Clowhom Lake Reservoir Kokanee Assessment

(FWCP Project No. COA F19-F-2771)

Report CAQ – 034

28 Jun 2019

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Prepared with financial support of the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations and Public Stakeholders.

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Prepared for

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Glossary of Terms

Allele	One of two or more alternative forms of a gene or microsatellite that arise by mutation. In the case of microsatellite, this refers to the number of repeats of a short nucleotide sequence.
Allelic Frequency	Number of copies of a particular allele divided by the number of copies of all alleles at a specific genetic or microsatellite locus in a population of interest.
Allelic Richness	Total number of alleles in a population and is a measure of genetic diversity. In present context, averaged across multiple microsatellite loci.
Expected Heterozygosity (H_T):	Refers to the theoretical proportion of individuals in a population that have heterozygotic alleles based on the Hardy–Weinberg Principle. Calculated for each microsatellite locus and either summed or averaged.
Fixation	The change in a gene pool from a heterozygotic condition (where there are two or more alleles in a given population) to a homozygotic condition (where only one allele remains), i.e., the dominant allele becomes fixed (permanently established at 100% frequency) in the population. Fixation can refer to a genes or microsatellites.
Fixation Index (F)	The degree of reduction (usually) in heterozygosity (i.e., fixation) when compared to the Hardy–Weinberg expectation (expected heterozygosity H). When referring to sub-populations, it is denoted as F_{ST} and is calculated as $(H_T – H_S)/H_T$ where H_T = expected heterozygosity based all populations and H_S = observed heterozygosity within a given sub-population. Values range from 0 (panmictic; complete inbreeding; no genetic divergence) to 1 (no shared genetic diversity; complete isolation; full genetic divergence). Values < 0 suggest outbreeding. Often referred to as an inbreeding coefficient.
Gene	The basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides coded instructions for the synthesis of RNA, which, when translated into protein, leads to the expression of hereditary character.
Hardy–Weinberg Principle:	States that allele and genotype frequencies in a population will remain constant from generation to generation (in equilibrium) in the absence of other evolutionary influences. As a result, allele frequencies in a population are predictable. This is the foundation for calculating/estimating expected allele frequencies in a heterozygotic population.
Heterozygosity	The possession of two (or more) alleles of a particular gene or microsatellite in an individual.
Homozygosity	The possession of single allele of a particular gene or microsatellite in an individual.
Locus	The position of a gene, microsatellite, or mutation on a chromosome

Loci	Plural of locus
Microsatellite	A section of DNA consisting of very short nucleotide sequences (1 to 6 nucleotides) repeated many times (5 – 50 times), the number of repeats (alleles) varies between members of the same species: used as a marker in determining genetic diversity (e.g., TATATATATA or CTGCTGCTGCTGCTG)
Sympatry	Related species or populations occurring within the same geographical area; overlapping in distribution.
Wahlund Effect	Refers to the reduction of heterozygosity in a population caused by subpopulation structure. Namely, if two or more subpopulations have different allele frequencies then the overall heterozygosity is reduced, even if the subpopulations themselves are in a Hardy-Weinberg equilibrium. The underlying causes of this population subdivision could be geographic barriers to gene flow followed by genetic drift in the subpopulations.

Executive Summary

Very little was known about the kokanee (*Onchorynchus nerka*) in Clowhom Lake Reservoir (CLM) until the capture of a few individuals in a fall survey carried out in 2002 (Bruce 2003). The only other scientific survey prior to this was done in 1950 by Smith and Larkin (1950) which, because of their shore-based sampling methods, did not encounter any kokanee. Clowhom Lake reservoir was formed in 1952 by a 21 m high dam that resulted in the impoundment of two small lakes. Access of in-migrating salmonid spawners to these lakes had been poorly documented, leading some to speculated that historically, there may have been a sockeye run into the deep waters of the upper lake and that this run had been extirpated by the formation of Clowhom Dam. It followed that residual sockeye that did not leave the lake formed the kokanee population presently found in the reservoir and that they represent a land-locked sockeye population rather than 'true' kokanee. Such extirpation events have been documented for both Alouette and Coquitlam Lake Reservoirs (Godbout et al. 2011), and in both cases have what are now believed to be land-locked sockeye populations that readily re-anadromize (survive and grow at sea to maturity) if given the opportunity. The objective of this study was to determine whether, though genetic analysis, Clowhom Lake kokanee may have followed the same fate, or were always an isolated lacustrine population prior to impoundment; i.e., are 'true' kokanee. A second objective was to develop a basic understanding of kokanee condition and size-at-age in the reservoir. Though there have been several fish surveys in the reservoir since Bruce (2003), catches have been very low due to the shore-based sampling methods used (Bates and Coombs 2012). In the present study, capture methods were deployed that specifically targeted kokanee.

Genetic analysis of the 34 fish caught in CLM revealed that they were highly divergent (unrelated) from neighboring sockeye and kokanee populations, suggesting a period of isolation that was likely longer than the period of impoundment. Genetic diversity, as defined by expected heterozygosity and average allelic richness found among 14 microsatellite loci, showed no indication of a recent bottleneck event. It was hypothesized that following impoundment, the inability of returning sockeye salmon to spawn in their native areas would cause genetic diversity to drop considerably. However, both indices had near normal values, suggesting that this was not the case. Rather, it would appear that CLM kokanee are not the result of a recently residualized sockeye population, but were always 'true' kokanee. Though not conclusive, these results address Priority Action COM.RLR.RI.09.01 (Conduct research to determine if the Clowhom lakes used to support anadromous salmon-P2) of the Clowhom River Watershed Action Plan (FWCP 2017) and no further study is recommended until such time new evidence arises to suggest otherwise.

A comparison of CLM kokanee condition factors and size-at-age data with reference populations in the Coquitlam and Alouette Lake reservoirs revealed that these fish tended to be smaller in size and in poorer condition. In fact, size-at-age and fish condition was similar to that found in Stave Lake Reservoir. Both reservoir systems have similarly low total dissolved phosphorus levels, an indicator of reservoir productivity that is linked to kokanee biometrics through its effect on available food resources. Coquitlam and Alouette Lake reservoirs have much higher total dissolved phosphorus levels which were correlated with both better fish condition and size-at-age statistics. The limited data suggests that CLM kokanee growth is limited by low reservoir productivity and its effect of food availability. This however is circumstantial and thus only partly addresses Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1). More study is required including seasonal zooplankton abundance surveys, kokanee stomach content analyses and kokanee abundance studies. The latter may also apply to the piscivorous salmonids in the reservoir as kokanee appear to be their main prey.

Little is known of CLM kokanee biology. Limited anecdotal information suggests these may be a type knows as 'black' kokanee (Moirera and Taylor 2015). Future study efforts should be focused on determining the timing, location and relative fecundity of these fish to determine in they are indeed black kokanee. These fish are believed to have a biology that is different from 'true' kokanee, though it is not well documented. It is unclear whether these fish have been impacted by dam construction. This work aligns with Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1) with the emphasis on the availability of suitable spawning habitat.

The Clowhom Lake Reservoir lies within the shíshálh Nation Swiya (territory) and key non-scientific objective of this study was to provide an opportunity to engage and train shíshálh Nation fisheries technicians, as well as provide some new insight regarding the reservoir system to band members in general. This objective was successfully met.

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1 Introduction

1.1 Background

Clowhom Lake Reservoir, located in the shíshálh Nation Swiya (territory) on the Sunshine Coast, was formed in 1952 by the construction of a 21 m high dam (crest elevation 55.78 m) at its outlet into Sechelt Inlet, BC (BC Hydro, 2005). It was formerly two lakes separated by a narrow channel; one small and shallow (about 20 m deep) and the other several times larger and > 100 m deep (Figure 1). Prior to impoundment, the outlet included a large water fall believed to be impassable for anadromous (sea going) salmonids (FWCP, 2017). There was however, a smaller side channel that some believe could have been passable, leading to speculation that some of the reservoir's salmonid fishes may have been anadromous prior to dam construction - in particular the kokanee now found in the reservoir.

Clowhom Lake Reservoir is part of the BC Hydro Clowhom hydroelectric project consisting of a 33 MW generating station and spillway facility. All water releases from the project flow directly into Salmon Inlet. There is no riverine habitat below the facility except for a short, steep bedrock lined channel that forms part of the spillway. This channel was formerly part of Clowhom Falls, the main outlet of the Clowhom lakes system, but was excavated to form the present-day spillway during dam construction. Except for a few isolated pools, there is no usable fish habitat in the channel and as a result, there is no potential for downstream freshwater fish habitat enhancement.

Clowhom Falls was believed to be largely impassable to all anadromous salmonids prior to impoundment. However, there are anecdotal reports indicating that a side channel to the left of the present-day powerhouse may have provided lake access for at least some of these fish. The reservoir currently supports three salmonid species; cutthroat trout, rainbow trout and kokanee (Bruce 2003). It is uncertain how long these populations have been isolated from the marine environment. Resolving this uncertainty is considered to be a critical step for priority setting of potential enhancement objectives and deciding the best approach to future enhancement works in the Clowhom Lakes Reservoir.

Although many fish surveys have been carried out in Clowhom Lake Reservoir, most have relied on gillnets set from shore as the main fish capture method. Few kokanee have been caught over the years (2003 to 2012; Bates and Coombs 2012) and none were caught during fish surveys carried out at the time of dam construction (Smith and Larkin 1950). As a result, size-at-age and condition factors remain poorly defined for these fish. One of the goals of this study was to enhance the existing dataset of kokanee biometrics by specifically targeting kokanee using gillnets sets away from shore. The data collected through this fish survey will be appended to the existing data and compared to other BC Hydro reservoirs of similar size. These include Coquitlam Lake (COQ), Alouette Lake (ALU) and Stave Lake (SFN) reservoirs which have been studied extensively. Each reservoir is unique and such comparisons could provide some insight on CLM's potential capacity for kokanee/sockeye production.

Whether CLM kokanee represent an extirpated anadromous population impacted by impoundment remains uncertain. Before embarking on more complex paleolimnological studies to address this uncertainty, this study will attempt to assess the degree of relatedness to other coastal populations, in particular the Sakinaw, Powell and Lois lake populations, using comparative genetic analyses. Borrowing from the techniques used in the COQ, ALU and SFN populations, genetic samples taken from CLM kokanee will be compared to the DFO database of other sockeye/kokanee populations.

A high degree of relatedness would indicate past cohorts intermingled through straying, implying an anadromous pre-impoundment life history. Conversely, a unique stock would suggest an extended period of isolation, indicating they were more likely landlocked.

1.2 Objectives

Objectives of this study were two-fold:

1. Expand the existing set of kokanee biometric information on fish condition and size-at-age characteristics and compare them to those of the COQ, ALU and SFN reservoirs; providing some insight on CLM's kokanee/sockeye potential productive capacity

2. Compare the genetic data of CLM kokanee to assess extent of genetic diversity and degree of relatedness with other populations found in the lower mainland, south coast and east coast of Vancouver Island. Low diversity combined with high relatedness (low divergence) would suggest an anadromous population that was recently extirpated; in this case by dam construction on the lower Clowhom River.

The first objective aligns with the Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1) of the Clowhom River Watershed Action Plan (FWCP 2017) while the second aligns with Priority Action COM.RLR.RI.09.01 (Conduct research to determine if the Clowhom lakes used to support anadromous salmon-P2).

2 Methods

2.1 Study Area

The Clowhom hydroelectric project (CLM) is located in the shíshálh Nation Swiya (territory) on the Sunshine Coast and is also within the Sunshine Coast Regional District. It was commissioned in 1958 and consists of a 33 MW generation station capable of discharging up to 100 m^3/s, a 21 m high concrete gravity dam, a spillway facility capable of discharging 1080 m^3/s, and an approximately 800 ha reservoir when at full pool. The dam itself was constructed in 1952 as part of an earlier project. The reservoir occupies a steep-sided valley at the end of Salmon Inlet, and is located at the edge of the Tantalus Range north of Sechelt, BC (Lat 49°42'44" N, Long 123°32'8" W). The Clowhom Lake Reservoir is supplied by a 382 km^2 drainage basin that includes the Clowhom River at its upper end as well as a number of smaller tributaries that discharge directly into the reservoir (Figure 1). Before impoundment, the reservoir area consisted of two smaller lakes, one shallow (~ 20 m deep at full pool) and the other very deep (> 110 m at full pool), connected by a short section of riverine habitat located at the confluence of Bear Creek (Figure 1).

The Clowhom Lake Reservoir is an isolated system accessible only by boat (via Salmon Inlet) or float plane. This presented a challenge when sampling as all equipment, including sampling boat, outboard engine and fuel, had to be brought in. To do so, it had to be light weight, which limited the range of travel when on the reservoir. As a result, only the bottom half of the reservoir could be sampled during the September 6 - 8, 2018 study period. Sampling locations are provided in Figure 1.

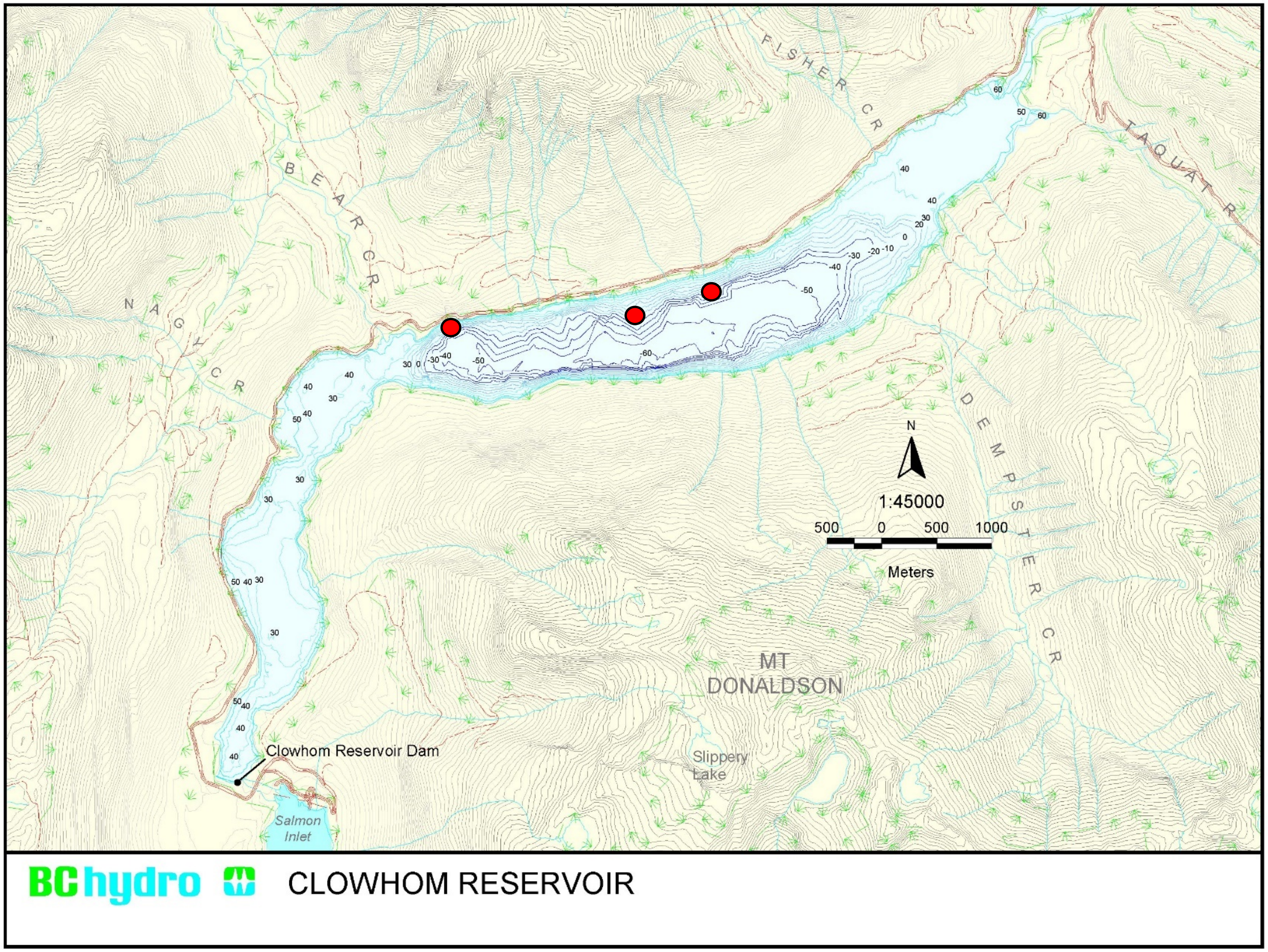

Figure 1. Bathymetric map of Clowhom Lake Reservoir. Outline of the pre-impoundment lakes is approximated by the 40 m elevation isoline. Red dots denote location of successful, open water gillnet sets.

2.2 Field Methods

All kokanee samples from Clowhom Lake Reservoir were collected by standard RIC (1997) gillnets deployed away from shore at a depth of 5 m for 2 successive overnight sets (approximately 18 and 17 hours respectively). The nets consisted of six panels of different mesh sizes that are strung together to form a gang net 91.2 m long and 2.4 m deep. Each panel was 15.9 m long and had 25, 76, 51, 89, 38, and 64 mm mesh sizes that were strung in that order to form each gang net. All gillnets were of the floating type with a float line at the top and a lead line at the bottom.

When the Clowhom Lake reservoir was impounded, no effort was made to remove shoreline vegetation, thus the nets were at high risk of snagging on to submerged and floating woody debris. To minimize this occurrence, as well as focus sampling on more open water where kokanee are likely to be found, the bottom ends of each gang net were tied to cinder block anchors set a considerable distance away from shore in what was judged to be pre-impoundment lake bottom. The tops of each net were tied to a large buoy. Both the anchors and buoys were equipped with a pully system so that the top line of each gillnet could be set to a 5 m depth regardless bottom topography. Once the height was set, the anchor rope was tied to the float to prevent further movement. The distance between anchors for each gillnet was such that it kept the net as taught as possible.

Strong winds prevailed though-out the sampling period. Because of the reservoir's orientation, this created large waves. While this presented a challenge when setting the nets, once set, they initially appeared sufficiently stable to fish successfully. However, over the course of the deployment period, it appeared each wave was large enough to lift the anchor and float system off the bottom and incrementally drag it closer to shore. Once close to shore, the nets quickly became entangled in the uncleared wood debris. Fortunately, all nets were retrieved. However, many were so close to shore and bunched together that the sets were considered ineffective. In fact, no fish were caught in these nets. In future deployments, smaller buoys should be used so that its buoyancy does not overpower the weight of the anchors during such high-wind events. Because of the reservoir's isolation, the crew were unable to modify their deployment system accordingly. Nonetheless, three gillnets sets were successfully fished to capture kokanee for sampling (Figure 1)

All captured fish were taxonomically identified to the species level, wet weighted (WW; g) and measured for fork length (FL; mm). Scale samples were collected from the dorsal fin area of each kokanee and placed between microscope slides for later ageing in the laboratory. A tissue sample (fin clip) for genetic analysis was also obtained from the caught kokanee. These were placed in individual vials containing a 10% ethanal solution for preservation. All tissue samples were sent to the Pacific Biological Station (PBS) in Nanaimo, BC for genetic analysis. All biometric data were collected as per RIC (1997) standards.

2.3 Laboratory Methods

At the PBS laboratory, microsatellite DNA was extracted from the kokanee tissue samples as described by Withler et al. (2000). Fourteen microsatellite loci (Ots2, Ots3, Ots100, Ots103, Ots107, Ots108, Oki1a, Oki1b, Oki6, Oki10, Oki16, Oki29, One8, and Omy77; Beacham et al. 2005) were assayed for polymorphism by size fractioning their respective polymerase chain reaction (PCR) products on denaturing polyacrylamide gels. The number of alleles was determined using the ABI 377 automated DNA sequencer. The individual assay results for CLM kokanee were then collated to derive a multi-locus

Table 1. List of reference lakes known to have either sockeye and/or kokanee populations with genetic data that are in the general vicinity of Clowhom Lake Reservoir. Data provided by DFO, Pacific biological Station.

Gazetted Name	General Location	UTM Zone	UTM Easting	UTM Northing	Lake Perimeter (km)	Lake Area (ha)	Lake Elevation (m)	Type	Expected Heterozygosity	Allelic Richness
ALOUETTE LAKE	Lower Mainland	10	537287	5459223	39.8	1625.3	122	KO	63	5.3
COQUITLAM LAKE	Lower Mainland	10	516343	5466907	38.6	1188.4	154	KO	67	6.2
STAVE LAKE	Lower Mainland	10	546885	5453146	92	5686.6	82	KO	62	6.9
CLOWHOM LAKE	South Coast	10	461329	5506743	25.2	739.5	57	KO	66	6.9
LOIS LAKE	South Coast	10	405249	5516765	58.6	2252	152	KO	71	7.8
POWELL LAKE	South Coast	10	389165	5526945	190.6	12325	56	KO	75	9.0
SAKINAW LAKE (KO)	South Coast	10	423132	5500638	35.3	681.3	7	KO	63	6.6
GLENDALE LAKE	East Coast Van Is	10	312921	5610967	5.4	122.5	74	SK	55	5.6
HEYDON LAKE	East Coast Van Is	10	313526	5603819	19.1	799.1	36	SK	65	5.5
NAHWITTI LAKE	East Coast Van Is	9	579611	5618094	9.9	244.6	199	SK	69	6.3
NIMPKISH LAKE	East Coast Van Is	9	640426	5598087	57	3678.8	25	SK	71	7.0
PHILLIPS LAKE	East Coast Van Is	10	331667	5606279	11.5	309.2	14	SK	67	7.2
QUATSE LAKE	East Coast Van Is	9	602729	5610695	9.1	151.6	83	SK	56	5.2
SCHOEN LAKE	East Coast Van Is	9	692467	5560404	13.1	246.3	402	SK	64	6.4
VERNON LAKE	East Coast Van Is	9	682657	5548571	19.2	801.6	207	SK	74	7.2
WOSS LAKE	East Coast Van Is	9	669641	5561804	38.3	1378.5	147	SK	71	6.9
PITT LAKE	Lower Mainland	10	528068	5466523	70.9	5348.2	4	SK	73	9.4
SAKINAW LAKE (SK)	South Coast	10	423132	5500638	35.3	681.3	7	SK	68	6.3
TZOONIE LAKE	South Coast	10	458830	5528831	2	19.3	1346	SK	-	-
VILLAGE BAY LAKE	South Coast	10	343769	5559913	11	101.4	14	SK	72	7.1

genotype that could be compared to that of 17 reference sockeye/kokanee populations that have been similarly analysed in the lower mainland, south coast, and east coast of Vancouver Island, BC (Table 1). Results of the genetic assays and reference genetic data were all provided by PBS (Withler R. and L. Godbout; Pers. Comm, PBS, 2019).

2.4 Data Analysis

2.4.1 Kokanee Biometrics

In addition to basics descriptive statistics, Kokanee biometric data were first assessed in terms of length-weight relationships of the form:

$$WW = a \cdot FL^{b} \qquad \text{Eq. 1}$$

where the '*a'* coefficient is the intercept when plotted using log-log axes, and the '*b'* coefficient the slope. Coefficients were compared to those of reported for the Coquitlam Lake (COQ), Alouette Lake (ALU) and Stave Lake (SFN) reservoirs for which there is comparable data. Because variance statistics were not provided in the reference reservoirs, statistical comparisons could not be made. However, 95% confidence intervals were derived for the CLM data set, which were used as an informal indicator of 'significance'. Reference coefficients found outside these calculated confidence intervals were considered to be "meaningfully" different.

Fulton Condition factors (K_F) were also calculated for each fish in CLM, and compared the reference reservoirs. These data were calculated as:

$$K_F = 10^5 \cdot WW/FL^3 \qquad \text{Eq. 2}$$

Mean K_F values and corresponding standard deviations (SD) and sample sizes (n) were only available for the COQ and ALU reservoirs. Welch's t-test was used to assess the statistical significance of differences between reservoir means (Welch 1947). This t-test makes no assumption regarding homoscedasticity and is robust against large deviations in normality (Fagerland, 2012). The t statistic was calculated as:

$$\text{Welch's } t = (X_{Avg} - X_{Hyp})/SE_{Pooled} \qquad \text{Eq. 3}$$

where pooled standard error (SE_{Pooled}) was calculated as:

$$SE_{Pooled} = (SD_1^2/n_1 + SD_2^2/n_2)^{0.5} \qquad \text{Eq. 4}$$

and the degrees of freedom (df) as:

$$df = (SD_1^2/n_1 + SD_2^2/n_2)^2 / (SD_1^4/n_1^2(n_1 - 1) + SD_2^4/ n_2^2(n_2 - 1)) \qquad \text{Eq. 5}$$

Because multiple comparisons were often made, the Bonferroni correction was applied as required where the significance level of each comparison (α) was adjusted by the total number of comparisons (m); i.e. α/m. For the present study, $\alpha = 0.05$.

Standard weights (W_s) were also calculated and compared to the measured wet weight of individuals. W_s was derived from the following relationship provided by Hyatt and Hubert (2000):

$$\text{Log}_{10}(W_s) = -5.062 + 3.033 \cdot \text{Log}_{10}(1.065 \cdot FL) \qquad \text{Eq. 6}$$

The ratio of weights $W_r = WW/W_s$ are considered indicators of fish condition relative to what would be considered an idealized kokanee for a given FL growing in near ideal, but natural conditions. Values of $W_r < 1$ suggest fish that are in relatively poor condition overall.

The last biometric component under consideration was size-at-age. Mean size at age data were available for all reference reservoirs, however, only COQ and ALU provided the SD statistics needed for statistical comparison. As with the K_F data, the Welch's t test was used to test for significant differences in mean FL for the 1+, 2+ and 3+ age classes where valuable data allowed. Bonferroni corrections were applied as required.

Attributes to consider when interpreting the comparative biometric data, along with a list of comparisons that were made with the available data, are summarized in Table 2.

Table 2. Key reservoir attributes to consider when interpreting CLM comparisons with reference reservoir kokanee biometrics.

Reservoir	**Attributes**			**Biometric Comparisons with CLM**		
	Fishing Pressure	**Drawdown Range**	**Nutrient Addition**	**Length-weight**	**K_F**	**Size at Age**
Coquitlam Lake	None	14.6	No	✓	✓	✓
Alouette Lake	Present	12.9	Yes	X	✓	✓
Stave Lake	Present	9	No	✓	X	✓
Clowhom Lake	Negligible	4.3	No	-	-	-

2.4.2 Genetics

To evaluate within-population variation, basic population genetic summary statistics (number of alleles per locus, allelic richness, observed heterozygosity, and expected heterozygosity) were performed in FSTAT version 2.9.3.2 (Goudet 2001). Of particular interest to this study were the allelic richness (average number of alleles/loci) and expected heterozygosity statistics as these were considered general indicators of genetic diversity (Allendorf 1985) and could be compared to the reference stocks. The reference lakes and corresponding genetic diversity data are provided in Table 1.

The genetic divergence between sockeye/kokanee populations was assessed by calculating pairwise F_{ST} statistics that test for differences in allelic frequencies between paired populations. The F_{ST} values range from 0 (no detectable divergence) to 1 (complete divergence) and are considered statistically significant (i.e., $F_{ST} > 0$) when $P < 0.05$ following Bonferroni correction ($0.05/153 \times 2 = 0.000653$). The pairwise F_{ST} calculations were done using FSTAT version 2.9.3.2 (Goudet 2001). The pairwise F_{ST} values are presented in the form of an unrooted, consensus neighbor-joining tree based on 1000 replicate trees generated with the CONSENSE program from PHYLIP (Felsenstein 2005).

3 Results

3.1 Catch

A total of 66 fish were caught in the three gillnets that were successfully set in open waters. The majority were salmonids, comprising of 34 kokanee (*Oncorhynchus nerka*), 17 Dolly Varden (*Salvelinus malma*) and 8 cutthroat trout (*Oncorhynchus clarki*). Although rainbow trout (*Oncorhynchus mykiss*) are known to reside in the reservoir (Bruce 2003), none were caught in the present study. This is consistent with their general close association with shoreline habitats rather than the more open waters targeted here. Sculpins (Cottus sp.; of which there were 6) were the only other fish captured. These latter fish were likely captured when the nets drifted into shore. The catch, along with biometric information, is summarized in Appendix A. Because the gillnets sets were inconsistent across sites, days and over time because of the high winds, no attempt was made to calculated abundance metrics (i.e., catch per unit effort statistics).

3.2 Kokanee Biometrics

3.2.1 Length-weight relationship

The kokanee captured in this this study ranged in size from 134 to 210 mm FL and were 1+ to 3+ years in age. None of the fish captured were sexually mature nor displayed secondary sexual characteristics; an outcome not entirely unexpected given the time of year these fish were caught. Regression analysis of wet weight (WW) as a function of fork length (FL) resulted in the following power relationship (Figure 2):

$$WW = 6.7 \times 10^{-6} \cdot FL^{0.3106} \qquad (R^2_{Adj} = 0.987, P < 0.0001) \qquad \text{Eq. 7}$$

This is in line with the 7 observations collected by Bruce (2003) as well as the collection of 7 observations obtained over a three-year period during a Water Use Plan monitoring study (Figure 2; Bates and Coombs 2012). When combined, the resulting regression equation was determined to be:

$$WW = 8.0 \times 10^{-6} \cdot FL^{0.3068} \qquad (R^2_{Adj} = 0.983, P < 0.0001) \qquad \text{Eq. 8}$$

which spans the range of FL from 99 to 210 mm. 95% Confidence Interval for the intercept coefficient (*a*) was 4.4×10^{-6} – 14.7×10^{-6} while that for the exponential slope coefficient (*b*) was 2.951 – 3.186. The length-weight relationship was similar to that reported for Stave Lake Reservoir (SFN) kokanee where $a = 6.3 \times 10^{-6}$ and b = 3.122 for data collected from 2005 to 2014 (Stables and Perrin 2016). It was however significantly different from that reported for Coquitlam Lake Reservoir (COQ) by Bussanich et al. (2005) in 2004, where $a = 3.0 \times 10^{-5}$ and $b = 2.851$. Both coefficients were outside their respective CLM confidence intervals and more importantly, the lower *b* coefficient among the COQ kokanee suggested these fish generally became thinner as they grew in length than their CLM counterparts. The higher intercept coefficient also indicated that COQ kokanee generally had larger girths (more mass) across all lengths, particularly the smaller fish. This trend however was not repeated with the COQ length-weight relationships derived in 2010 and 2011, where $a = 2.0 \times 10^{-6}$ and 4.0×10^{-6} and $b = 3.317$ and 3.207 respectively (Plate et al. 2012). In this case, COQ kokanee grew significantly more in girth with increasing length than COM kokanee. These fish however, were generally smaller across all

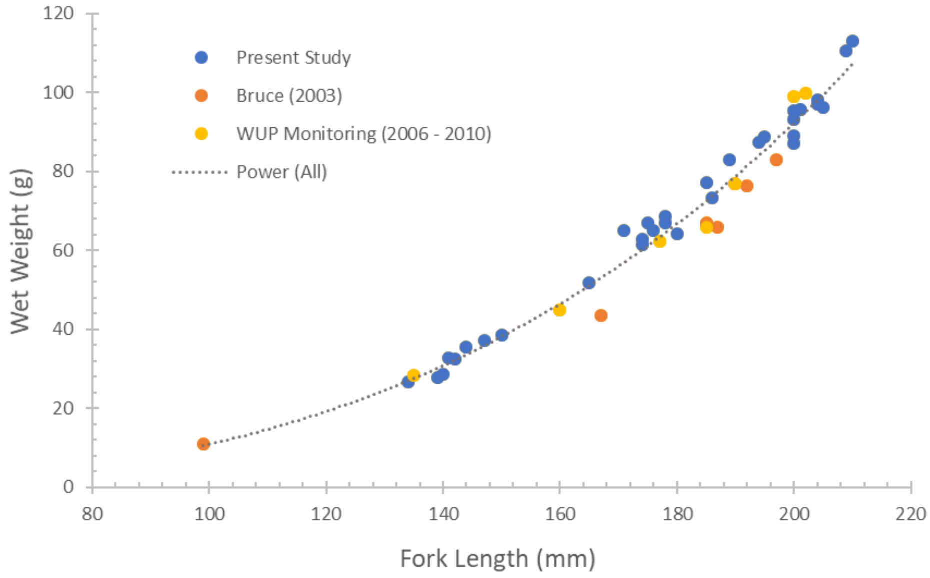

Figure 3. Plot of wet weight (WW) as a function of fork length (FL) for kokanee caught in the Clowhom Lake Reservoir. The regression line ($WW = 8.0 \times 10^{-6} \cdot FL^{0.3068}$) is for all observations combined.

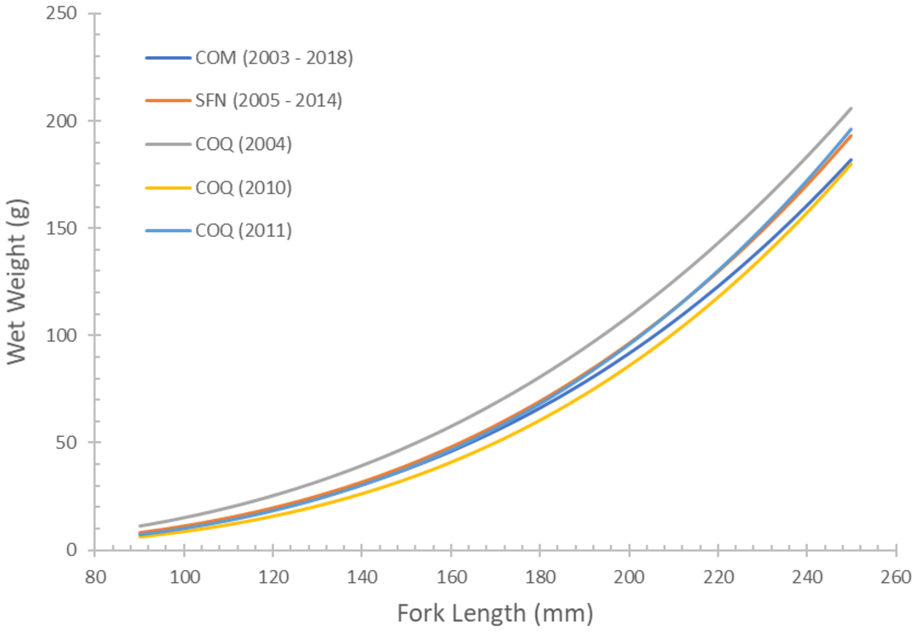

Figure 2. Plot of kokanee length-weight relationships for COM in comparison to that of the SFN and COQ reservoir systems.

lengths. Its worth noting that COQ relationships in 2010 and 2011 were done in late October/November and likely captured the effects of gonadal growth prior to spawn in older fish, along with a post-summer, dwindling food supply for the younger individuals. The other relationships were all based on fish collected in September and are likely better direct comparisons (Figure 3).

3.2.2 Condition Factor (Fulton)

For the CLM kokanee in the present study, K_F ranged from 1.04 to 1.30 and averaged 1.16 (SD = 0.06). For the seven fish caught by Bruce (2003), K_F averaged 1.06 (SD = 0.07) while the observations collected during the WUP monitoring program (2006 – 2010) by Bates and Coombs (2012) averaged 1.14 (SD = 0.07). ANOVA confirmed that the average condition of fish collected by Bruce (2003) in 2002 was significantly lower than either the present study or the WUP data, indicating the potential for significant between-year variation. Nonetheless, when all the data were combined, K_F averaged 1.15 (SD = 0.07). This was significantly lower than the average K_F for COQ fish caught by Bussanich et al. (2005) and Plate et al. (2012) (K_F = 1.25, SD = 0.12; Welch's $t_{2,132} = 7.308$, $P < 0.0001$). This was also the case for ALU kokanee where K_F averaged 1.24 (SD = 0.34) across yearly surveys from 2002 to 2008 (Harris et al. 2010; Welch's $t_{2,342} = 4.634$, $P < 0.0001$).

In the case of the COQ fish, the difference in K_F may simply be due to the mature/maturing kokanee caught in the 2010/2011 surveys (Plate et al. 2012). Limiting the comparison to the Bussanich et al. (2005) fish caught at the same time frame as the present study, the difference in K_F narrows considerably (COQ K_F = 1.18, SD = 0.10, Welch's $t_{2,74} = 1.802$, $P < 0.0793$). This explanation however, may not apply to the ALU fish which were also captured in September. In this case, it likely reflects the impact of nutrient addition to the reservoir and thus far richer food web (Harris et al. 2010). Unfortunately, K_F data were not provided for the SFN kokanee to permit similar comparisons (Stables and Perrin 2016).

3.2.3 Relative Weight

Standard weight (W_s) was calculated for each CLM kokanee (Eq. 6) and compared to the corresponding measured wet weight to derive a relative weight index value. In most cases, WW < W_s for most FL observations and tended to worsen as FL increased (Figure 4). This suggests that not only are CLM kokanee in poorer condition than the COQ and ALU as indicated by K_F, they are also considered to be in poor condition over all. Fish in good condition are thought to have a W_r (i.e., WW/W_s ratio) approaching 1. For the CLM kokanee, W_r = 0.919 (SD = 0.056), which was significantly below this value ($t_{47,1} = 9.993$, $P < 0.0001$).

3.2.4 Size at Age

Size at age data were available for all reservoir systems, though raw data or measures of variability were not always given. However, a plot of mean size (i.e., FL) at age for each reservoir were able to show clear differences in growth (Figure 5). As would be expected from to the addition of nutrients, ALU kokanee were the largest by far for both 2+ and 3+ age classes (Table 3). This was followed by COQ fish, which also had age 4+ individuals (not shown in Table 3 or Figure 5), the only reservoir to do so. These 4+ fish were near identical in size to the 3+ fish (FL_{Avg} = 243 mm, SD = 9 mm). Both SFL and COM had similar size-at-age characteristics. Unfortunately, because variance statistics were not given, this latter comparison could not be assessed statistically. With the exception of age 1 fish, differences in FL_{Avg} between COM, COQ and ALU were statistically significant for both the 2+ and 3+ age classes ($P < 0.0001$ in all cases using Welche's t-test).

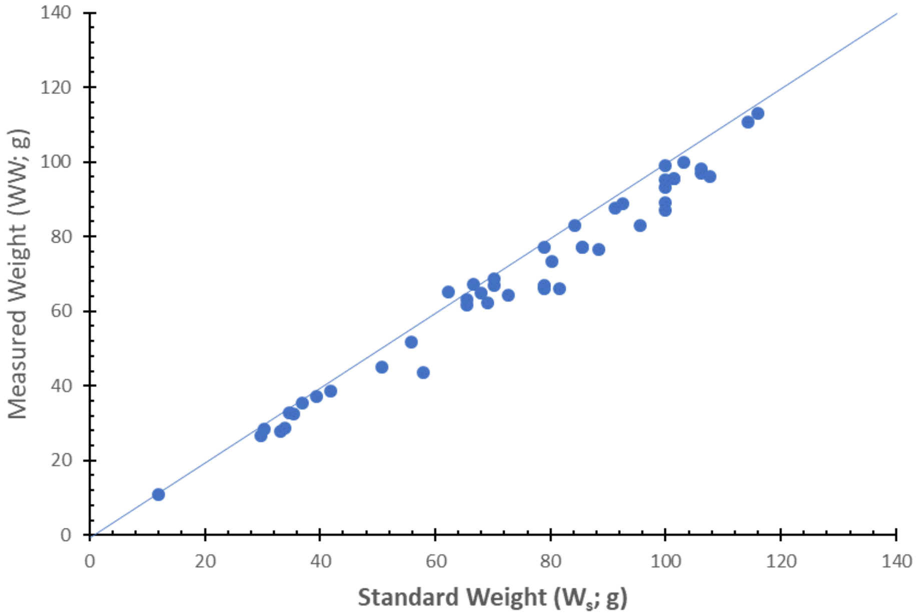

Figure 4. *Comparison of standard weight as calculated from Eq. 6 and measured wet weight. Diagonal line is the line of equality and is equivalent to $W_r = 1$. Note from Eq. 6 that W_s increases with FL.*

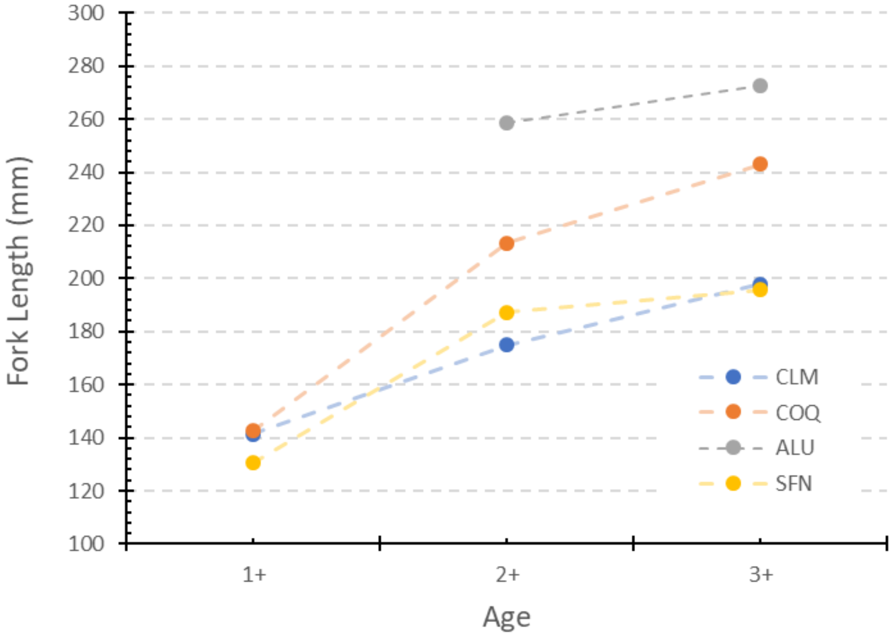

Figure 5. *Plot of mean fork Length as a function of age for CLM kokanee compared to those in the COQ, ALU and SFN reference reservoirs*

Table 3. Summary size-at-age statistics for CLM kokanee as well as those for the COQ, ALU and SFN reference reservoirs

System	1+			2+			3+		
	n	**FL_{Avg}**	**SD**	**n**	**FL_{Avg}**	**SD**	**n**	**FL_{Avg}**	**SD**
CLM	10	141	19	17	175	14	21	198	7
COQ	11	150	18	46	221	16	71	244	8
ALU	-	-	-	195	259	19	260	272	18
SFL	33	130	-	77	187	-	38	196	-

3.3 Genetics

3.3.1 Diversity (Expected Heterozygosity)

Expected heterozygosity of CLM kokanee was found to be 66.3, a value roughly mid-way in the range of possible values across the reference lakes (Table 1, Figure 6). A Shapiro-Wilks test confirmed the reference data set followed a normal distribution (W = 0.944, P = 0.315), allowing a simple t test to in turn confirm that the CLM value is not significantly different from the reference mean (H_{Ref} = 67.0, SD = 5.57; $t_{2,18}$ = 0.523, P = 0.607). It would appear that the expected heterozygosity of CLM kokanee is typical for both kokanee and sockeye populations found in the east Vancouver Island and lower mainland areas.

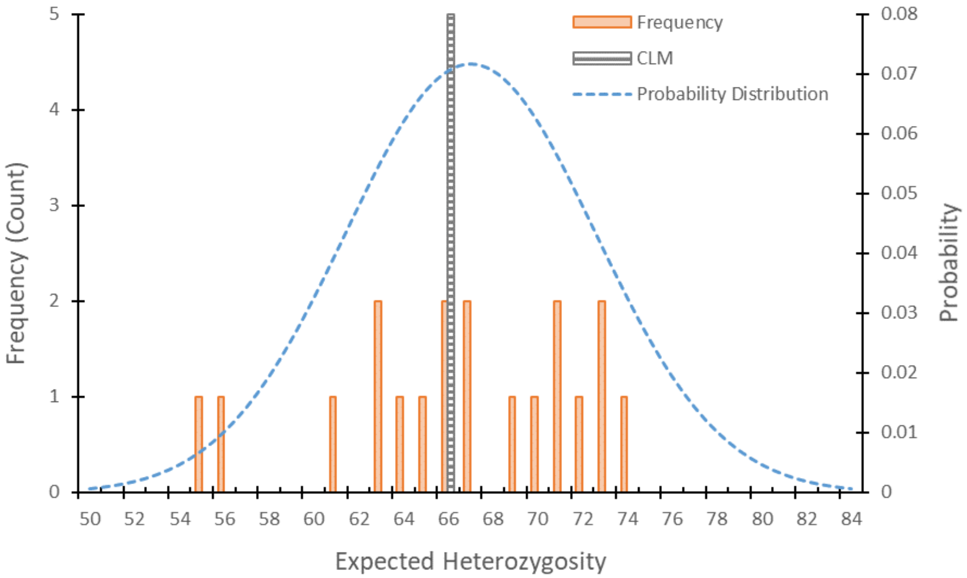

Figure 6. Expected heterozygosity of reference (Table 1, orange bars) and CLM (striped bar) sockeye/kokanee populations expressing degree of genetic diversity in each population. The higher the heterozygosity, the greater the diversity. The probability distribution function is based on the mean and standard deviation of the reference heterozygosity data in Table 1.

3.3.2 Diversity (Allelic Richness)

Allelic richness for CLM kokanee was found to be 6.78 alleles. Like expected heterozygosity, this value was roughly in the middle of the range of reference values (Table 1, Figure 7). The reference values were considered to be normally distributed (Shapiro-Wilks W = 0.916, P = 0.0944), thus allowing for a simple t-test to confirm that the CLM allelic richness value was not significantly difference from the reference mean (Mean richness = 6.78, SD = 1.11; $t_{2,18} = 0.566$, $P = 0.628$). Like expected heterozygosity, it would appear that the allelic richness of CLM kokanee is typical for both kokanee and sockeye populations found in the east Vancouver Island and lower mainland areas.

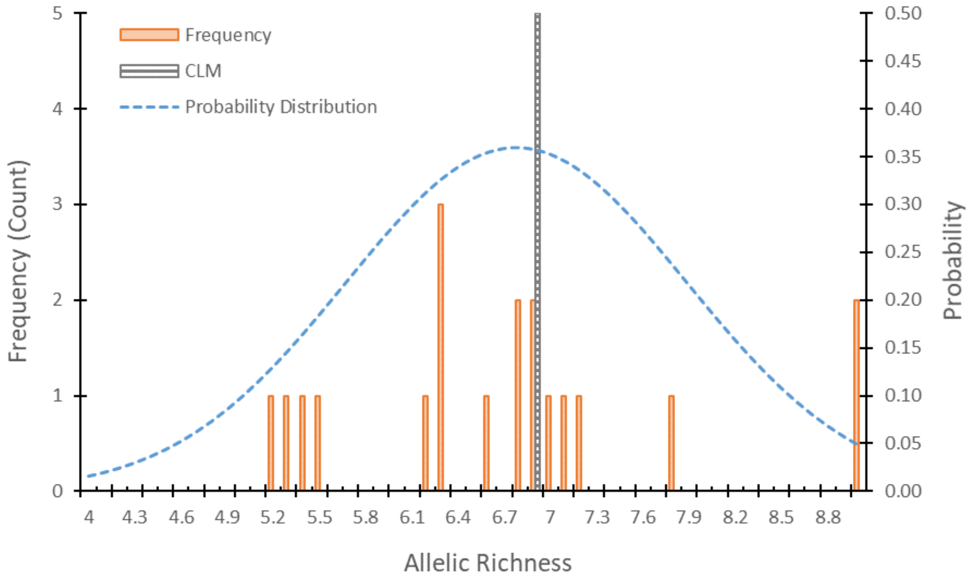

Figure 7. Allelic richness of reference (Table 1, orange bars) and CLM (striped bar) sockeye/kokanee populations expressing degree of genetic diversity in each population. The higher the richness value, the greater the diversity. The probability distribution function is based on the mean and standard deviation of the reference allelic richness data in Table 1.

3.3.3 Population Divergence (Pairwise F_{ST})

Pairwise F_{ST} values for the available reference genetic data, including the CLM data, are provided in Appendix B. Of particular note, all but one pairwise F_{ST} comparison were found to be statistically significant ($P < 0.05$), indicating significant genetic divergence between populations. The only exception was between the Nimpkish and Woss lake populations on Vancouver Island. A phylogenetic dendrogram based on the pairwise F_{ST} data of all reference population, including CLM, is presented in Figure 8. Although the clustering procedure was able to find groupings of what may appear to be populations with the least divergence, these groupings did not have a strong correlation with geographic location as one would expect. Most importantly, CLM kokanee did not appear to have a close relationship with any of the reference populations except perhaps for Schoen Lake sockeye, a small lake population on Vancouver Island that is part of the headwaters to Nimpkish Lake (Woss and Vernon lakes are part of this drainage system as well). This isolation is more apparent when the kokanee types are considered alone (Figure 9). The CLM kokanee immediately separates into a group of its own with all the other populations clustering into another, less divergent group.

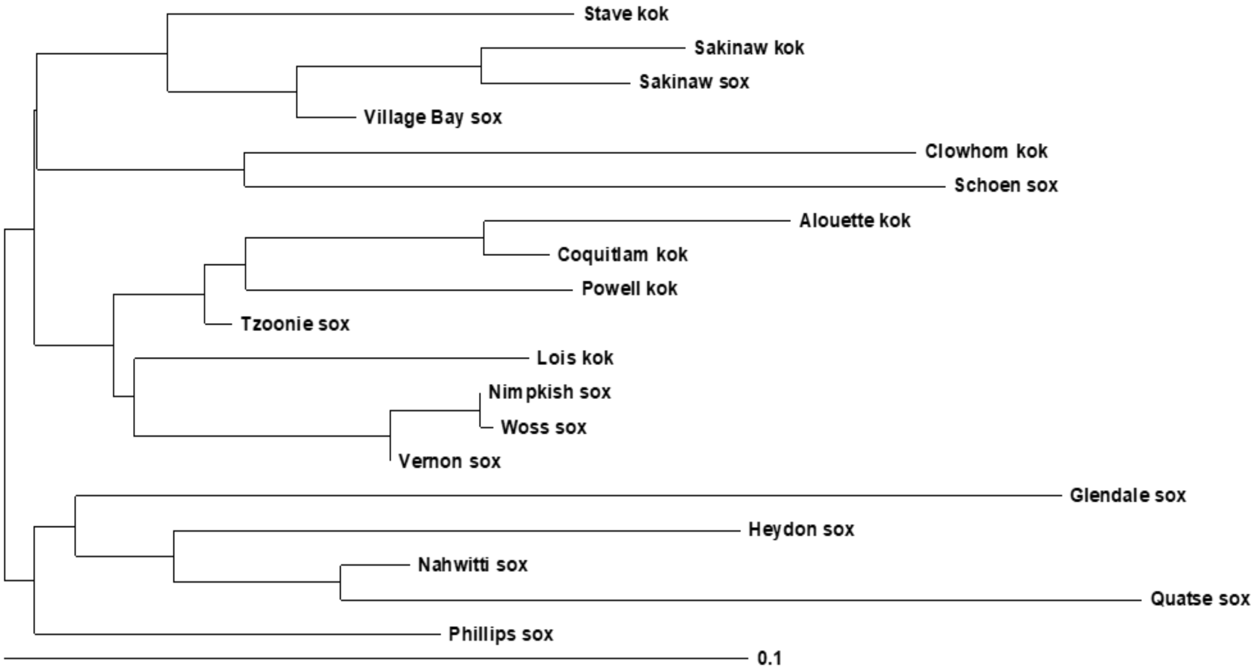

Figure 8 Phylogenetic dendrogram illustrating the divergence of sockeye/kokanee populations located in the lower mainland, south coast and each Vancouver Island (Table 1) based on pairwise F_{ST} values.

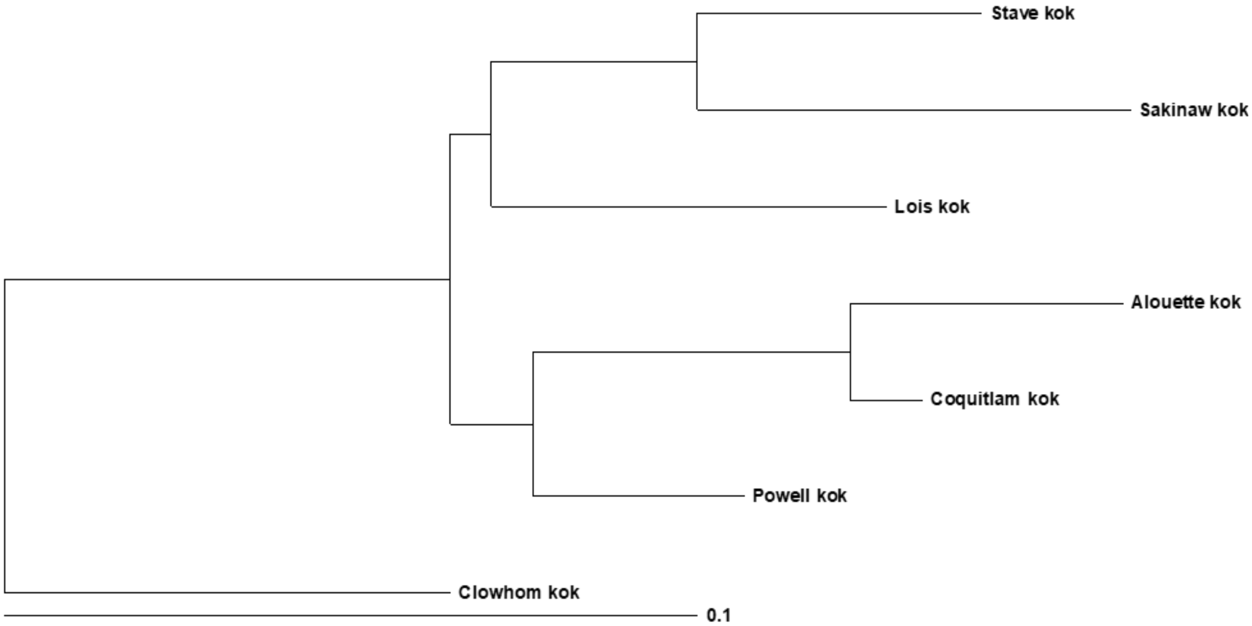

Figure 9. Phylogenetic dendrogram illustrating the divergence of kokanee populations located in the lower mainland and south coast regions (Table 1) based on pairwise F_{ST} values. Note the immediate divergence of Clowhom kokanee from the rest of the population.

4 Discussion

Results of this study are consistent with the notion that CLM kokanee were isolated well before impoundment. Neither expected heterozygosity or allelic richness values are indicative of a population that recently lost a large proportion of its individuals through smolt outmigration and then had its in migrating spawners blocked from re-entering the Clowhom river by an impassable dam. In the case of Alouette Lake Reservoir where this had indeed occurred, both expected heterozygosity (63) and allelic richness (5.3) values were at the lower end of the range of possible values. In fact, a more robust test of this hypothesis was carried out by Godbout et al. (2011) where they were able show that there was a high probability that the ALU kokanee population had recently experienced a bottleneck event, most likely the result of impoundment. Among the CLM kokanee, expected heterozygosity (66) and allelic richness (6.9) values were more mid-ranged, indicating a relatively stable population over time. Its should be stressed that this test is far from definitive. In the same study by Godbout et al. (2011), they were able to detect a similar high probability of a recent population bottleneck among COQ kokanee, also likely linked to impoundment. Expected heterozygosity (67) and allelic richness (6.2) values however, were closer to those of CLM kokanee. The reason for this is uncertain. Its possible that this may be related to the time since impoundment.

Coquitlam Lake was first impounded in 1892 by a log crib dam, which was later replaced by a concrete structure in 1915 (CRWR, 2019). Alouette Lake however, was impounded in 1925; 31 years later (ARMS, 2019). Thus, COQ kokanee may have had more time (i.e., generations) to recover from the bottleneck event than ALU kokanee; assuming that mutation rates can exceed the effects of genetic drift in the newly isolated kokanee population. Genetic recovery following a founding or bottleneck event, in the absence of in-migration, is a well stablished theory in genetics (Nei et al. 1975) and has been noted in many founding or bottlenecked populations (e.g., Combs et al. 2001, Du et al. 2016 and Demastes et al. 2018.). It is therefore likely in fish as well, though the rate of recovery remains unknown. Nonetheless, if genetic recovery is indeed taking place, a much stronger reduction in allelic richness and expected heterozygosity would have been be expected in the CLM kokanee than either the COQ and ALU populations, simply because there has been less time for recovery. This was clearly not the case and lends credence to the idea that LM kokanee had not recently undergone a bottleneck event related to impoundment and that in turn were likely kokanee pre-impoundment and not a seagoing sockeye.

Genetic divergence analysis found the CLM kokanee to be a unique population largely unrelated to kokanee or sockeye populations in neighboring watersheds, suggesting they may have been isolated for an extended period of time. In other words, there has been little to no gene flow into the population by sockeye stays from nearby systems. The gene flow from straying fish has been demonstrated in the Alouette watershed where COQ sockeye spawners were found (ALU and COQ are neighboring watersheds), and is captured in the divergence dendrogram of Figure 8 with the close pairing of ALU and COQ populations. This was also found between the sympatric sockeye and kokanee populations of Sakinaw Lake and the clustering of the Nimpkish, Woss and Vernon lake populations (all in the same watershed). The closest paring one would have expected with the CLM population was with the Tzoonie River Sockeye, located in the headwaters of Narrows Inlet. These two populations however, showed a high degree of divergence, indicating that there hasn’t been significant gene flow between populations. This further supports the notion that CLM kokanee were an isolated population prior to impoundment and were unlikely residualized anadromous sockeye. The fact that CLM kokanee are so unique compared to all other reference kokanee populations (Figure 9) suggests that the potential existence of a sympatric sockeye population at the time of impoundment was also unlikely.

Analysis of CLM kokanee biometric data found these fish to be slow-growing compared to ALU and COQ populations, and in poorer condition. Size-at-age and conditions factors were however similar to that of SFN kokanee. The implications of this are uncertain as little is known of the population's abundance, availability of food resources and potential reproductive capacity. A small size-at-age could be solely the result of a nutrient limitation in the lake, limiting fish growth by acting on it's zooplankton population, their primary source of food (Harris et al., 2010, Hyatt et al., 2011). Alternatively, small sized fish could occur independently of nutrient concentration, where there is strong annual recruitment and low predation, creating a situation where there may too many fish foraging for a finite food resource. Hyatt et al. (2011) was able to show that the latter, commonly referred to as density dependent growth, is atypical for natural lake systems, and that it is only possible with exceptionally high fish densities. This was indeed found to be the case for ALU kokanee, where the size of age 3+ fish was found to be independent of their abundance, despite a 5-fold range (Andrusack and Irvine 2010). This is likely the case for CLM as well, though the potential for density dependence growth cannot be ruled out entirely due to the absence of population data suitable for hypothesis testing. The effects of food limitation however, can be explored at a cursory level if one were to assume a direct correlation with the concentration of total dissolved phosphorus (Randall et al. 1995, Wetzel 2001).

Total dissolved phosphorus concentrations in CLM averaged 1.5 μg/L in February 2002 (Bruce 2003), while the multi-year/season averages for SFN, ALU and COQ were 1.3 μg/L (2001 – 2014; Stables and Perrin 2016), 2.7 μg/L (2003 – 2008; Harris et al. 2010) and 1.8 μg/L (2003 – 2008; Mavinic et al. 2012) respectively. A plot of these nutrient concentrations and respective size-at-age data for 2+ and 3+ kokanee (Figure 10) suggests a strong correlation between the two variables, indicating nutrient limitation is a likely factor. Furthermore, it would appear that CLM is similarly nutrient deficient to SFN, explaining the similarity in size-at-age. This could also explain the differences in condition factor between CLM, COQ and ALU kokanee populations. While density dependent growth is unlikely but

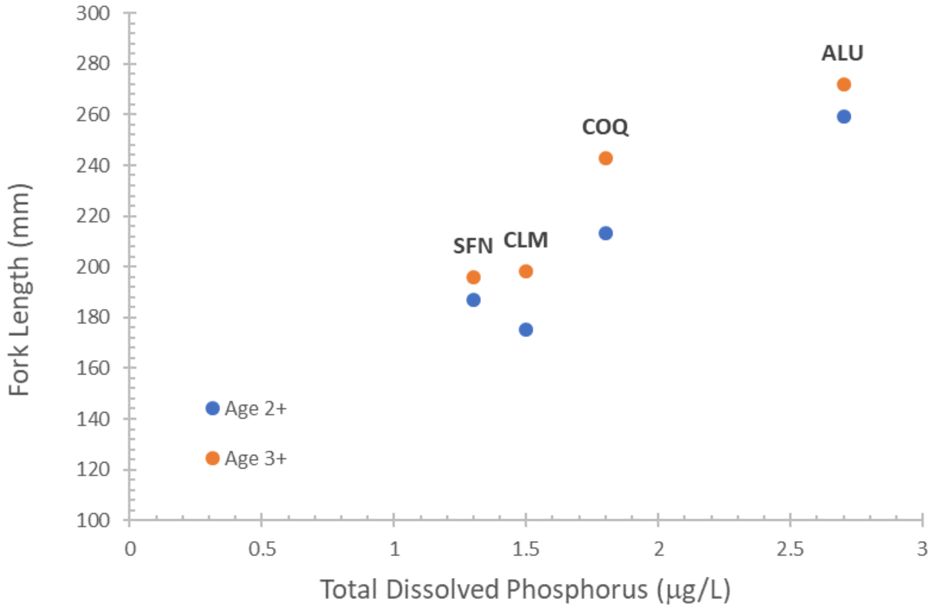

Figure 10. Plot of fork average fork length of kokanee in the Stave Lake (SFN), Coquitlam Lake (COQ), Alouette Lake (ALU) and Clowhom Lake (CLM) reservoirs as a function of average total dissolved phosphorus concentration for age 2+ and 3+ fish.

cannot be ruled out, the available evidence does suggest a strong link between small size-at-age statistics and nutrient limitation. It should be stressed that other density independent factors such as, nitrogen availability, water temperature and water residence times also play a role on zooplankton species composition and availability (Wetzel 2001, Obereggen et al. 2007) and hence the availability of food for growing kokanee. Terrestrial insects may also play a role.

Recently, researchers have begun to differentiate between two phenotypes of kokanee; a 'regular kokanee which become brightly colored prior to spawn (green heads and red bodies), and 'black' kokanee which as the name suggests, turn black (Moreira and Taylor 2015). Black kokanee have been observed in several lakes in the interior of BC, as well as in the lower mainland and south coast (Bartlett, 2017). In addition to the lack of pre-spawn coloration, black kokanee tend to spawn later in the winter than regular kokanee, and are deep water lake spawners rather that creek spawners (Moreira and Taylor 2015). The phylogenetic type of kokanee in CLM is unknown. However, that fact that no fish has ever been caught (in September) with pre-nuptial colouration suggests a later spawning window, and no late fall/winter spawning has ever been reported in any of the to tributaries to the CLM reservoir. Both anecdotal observations suggest CLM kokanee may be the black type. However, regular fish sampling and creek surveys done throughout the fall and winter would be needed to confirm whether this is the case.

5 Conclusions

Results of the genetic analysis done on CLM kokanee showed that these fish were a distinct population unrelated to kokanee or sockeye population in nearby lake systems, suggesting these fish have been isolated for an extended period of time. Indices of genetic diversity were typical of other populations and did not show evidence of a recent bottleneck event (in this case impoundment) that would have resulted in a dramatic loss of diversity. These results are consistent with the notion that CLM kokanee are not the result of an anadromous sockeye population that was residualized as a result of impoundment. The uniqueness of the CLM kokanee compared to neighboring populations also suggests that there was no sympatric sockeye population using the lake at the time of impoundment. Though the results of this study are not conclusive, the genetic evidence does strongly suggest that CLM's nerkid population consists entirely of kokanee that were isolated long before impoundment of this lake system. It also addresses Priority Action COM.RLR.RI.09.01 (Conduct research to determine if the Clowhom lakes used to support anadromous salmon-P2) as it pertains to kokanee; i.e., in Clowhom Lake reservoir, there is no evidence to suggest that resident kokanee were anadromous prior to impoundment. There are more conclusive paleolimnological methods that can be employed to be more certain, but use of such methods - due to the high costs involved - should wait until new evidence surfaces that counters or offers an alternative explanation for the present study results.

CLM kokanee were considered small for their age compared to those of the reference populations in ALU and COQ, but were similar in size to the SFN kokanee. They were also in poorer condition. The differences appeared to be related to the availability of nutrients (in particular, total dissolved phosphorus) and its effects of food availability. The evidence for this however, is circumstantial. Little is actually known about the food resources available to kokanee in the reservoir, nor the abundance of these individuals in relation to this food availability. Furthermore, no kokanee fry have ever been caught in the reservoir and few 1+ fish. As a result, this study only partly addresses Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1). It should be noted that kokanee appear to be the main source of prey for cutthroat and Dolly Varden in the reservoir

(Bruce 2003). Few non-sport fish other than cottids and a few three-spine sticklebacks have been caught in the reservoir, despite the years of survey carried out (Bruce 2003, Bates and Coombs 2012). Thus, the health and abundance of kokanee may also have implications on the productivity of other salmonid species in the reservoir. Clearly more study is required in this area.

Little is known about CLM kokanee ecology. Anecdotal evidence suggests that they may be a rare type of kokanee referred to a 'black' kokanee that do not become brightly colored at the time of spawn, and possibly spawn much later in the winter than most other sockeye/kokanee populations. Where they spawn is unknown, as is their fecundity and recruitment success in light of reservoir operations. Because these aspects are unknown, it is unclear whether impoundment has had an impact on these fish, particularly if they are shoal spawners as some populations appear to be (Moreira and Taylor 2015, Bartlett 2017). Future studies should focus on resolving these uncertainties, along with a general indication of population size that can be compared to the COQ, ALU and SFN reservoirs, to help assess whether conservation action is warranted. Such work would align with Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1) with particular emphasis on the availability of suitable spawning habitat.

The gillnetting techniques used in this study were only marginally successful due to the effects of unexpectedly high winds on the buoys used to mark the gill net ends. Use of smaller buoys and perhaps heavier anchors should resolve this problem in future applications. When successfully implemented, the gillnet techniques used did prove to be highly selective for kokanee of all ages ≥ 1+ years.

6 Recommendations

Results of the present study have led to the following recommendations:

1. No further study be considered regarding past anadromy of Clowhom Lake Reservoir salmon (Priority Action COM.RLR.RI.09.01 (Conduct research to determine if the Clowhom lakes used to support anadromous salmon-P2)) unless new evidence surfaces to suggest these fish were indeed anadromous.
2. Carry out future studies to assess food abundance for kokanee in their pelagic habitat through repeated zooplankton sampling and kokanee stomach content analyses over a single summer growing period (April – October). Aligns with Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1)
3. Carry out kokanee abundance surveys – preferably using hydro-acoustic sampling techniques. This not only aligns with Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1) for kokanee, but also for the piscivorous salmonids in the reservoir that rely on kokanee as prey (Cutthroat trout, Dolly Varden, and maybe rainbow trout)
4. Carry out regular spawning surveys over a single winter to determine: 1) timing and location of kokanee spawning and whether spawning habitats were impacted by impoundment and/or at risk due to reservoir operations; 2) fecundity; and 3) morphological changes resulting from maturation. The first two aspects align with Priority Action COM.ALL.RI.02.02 (Conduct a limiting factors analysis-Clowhom Lake & tributaries-P1) with an emphasis on spawning habitat. The third aspect will be used to identify kokanee type.

7 References

Alouette River Management Society, Historical timeline of the Alouette Watershed. Accessed May 2019. http://www.alouetteriver.org/history-watershed.html

Allendorf, F.W. 1985. Genetic drift and the loss of alleles versus heterozygosity. Zoo. Biology 5(2):181 – 190.

Andrusack, G. and J. Irvine. 2010. Alouette Project Water Use Plan: Kokanee age-structure population analysis (ALUMON # 6). Report prepared by Redfish Consulting Ltd. and Poisson Consulting Ltd. for BC Hydro, Water Use plans and BC Ministry of Environment. April 2010. 22p.

Bartlett, M.C. 2017. Minihumps: Characterizing a Coastal British Columbia Kokanee Population Recently Derived due to Anthropogenic Environmental Change. Master's of Science Thesis. Department of Ecology and Evolutionary Biology, University of Toronto. 91 p.

BC Hydro. 2003. Consultative Committee Report: Clowhom Water Use Plan. Executive Summary available at: https://www.bchydro.com/content/dam/hydro/medialib/internet/documents/environment/pdf/wup_clowhom_executive_summary_pdf.pdf

BC Hydro. 2005. Clowhom Project Water Use Plan. Revised for Acceptance by the Comptroller of Water Rights. Available at: https://www.bchydro.com/content/dam/hydro/medialib/internet/documents/environment/pdf/wup_clowhom_water_use_plan_pdf.pdf

Fish and Wildlife Compensation Program (FWCP). 2017. Clowhom River Watershed Action Plan. September 28, 2017. Version 9.0 (FINAL DRAFT). Available at: fwcp.ca/app/uploads/2017/10/Action-Plan-Coastal-Region-Clowhom-River-Watershed-FINAL-DRAFT-Sept-28-2017.pdf

Beacham, T.D., Candy, J.R., McIntosh, B., MacConnachie, C., Tabata, A., Kaukinen, K., Deng, L., Miller, K.M., Withler, R.E., and Varnavskaya, N.V. 2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Trans. Am. Fish. Soc. 134(5): 1124–1146.

Bates, D.J. and O. Coombs. 2012. Clowhom Lake Water Use Plan: Fish Productivity Monitoring COMMON-2. Implementation Year 5 (Study Period: 2006 – 2010). Prepared by FSCI Biological Consultants and Sechelt Indian Band, Resource Management Department. Sechelt, BC. May 2012. 14 p.

Bruce, J., 2003. Results of fish studies – Clowhom Lake Water Use Plan. BC Hydro Technical Note Com-FTC-TN-001. Burnaby, BC. 31 p.

Bussanich, J., Murray, B., and R. Bocking. 2005. Assessment of fish populations & lakeshore spawning habitat in Coquitlam reservoir – year 1; Studies to address fish populations and reservoir ecology,

2004. Prepared by LGL Limited for BC Hydro Fish and Wildlife Compensation Program. BCRP Report No. 04.Co.03. 38p. + App.

Comps, B., Gomory, D., Letouzey, J., Thiebaut B., and R. J. Petit. 2001. Diverging Trends Between Heterozygosity and Allelic Richness During Postglacial Colonization in the European Beech. Genetics 157: 389–397.

Coquitlam River Watershed Round Table (CRWR), Coquitlam Lake Dam. Accessed May 2019. https://www.coquitlamriverwatershed.ca/coquitlam-lake-dam/

Demastes, J.W., Hafner, D.J., Hafner, M.S., Light, J.E. and T.A. Spradling. 2018. Loss of genetic diversity, recovery, and allele surfing in a colonizing parasite, Geomydoecus aurei. Molecular Ecology 28(4). DOI: 10.1111/mec.14997. Available at: http://works.bepress.com/james_demastes/32/

Du, Y., Zou, X., Xu, Y., Guo, X, Li, S., Zhang, X., Su, M., Ma, J. and S. Guo. 2016. Microsatellite Loci Analysis Reveals Post-Bottleneck Recovery of Genetic Diversity in the Tibetan Antelope. Scientific Reports 6:35501. 7 p. DOI: 10.1038/srep35501. (https://www.nature.com/articles/srep35501.pdf)

Fagerland, M. W. 2012. T-tests, non-parametric tests, and large studies—a paradox of statistical practice? BioMed Central Medical Research Methodology. 12: 78. https://doi.org/10.1186/1471-2288-12-78.

Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) Version 3.6. Department of Genome Sciences, University of Washington, Seattle, Wash.

Fish and Wldlife Compensation Progran (FWCP). 2017. Clowhom River Watershed Action Plan. Version 9.0. Available at: https://www.fwcp.ca/app/uploads/2017/10/Action-Plan-Coastal-Region-Clowhom-River-Watershed-FINAL-DRAFT-Sept-28-2017.pdf

Godbout, L., Wood, C.C., Withler, R.E., Latham, S., Nelson, R.J., Wetzel, L., Barnett-Johnson, R., Grove, M.J., Schmitt, A.K., and K.D. McKeegan. 2011. Sockeye salmon (*Oncorhynchus nerka*) return after an absence of nearly 90 years: a case of reversion to anadromy. Can. J. Fish. Aquat. Sci. 68: 1590–1602.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3) [online]. Available from http://www2.unil.ch/popgen/softwares/fstat.htm [accessed April 2018].

Harris, S.L., Andrusak, H., Andrusak, G., Sebastian, D., Scholten, G., Weir, T., Vidmanic, L., Johnson, E.J. and N.E. Down. 2010. The Alouette Reservoir nutrient restoration program, 2003-2008. Fisheries Project Report No. RD129. BC Ministry of Environment, Aquatic Ecosystem Protection Branch. 102p.

Hyatt, M.H. and W.A. Hubert. 2000. Proposed Standard-Weight (W_s) Equations for Kokanee, Golden Trout and Bull Trout, Journal of Freshwater Ecology, 15:4, 559-563.

Hyatt, K.D., McQueen, D.J., Rankin, D.P., and E. Demers. 2011. Density-Dependent Growth in Juvenile Sockeye Salmon (Oncorhynchus nerka). The Open Fish Science Journal 4: 53-65.

Mavinic, D.S., Stockner, J.G., Ward, P.R.B., and S.E. Hrudey. 2012. Drinking Water Quality with Sockeye Salmon Introduction in Coquitlam Reservoir. Metro Vancouver Expert Panel Report, October 2012. 75 p.

Moreira, AL and E.B. Taylor. 2015. The origin and genetic divergence of “black” kokanee, a novel reproductive ecotype of *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 72: 1–12

Nei, M., T. Maruyama and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution. 29:1-10.

Plate, E.M., Johnson, P.N., Bocking, R.C. and D. Robichaud. 2012. Assessment of kokanee abundance and biomass in Coquitlam reservoir, 2011. Prepared by LGL Limited Environmental Research Associates and D. J. Degan, Aquacoustics, Inc. for BC Hydro Fish and Wildlife Compensation Program. Report No. EA3226A. 57p.

Randall, R.G., Kelso, J.R.M. and C.K. Mimns. 1995. Fish production in freshwaters: Are rivers more productive than lakes? Can. J. Fish. Aquat. Sci. 52: 631-643.

RIC. 1997. Fish Collection Methods and Standards. Version 4. Prepared by the B.C. Ministry of Environment, Lands and Parks, Fish Inventory Unit for the Aquatic Ecosystems Task Force, Resources Inventory Committee (RIC). January 1997. 58 p.

Smith, S.B. and P.A. Larkin. 1950. Report on the Clowhom lakes investigation. Unpubl. Report, B.C. Game Branch, Vancouver, BC. 3 p

Stables, T.B., and C.J. Perrin. 2016. Abundance and biomass of fish in Stave Reservoir 2005-2014. Report prepared by Limnotek Research and Development Inc. and Shuksan Fisheries Consulting for BC Hydro. 105 p.

Obereggen, U., Flaim, G., Braioni, M.G., Sommaruga, R., Corradini, F. and A. Borsato. 2007. Water residence time as a driving force of zooplankton structure and succession. Aquat. Sci. 69: 575–583.

Welch, B. L. 1947. The generalization of "Student's" problem when several different population variances are involved. Biometrika. 34 (1–2): 28–35

Withler, R. E., K. D. Le, R. J. Nelson, K. M. Miller, and T. D. Beacham. 2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon, *Oncorhynchus nerka*, populations of the Fraser River, British Columbia, Canada. Can. J. of Fish. and Aquat. Sci. 57:1985–1998.

8 Appendices

Appendix A. Summary of catch and fish biometric data from all fish surveys in Clowhom Lake Reservoir (2003 – 2018)

Fish #	Date	Net	Panel	Mesh	Length (mm)	WW (g)	K_F	W_s	W_r	Age
1	2018-09-06	1	5	3	150	38.5	1.14	41.8	0.92	1+
2	2018-09-06	1	5	3	144	35.5	1.19	36.9	0.96	1+
3	2018-09-06	3	2	1.5	204	97.0	1.14	106.2	0.91	3+
4	2018-09-06	3	4	2	209	110.6	1.21	114.3	0.97	3+
5	2018-09-06	3	3	3.5	195	88.7	1.20	92.6	0.96	3+
6	2018-09-06	3	3	3.5	185	77.2	1.22	78.9	0.98	2+
7	2018-09-06	3	3	3.5	205	96.1	1.12	107.8	0.89	3+
8	2018-09-06	3	3	3.5	201	95.6	1.18	101.5	0.94	3+
9	2018-09-06	3	3	3.5	200	89.2	1.12	100.0	0.89	3+
10	2018-09-06	3	3	3.5	180	64.2	1.10	72.6	0.88	2+
11	2018-09-06	3	3	3.5	186	73.4	1.14	80.2	0.91	2+
12	2018-09-06	3	3	3.5	178	68.6	1.22	70.2	0.98	2+
13	2018-09-06	3	3	3.5	200	87.0	1.09	100.0	0.87	3+
14	2018-09-06	3	3	3.5	189	82.9	1.23	84.2	0.98	2+
15	2018-09-06	3	5	3	200	95.3	1.19	100.0	0.95	3+
16	2018-09-06	2	4	2	141	32.8	1.17	34.6	0.95	1+
17	2018-09-07	3	2	1.5	174	61.6	1.17	65.5	0.94	2+
18	2018-09-07	3	2	1.5	204	98.2	1.16	106.2	0.92	3+
19	2018-09-07	3	2	1.5	210	113.1	1.22	115.9	0.98	3+
20	2018-09-07	3	2	1.5	165	51.8	1.15	55.8	0.93	2+
21	2018-09-07	3	2	1.5	176	65.0	1.19	67.9	0.96	2+
22	2018-09-07	3	2	1.5	175	67.1	1.25	66.7	1.01	2+
23	2018-09-07	3	2	1.5	174	63.0	1.20	65.5	0.96	2+
24	2018-09-07	3	2	1.5	201	95.6	1.18	101.5	0.94	3+
25	2018-09-07	3	2	1.5	178	66.9	1.19	70.2	0.95	2+
26	2018-09-07	3	2	1.5	200	93.2	1.17	100.0	0.93	3+
27	2018-09-07	3	2	1.5	171	65.1	1.30	62.2	1.05	2+
28	2018-09-07	3	2	1.5	185	66.6	1.05	78.9	0.84	2+
29	2018-09-07	1	6	1	139	27.8	1.04	33.2	0.84	1+
30	2018-09-07	1	6	1	194	87.5	1.20	91.2	0.96	2+
31	2018-09-07	1	6	1	147	37.2	1.17	39.3	0.95	1+
32	2018-09-07	1	6	1	142	32.4	1.13	35.4	0.92	1+
33	2018-09-07	1	6	1	134	26.7	1.11	29.7	0.90	1+
34	2018-09-07	1	6	1	140	28.6	1.04	33.9	0.84	1+
35	2002-09-04	-	-	-	185.0	67.00	1.06	78.9	0.85	3+
36	2002-09-05	-	-	-	99.0	11.00	1.13	11.8	0.93	1+
37	2002-09-07	-	-	-	187.0	66.00	1.01	81.6	0.81	3+
38	2002-09-07	-	-	-	192.0	76.50	1.08	88.3	0.87	3+
39	2002-09-07	-	-	-	190.0	77.00	1.12	85.6	0.90	3+
40	2002-09-07	-	-	-	167.0	43.50	0.93	57.9	0.75	2+
41	2002-09-07	-	-	-	197.0	83.00	1.09	95.5	0.87	3+
42	2006-09-19	-	-	-	177	62.2	1.12	69.0	0.90	1+
43	2008-09-24	-	-	-	135	28.3	1.15	30.4	0.93	2+
44	2008-09-24	-	-	-	202	99.8	1.21	103.1	0.97	3+
45	2010-09-17	-	-	-	200	99.0	1.24	100.0	0.99	3+
46	2010-09-17	-	-	-	190	77.0	1.12	85.6	0.90	3+
47	2010-09-17	-	-	-	185	66.0	1.04	78.9	0.84	3+
48	2010-09-17	-	-	-	160	45.0	1.10	50.8	0.89	2+

Appendix B. Pairwise F_{ST} values (above diagonal) and significance of allele frequency differentiation (below diagonal) between lower mainland, south coast and east coast Vancouver Island kokanee and sockeye populations. Note that all populations have significantly different allele frequencies ($P < 0.05$) except Nimpkish and Woss sockeye (N.S. $P > 0.05$). The two tables are a continuation on one another.*

	Coquitlam Kok	**Stave Kok**	**Alouette Kok**	**Clowhom Kok**	**Lois Kok**	**Powell Kok**	**Sakinaw Kok**	**Glendale Sox**	**Heydon Sox**
Coquitlam Kok	-	0.1248	0.0466	0.1842	0.1243	0.0909	0.1377	0.2097	0.1630
Stave Kok	*	-	0.1579	0.1853	0.1088	0.1319	0.0982	0.1911	0.1975
Alouette Kok	*	*	-	0.1992	0.1517	0.0992	0.1790	0.2286	0.1797
Clowhom Kok	*	*	*	-	0.1763	0.1544	0.1989	0.2256	0.2055
Lois Kok	*	*	*	*	-	0.0908	0.1491	0.1819	0.1304
Powell Kok	*	*	*	*	*	-	0.1339	0.2315	0.1542
Sakinaw Kok	*	*	*	*	*	*	-	0.1855	0.2268
Glendale Sox	*	*	*	*	*	*	*	-	0.2040
Heydon Sox	*	*	*	*	*	*	*	*	-
Nahwitti Sox	*	*	*	*	*	*	*	*	*
Nimpkish Sox	*	*	*	*	*	*	*	*	*
Phillips Sox	*	*	*	*	*	*	*	*	*
Quatse Sox	*	*	*	*	*	*	*	*	*
Sakinaw Sox	*	*	*	*	*	*	*	*	*
Schoen Sox	*	*	*	*	*	*	*	*	*
Vernon Sox	*	*	*	*	*	*	*	*	*
Village Bay Sox	*	*	*	*	*	*	*	*	*
Woss Sox	*	*	*	*	*	*	*	*	*

	Nahwitti Sox	**Nimpkish Sox**	**Phillips Sox**	**Quatse Sox**	**Sakinaw Sox**	**Schoen Sox**	**Vernon Sox**	**Village Bay Sox**	**Woss Sox**
Coquitlam Kok	0.1094	0.0951	0.1048	0.1930	0.1124	0.1702	0.0773	0.0907	0.1000
Stave Kok	0.1308	0.1427	0.1161	0.2108	0.0943	0.1712	0.1272	0.0916	0.1465
Alouette Kok	0.1327	0.1263	0.1581	0.2201	0.1480	0.2088	0.1062	0.1286	0.1327
Clowhom Kok	0.1539	0.1649	0.1659	0.2387	0.1782	0.1682	0.1434	0.1473	0.1567
Lois Kok	0.1127	0.0885	0.1366	0.1878	0.1420	0.1670	0.0808	0.1146	0.0886
Powell Kok	0.1135	0.0923	0.1510	0.2040	0.1093	0.1657	0.0771	0.0911	0.0994
Sakinaw Kok	0.1556	0.1586	0.1341	0.2210	0.0365	0.1504	0.1420	0.0622	0.1632
Glendale Sox	0.1841	0.2048	0.1837	0.2174	0.1828	0.2232	0.1783	0.1722	0.2054
Heydon Sox	0.1003	0.1361	0.1345	0.1802	0.1782	0.2418	0.1193	0.1583	0.1334
Nahwitti Sox	-	0.1267	0.0957	0.1039	0.1146	0.1686	0.1034	0.1038	0.1231
Nimpkish Sox	*	-	0.1201	0.2017	0.1434	0.1543	0.0044	0.1063	0.0011
Phillips Sox	*	*	-	0.1918	0.1080	0.1750	0.1041	0.1016	0.1156
Quatse Sox	*	*	*	-	0.1811	0.2599	0.1843	0.1977	0.1956
Sakinaw Sox	*	*	*	*	-	0.1627	0.1277	0.0325	0.1453
Schoen Sox	*	*	*	*	*	-	0.1238	0.1436	0.1606
Vernon Sox	*	*	*	*	*	*	-	0.0889	0.0074
Village Bay Sox	*	*	*	*	*	*	*	-	0.1081
Woss Sox	*	N.S	*	*	*	*	*	*	-