* **98**, 1583-1597.

Coxson D, Huber D, Shrimpton M, Wiensczyk A (2017) Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir watershed. Natural Resources and Environmental Studies Institute, University of Northern British Columbia. Prepared for: Fish & Wildlife Compensation Program – Peace Region, Report No. PEA-F17-F-1471.

Coxson DS, Huber DPW, Shrimpton JM, Thielman AC, Wiensczyk A (2018) Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed. Natural Resources and Environmental Studies Institute, University of Northern British Columbia, Prepared for:

Fish & Wildlife Compensation Program – Peace Region, Report No. PEA-F18-F-2296.Correa C, Gross MR (2008) Chinook salmon invade southern South America. *Biological Invasions* **10**, 615–639.

Craig JK, Foote CJ (2001) Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of Sockeye salmon (*Oncorhynchus nerka*). *Evolution* **55**, 380-391.

Crowder LB (1984) Character Displacement and Habitat Shift in a Native Cisco in Southeastern Lake Michigan: Evidence for Competition? *Copeia* **1984**, 878–883.

Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359-361.

Elgar MA (1990) Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* **59**, 283-287.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.

Fielden RJ (1991) Finlay River Kokanee (*Oncorhynchus nerka*) spawning survey 1990. Peace / Williston Fish & Wildlife Compensation Program Report No. 59. 40 pp plus appendices.

Fielden RJ (1992) Finlay River kokanee (*Oncorhynchus nerka*) spawning survey, 1991. Peace / Williston Fish & Wildlife Compensation Program, Report No. 63. 27 pp plus appendices.

FWCP – Fish & Wildlife Compensation Program Peace Region (2014) Peace Basin Reservoirs Action Plan, BC Hydro. Available at: https://fwcp.ca/app/uploads/2020/06/Archived-AP-Peace-Region-Reservoirs-Action-Plan-2014.pdf

Florin A-B, Lingman A (2008) Shrinkage of flounder *Platichthys flesus* (L.) and turbot *Psetta maxima* (L.) following freezing. *Journal of Fish Biology* **72**, 731–736.

Foote CJ, Moore K, Stenberg K, Craig KJ, Wenburg JK, Wood CC (1999) Genetic differentiation in gill raker number and length in sympatric anadromous and nonanadromous morphs of sockeye salmon, *Oncorhynchus nerka*. *Environmental Biology of Fishes* **54**, 263–274.

Goudet J, Jombart T, Kamvar ZN, Archer E, Hardy O (2020) hierfstat: Estimation and tests of hierarchical F-statistics.

Guo S, Thompson EA (1992) Performing the Exact Test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361-372.

Hard JJ, Heard WR (1999) Analysis of straying variation in Alaskan hatchery Chinook salmon (*Oncorhynchus tshawytscha*) following transplantation. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 578–589.

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**(5491), 516-519.

Holm LE, Loeschcke V, Bendixen C (2001) Elucidation of the molecular basis of a null allele in a rainbow trout microsatellite. *Marine Biotechnology* **3**, 555-560.

Jombart T (2008) *adegenet*: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Jombart T, Ahmed I (2011) *adegenet* 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27**, 3070-3071.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics* **11**, 94.

Kalinowski S T (2009) How well do evolutionary trees describe genetic relationships among populations? *Heredity* **102**, 506-513.

Khrustaleva AM, Klovach NV, Seeb JE (2017) Genetic variability and population structure of Sockeye salmon from the Asian Coast of Pacific Ocean. *Russian Journal of Genetics* **53**, 1126-1136.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* **15**, 1179-1191.

Langston AR, Zemlak RJ (1998) Williston Reservoir stocked Kokanee spawning assessment, 1994, Peace / Williston Fish & Wildlife Compensation Program Report No. 176. 13 pp plus appendices.

Langston AR, Murphy EB (2008) The History of fish introductions (to 2005) in the Peace / Williston Fish and Wildlife Compensation Program Area. Peace / Williston Fish and Wildlife Compensation Program Report No. 325. 59p.

Langston AR (2012) Williston Watershed Kokanee spawner distribution and enumeration surveys (2002 – 2006). Fish and Wildlife Compensation Program – Peace Region Report No. 357. 11 pp plus appendices.

Leonard KC, Worden N, Boettcher ML, Dickinson E, Hartstone-Rose A (2021) Effects of freezing and short-term fixation on muscle mass, volume, and density. *The Anatomical Record* **2021**, 1–10.

McDermot-Fouts A (2019) Williston Watershed Kokanee spawner distribution and aerial enumeration surveys (2018). DWB Consulting Services Ltd., FWCP Project No. PEA-F19-F-2895-DC-103364. 23 pp plus appendices.

McDowall RM (1994) The origins of New Zealand's chinook salmon, *Oncorhynchus tshawytscha*. *Marine Fisheries Review* **56**, 1–7.

McGurk MD (2000) Comparison of fecundity–length–latitude relationships between nonanadromous (kokanee) and anadromous sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* **78**, 1791–1805.

McPhail JD, Lindsey CC (1986) Zoogeography of freshwater fishes of Cascadia (the Columbia System and rivers north to the Stikine). Pages 615-637 *in* The zoogeography of North America freshwater fishes. Edited by C.H. Hocutt and E.O. Wily. John Wiley and Sons, Toronto, Ontario.

McQueen K, Mion M, Hilvarsson A, Casini M, Olesen HJ, Hüssy K, Radtke K, Krumme U (2019) Effects of freezing on length and mass measurements of Atlantic cod Gadus morhua in the Baltic Sea. *Journal of Fish Biology* **95**, 1486–1495.

McRae CJ, Warren KD, Shrimpton JM (2012) Spawning site selection in Interior Fraser River coho salmon (*Oncorhynchus kisutch*): an imperiled population of anadromous salmon from an interior, snow-dominated watershed. *Endangered Species Research* **16**, 249-260.

Morris DB, Richard KR, Wright JM (1996) Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 120–126.

Nelson RJ, Beacham TD (1999) Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. *Animal Genetics* **30**, 228–229.

Nelson RJ, Wood CC, Cooper G, Smith C, Koop B (2003) Population structure of sockeye salmon of the central coast of British Columbia: Implications for recovery planning. *North American Journal of Fisheries Management* **23**, 703–720.

O'Dell ADC, Flores A-M, Schwarzfeld MD, Erasmus DJ, Heath DD, Huber DPW, Shrimpton JM (2020) Determining diets for fishes (Actinopterygii) from a small interior British Columbia stream: a comparison of morphological and molecular approaches. *The Canadian Entomologist* **152**, 702–720.

Palstra FP, O'Connell MF, Ruzzante DE (2007) Population structure and gene flow reversals in Atlantic salmon (*Salmo salar*) over contemporary and long-term temporal scales: effects of population size and life history. *Molecular Ecology* **16**, 4504–4522.

Park S (2001) Microsatellite Toolkit v. 3.1, Animal Genomics Lab, University College, Dublin, Ireland.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* **28**, 2537-2539.

Pillipow RA, Langston AR (2002) Williston Reservoir fish assessments 2000, pelagic netting summary. *Peace / Williston Fish & Wildlife Compensation Program* Report No. **261**

Porras-Hurtado L, Ruiz Y, Santos C, Phillips C, Carracedo Á, Lareu MV (2013) An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in Genetics* **4**, 1-13.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155**, 945–959.

Quinn TP (2005) The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, WA.

Quinn TP, Nemeth RS, McIsaac DO (1991) Homing and straying patterns of fall Chinook salmon in the lower Columbia River. *Transactions of the American Fisheries Society* **120**, 150–156.

Quinn TP, Kinnison MT, Unwin MJ (2001) Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. *Genetica* **112–113**, 493–513.

Robinson M (2020) Williston Watershed Kokanee spawner distribution and aerial enumeration surveys (2019). DWB Consulting Services Ltd., FWCP Project No. PEA-F20-F-3359-DC-106374. 34 pp plus appendices

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rambaut A (2018) FigTree v1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Ricker WE (1938) "Residual" and kokanee salmon in Cultus lake. *Journal of the Fisheries Research Board of Canada* **4**, 192-218.

Roesch C, Lundsgaard-Hansen B, Vonlathen P, Taverna A, Seehausen O (2013) Experimental evidence for trait utility of gill raker number in adaptive radiation of a north temperate fish. *Journal of Evolutionary Biology* **26**, 1578–1587.

Rousset F (2008) Genepop'007: A complete re-implementation of the Genepop Software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.

Schlaepfer MA, Sax DF, Olden JD (2011) The potential conservation value of non-native species. *Conservation Biology* **25**, 428–437.

Scribner KT, Gust JR, Fields RL (1996) Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 833–841.

Sebastian DC, Scholten GH, Woodruff PE (2003) Williston Reservoir fish assessment: Results of hydroacoustic, trawl and gill net surveys in August 2000. Peace / Williston Fish & Wildlife Compensation Program Report No. 274. 34pp plus appendices.

Sebastian D, Andrusak G, Scholten G, Langston A (2009) Peace Project water use plan Williston Fish Index, GMSMON-13. An index of fish distribution and abundance in Peace Arm of Williston. 58 + appendices pp.

Shrimpton JM, Roberts SL, Clarke AD (2012) Genetic analysis of Arctic grayling population structure in the Williston Reservoir. Fish and Wildlife Compensation Program – Peace Region Report No. 311. 12pp.

Smith CT, Koop BF, Nelson RJ (1998) Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. *Molecular Ecology* **7**, 1613–1621.

Stamford MD, Taylor EB (2004) Phylogeographical lineages of Arctic grayling (*Thymallus arcticus*) in North America: divergence, origins and affinities with Eurasian *Thymallus*. *Molecular Ecology* **13**, 1533–1549.

Stamford MD, Taylor EB (2005) Population subdivision and genetic signatures of demographic changes in Arctic grayling (*Thymallus arcticus*) from an impounded watershed. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2548–2559.

Takesaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**, 389-399.

Templeton AR (1980) The theory of speciation *via* the founder principle. *Genetics* **94**, 1011-1038.

Tuor KMF, Shrimpton JM (2019) Differences in water temperatures experienced by embryo and larval Coho Salmon (*Oncorhynchus kisutch*) across their geographic range in British Columbia. *Environmental Biology of Fishes* **102**, 955-967.

Tuor KMF, Heath DD, Shrimpton JM (2020) Spatial and environmental effects on Coho Salmon life-history trait variation. *Ecology and Evolution* **10**, 13198-13210.

Turcotte LA, Shrimpton JM (2020) Assessment of spawning site fidelity in Interior Fraser River Coho Salmon *Oncorhynchus kisutch* using otolith microchemistry, in British Columbia, Canada. *Journal of Fish Biology* **97**, 1833-1841.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Veale AJ, Russello MA (2016) Sockeye salmon repatriation leads to population re-establishment and rapid introgression with native kokanee. *Evolutionary Applications* **9**, 1301–1311.

Vernon EH (1957) Morphometric comparison of three races of Kokanee (*Oncorhynchus nerka*) within a large British Columbia Lake. *Journal of the Fisheries Research Borad of Canada* **14**, 573–598.

Warnock WG, Rasmussen JB, Taylor EB (2010) Genetic clustering methods reveal Bull Trout (*Salvelinus confluentus*) fine-scale population structure as a spatially nested hierarchy. *Conservation Genetics* **11**, 1421–1433.

Whitlock SL, Campbell MR, Quist MC, Dux AM (2018) Using genetic and phenotypic comparisons to evaluate apparent segregation among Kokanee spawning groups. *Transactions of the American Fisheries Society* **147**, 43–60.

Wilson PN, Shrimpton JM (2020) Genetic population structure and demographics of Kokanee introduced to the Williston Reservoir. Fish & Wildlife Compensation Program – Peace Region Project Report PEA-F19-F-2870-DCA. 15 pp.

Witt JDS, Zemlak RJ, Taylor EB (2011) Phylogeography and the origins of range disjunctions in a north temperate fish, the pygmy whitefish (Prosopium coulterii), inferred from mitochondrial and nuclear DNA sequence analysis. Journal of Biogeography 38, 1557–1569.

Wood CC, Foote CJ (1996) Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* **50**, 1265–1279.