Minihumps: Characterizing a Coastal British Columbia Kokanee Population Recently Derived due to Anthropogenic Environmental Change

by

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Department of Ecology and Evolutionary Biology
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Abstract

Sockeye Salmon have high levels of intraspecific diversity exhibiting multiple life histories and forms that allow it to make use of diverse and dynamic environments. Kokanee is the freshwater resident form of Sockeye Salmon. The Mini Hump Creek kokanee population, which inhabits a coastal lake in British Columbia, shares morphological features similar to the unique Black kokanee ecotype found in three populations from Japan and British Columbia. Unlike black kokanee, Mini Hump Creek kokanee spawn in shallow creeks. The evolution of the Mini Hump kokanee life history and morphological traits are potentially a response to environmental changes including water level changes and barriers to migration, which originated with logging and dam building in the 20th century. Genetic analysis using microsatellites confirms that the population clusters with other coastal kokanee in British Columbia, yet is genetically distinct from the coastal kokanee populations and is independent from other black kokanee populations.

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1 Introduction

Intraspecific diversity is important to conserve biodiversity (Moritz, 2002). Species with high intraspecific diversity can remain at a steady abundance and buffer inter-annual changes in climate by offering a portfolio of phenotypic variation that respond differentially to environmental variation (Schindler et al., 2010). For example, the Sockeye Salmon (*Oncorhynchus nerka*) stock complex in Bristol Bay contains hundreds of locally adapted populations that are responsible for the most valuable fishery in the United States of America (Schindler et al., 2010). Each year, certain locally adapted stocks return in high abundance while others return in low abundance which is the result of interannual environmental variation, yet the overall abundance of Sockeye Salmon in the Bristol Bay stock complex remains high (Schindler et al., 2010). Contemporary changes to an environment, natural or anthropogenic, can result in local adaptations, adding to the intraspecific variation that enhances population persistence in relation to environmental change (Reznick & Ghalambor, 2001; Stockwell et al., 2003). Standing intraspecific variation in peppered moth (*Biston betularia*) populations in the United Kingdom allowed the populations to avoid extirpation by selecting for different cryptic pigmentation patterns due to environmental change brought on by air pollution and its reversal (Clarke, Mani, & Wynne, 1985). Isolation can also increase intraspecific variation by reducing gene flow and increasing divergence between populations (Garant et al., 2007). For example, sympatric resident (fish eating) and transient (mammal eating) killer whale (*Orcinus orca*) populations in the Eastern Pacific Ocean are genetically distinct and maintain reproductive isolation through foraging specialization which can reduce extirpation risks by diversifying prey dependence (Hoelzel et al., 1998).

Sockeye Salmon exhibit extensive levels of intraspecific diversity that is most notable in the diverse life histories of often sympatric populations (Lemay & Russello, 2015; Taylor & Foote, 1996). Sockeye Salmon and kokanee, the freshwater resident form of Sockeye Salmon, populations can be sympatric, overlapping spatially and temporally during spawn, yet are genetically isolated (Lemay & Russello, 2015; Taylor et al., 1997). This isolation is the result of varied life histories, spawning site fidelity and differing sexual selective pressures that can result

in outbreeding depression when hybridization does occur (Raig, 2001; Wood et al., 2008; Wood & Foote, 1996). Anadromous Sockeye Salmon select for large individuals to mate with, which limit the potential of breeding with kokanee, yet some kokanee males take the role of "sneak" males fertilizing eggs without directly competing with the large anadromous males (Craig & Foote, 2001). Hybrid anadromous Sockeye Salmon and kokanee progeny are less fit swimmers than anadromous Sockeye Salmon and are likely maladapted to marine survival and long migration events (Taylor & Foote, 1991). Sockeye Salmon populations are found in reproductive or ecological isolation throughout their range, which has led to a vast array of life histories and intraspecific variation (Blair et al., 1993; Nakabo et al., 2011; Taylor & Volpe, 1997). Here, I examine a novel population of Sockeye Salmon, which my results suggest was isolated due to dam building in the early 20th century, and characterize the phenotypic changes involved in its adaptation to a novel environment caused by the environmental changes associated with the dam building and logging.

Sockeye Salmon have high socioeconomic and ecological importance and is considered a keystone species (Noakes & Beamish, 2011; Willson & Halupka, 1995). Salmon returns, and specifically Sockeye Salmon, have been a subsistence food source for indigenous peoples living throughout the species range for thousands of years (Campbell & Butler, 2010). Sockeye Salmon continue to be important culturally and economically with commercial, recreational and First Nations Food, Social and Ceremonial (FSC) fisheries occurring annually. For example, in 2010 the Bristol Bay commercial Sockeye Salmon fishery was valued at over $1.5 billion in sales and associated activities (Knapp et al. 2013). The largest commercially important Sockeye Salmon Runs are located in Bristol Bay, Alaska, the Fraser, Skeena and Nass rivers in British Columbia and in Ozernaya River on the Kamchatka peninsula (Smith et al., 1987). Sockeye Salmon is ecologically important as with its anadromous and semelparous life history, it transfers large amounts of marine-derived nutrients from the ocean to its often oligotrophic, low nutrient, spawning watersheds each year (Naiman et al., 2002; Willson & Halupka, 1995). Salmon in all life stages support diverse marine, terrestrial and aquatic ecosystems including wolves, black bears, grizzly bears, marine mammals, bald eagles and countless shore and sea bird species as well as terrestrial invertebrates, aquatic invertebrates and freshwater fishes, which subsequently deposit nutrients throughout the ecosystems (Cederholm et al., 1999; Helfield & Naiman, 2006;

Willson & Halupka, 1995). Salmon predation by terrestrial carnivores can account for up to 24% of nitrogen input to riparian vegetation (Willson & Halupka, 1995).

Sockeye Salmon range throughout the northern Pacific basin using spawning rivers from northern Japan to northern California. Sockeye Salmon in its most common ecotype is a relatively large anadromous salmon spending one or two years in fresh water followed by two to three years in the ocean before returning to spawn (Healey, 1987). The intraspecific variation of anadromous Sockeye Salmon populations is structured by both the spawning grounds used and the way in which juveniles use fresh water. Anadromous Sockeye Salmon can spawn in rivers and lake shores while juvenile Sockeye Salmon often rear in lakes for a year. However, some populations have juveniles that rear in rivers for a year and others that migrate immediately to estuaries and the ocean in their first year (Groot & Margolis, 1991b).

1.1 **Kokanee**

Kokanee are non-anadromous Sockeye Salmon populations that spend their entire lives in fresh water (Ricker, 1938). Kokanee populations have independently arisen throughout the Sockeye Salmon range and are also present in introduced locations (Quinn, Graynoth, Wood, & Foote, 1998; Taylor & Foote, 1996; Taylor et al., 1997). Kokanee has been found to be genetically more closely related to sympatric anadromous Sockeye Salmon populations than to other geographically close kokanee populations (Taylor et al., 1997). This relationship implies that kokanee populations have formed independently and in parallel throughout its range as opposed to radiating from founding Sockeye Salmon populations after the last glacial maxima (Taylor & Foote, 1996). Some kokanee populations are believed to be recently formed by the introduction of migration barriers such as dams (Godbout et al., 2011). Kokanee are known to spawn in gravel substrate in rivers, on lake shores and even at depth in lakes. Kokanee are usually smaller and differ morphologically from sympatric Sockeye Salmon (Wood & Foote, 1996). Sympatric kokanee populations can exhibit smaller body size, higher gill raker numbers, reduced egg size and number and increased efficiency in carotenoid uptake (Foote et al., 1999; McGurk, 2000; Quinn et al. 2015; Raig, 2001; Wood & Foote, 1996).

1.2 **Black kokanee**

Black kokanee are another facet of Sockeye Salmon diversity, known from genetically distinct populations in Japan and British Columbia (Moreira & Taylor, 2015; Nakabo et al., 2011). Black kokanee are most easily differentiated from other kokanee by their dark colouration at spawn. They spawn at depth, on the lake bottom up to 50 m from the surface. The black kokanee spawning season stretches from September to February in contrast to regular Sockeye Salmon and kokanee that spawn in the fall. There are both historic and what are believed to be recently derived populations of black kokanee in BC (Godbout et al., 2011; Moreira & Taylor, 2015). The Anderson and Seton Lake populations (Figure 1), located in the central Fraser River drainage, known as Gwenish, they are sympatric with anadromous Sockeye Salmon populations, and are traditionally important to the First Nations that inhabited the region, which implies they have persisted in this ecotype and are not a recently derived population. Alouette Lake, located near the mouth of the Fraser River, contains what is believed to be a contemporary black kokanee population created by the introduction of a hydroelectric dam that created a full migration barrier for a pre-existing anadromous Sockeye Salmon population that inhabited the lake (Godbout et al., 2011). The dark and drab colouration is believed to be a result of spawning at depth where red colour no longer attenuates and a bright spawning colouration would not be visible (Moreira & Taylor, 2015). Transport of carotenoids to externally pigment skin is metabolically expensive so may be selected against if it is no longer advantageous (Moreira & Taylor, 2015). Sockeye Salmon exhibit mate choice at spawn associated with the secondary sexual characteristic of external red colouration (Craig & Foote, 2001). Mate choice associated with colouration has been observed across taxa and is associated with measures of foraging ability, disease resistance or other measures of fitness and is potentially responsible for driving speciation events (Maan & Seehausen, 2011). Thus, black kokanee appear to have lost their red colouration because the mate-choice benefit was outweighed by the cost of maintaining bright nuptial coloration when the trait was no longer visible when spawning at depth.

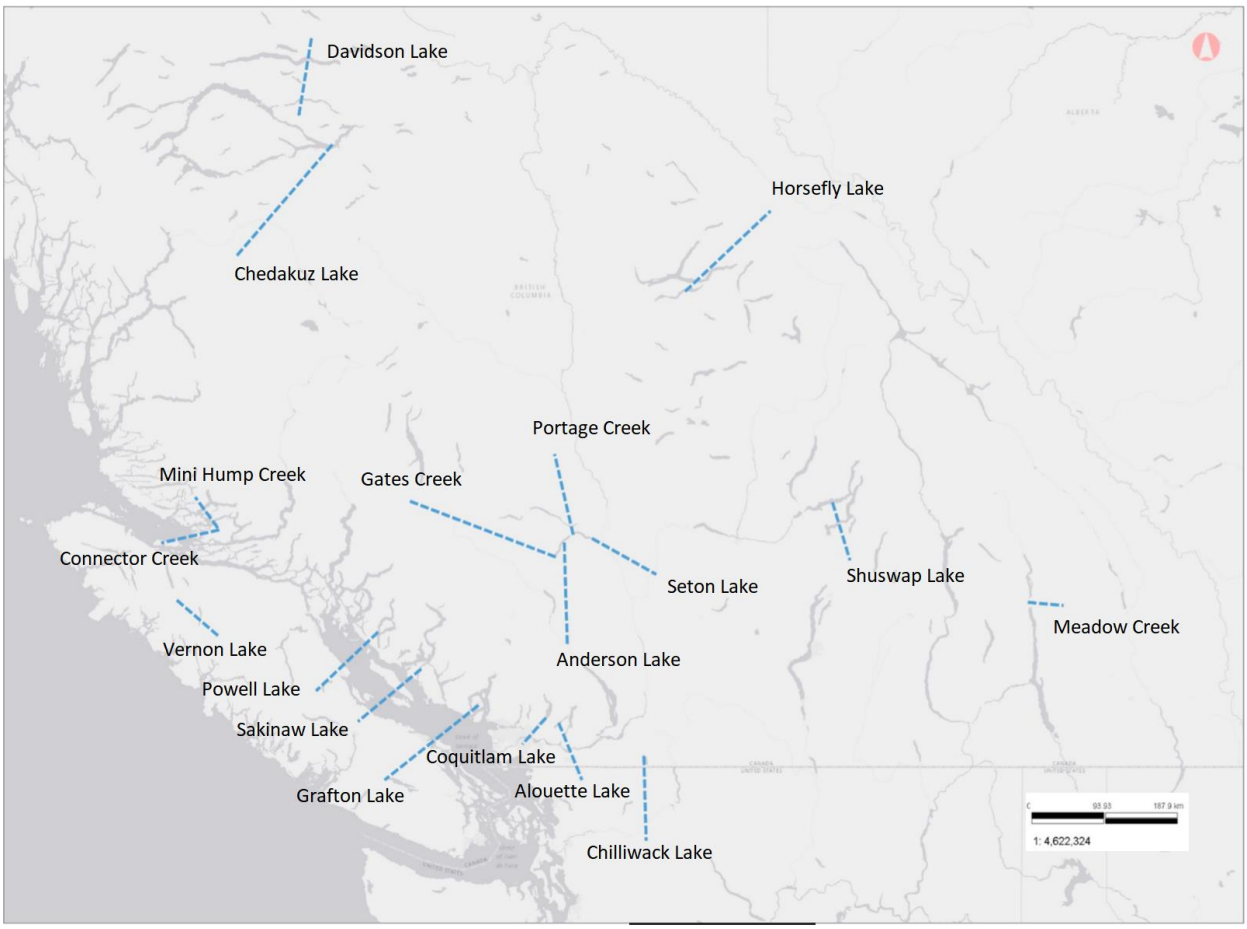

Figure 1 Geographic locations of the Sockeye Salmon populations used in the analysis. The Hansen Creek, Alaska and the Lake Saiko, Japan populations are from locations beyond this map.

1.3 Residual Sockeye Salmon

Residual Sockeye Salmon are kokanee that are the progeny of anadromous Sockeye Salmon that remain in the freshwater environment instead of smolting and heading out to sea (Ricker, 1938). They are drab in colouration at spawn and are smaller than the anadromous component of the spawning year class. Residual kokanee are thought to be a small component of an anadromous Sockeye Salmon population and consist mostly of males. Residual Sockeye Salmon are much like “jacks” or “jill’s”, a salmon that returns to spawn a year earlier than expected, except that they are acting as a potential evolutionary link between anadromous Sockeye Salmon and kokanee (Flain, 1970; Groot & Margolis, 1991a). Mature male parr have been found in Masu

Salmon (*Oncorhynchus masou*) populations in Japan, that are normally anadromous, where the males will mature at the end of their first year in fresh water and spawn before heading out to sea (Yamamoto, Edo, & Ueda, 2000). Some iteroparous Atlantic Salmon (*Salmo salar*) and introduced Chinook Salmon (*Oncorhynchus tshawytscha*) populations also exhibit mature male parr that can spawn in multiple events (Thomaz et al. , 1997; Unwin et al. , 1999). That there are some species such as Masu Salmon and Chinook Salmon that have populations with mature male parr indicates that these species, considered to be strictly anadromous, can nonetheless complete their life cycle entirely in fresh water. Therefore, residual Sockeye Salmon, mature male parr, and jacks highlight some of the phenotypic plasticity of salmon populations and their ability to respond to environmental variation.

1.4 Variations of anadromy in salmonid species

Several trout and salmon species exhibit both anadromous and freshwater resident populations, which indicates the adaptability and plasticity of salmon and trout populations to respond to differing environments. *Oncorhynchus mykiss* have both an anadromous form, steelhead and a freshwater resident form, Rainbow Trout, which can occur in sympatry and genetic analysis supports that they are polyphyletic and the two forms have arisen independently and in parallel throughout their range (Pearse et al., 2009). Chinook Salmon, Coho Salmon (*Oncorhynchus kisutch),* Pink Salmon (*Oncorhynchus gorbuscha)* and Chum Salmon (*Oncorhynchus* keta) typically anadromous species, have been reported to form freshwater resident populations when introduced to non-native lake systems, including Chinook Salmon in the Great Lakes and Coho Salmon in Kawkawa Lake, near Hope, BC (Hendry & Stearns, 2004; Hsu, 2016). Atlantic Salmon populations have extensive levels of phenotypic plasticity and exhibit diverse life histories with extremes ranging from freshwater resident females maturing at 10 cm in length and anadromous individuals spending upwards of five years at sea before returning to spawn and single populations displaying multiple life-history types in a single spawning year (Klemetsen et al., 2003). Brown Trout (*Salmo trutta*) has distinct freshwater and anadromous forms, spawn in brackish water and also have populations that have both resident and migratory components, which are polymorphic and contingent on growth rates and body size, and take advantage of diverse feeding habits from near-shore to off-shore (Klemetsen et al., 2003). Arctic Char

(*Salvelinus alpinus)*, Masu Salmon, Cutthroat Trout (*Oncorhynchus clarkii)* and Dolly Varden (*Salvelinus malma)* among other trout, whitefish and salmon species also exhibit diverse life history employing anadromy as well as freshwater residence in distinct populations or as components of single populations (Hendry & Stearns, 2004). Intraspecific variation and plasticity has allowed salmonids to expand beyond their native ranges and form persistent populations when introduced to non-native locations.

1.5 Mini Hump Creek kokanee

Mini Hump Creek kokanee, located on Gilford Island BC (Figure 2), represent another facet of Sockeye Salmon diversity sharing morphological similarities to black kokanee and residual Sockeye Salmon. The Mini Hump Creek population is relatively small with 186 to 2204 spawning mature salmon reported between 2009 and 2016 (Gagnon, 2016). Mini Hump Creek kokanee were first discovered in the 1970s during logging assessments by a local resident of Gilford Island (Proctor, pers. comm., 2016). The population was considered unique by local residents and fishers for their small body size, odd colouration, distinct morphology and low fecundity. The population was previously thought to be a landlocked Pink Salmon or Chum Salmon population or potentially a hybrid of the two species. Samples were collected and morphological assessments were completed by the fisheries guardian of the region and by Fisheries and Oceans Canada technicians at the Pacific Biological Station. The morphology of the sampled population did not match that of the identification keys at hand (Proctor, pers. comm., 2016). The population was thought to be related to Pink Salmon because of their small body size and being sexually dimorphic with the males developing a large dorsal hump at spawn and both sexes displayed dark vertical striations like Chum Salmon at spawn. Hump or humpy is a colloquial name for Pink Salmon, which led to the name of Mini Hump for the population. In 2010, genetic samples were collected from the spawning population and sent for genetic analysis by researchers from Salmon Coast Field Station; the results were at first inconclusive and initial interpretations were that there was a potential hybridization occurring with pink salmon (Rogers, pers. comm. 2015). On further analysis the Molecular Genetics Lab at Pacific Biological Station was quite certain the population was a Sockeye Salmon population.

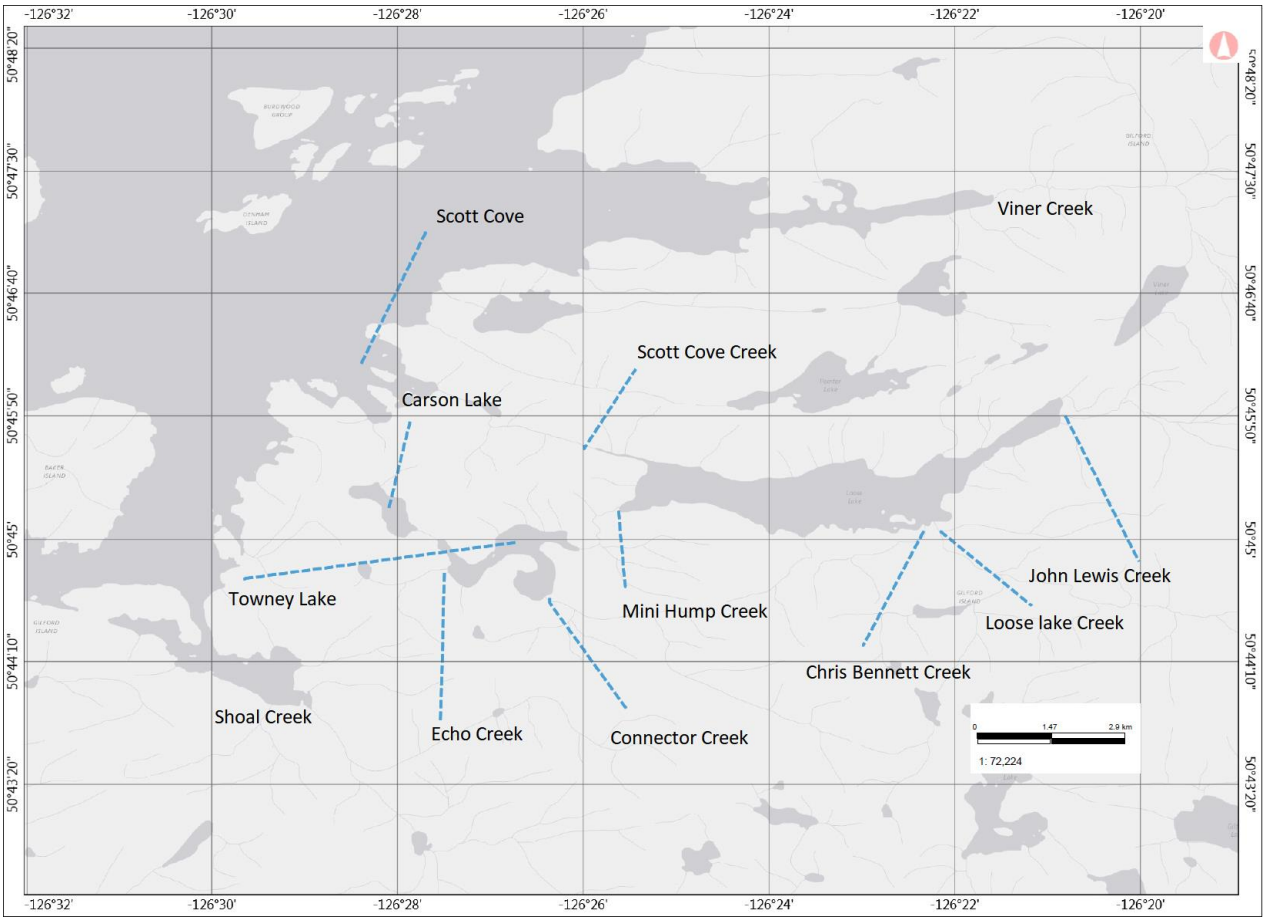

Figure 2 The Loose Lake system including streams enumerated 2009-2016. Mini Hump kokanee have only been sighted in Mini Hump Creek and Connector Creek.

1.6 Loose Lake system

Loose Lake is a coastal system located on Gilford Island, BC (50°45'15" N 126°23'40" W). The lake is 46 m above sea level, 280 ha in area and reaches a depth of 73 m.

A log-crib dam was built in Scott Cove Creek in 1918 (Figure 3) and stood until 1980, when the dam and major log jams were removed from the creek (Youds, 1982). The dam formed a migration barrier between the ocean and Loose Lake, which would have affected all salmonids that migrated into the Loose Lake system. The dam originally raised the water level by 5.2 m in Loose Lake. There are no anecdotal reports of anadromous Sockeye Salmon runs in the system

before logging operations in 1918 but runs of Coho, Chum and Pink salmons were reported (Rogers, 2009, unpublished data).

Figure 3 Log trough cradle piling remaining in Scott Cove Creek in 2016 after dam and remaining structures were removed in 1980 (Photo: Mack Bartlett).

A dam was also built in Mini Hump Creek (Figure 4) to raise the water level and aid movement of logs from Towney Lake to Loose Lake in 1939 (Proctor, pers. comm., 2016). The dam was active until 1945 and left in place until it was removed in 1995. A marshy area and woody debris remains at the head of Mini Hump Creek at Towney Lake as a result of logging operations. Adult Pink, Chum and anadromous Sockeye salmons salmon have been observed returning to the system in small numbers since the dams and jams were removed (Department of Fisheries, 2014; Gagnon, 2016; Youds, 1982).

Mini Hump Creek is approximately 1000 m in length (Rogers, 2009, unpublished report). The low end of the creek has a sandy substrate and a low gradient and flow rate. The central portion

has a higher flow rate and gradation. It has bedrock cascades, a small canyon and pools formed by woody debris. The upper section is marshy, which was formed during logging operations.

Figure 4 Logging dam trough pilings at the outflow of Mini Hump Creek into Loose Lake (left). Logging debris left in Mini Hump Creek at the inflow from Towney Lake (right) (PC: Mack Bartlett).

Abiotic measurements from a 2009 spring survey, conducted by the Mainland Enhancement of Salmonid Species Society (MESSS), found a pH of 5.7, temperature of 12.6 °C and a flow rate of 0.25 m/s (Rogers, 2009, unpublished report). The average depth was 0.37 m, with a bankfull width of 7.7 m and a wetted width of 6.4 m. Predominant substrates are sand, fines and pebbles at 54%, 14% and 14% composition respectively of the total substrate. Invertebrate surveys based on the BC Stream Keepers program found overall good water-quality characteristics based on diversity (18 species), abundance (85 individuals) and density (396.3/m^2) of invertebrates (Rogers, 2009; Taccogna & Munro, 1995). The ratio of pollution intolerant or EPT (Ephemeroptera, Plecoptera, Trichoptera) invertebrates to total invertebrates was 0.79, which is considered an indicator of good water quality and oxygen levels (Taccogna & Munro, 1995). Coho Salmon fry and parr were reported during the survey.

1.7 Geographically close Sockeye Salmon and kokanee

There are contemporary runs of Sockeye Salmon in the region around Gilford Island (Figure 5). These include Sockeye Salmon from Mackenzie Lake, Quatse River and the Nimpkish River. No major Sockeye Salmon runs have been reported to occur on Gilford Island or proximate to the Loose Lake system since 1953 when the Federal Department of Fisheries and Oceans salmon enumerations began in area 12 (Department of Fisheries, 2014). Nimpkish River Sockeye

Salmon smolts are often found within the Broughton Archipelago, within 17 km of Scott Cove during their out migration (Peacock et al., 2016). River systems on Gilford Island including Shoal Creek and Scott Cove Creek and Viner Creek (Figure 2), often have Sockeye Salmon spotted during the fall spawning enumerations (Gagnon, 2016). Occurrences of Sockeye Salmon in these systems are usually less than 10 individuals.

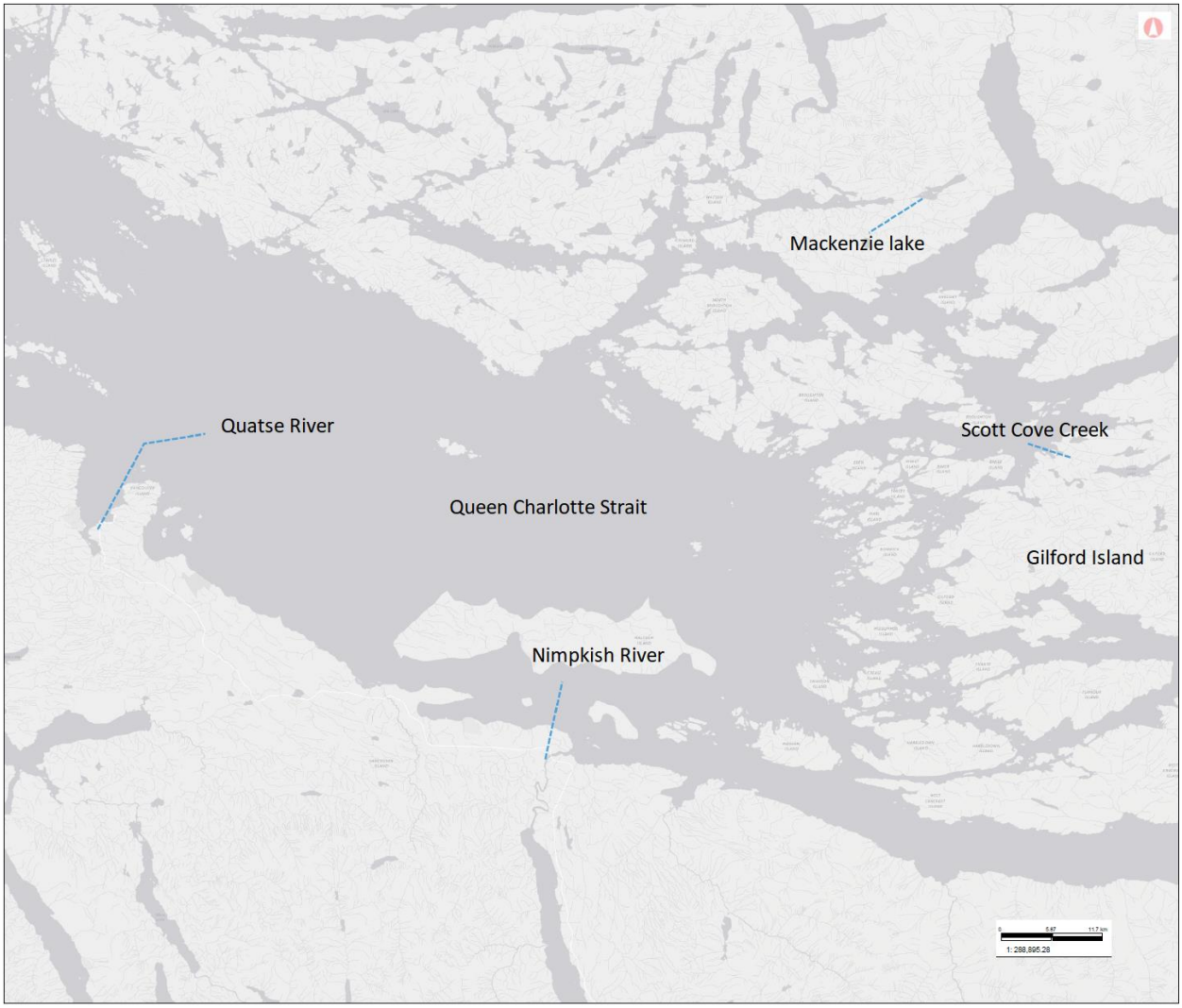

Figure 5 Extant Sockeye Salmon populations in proximity to Scott Cove Creek and the Loose Lake system on Gilford Island.

The Quatse River Sockeye Salmon are genetically or morphologically different compared to other coastal lake Sockeye Salmon populations (Beacham, Mcintosh, & Macconnachie, 2005). In an analysis of 40 lake Sockeye Salmon populations from the south coast region of British

Columbia, the Quatse River population was found to be the most genetically distinct. Quatse Sockeye Salmon had the lowest number of alleles and lowest heterozygosity of any population. Quatse River Sockeye Salmon are also small, less than 2 kg and return to freshwater as early as April for spawn. The Quatse River is approximately 80 km from Mini Hump Creek. Some of the geographically closest kokanee populations to the Loose Lake kokanee include Vernon Lake, a lake on the Nimpkish system, and Powell Lake at 120 km and 230 km, respectively (Figure 1).

1.8 Kokanee bearing lake systems with similar anthropogenic ecosystem alterations

Alouette Lake (Figure 5), has a population of black kokanee believed to be recently derived and caused by a dam built in 1928 (Godbout et al., 2011). The black kokanee spawn at depth in Alouette Lake and are believed to use spawning grounds that would have been stream spawning locations before the reservoir increased water levels. Although anadromous Sockeye Salmon were present in the lake prior to the installation of the dam, the dam has formed a full migration barrier and no anadromous Sockeye Salmon have been observed since the dam was built.

Grafton Lake on Bowen Island, BC also contained a kokanee population of unknown origin. The outflow of Grafton Lake was affected by a drinking-water dam and logging operations that may have created a barrier to migration. The Grafton Lake population was known for decades but no enumerations were completed and was last observed in 2002 and is now thought to be extirpated (Bell-Irving, pers. comm., 2016). The origin of the Grafton Lake kokanee is uncertain, the only salmon records for the Grafton Lake system were of “red” salmon using the estuary during the 1920s to 1940s during the time that logging operations and damming was occurring in the system. Grafton Lake is only 4 ha in size and may be too small to support a population over an extended period of time.

The Mini Hump Creek kokanee is a population from which we can expand our understanding of adaptation and intraspecific variation within Sockeye Salmon. Genetic, historical and morphological data suggest that the Mini Hump Creek population, and others with similar histories, may be contemporary kokanee populations formed more recently than the last glacial

maxima. I propose that the novel morphological features and associated life history of the Mini Hump Creek population are the result of recent changes to their ecosystem due to damming, a barrier to migration, and resulting water-level changes that caused recent adaptation.

In this study I have included morphological measurements, spawning and enumeration data, genetic results, limnology data and historical information with the objective of characterizing the Mini Hump Creek population and understand if their current life-history and morphology is the result of recent anthropogenic environmental change.

2 Methods

2.1 Limnology survey

During September and October 2016 I conducted a limnological survey on Loose Lake, Gilford Island BC, which is the lake into which Mini Hump and Connector Creek drain and thought to be where the kokanee reside. A Garmin Echo 150 fishfinder was used to collect depth data and a Garmin 64 GPS unit was used to record latitude and longitude of depth positions. A Hanna HI98194 multiparameter meter was used to collect pH, dissolved oxygen, temperature and total dissolved solids from depths 0, 1, 5, 10 and 20 m. A Secchi disk was used to measure water transparency.

2.2 Spawner survey

I took part in spawning abundance enumerations and observations throughout the spawning season, from first appearance September 20 2016 until the end of enumeration November 5 2016. Observations included spawner abundance, species composition including predators of kokanee, observational visibility, and presence of mortalities both pre and post spawn. Enumerations were completed in conjunction with the local salmon enumeration society on Gilford Island, the Mainland Enhancement of Salmonid Species Society. Enumerations were made in six streams in the Loose Lake system (Figure 2), but kokanee were only found in two of the streams. Enumeration data are available from 2009 to 2016 seasons.

2.3 Sample collections and storage

To compare the Mini Hump Creek population to other kokanee populations in British Columbia, Alaska and Japan, I collected adults from the population. Collected samples were used for morphological and genetic analysis of the populations. In the fall of 2015 and 2016, I collected kokanee from Connector Creek, Mini Hump Creek and Loose Lake on Gilford Island BC. I also

received 40 kokanee collected by members of the Salmon Coast Field Station Society from Mini Hump Creek in 2015. During the 2015 collections, 40 individuals were administered a lethal dose of clove oil in the field and then individually frozen flat and stored at -20 °C shortly after collection while all others were released alive. The frozen samples were transported to the Taylor lab at UBC where I conducted genetic and morphological analyses.

To examine the diet and colouration of Mini Hump Creek kokanee, collections were made of individuals in Loose Lake. In 2016, I received 10 kokanee which were captured in Loose Lake by recreational fishers. I removed tissue samples as above and individually froze and stored the samples for further analysis. In 2016, I also collected four post-spawn mortality fish from the banks of Mini Hump Creek and captured 11 kokanee in Connector Creek. I used a seine net to live capture kokanee from Mini Hump Creek and Connector Creek. The seine net has the dimensions of 120 cm high by 10 m long with a mesh of 5 mm. I took 6 mm tissue punches from the caudal fin of all fish and stored them in 70% ethanol while storing another punch dry on Whatman paper to be used for genetic analysis. I used both ethanol and Whatman paper for tissue storage as the Taylor lab prefers ethanol storage, whereas the DFO Molecular Genetics Lab at PBS prefers Whatman paper storage.

At the Taylor lab, I thawed the previously frozen samples to remove otoliths and take 2 g tissue punches to be used for age and stable isotope analysis, respectively. I removed and stored stomach and intestinal contents in 45% ethanol for analysis of consumed prey items. Otoliths and tissue punches were stored dry at -20 °C. To analyze fecundity of the population, I removed eggs if present and stored them in 45% ethanol. I then fixed the sampled fish in a solution of 10% formalin for 7 days. After fixation the formalin solution was drained and the specimens were rinsed in water for 1 hour. The samples were stored in 45% ethanol.

2.4 **Morphological measurements**

I measured the following characteristics: standard length, maximum body depth, orbit diameter, upper-jaw length, gill raker counts and colouration. These measurements were made to characterize several standard morphological traits of Mini Hump kokanee that can be used for

comparison to other populations. I chose these measurements for the analysis of the Mini Hump Creek population as they are considered to have implications for life-history traits including spawning grounds used and feeding habits (Moreira & Taylor, 2015; Taylor & Foote, 1996; Taylor et al., 1997). Also, red colouration is of importance to spawning Sockeye Salmon so I measured colouration as the presence or absence of red pigmentation on the skin or flesh of the abdominal cavity along with the presence or absence of conspicuous external markings (Craig & Foote, 2001). I made all measurements on the left side of each individual for consistency.

Standard length is measured as the distance from the tip of the snout to the posterior end of the hypural plate. Maximum depth is measured from the anterior insertion of the pectoral fin to the anterior insertion of the dorsal fin. Orbit diameter, a measure of the eye size, is taken from anterior to posterior of the orbital cavity. Upper-jaw length is measured from the ventral end of the maxillary to the anterior end of the pre-maxillary. Gill rakers may reflect the diet of kokanee and anadromous Sockeye Salmon and vary widely both within and among populations (Vernon, 1957). To analyze the gill rakers, I excised the first arch of the left gill raker from each individual. I rinsed the gill rakers in fresh water to remove sediment and ethanol and then counted them using a dissecting microscope at 160x magnification.

To provide a reference point for Mini Hump Creek kokanee external morphology, I compared them to individual kokanee specimens from two black kokanee populations from the Fraser River drainage in British Columbia which I obtained from the Beaty Biodiversity collection at the University of British Columbia. Mini Hump Creek Kokanee share similarities in external morphology to black kokanee. The black kokanee populations are located in Anderson Lake and Seton Lake, BC a set of lakes linked by Portage Creek. For analysis of the Mini Hump Creek, Anderson Lake and Seton Lake populations I measured standard length, maximum depth, orbit diameter, upper jaw length and gill raker numbers.

2.5 **Egg analysis**

To measure female spawning investment, I removed eggs from mature and post-spawn females. I measured the diameter of each egg along the widest axis. Eggs that had collapsed, been

punctured or were not fully developed were not included in measurements, but were included in egg number counts. Individual egg weights were collected for each mature female. Eggs were first blotted dry. Crushed eggs and ovarian tissue were removed before weighing. Females were categorized as ripe if the ovum casing was intact, partially spent if the ovum casing was ruptured and the eggs were loose, and spent if no eggs or very few eggs remained but it was clear the individual had spawned. No immature females were present. Egg size and number vary by population, spawning ground conditions, ecotype latitude and body size (Hendry et al. , 2001; Kaeriyama et al. , 1995; McGurk, 2000; Quinn et al., 2015). There is a positive correlation with body size and both egg number and mass in Sockeye Salmon (Kaeriyama et al., 1995; Quinn et al., 2015). There is a negative correlation between egg size and increasing latitude in kokanee but not anadromous Sockeye Salmon (McGurk, 2000). As these studies of Sockeye Salmon eggs report female length in fork length, I measured the females in fork length so I could make comparisons of the Mini Hump Creek population's egg diameter, mass and numbers to what is expected for kokanee of a similar size or at similar latitude.

2.6 **Otolith analysis**

To determine the ages of the fish collected, I analyzed otoliths for number of annual growth rings (annuli) (Miller & Simenstad, 1994). I re-hydrated the otoliths from 20 individuals in distilled water for 3 days prior to counting the annuli. To aid in counting the otoliths I used a dissecting microscope at 160x magnification, and placed the otoliths in a drop of water and side illuminated them to count the annular rings. I used the left otolith for reading age but I counted annuli on both otoliths as a confirmation of age. If the left otolith was damaged or crystalline, I used the right otolith for age determination.

2.7 **Diet**

To analyze the diet composition of the fish collected, I identified and counted the invertebrates in the stomach contents using a dissecting microscope, at between 64x and 400x magnification. Organisms not overly digested were identified down to the lowest taxonomic unit, typically

genus. Stomach contents were measured for abundance in the diet and length measurements were taken to determine relative proportions of each organism type in the diet. Ten individuals were found to have identifiable prey items in their stomachs.

2.8 Morphological data analysis

To compare the morphological measurements from each population, I had to determine if the averaged physical characteristics of each population were significantly different among populations. Sockeye Salmon is known to exhibit sexual dimorphism so morphological measurements were separated by sex (Hendry & Berg, 1999; Hendry & Quinn, 1997). I found maximum body depth, orbit diameter and upper-jaw length correlated with body length while number of gill rakers was independent of body length of the analyzed samples. Size adjusted measurements of morphological features was therefore required to directly compare populations with different mean lengths (Elliott 1995). First, An ANCOVA was completed between populations for maximum body depth, orbit diameter and upper-jaw length against length to ensure there is no significant difference in the slope of compared measurements between populations.

When completing the ANCOVA's I was observing the slope of each morphological measure, when put against standard length, to see if they varied significantly among the populations. Significantly different slopes of a morphological trait's relationship to body size among populations would mean that the morphological trait in question was getting larger or smaller per unit of body length when compared to the other populations. I did not find that the slopes of max depth, orbit diameters or upper jaw length against standard length were significantly different between any populations among sex groupings. This meant that I could subsequently use a standardized length measurement among populations to adjust and then compare the other morphological measurements that scaled with length.

Standard length was used to compute adjusted measures of maximum body depth, orbit diameter and upper jaw length using the formula (Reist, 1985):

$$M_{adj} = M\left(\frac{\bar{L}}{L}\right)^{b}$$

Where M_{adj} is the adjusted morphological measurement, M is the recorded measurement of the sample, $\bar{L}$ is the mean length among populations, L is the recorded length of the sample. b is the allometric coefficient or slope of the variable against the length measurements.

To test for significant differences of the mean unaltered and size-adjusted morphological measurements between sexes and populations, I used several-sample tests with one-way analysis of variance ($p<0.05$).

2.9 **Microsatellite analysis**

To examine the genetic relationship of The Mini Hump Creek population to other salmon populations, I had to complete a molecular genetic analysis. I analyzed Genetic Samples in the Taylor Lab at The University of British Columbia and also sent samples to the Molecular Genetics Lab at the Pacific Biological Station for independent analysis. The Taylor Lab has genetic samples of black kokanee populations in its database. These were useful for comparison as the Mini Hump kokanee are morphologically similar to black kokanee. The Molecular Genetics Lab has a large Sockeye Salmon database which includes kokanee populations geographically close to the Mini Hump Creek and black kokanee from Anderson Lake, Seton Lake and Alouette Lake which are useful in deciphering their relationship to other Sockeye Salmon and kokanee populations in the region.

To extract and isolate DNA from the tissue samples previously stored in ethanol, I used the Qiagen Dneasy blood and tissue extraction kit following the protocol provided by the manufacturer (Qiagen, 2006). Forty Mini Hump Creek samples were processed along with 40 black kokanee from Alouette Lake and eight Pink Salmon from the Broughton Archipelago. The Mini Hump Creek kokanee share morphological similarities with other black kokanee so were analyzed along with the Alouette Lake population, which are also black kokanee. Pink Salmon from the Broughton Archipelago were also used in the analysis because it was previously

believed that the Mini Hump Creek kokanee were potentially Pink Salmon hybrids. Extraction and analyses were completed at the Taylor Lab at the University of British Columbia.

I sent 60 kokanee samples to the Molecular Genetics Lab at the Pacific Biological Station to be independently analyzed. Of these 60 samples, 50 were from Mini Hump Creek, 40 of which were collected by Salmon Coast Field Station researchers in 2015 and 10 were collected by me in 2016. Ten of the samples were from Connector Creek, which I collected in 2015. Digested and extracted DNA has to be amplified for genetic analysis so I used Polymerase chain reactions (PCR) to amplify microsatellites of interest. Once PCR was completed on all individual samples I was then able to visually score, assign a value, for each individual at each of the nine microsatellites. Thes were the data that was then used for all further genetic analysis. I ran PCR's using the Qaigen PCR Multiplex kit following the manufactures protocols (Qiagen, 2010). Nine microsatellite loci were assayed to assess the genetic divergence of Mini Hump Creek kokanee from "black" kokanee and other Sockeye Salmon populations. I used these nine microsatellites as they were previously found to have a high level or clarity in scoring resolution and offer a degree of polymorphism in kokanee studies (Moreira & Taylor, 2015). The DNA microsatellite loci were: Omy77, Ots100, Ots103, Ots108, Oki10, Oki29, One103, One103, One108, and One110. To make scoring easier, PCR reactions were split into three separate PCR multiplex groups. Locus Oki29 was run by itself due to the need for a higher annealing temperature (58C). All the PCR reaction products were visualized on a Beckman-Coulter CEQ 8000 automated genotyper.

Samples run in the Molecular Genetics Lab at the Pacific Biological Station used 14 microsatellites loci developed for stock composition and differentiation of Sockeye Salmon (Beacham et al., 2001). The loci used were: 1b, 3dre, i1, oki10, oki16, oki1a, oki1b, oki29, oki6, omy77, one8, ots103, ots2, and ots3.

Table 1 Spawning populations of Sockeye Salmon used for genetic analysis. TL-Taylor lab at UBC and MGL-Molecular Genetics Lab at the Pacific Biological Station, n represents the number of samples in the analysis in each lab. All samples were pre-existing in each lab with the exception of the Mini Hump and Connector Creek samples which were provided by this study.

Spawning location	ecotype	coordinates	Watershed	Lab	n
MiniHump Creek	minihump kokanee	50.752, -126.428	South Coast, BC	TL, MGL	40, 91
Connector Creek	minihump kokanee	50.743, -126.439	South Coast, BC	MGL	10
Grafton Lake	kokanee	49.372, -123.364	South Coast, BC	MGL	26
Alouette Lake	black kokanee	49.338, -122.417	Fraser R, BC	TL, MGL	40, 104
Anderson Lake	black kokanee	50.642, -122.413	Fraser R, BC	TL, MGL	84, 36
Seton Lake	black kokanee	50.691, -122.109	Fraser R, BC	TL, MGL	48, 40
Meadow Creek	kokanee	50.201, -116.954	Kootenay R, BC	TL, MGL	30, 297
Chilliwack Lake	kokanee	49.051, -121.417	Fraser R, BC	MGL	100
Coquitlam Lake	kokanee	49.404, -122.782	Fraser R, BC	MGL	60
Horsefly Lake	kokanee	52.431, -121.001	Fraser R, BC	MGL	121
Powell Lake	kokanee	50.093, -124.413	South Coast, BC	MGL	31
Sakinaw Lake	kokanee	49.680, -124.007	South Coast, BC	MGL	70
Shushwap Lake	kokanee	50.919, -119.052	Fraser R, BC	MGL	98
Vernon Lake	kokanee	50.046, -126.448	Nimpkish R, BC	MGL	18

Chedakuz Creek	kokanee	53.414, -124.993	Fraser R, BC	TL	30
Davidson Lake	kokanee	53.634, -125.458	Fraser R, BC	TL	31
Portage Creek	Sockeye	50.708, -122.290	Fraser R, BC	TL	41
Hansen creek	Sockeye	59.324, -158.696	Bristol Bay, Ak	TL	20
Gates Creek	Sockeye	50.550, -122.477	Fraser R, BC	TL	41
Lake Saiko	black kokanee	35.499, 138.682	Japan	TL	13

2.10 **Microsatellite data analysis**

To compare the Mini Hump Creek population to other Sockeye Salmon, I had to access genetic data that contained populations with a broad geographic range and a diversity of ecotypes. The Taylor Lab's genetic database has 11 Sockeye Salmon populations while the Molecular Genetics Lab gave access to 14 populations for genetic analysis, I used these populations to geographically and genetically orient the Mini Hump Creek population. The Taylor Lab database contains highly distinct kokanee populations from the Pacific basin that share morphological similarities to those of the Mini Hump kokanee along with "normal" kokanee populations from Alaska, Fraser River and Columbia River to help orient the Mini Hump population. The Molecular Genetics Lab provided access to genetic data from a number of coastal kokanee populations along with kokanee populations from the Fraser River and Columbia River so I could compare the results from both analyses.

To analyze the microsatellite data, I first had to visually score the microsatellite markers for each individual. I was then had to confirm that the Mini Hump Creek population was a Sockeye Salmon population. I then had to ensure the data were error-free and conformed to Hardy-Weinberg equilibrium, a test that ensures the microsatellites are genetically neutral and not under selection. I used the program Micro-checker V2.2.3 (Van Oosterhout, 2004) to check data for

scoring errors, stuttering during PCR reactions or the presence of null alleles. Once the data were confirmed to be free of errors, I was then able to look at each population's genetic diversity and relatedness and each other population. I used summary statistics, such as allelic richness to identify variance and genetic diversity within each population. I then examined observed and expected heterozygosity, which compares genetic variance both within and between populations. I then created phylogenetic trees using chord distance measures to visualize the relatedness of kokanee populations. Due to the limitations of chord distance measures, I then used correspondence and principal component analysis to examine genetic relationships between populations without the use of genetic distance.

To test for departure from Hardy-Weinberg equilibrium, I used the program GENEPOP 4.5.1 (Raymond, 1995). An exact test for each locus in each population using P-values was completed using a Metropolis-Hastings Markov Chain algorithm (Guo & Thompson, 1992). To get exact tests for genotypic disequilibrium for each pair of loci within each population, I used the Markov chain method in GENEPOP 4.5.1.

To analyze within population variation, I compared the observed and expected heterozygosity, number of alleles per locus and allelic richness. These gene diversity statistics were generated using the program FSTAT 2.9.3.2. (Goudet, 2001) Among population variation (Fst) was examined using Nei's F statistic and Weir and Cockerham's F statistics using FSTAT 2.9.3.2. Weir and Cockerham's F stat uses pairwise population differentiation estimated as θ. G tests allowed me to compare Connector and Mini Hump Creek populations as G tests have been shown to be more effective than Fst to differentiate low level genetic Divergence (Balloux, 2002).

I used the program Populations 1.2.32 (Langella, 1999) to compute the population distances and create a phylogenetic tree by taking the microsatellite allele frequencies from the populations and applying the Neighbour-joining method and bootstrapped with 5000 replicates (Leblois, Estoup, & Rousset, 2003). Cavalli-Sforza and Edwards chord distance (Dc) measure is used as a genetic distance measure between populations in the program. Dc assumes genetic drift is the only divergent factor affecting the microsatellites so it is unaffected by model or mutation rates

applied to treatments. The program Treeview 1.6.6 (Page, 2001) was then used to visualize the output of Populations 1.2.32.

Chord distance measures can create unlikely population clusters when analyzing genetically distant populations with small sample size, so I used other methods to ordinate populations (Bergsten, 2005). I used the program GenAlEx 6.502 (Peakall, 2001) to compute a secondary assessment of population structure. Genaiex uses principle components analysis (PCA) based on Nei's genetic distance measure. PCA is useful to visualize genetic distance but not to further analyze genetic relationships as it does not give an indication of genetic distance or significance of relationships derived from the anlysis. This method ordinates populations and removes measures of genetic distance, reducing the effect of high genetic variability among populations from skewing phylogenetic outputs (Bryja et al., 2010; Chakraborty, 2010). This method was used to remove the potential for Long Branch attraction (LBA). LBA can occur when genetically distant populations are placed genetically close together in analysis due to both populations being highly genetic divergent compared to other populations within the analysis (Baldauf, 2003).

As a secondary method to ordinate the populations, I used Genetix 4.05.2 (Belkher, 1996). Genetix uses 3D factorial correspondence analysis (FCA), which uses the allele frequency data to cluster individuals by their relatedness and inertia from an average profile "centroid" for each population. I used this method as it allows the visualization of populations, each individual represented as a point so closely related populations can be observed overlapping.

3 Results

3.1 Limnology

I made abiotic measurements of Loose Lake in the fall of 2016 to assess the water conditions of the lake in which the Mini Hump Creek kokanee likely rear. Loose lake is 280 hectares in area, drops to a depth of 73 m which is approximately 30 m below sea level. In October surface temperatures averaged 13.5°C while temperatures at 20 m averaged 7.8 °C. Dissolved oxygen average 11.6 ppm, pH averaged 5.54 and total dissolved solids 6.8 ppm during the assessment. Secchi disk readings were 2 m throughout the assessment.

3.2 Spawning enumerations

I surveyed creeks in the Loose Lake watershed in fall 2016 for the presence of spawning salmon (Figure 2). Of the six creeks I surveyed, kokanee were only observed in two of the creeks. This result is consistent with enumerations from 2009 to 2015 spawning seasons. Kokanee were present in Mini Hump and Connector Creeks. Spawners first appeared September 24 with a peak spawn of 655 individuals on October 16. The last record of spawners was on November 4 with 225 individuals present. Connector Creek had spawners first observed on October 11, peak spawn was 22 individuals on October 24 and no fish were present by November 4. Seventeen dead kokanee were reported on the banks of Mini Hump Creek during enumerations.

3.3 Morphology

3.3.1 Overview

The Mini Hump Creek males were significantly larger than the females ($p<0.05$) by all measures with average standard lengths of 147.3 mm and 139.3 mm respectively (Table 2). The males of the sampled population had a deeper average maximum body depth than females at 51.9 mm and

47.4 mm respectively. Males had larger orbit diameters and upper jaw lengths than the females at 11.8 mm against 10.4 mm and 24.3 mm against 19.6 mm respectively.

To compare the Mini Hump kokanee to similar kokanee populations I had to adjust all measurements, which were found to scale with body length, to an among population mean length. I found maximum depth, orbit diameter and upper jaw length were sexually dimorphic among the populations and correlated positively with length. Therefore, these measures could be adjusted by each individuals standard length and then adjusted to fit the among populations size-adjusted mean length. Because of sexual dimorphism in the measurements, I adjusted each sex individually for the among population comparisons. The mean lengths among populations that I calculated were 168.11mm for females and 201.22mm for males. Gill raker numbers were not correlated to length measurements or sex so were not adjusted.

3.3.2 **Length, max depth, orbit diameter**

The Mini Hump Creek population showed significant sexual dimorphism in standard length, adjusted maximum depth and adjusted orbit diameter. Adjusted orbit diameter was not significantly different between female populations. I included the Anderson Lake and Seton Lake individuals in my morphological analysis as they are from black kokanee populations of a known origin, offering a basis for comparison, and appear morphologically similar to the Mini Hump Creek kokanee. Anderson and Mini Hump males were significantly larger than females (all, $p<0.05$) from the same populations (Table 2). Adjusted orbit diameter and maximum depth was significantly larger in males from each population. Anderson males were significantly longer than the Seton and Mini Hump males at 252.96 mm, 146.67 mm and 147.29 mm respectively.

Table 2 Morphological measurements of three populations of kokanee. Males and females measurements were size adjusted to a common length of 201.22 mm and 168.11 mm respectively. MH is the Mini Hump Creek population. Significant differences between populations but of the same sex are marked by *. Significant differences between sexes of the same population indicated by †

Females		MH	Anderson	Seton	**Males**		MH	Anderson	Seton
	n	28	19	24		n	23	30	6
length	mean	**139.26†**	**239.23†***	**145.48**		mean	**147.29†**	**252.955†***	**149.27**
	SE	1.21	1.91	0.72		SE	1.53	1.32	1.09
Max depth	mean	**57.91†**	**57.30†**	**55.85†**		mean	**74.63†**	**74.20†**	**71.28†***
	SE	0.44	0.52	0.40		SE	0.60	0.69	0.87
Orbit diameter	mean	**11.68†**	**11.45†**	**11.00†**		mean	**13.32†**	**13.09†**	**12.29†***
	SE	0.11	0.17	0.10		SE	0.10	0.17	0.29
upper jaw length	mean	**22.76†**	**21.37†**	**19.12*†**		mean	**32.17*†**	**30.36*†**	**24.31*†**
	SE	0.23	0.29	0.30		SE	0.40	0.36	1.05

3.3.3 **Gill rakers**

I found that the Mini Hump kokanee had significantly lower gill raker counts, 32.33 (n=36) on average than the Seton Lake, 38.03 (n=29) and Anderson Lake populations at 37.55 (n=45) ($P<.005$) (Table 3).

Table 3 Gill raker counts from the first gill arch on the left side of kokanee from Anderson Lake, Seton Lake and Mini Hump Creek.

gill rakers	Mini Hump	Anderson	Seton
n	36	45	29
mean	32.33	37.55	38.034
SE	0.211	0.255	0.524
Min-Max	30-35	34-42	31-42

3.3.4 **Otolith aging**

During analysis of the Otoliths, I found the nine males were all age 3 while eight of the females were age 3 and three were age 2.

3.3.5 **Stomach contents**

I removed stomach contents with identifiable prey items from 10 individuals. The bulk of prey items in both abundance and volume appeared to be *Daphnia* sp., comprising 80% of the combined stomach contents by volume and one stomach containing 1129 *Daphnia* (Table 5). *Chaoborus* larvae, or phantom midge larvae (Figure 6), were the most abundant item in two of the stomach samples and made up a 16% of the combined stomach contents. Nematocera or adult midge species made up 3% of the combined stomach contents, while Chironomid larvae, invertebrate eggs, ostracods and inert objects, such as stones, made up 1% of the combined stomach contents. There was not a clear relationship between sex or length of the individuals and prey item choice based on the stomach content analysis.

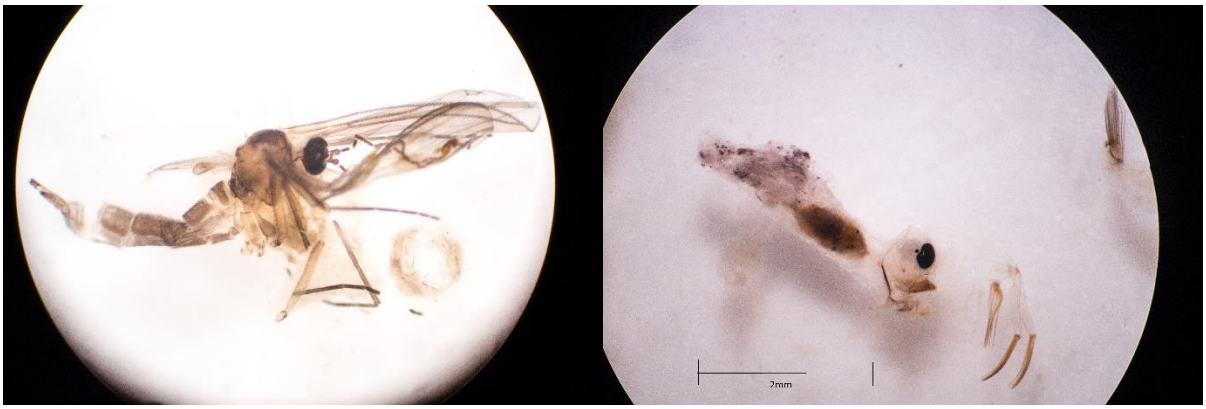

Figure 6 Adult *Chaoborus* (midge) and ghost midge larvae extracted from a stomach sample from one Mini Hump Creek kokanee sample viewed at 40x. (PC: Mack Bartlett).

Table 4 Stomach contents from 10 Mini Hump Creek kokanee individuals. Contents are listed by their proportion of the total stomach contents of each individual.

sex	length	*Daphnia*	*Chaoborus* midge	glass worm	Chironomid	eggs	other
M	143.4	0.814	0.070	0.093	0.023	0.000	0.000
F	150.1	0.352	0.000	0.625	0.000	0.023	0.000
F	150.3	0.896	0.000	0.096	0.000	0.000	0.008
M	164.0	0.978	0.000	0.000	0.000	0.006	0.016
F	144.3	0.974	0.000	0.000	0.000	0.026	0.000
F	140.3	0.955	0.000	0.041	0.000	0.002	0.002
F	157.0	0.983	0.017	0.000	0.000	0.000	0.000
M	140.0	1.000	0.000	0.000	0.000	0.000	0.000
F	135.9	1.000	0.000	0.000	0.000	0.000	0.000
M	142.0	0.025	0.230	0.746	0.000	0.000	0.000

3.3.6 **Colouration and markings**

The individuals I examined from the Mini Hump Creek kokanee population lacked strong red or orange pigmentation in the flesh or on the skin with the exception of ripe eggs (Figure 7). I found the flesh was white in all sampled individuals. Two of 11 individuals collected in Loose Lake and 20 of the 40 individuals collected at spawn in Mini Hump Creek displayed a light pink hue near the lateral line. Fourteen males and eight females displayed red colouration. Parr markings, similar to the vertical flank markings found on juvenile salmonids, were visible on the lateral surface of 27 of the 51 individuals. The parr markings were most notable on those fish examined in their non-spawning colouration and were present on 9 of the 11 individuals, while the dark vertical striations on the spawning individuals made observing parr marks difficult.

Figure 7 Colouration of individuals in lake (left); skin is light gray or white and translucent, parr-like marks observable. Ripe female with eggs extracted (right); eggs are carotenoid rich while skin and flesh lack the red pigments (PC: Mack Bartlett).

The skin of non-spawning individuals was white and almost clear with small soft translucent scales and a lack of metallic sheen or green pigmentation. I found that spawning Mini Hump Creek kokanee were dark in colouration having vertical striations over top of a gold or bronze pigmented skin (Figure 8).

Figure 8 A Mini Hump Creek kokanee in river spawning colouration (left) and lake non-spawning colouration (right) (PC: Mack Bartlett).

3.3.7 Egg size

Twenty-eight females were collected in 2015 and 2016 from Mini Hump Creek. Females had an average fork length of 147.70 mm (SE=1.09). Two of the females were completely spent and contained no eggs. Twenty-six females contained at least one egg and were in a spent or ripe state, no immature females were present. Fourteen of the females collected were ripe and unspent, meaning they had eggs contained within an unbroken ova. The average egg diameter among all individuals was 5.01 mm (SE=0.07) with an average weight of 0.061 g (SE=0.002). Of the ripe unspent individuals, the average egg number was 76.64 (SE=6.30) with a minimum of 41 and a maximum of 115 eggs.

3.4 Microsatellite analysis

Genetic analysis of the Mini Hump Creek kokanee samples confirmed that they were Sockeye Salmon rather than the preceding view of locals that they were a landlocked hybrid salmonid population. The Pink Salmon samples did not amplify properly using the microsatellite markers, while the Mini Hump population and the other kokanee populations in the analysis did. Therefore, the Pink Salmon were removed from further analysis. The Mini Hump Creek kokanee samples were amplified without issue and were not prone to excessive stuttering, scoring errors or null alleles, indicating they are a Sockeye Salmon population.

Further analysis of the Mini Hump Creek kokanee then allowed me to look at the genetic diversity of the population and how it compared to other kokanee and Sockeye Salmon populations. Finally, I looked at the relatedness of the Mini Hump Creek kokanee to the other kokanee and Sockeye Salmon populations in the analysis. Building phylogenetic trees and correspondence analysis allowed me to analyze the genetic and geographic relationship of the Mini Hump Creek population to other kokanee and Sockeye Salmon populations.

3.4.1 **Null alleles**

To determine if the genetic data contained stuttering, null alleles and scoring errors, I looked at all the population-locus pairs and the raw genetic data. If any of these signatures are identified than the raw genetic data could not be used effectively for further analysis. Previous analyses completed in the Taylor lab identified Ots108 as a potential locus containing null alleles, but found including or removing Ots108 from the analysis did not alter the results (Moreira, 2014). My analysis with the additional populations also found that OTS108 potentially contained non-amplifying or null alleles as identified using Microchecker 2.2.3. Null alleles do not properly amplify during PCR and are identified as containing no strong peaks during scoring, giving no value at the microsatellite locus. OTS108 containing null alleles makes it a poor candidate as a microsatellite marker for this specific analysis. Null alleles can appear as homozygotes at the suspect locus which then gives the population an artificially lower genetic variation and diversity at that locus (Paetkau & Strobeck, 1995).

3.4.2 **Hardy Weinberg equilibrium**

Testing for Hardy-Weinberg equilibrium allowed me to see if any of the microsatellite markers appeared to be under selective pressures and would, therefore, be a poor candidate as a neutral genetic marker. I tested each loci population pairing for the probability of being out of Hardy Weinberg equilibrium. A loci in a population is considered significantly out of equilibrium with a $p<0.018$ (Narum, 2016). Analysis of Ots108 indicated that it significantly departed from equilibrium in 10 of the 11 populations matching the null allele prediction from micro checker. Oki10 was out of equilibrium in 5 of 11 populations. Of the 99 tests for equilibrium throughout the analysis, 24 loci-population tests departed from equilibrium and the remaining loci did not cluster to a certain population or other loci besides OTS108. This reinforced that Ots108 was not

an effective microsatellite marker for analysis with these particular populations. I considered all the other loci as effective for use in further genetic analysis as they did not depart from equilibrium. Analysis was attempted both with and without Ots108. The inclusion of Ots108 did not alter the results.

While analyzing the data from the Molecular Genetics Lab at Pacific Biological Station, I did not find that significant departures from Hardy-Weinberg equilibrium clustered at any one of the 14 microsatellite loci or populations. Fourteen populations and 14 loci were included in the analysis for a total of 196 tests, of these tests, six did departed from equilibrium. Grafton Lake and Connector Creek had a result of "no result" for multiple tests of departure from equilibrium. This "no result" is displayed in the program when it cannot properly analyze the allele frequencies of certain loci. A low number of samples and multiple loci being at fixation in the analysis is likely responsible for the program producing "no result" for Grafton Lake and Connector Creek loci.

3.4.3 **Linkage disequilibrium**

Linkage disequilibrium can affect allele frequencies and thus alter the genetic results so I performed a test to assess the loci pairs from each population. A limited amount of genotypic linkage disequilibrium was detected within the Taylor lab analysis. Five of 396 tests showed significant linkage disequilibrium between loci pairs. Loci pair tests with "no information" occurred 29 times in the analysis meaning there was not enough genetic data for the linkage disequilibrium test to be completed. 26 of these tests occurred in the Lake Saiko population and were likely due to small sample size (n=13)

Linkage disequilibrium was not common in the analysis from the Molecular Genetics Lab. Significant linkage disequilibrium was found in 19 of 1274 tests. There were 166 tests that resulted in "no information", these tests came mostly from Grafton Lake and Connector Creek which had 76 and 55, respectively. Anderson Lake and Vernon Lake contained the remaining "no information" test results. All four populations have small sample sizes within the analysis, which is likely why locus-pair linkage disequilibrium tests could not be run.

3.4.4 **Summary statistics**

Summary statistics offer information on the number of alleles, allelic richness, observed heterozygosity and expected heterozygosity from each locus in each population. Analysis of heterozygosity can offer information on a populations structure or history (Allendorf, 1986). Lower observed heterozygosities than expected could be the result of inbreeding. When the results from each populations are averaged across all loci, we get a general comparison of the genetic diversity of each population. The Mini Hump Creek kokanee population had generally average values for the number of alleles, allelic richness and expected and observed heterozygosity compared to the other populations from both analyses. In the Taylor lab analysis, the Mini Hump Creek population did have a higher than average difference between the observed and expected heterozygosity 0.72 and 0.81, respectively, compared with the other populations. Therefore, there is potential inbreeding or another factor that has reduced diversity in the population. Connector Creek had a low average number of alleles, allelic richness and heterozygosity and this may be due to low sample size, although it should be noted that 10 individuals of a spawning population of 22 were sampled.

Taylor Lab analysis

The mean number of alleles for each locus across populations ranged from 7.4 (sd=2.67) at Omy77 (Appendix, table 1) to 24.1 (sd=12.16) at One103. Mean allelic richness across all loci and populations was 8.66 (sd=2.92). The genetic diversity, reported as observed heterozygosity, was between 0.63 (sd=0.16) for Omy77 and 0.90 (sd=0.08) for One103 averaged by loci across all populations. Genetic diversity by population across all loci ranged from 0.64 in Gates Creek (sd= 0.23) to 0.79 (sd=0.15) in Anderson Lake. Mini Hump Creek had below-mean allelic richness of 8.31 (sd=3.0) with a range from 4.54 (Omy77) to 13.7 (Oki10). Mini Hump Creek genetic diversity ranged from 0.60 (Omy77) and 0.95 (Oki10) with a mean Ho of 0.72 (sd=0.26). The Mini Hump population had 11 potentially unique alleles making up 9% of the total alleles within the population from the analysis. Two unique alleles represented 9% and 10% of the population at the loci Ots108 and Ots100, respectively.

The highest level of mean genetic differentiation at a single loci among populations was noted in Ots100 (Fst= 0.179, range 0.004-0.459). The mean genetic pairwise divergence across all populations and loci was Fst=0.083 (95%CI= 0.051-0.120).

Table 5 Values for the N (number of individuals), a (number of alleles), ar (allelic richness), alleles averaged by population size, and measures of He (heterozygosity expected), and Ho (heterozygosity observed). All values were averaged across all loci for each population from the analysis completed in the Taylor Lab.

	Minihump	Alouette	Anderson	Seton	Meadow	Chedakuz	Davidson	Portage	Hansen	Gates	Saiko
n	37	37	79	46	30	30	31	35	19	39	12
a	13.8	11.1	24.6	19.2	16.6	11.2	10.8	15.9	11.3	11.8	7.9
ar	8.3	7.5	10.4	10.4	9.7	7.5	7.6	9.5	9.0	6.7	7.5
He	0.81	0.80	0.87	0.87	0.85	0.77	0.78	0.84	0.84	0.77	0.81
Ho	0.72	0.74	0.79	0.78	0.77	0.70	0.66	0.77	0.76	0.64	0.70

Molecular Genetics Lab analysis

The mean number of alleles for each locus across populations ranged from 2.71 (sd=1.14) at Oki1a (Appendix, Table 2) to 16.79 (sd=6.23) at Oki10, while mean allelic richness across all loci and populations was 4.69 (sd=2.39). Mean genetic diversity across populations was between 0.29 (sd=0.21) at Oki1a and 0.90 (sd=0.11) at Oki10. Mean genetic diversity averaged across loci was between 0.12 (sd=0.22) at Grafton Lake and 0.74 (sd=0.20) in Powell Lake. Among populations and across loci, Mini Hump Creek had above average allelic richness, while Connector Creek had below average allelic richness at 4.87 (sd=2.07) and 2.56 (sd=1.56) respectively (Table 7). Mini Hump Creek allelic richness ranged from 2.19 (Oki1a) to 9.25 (Oki10). Mini Hump Creek genetic diversity ranged from 0.30 (Oki1a) to 0.92 (Oki10). Mini Hump Creek potentially had six unique alleles, none of which made up a major component of the loci allele frequencies. Using exact G-tests to compare allele frequencies differences between

population pairs, Connector and Mini Hump Creek populations were significantly different at 13 of 14 loci ($P<0.05$).

The highest level of mean genetic differentiation at a single loci across all populations was at Oki16, Fst=0.417 (range 0.008-0.807). The mean pairwise divergence across all loci and populations had a Fst=0.162 (95% CI= 0.117-0.215).

Table 6 Values for the n (number of individuals), a (number of alleles), ar (allelic richness), alleles averaged by population size, and measures of He (heterozygosity expected), and Ho (heterozygosity observed). All values were averaged across all loci for each population from the analysis completed in the Molecular Genetics Lab at the Pacific Biological Station.

	n	a	ar	He	Ho
Mini Hump	90	9.2	4.9	0.68	0.67
Connector	10	2.6	2.6	0.34	0.4
Alouette	102	12	4.3	0.63	0.63
Grafton	25	2.1	1.6	0.13	0.12
Anderson	15	26	5.1	0.67	0.7
Seton	31	9.5	5.5	0.65	0.66
Meadow	292	15.2	5.6	0.69	0.7
Chilliwack	93	26	4.3	0.62	0.63
Coquitlam	56	7.6	4.9	0.66	0.65
Horsefly	111	10.9	5	0.66	0.66
Powell	31	11	6.2	0.74	0.74
Sakinaw	67	9.4	4.8	0.63	0.63
Shushwap	84	11.9	5.8	0.71	0.69
Vernon	18	7.3	5.1	0.64	0.68

3.4.5 **Fst pairwise table**

Pairwise comparisons of fixation rates, reported as Fst values, gave me estimates of genetic differentiation of each population pair (M. Nei & Chesser, 1983). Lower Fst values mean populations have more genetic information in common. The Mini Hump Creek population paired closest with the population from Powell Lake, Connector Creek and Hanson Creek populations with Fst values of 0.11, 0.13 and 0.06, respectively (Table 8). High Fst values imply that the populations are genetically distinct and they do not share genetic diversity. Mini Hump Creek had relatively high average Fst values in both the Taylor Lab and Molecular Genetics Lab analysis at 0.11 and 0.18, respectively.

Taylor Lab Analysis

Lake Saiko, Mini Hump Creek and Alouette Lake all shared the greatest levels of genetic pairwise differentiation Fst=0.116 (0.076-0.154), Fst=0.114 (0.061-0.153) and Fst=0.115 (0.085-0.169), respectively (Table 8). The lowest mean level of genetic differentiation was in Anderson Lake Fst=0.059 (0.012-0.098). Mini Hump Creeks pairwise comparison with the lowest genetic differentiation was Hansen Creek at Fst= 0.061.

Table 7 Pairwise Fst of Sockeye Salmon populations from the Taylor lab analysis

	Alouette	**MiniHump**	Saiko	Portage	Gates	Hanson	Seton	Anderson	Meadow	Davidson
Chedakuz	0.1685	**0.1533**	0.1536	0.06027	0.1102	0.124	0.06539	0.06192	0.1093	0.01476
Davidson	0.1541	**0.1508**	0.1397	0.05409	0.1218	0.1199	0.06694	0.06084	0.09976	
Meadow	0.06991	**0.09274**	0.09003	0.05423	0.09894	0.05932	0.04966	0.0399		
Anderson	0.09107	**0.08741**	0.09821	0.01898	0.06748	0.04837	0.01226			
Seton	0.08952	**0.09288**	0.09387	0.03554	0.05746	0.05517				
Hanson	0.08502	**0.06082**	0.07568	0.06286	0.1053					
Gates	0.1532	**0.1355**	0.1618	0.0797						
Portage	0.1025	**0.1036**	0.1128							
Saiko	0.106	**0.133**								
Miniump	**0.132**									

Molecular Genetics Lab analysis

In pairwise comparisons of the Molecular Genetics Lab analysis, Grafton Lake had the greatest mean level of differentiation followed by Connector Creek at Fst=0.426 (0.306-0.710) and Fst=0.318 (0.131-0.710), respectively (Table 9). The lowest mean level of genetic differentiation was in Coquitlam Lake and Powell Lake Fst=0.147 (0.049-0.272) and Fst=0.147 (0.086-0.398). Mini Hump Creek and Connector Creek had a pairwise Fst value of 0.131 while Mini Hump Creek and Powell Lake had a pairwise Fst of 0.110.

Table 8 Pairwise Fst of kokanee populations from the Molecular Genetics Lab analysis

	Connector	MiniHump	Vernon	Shushwap	Seton	Sakinaw	Powell	Meadow	Horsefly	Grafton	Coquitlam	Chilliwac	Anderson
Alouette	0.26	**0.1565**	0.1862	0.1334	0.1299	0.1784	0.09867	0.1286	0.1585	0.3517	0.0488	0.1899	0.1495
Anderson	0.3342	**0.1562**	0.1851	0.08632	0.05183	0.1164	0.1184	0.09296	0.1325	0.5151	0.09795	0.1693	
Chilliwac	0.3323	**0.2249**	0.2406	0.1836	0.1378	0.1889	0.1815	0.1667	0.2063	0.4395	0.1504		
Coquitlam	0.2722	**0.1557**	0.1751	0.1024	0.09162	0.1339	0.09267	0.1083	0.1162	0.37			
Grafton	0.7095	**0.3924**	0.4868	0.3461	0.4823	0.393	0.3977	0.3064	0.3477				
Horsefly	0.3222	**0.187**	0.1649	0.09971	0.1442	0.1467	0.1376	0.1131					
Meadow	0.2993	**0.1745**	0.1464	0.0932	0.1175	0.1121	0.109						
Powell	0.2325	**0.1095**	0.0967	0.0866	0.121	0.1337							
Sakinaw	0.324	**0.185**	0.178	0.13	0.133								
Seton	0.3008	**0.1532**	0.1896	0.1156									
Shushwap	0.2853	**0.151**	0.1438										
Vernon	0.333	**0.1684**											
MiniHump	**0.1307**												

3.4.6 **Neighbor joining trees**

A neighbor joining tree (Figure 9) developed using the Taylor lab data grouped Mini Hump Creek with Hansen Creek in Alaska, which is likely due to a lack of geographically closer populations to the Mini Hump Creek population. The Fraser River populations clustered together with the exception of Gates Creek, which is an anadromous Sockeye Salmon population. Meadow Creek on the Columbia River clustered near to the Mini Hump Creek, Lake Saiko (Japan), Alouette Lake and Hansen Creek (Alaska). The Japanese population, Lake Saiko clustered with Alouette Lake, a Fraser River population. This last result is likely due to long-branch attraction and the populations are not likely to be closely related.

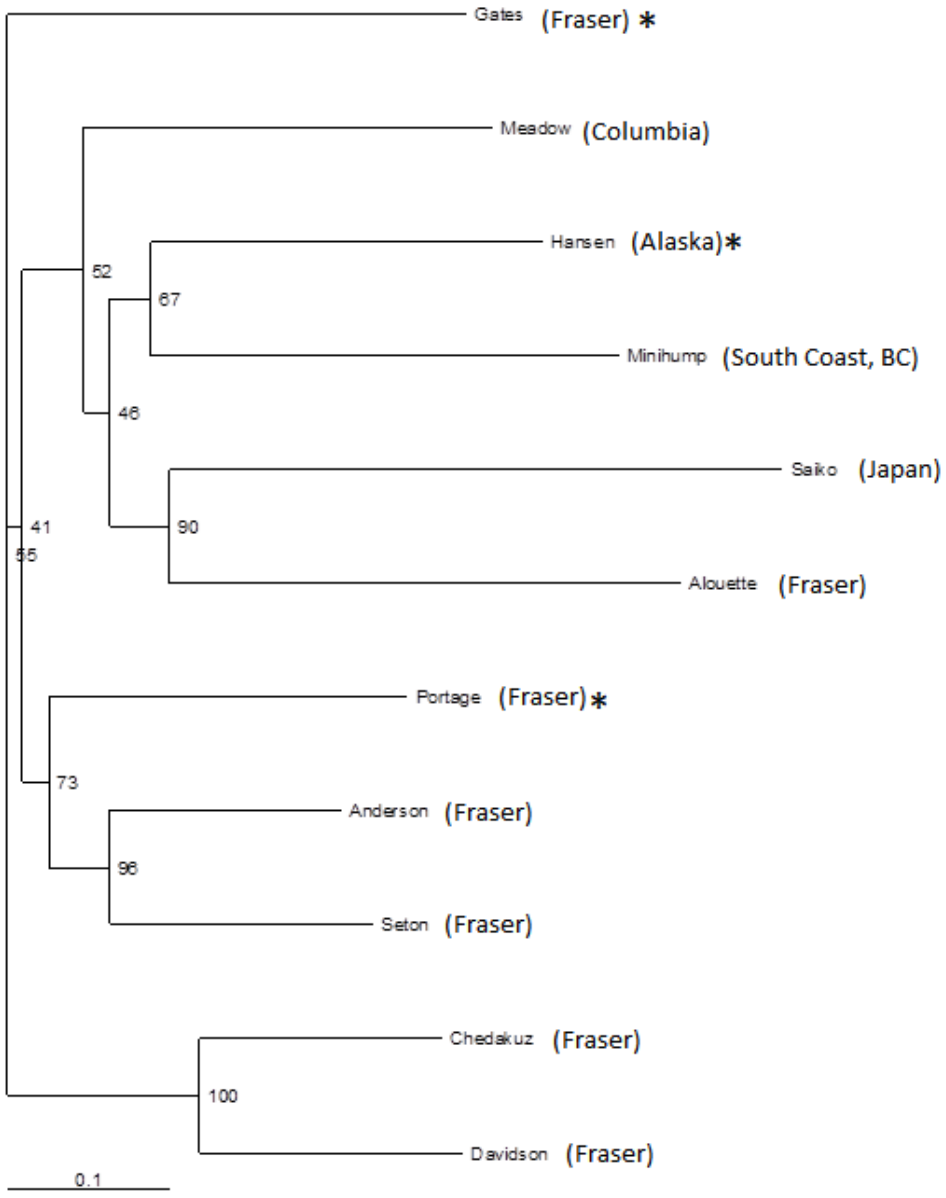

Figure 9 Neighbor joining tree including bootstrap values (x4000) at nodes for the percentage of runs which included that grouping and chord distance by arm length for populations included in analysis completed at the Taylor Lab. The larger watershed or region of each system is in parenthesis. Asterisks represent anadromous Sockeye Salmon populations.

3.4.7 **Genalex tree**

The results of the neighbor joining tree using chord distance measures gave some unlikely results, likely due to long branch attraction. Removing measures of genetic distance from the analysis using Principal components analysis (Figure 10) or ordination in GenaIex, placed Lake Saiko (Japan) as an outgroup to all North American populations. Mini Hump Creek and Hansen Creek still clustered together and were clustered within a group containing Alouette Lake and Meadow Creek. The Fraser River populations clustered together with the exception of Alouette Lake (Figure 10).

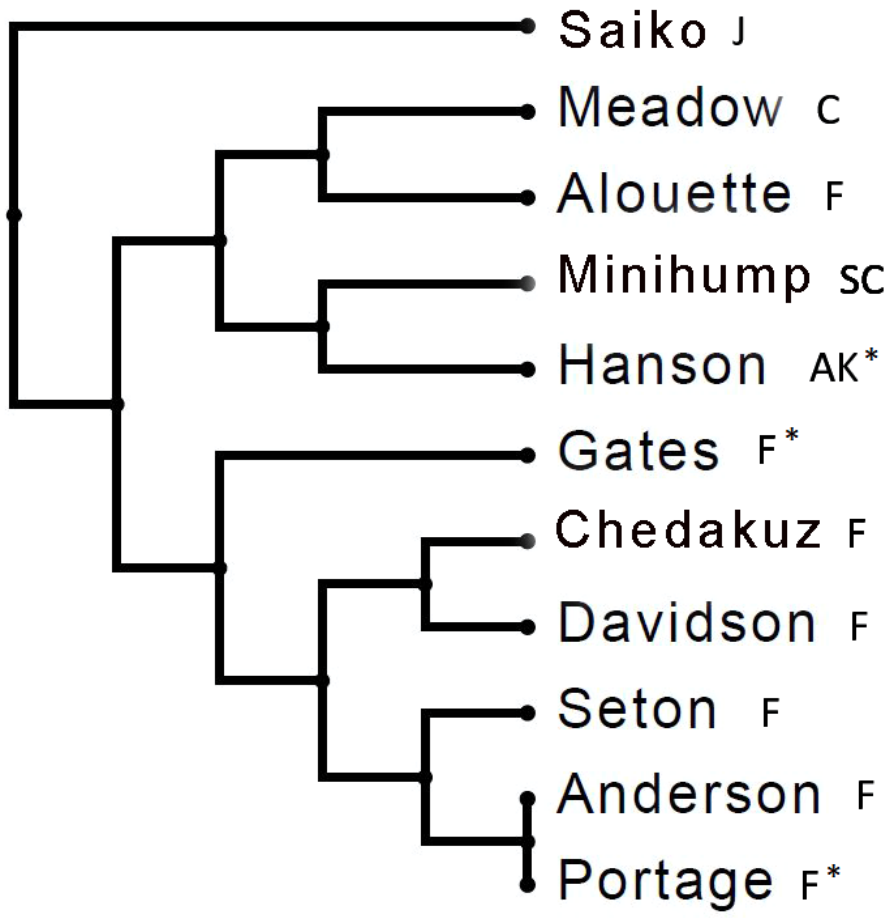

Figure 10 Phylogenetic map of populations of Sockeye Salmon from the Taylor Lab analysis removing measures of genetic distance. Watershed or area of origin represented by: J (Japan), C (Columbia River), AK (Alaska) and F (Fraser River). Asterisks represent anadromous Sockeye Salmon populations.

3.4.8 **Molecular Genetics Lab tree**

A neighbor joining tree developed from the Molecular Genetics Lab data clustered Grafton Lake and Connector Creek together, within a group with Mini Hump Creek (Figure 11). Grafton and Connector both have long-branch arms, which is likely why they are grouped together using Dc measures of a neighbor joining tree. The Mini Hump cluster shared a branch with Powell Lake and Vernon Lake, two geographically close coastal kokanee populations. The bootstrap values for the Mini Hump Creek, Grafton Lake, Connector Creek and the branch with Powell Lake and Vernon Lake were relatively low at 48, 52 and 36, respectively. Therefore, the structure between the coastal kokanee populations is potentially different than is reported here as the Connector Creek and Mini Hump Creek kokanee populations share the same lake system. The Meadow Creek node with Horsefly Lake and Shuswap Lake also had a low bootstrap value of two putting the relationship between the groups in question. Meadow Creek is supposed to act as an outgroup as the creek is part of the Columbia River system.

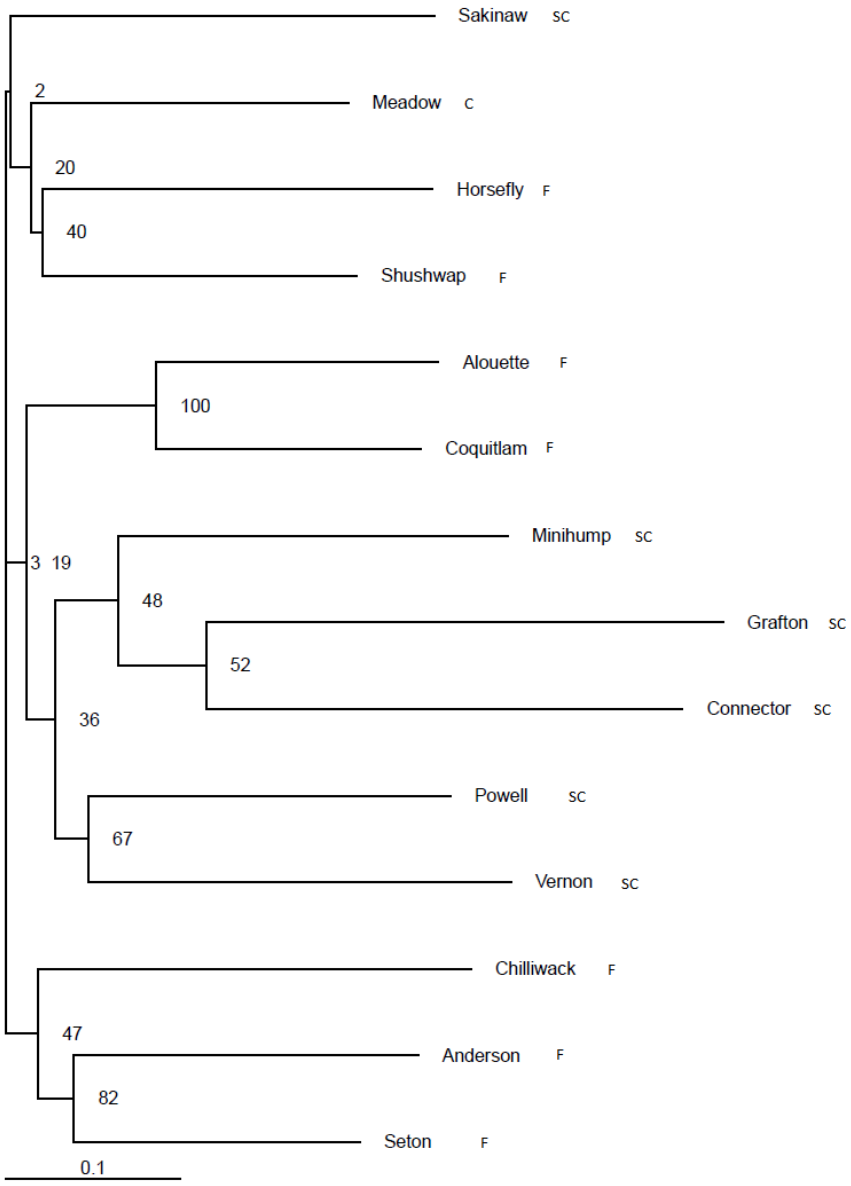

Figure 11 Neighbor joining tree including bootstrap values (x5000) at nodes for the percentage of runs which included that grouping and chord distance, as measured by length of arms for the 14 kokanee populations included in the Molecular Genetics Lab Analysis. Watershed or area of origin represented by: SC (South Coast, BC), C (Columbia River) and F (Fraser River).

A neighbor joining tree developed by the Molecular Genetics Lab (figure 10) including kokanee populations and anadromous Sockeye Salmon populations found that Mini Hump Creek and Connector Creek kokanee did not cluster with the geographically closest anadromous population, Quatse River, approximately 80 km away (Figure 5). The analysis indicated a genetic closeness to Powell Lake and Vernon Lake kokanee populations. Vernon Lake is the geographically closest kokanee population to Loose Lake at approximately 125 km away. No bootstrapping was completed, so the significance of the genetic relationships cannot be derived from this analysis as it is not part of the methodology of the Molecular Genetics Lab.

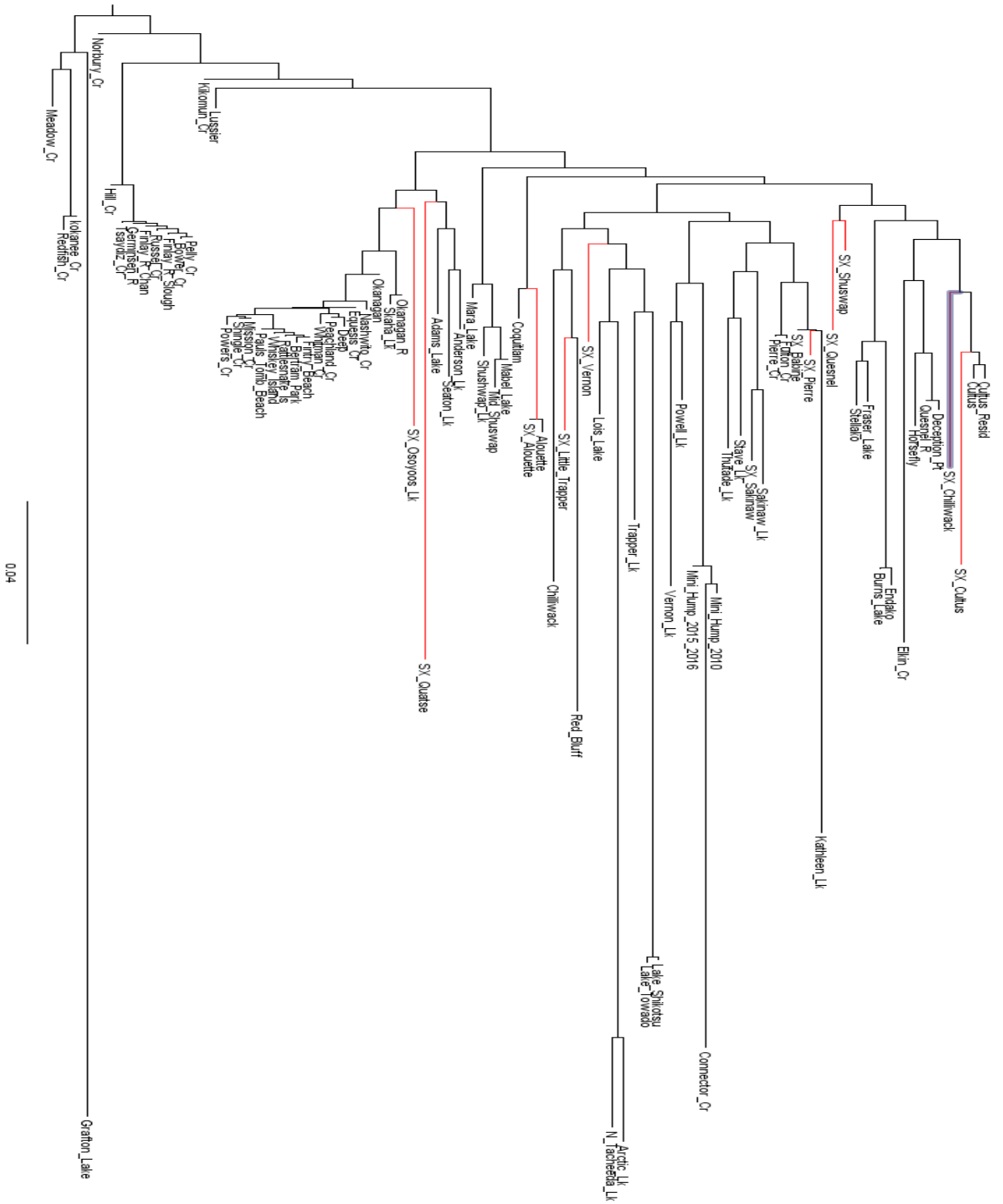

Figure 12 Phylogenetic neighbor joining tree developed by the Molecular Genetics Lab including kokanee populations (black arms) and anadromous Sockeye Salmon populations (red arms).

3.4.9 **Genetix FCA plots**

Factorial correspondence analysis using all 14 loci and 14 populations from the Molecular Genetics Lab data set allowed me to visualize how individuals from each population group among the entire data set. FCA finds the best fitting relationship between allele frequencies at loci and presents this variation graphically for each individual (Bryja et al., 2010) (Figure 13). Mini Hump Creek and Connector Creek populations group together and farthest from the other populations. Chilliwack (white, top-centre) and Horsefly (teal, bottom right) also show differentiation from the other populations. Anderson Lake and Seton Lake both show outliers.

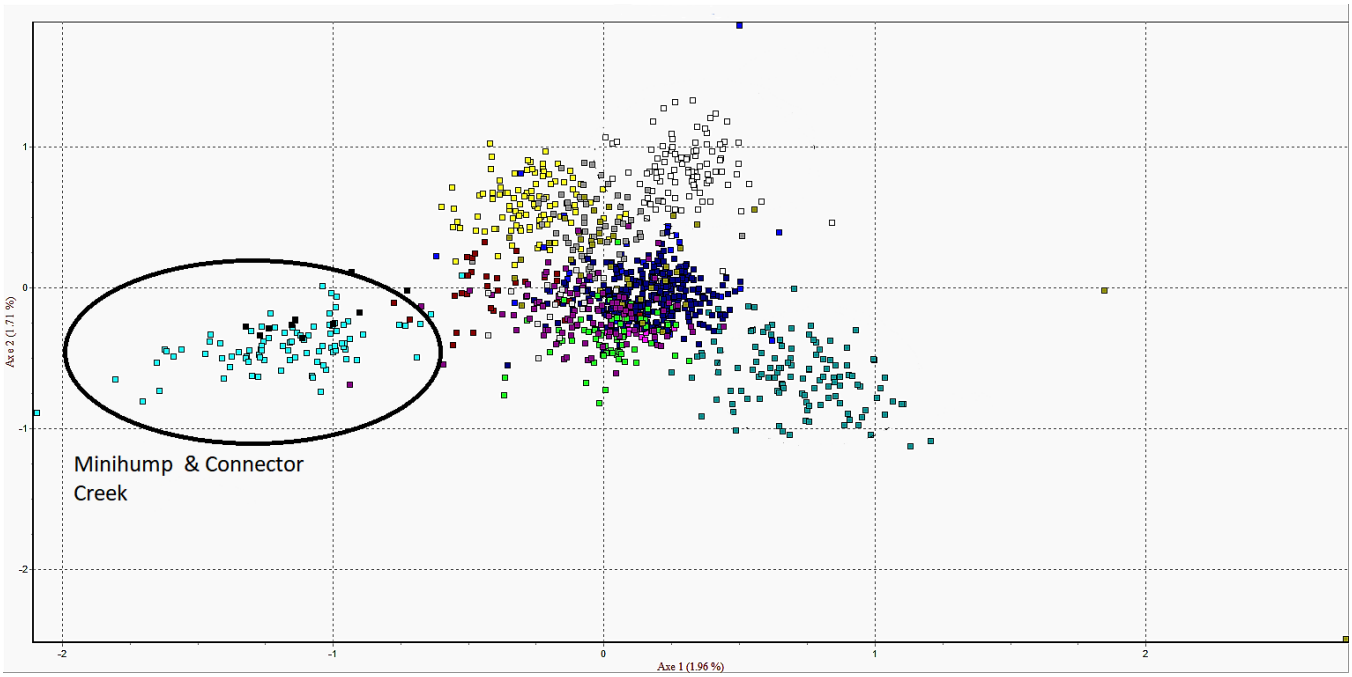

Figure 13 FCA results including 14 microsatellite loci and 14 populations of Sockeye Salmon. Each dot represents an individual and each colour represents a population. The distance from 0 on both axis represents the inertia of differentiation from each Loci pairing. Turquoise (Mini Hump Creek), black (Connector Creek), yellow (Alouette), blue (Anderson), white (Chilliwack), dark gray (Coquitlam), pink (Grafton), teal (Horsefly), dark blue (Meadow), burgundy (Powell), green (Sakinaw), bronze (Seton), purple (Shuswap), light gray (Vernon).

The 3D FCA grouped Mini Hump and Connector Creek together (Figure 14). Meadow Creek, a population from the Columbia River system, was differentiated from the Fraser and coastal

populations. Horsefly Lake, a geographically distant system in the Fraser River, also grouped away from the Fraser and coastal populations but not to the same extent the Loose Lake populations. The coastal populations, which are not circled on the plot, grouped towards the Alouette and Loose Lake populations while the Fraser populations (not circled) cluster towards Horsefly Lake.

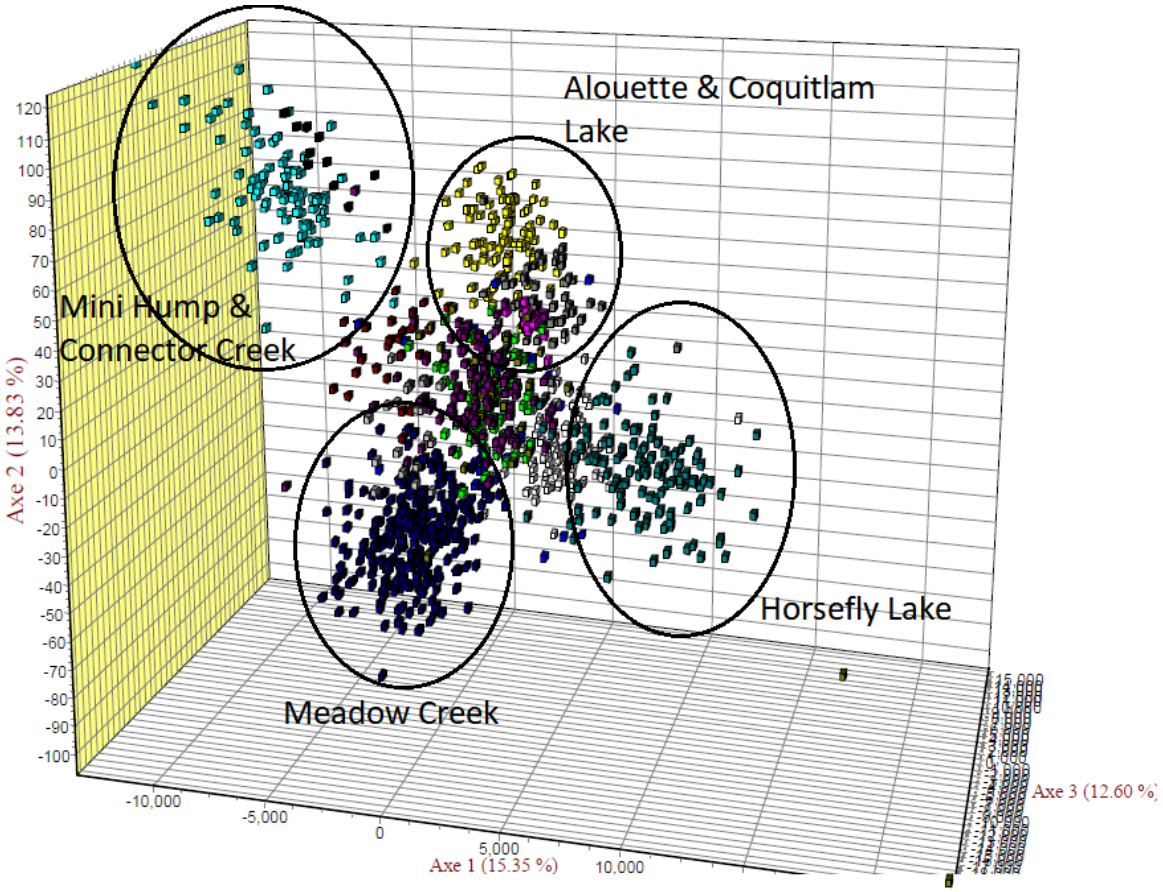

Figure 14 3D FCA visualization of all 14 Sockeye Salmon populations and microsatellite loci with an emphasis on population of origin included. Each dot represents an individual and each colour represents a population. The distance from 0 on both axis represents the inertia of differentiation from each Loci pairing. Coastal populations: Turquoise (Mini Hump Creek), black (Connector Creek), pink (Grafton), burgundy (Powell), green (Sakinaw), light gray (Vernon). Fraser populations: yellow (Alouette), blue (Anderson), white (Chilliwack), dark gray (Coquitlam), teal (Horsefly), bronze (Seton), purple (Shuswap). Columbia population: dark blue (Meadow).

3.4.10 **Molecular Results Summary**

The Mini Hump Creek kokanee were confirmed to be a Sockeye Salmon population by the microsatellite analysis. The population had a below-average number of alleles and allelic richness across loci and analysis. Mini Hump Creek kokanee had lower than expected heterozygosity when averaged across loci and populations. Pairwise estimates of genetic differentiation from both analysis found the Mini Hump population was relatively distinct from the other populations in the analysis. Mini Hump Creek kokanee had levels of genetic differentiation similar to that of the Lake Saiko, a population from Japan, when compared with Fraser River, Columbia River and an Alaskan population. When compared to other coastal kokanee populations along with those from the Fraser and Columbia rivers, the Mini Hump Creek population had relatively high genetic differentiation, which was similar to the levels found in other coastal kokanee populations. In the pairwise comparisons, the Mini Hump population had the most genetic information in common with Hansen Creek in Alaska. This result is likely due to the populations used in the initial analysis and may represent a lack of geographically close populations in the analysis. When other coastal kokanee populations were included in the analysis, the Mini Hump population shared the most genetic information with Powell Lake, another coastal kokanee population, and Connector Creek, the other population present in the Loose Lake system. These results were confirmed with chord distance and PCA analysis, which initially placed the Mini Hump population with Hansen Creek in Alaska on phylogenetic trees, again likely due to a lack of geographically closer populations present in the analysis. When coastal kokanee were included in the analysis, the Mini Hump population clustered closer to Connector Creek, Grafton Lake, Powell Lake and Vernon Lake, which are all coastal kokanee populations on the southern coast of BC. FCA analysis also showed the genetic relatedness of kokanee from Mini Hump and Connector Creek while differentiating them from the Fraser River, Columbia River and Coastal kokanee populations. Powell Lake kokanee was the next closest population to Mini Hump and Connector Creek in the FCA analysis, which was similar to the chord distance phylogeny results.

4 Discussion

To characterize the Mini Hump Creek kokanee population and make predictions about the origins of the population I analyzed their morphology, life history, genetics, ecology and recent history of the region. My results indicate the Mini Hump population is kokanee and is morphologically similar to black kokanee, yet has a life history that is more similar to a normal kokanee population.

The Loose Lake system has been drastically altered by logging and land use over the last century. A logging dam, acting as a barrier to salmon migration, was placed on Scott Cove Creek and Mini Hump Creek in 1918 and 1939 respectively. Both dams stood for nearly 60 year and would have prevented anadromous Sockeye Salmon from entering the system. There are no anecdotal reports of anadromous Sockeye Salmon using the Loose Lake system prior to the dams being built in 1918. However, the river slope is low enough to allow Coho Salmon into the lake and the system is similar to those which contain runs of anadromous Sockeye Salmon, so it is possible, that an anadromous Sockeye Salmon population was present in the Loose Lake system prior to logging since observations and records preceding 1918 are inadequate to confirm the absence of such a run (Groot & Margolis, 1991b; Youds, 1982). The dams were used to raise lake water levels and to level slope between the systems. The currently used spawning grounds would likely have been submerged and blocked by logging activities as a dam was placed at the mouth of Mini Hump Creek at the outflow to Loose Lake and the water level was raised on both sides of the dam to lessen gradations between the Towney Lake and Loose Lake, which was raised up to 5.2 m. The water in Loose Lake is dark with Secchi disk measures of clarity averaging 2 m in depth, therefore, it is possible that a limited amount of light would attenuate to 5.2 m potentially reducing the sexually selective pressure on bright spawning colouration.

The Mini Hump Creek population shares a similar external morphology to black kokanee from Anderson and Seton Lake BC. The Mini Hump Creek population have standard lengths that are relatively similar to those from Connector Creek, the smaller of the two populations, but have larger size-adjusted body depth, eye size and upper-jaw lengths closer to those of the Anderson Lake population. In sympatric comparative studies of kokanee body depth it was noted that larger maximum depth of males and longer jaws were indications of populations that spawn at

depth or on beaches relative to sympatric river spawning individuals (Blair et al., 1993; Hendry & Quinn, 1997). The Mini Hump Creek population has no sympatric population for comparison, but, based on the land use surrounding the watershed, the Mini Hump Creek spawning grounds would likely have been submerged from the current clear shallow depth of approximately 0.3 m depth to match the height of the dam offshoot at 5.2 m in height and would have been blocked by the logging dam located at the mouth of the river. The dam would have prevented fish from entering Mini Hump Creek from Loose Lake but would not have prevented fish from moving from Towney Lake into Mini Hump Creek or Loose Lake.

The Mini Hump Creek population had much lower gill raker counts compared to the black kokanee of Anderson and Seton Lake with an average of 32 and 38 (combined average for Anderson and Seton), respectively. Gill rakers are diet dependent and the numbers vary widely by population with those that have fewer gill rakers eating larger prey items and those with more gill rakers having a diet composed mostly of plankton (Beacham, 1985; Foote et al., 1999; Kurenkov, 1977; Wood et al., 2008). Anadromous Sockeye Salmon are noted to have lower numbers of gill rakers than sympatric kokanee populations and a trend in increasing gill raker numbers with latitude was also reported (Beacham, 1985; Foote et al., 1999). I found the Mini Hump kokanee had diets that ranged from individuals eating almost purely *Daphnia* to subsisting on adult insects and glass worms. The gill raker numbers did not vary widely, 30-35, a relatively low count for kokanee. It would be expected that the predominant prey choice would be larger items than *Daphnia*. The low gill raker count could be a hold over trait from a recent founding population in Loose Lake as gill rakers have been reported to be a heritable trait (Vernon, 1957).

Seton Lake shares similarities in limnology to Loose Lake as the lake is reported to be tannic and dark. Anderson Lake is reported to be clear and have a much higher index of plankton than Seton Lake, which is thought to be responsible for the difference in size between the populations (Moreira & Taylor, 2015). I completed a small plankton survey in Loose Lake but the data has not yet been analyzed to make comparisons to other systems. Plankton abundance and composition data can inform if the Mini Hump kokanee pigmentation is related to prey choice when compared with stomach contents and ultimately related to recently being isolated.

The Mini Hump Creek kokanee spawn in clear shallow streams, like regular kokanee populations, which is in contrast to true black kokanee populations that spawn at depth in lakes. Alouette Lake kokanee, a contemporary black kokanee population, spawn at depth in what are submerged creek spawning sites altered by reservoir building (Andrusak & Irvine, 2013). Red colouration due to emplacing carotenoids in the skin at spawn is an important sexually selected trait in Sockeye Salmon (Craig & Foote, 2001; Hendry & Berg, 1999). Black kokanee are hypothesized to have lost nuptial red colouration as red light attenuates rapidly with increasing depth and so expending energy to put carotenoids into the skin would be a potentially maladapted trait (Moreira & Taylor, 2015). Dark, tannin rich, water decreases light attenuation (Reimchen, 1989). In clear water, most visible light is absorbed within the first 10 m and red light, which attenuates faster than blue and green light, is absorbed entirely by 15 m (Davis, 1991). The Mini Hump Creek population lacks this nuptial red colouration in both the skin and flesh, yet the eggs are bright red and orange. Small rosy patches on the skin were noted in 31 of 51 individuals, so it was apparent carotenoids were being transplanted to the skin to some extent. With the changing depths of spawning grounds due to dams and water-level changes, the Mini Hump Creek kokanee may have been forced to spawn at depth while the logging dams remained in place and nuptial red colouration could have been selected against because of its high energetic cost (Craig & Foote, 2001; Hendry & Berg, 1999). Since the dams have been removed and spawning grounds were returned to clear shallow streams, perhaps red nuptial colouration has become important again and is now being selected for.

Coastal Cutthroat Trout and Dolly Varden caught in Loose Lake displayed bright red markings, skin pigmentation and red flesh not found in the Mini Hump Creek kokanee. Therefore, there are carotenoids in the Loose Lake system, yet the kokanee of the system are not up taking them at the same rate as the trout. Potentially, the kokanee are eating a diet consisting mostly of different prey than the trout of the lake. I collected Cutthroat Trout tissue samples so future stable isotope analysis can be used to compare the diet of the Cutthroat Trout and kokanee of Loose Lake. However, an alternative explanation may be that Mini Hump Creek kokanee, are inefficient at extracting carotenoids if they are a recently derived kokanee population. Because lakes are deficient in carotenoids compared to the marine environment (Craig & Foote, 2001) anadromous Sockeye Salmon are inefficient at extracting carotenoids relative to long-established kokanee as anadromous Sockeye Salmon have a surplus of carotenoids in their diet whereas

kokanee eventually evolve higher efficiency at extracting carotenoids from their diets (Craig & Foote, 2001).

Daphnia are a major component of the Mini Hump Creek kokanee diet. *Daphnia* create and store less carotenoids at low light levels (Green, 1957). Loose Lake is relatively acidic at a pH of 5.5. Daphnia do not do well in acidic environments yet their populations expand likely due to a lack of competition (Schindler et al., 1985). Since the Mini Hump kokanee depend on *Daphnia* and the lake is dark and acidic, they are potentially ingesting low levels of carotenoids due to the acidic environment.

Residual Sockeye Salmon are believed to be the evolutionary intermediate step between anadromous Sockeye Salmon and kokanee (Ricker, 1938). Residual Sockeye Salmon are a component of an anadromous population that are reported to be smaller, drabber in colouration than their anadromous counterparts and remain in the spawning lake instead of migrating out to sea. If the anadromous component of a population can no longer migrate back to natal lake or stream due to a barrier, the residual component of the population would potentially give rise to a kokanee population. Anadromous populations used the Alouette Lake system and a sympatric kokanee population was not known before the dam was built on the system in the 1928 (Andrusak & Irvine, 2013; Godbout et al., 2011). It is believed the building of the reservoir created the Alouette black kokanee population as the anadromous component of the population could no longer reach the natal lake and spawning sites. Recently, re-anadromy experiments have been attempted with anadromous Sockeye Salmon returning to the dam after kokanee smolts were released from the system, parentage confirmed by genetic analysis (Andrusak & Irvine, 2013; Godbout et al., 2011).

Grafton Lake and Loose Lake are both systems that have been dammed over the last century and have kokanee populations of unknown origin. The Grafton Lake and Mini Hump Creek populations could have similar origins to those of Alouette Lake, being derived from anadromous Sockeye Salmon populations extirpated by the installation of migration barriers. Both lake systems may have had anadromous Sockeye Salmon populations using the lake, which were extirpated by man-made barriers to migration, leaving the populations to be maintained by the remaining residual component. Conversely, both the Mini Hump and Grafton populations

could be kokanee populations that have independently arisen and diverged from anadromous Sockeye Salmon populations at a time closer to the last glacial maxima (Berg, 1985; Ricker, 1938, 1959).

The Mini Hump Creek spawning population is small, since enumerations began in 2009 the peak spawn has ranged from 183 to 1962 spawning individuals (Gagnon, 2016). Females produce a low number of relatively large eggs, the maximum I observed was 115 from a single female with eggs averaging 5.0mm and 0.06g. Comparative egg and length study of kokanee and Sockeye Salmon populations found female kokanee the size of the Mini Hump population are expected to have eggs of 0.053g (McGurk, 2000; Quinn et al., 2015). The number of eggs appears to be limited by the female size as the body cavity if filled with eggs at spawn to the point the internal organs appear reduced and diminished. The trait of possessing larger and fewer eggs rather than smaller but more numerous eggs may be due to requiring a larger initial nutrient investment per individual to ensure survival or could be a result of the ideal egg size based on stream characteristics and migration (Johnston & Leggett, 2002; Patterson, 2004; Quinn et al., 2015). Neither McGurk (2000) nor Quinn et al. (2015) found populations with mean fork length or fecundities as low as the Mini Hump Creek population (McGurk, 2000; Thomas P. Quinn et al., 2015).

Loose Lake and Mini Hump Creek have a relatively low pH of 5.6, reported in this study (Rogers, 2009). Low pH levels can drastically reduce the growth in fresh water and the marine survival of juvenile Sockeye Salmon (Kennedy & Picard, 2012). Kennedy (2012) reported a 46% reduction in size of Sockeye Salmon fry exposed to a low pH (5.0) compared to a control pH (6.8) and comparative marine survival rate of 14%. Adult kokanee are impacted by slight increases in acidification and show reduced to no redd digging behavior as pH decreases from 6.6 to 6.0 (Kitamura & Ikuta, 2000). In comparison, Mini Hump Creek has a pH of 5.7 yet the females dig redds and spawn in the same manner as regular kokanee populations (Gagnon, 2016; Rogers, 2009). In a lab-based study of Sockeye Salmon and acidification, adult Sockeye Salmon , their deposited eggs and the resulting alevins were exposed to a pH pf 5.6 and compared to a control group exposed to pH of 7.1 (Parker & Mckeown, 1987). The salmon and eggs exposed to low pH had longer times to hatch, decreased egg survival, exhibited smaller sized alevins and reduced yolk to tissue conversion compared to the control group. It is possible the Mini Hump

population exhibit small body size as a result of the relatively low pH level in Loose Lake and Mini Hump Creek. The low pH level could also have greatly reduced the potential for successful smoltification and marine survival of any juvenile kokanee leaving the Loose Lake system, further enforcing a non-anadromous population.

With such low fecundity and low spawning abundance of the Mini Hump Creek population, it seems unlikely that the population has the potential to survive significant environmental disturbances. The Connector Creek population has a spawning abundance of 22 individuals, if the population was to be isolated then it would likely not survive inter-annual variation in environmental conditions and would be at high risk of extinction due to demographic stochasticity. Alternatively, the Connector Creek population is potentially a component of the Mini Hump Creek population. The genetic results from this analysis seem to support that the two populations are connected (FCA interpretation) and observed heterozygosity of the Connector Creek population, which was higher than expected. Yet, the populations show genetic distinction through G test and Fst results. The genetic differentiation may be the result of a low number of samples from Connector Creek, 10 collected, and the effect of family interactions on genetic results. Low sample size in microsatellite analysis can bias and artificially inflate the genetic distance between populations by only having a portion of the genetic diversity within a population included in a analyses (Ruzzante, 1998). Since the Connector Creek spawning population was observed to be 22 individuals, having sampled 10 for the genetic analysis should have encompass a large portion of genetic diversity for the spawning year.

One factor that may have facilitated the adaptation and persistence of the Mini Hump Creek kokanee was the removal of Coho Salmon from Loose Lake due to the dam, which could have greatly reduced Sockeye Salmon fry predation and offset some of the negative impacts of dam building and isolation. Juvenile Coho Salmon have been reported to eat Sockeye Salmon fry in lakes where both species are present (Ruggerone & Rogers, 1992). Ruggerone (1992) reported that Coho Salmon smolts consumed approximately 59% of the Sockeye Salmon fry population in the Chignik Lakes, Alaska during a three-year study. This reduction in fry ultimately resulted in lower adult Sockeye Salmon returns compared to other nearby lake systems with lower Coho Salmon abundance. Coho Salmon populations were present in the Loose Lake system prior to logging and have increased since the dams were removed from Scott Cove Creek (Youds, 1982).

Coho adults, fry and smolts have been recently observed in Loose Lake and Mini Hump during enumerations and recreational fishing (Gagnon, 2016; Youds, 1982). The Coho Salmon smolts in Loose Lake are the size of adult Mini Hump kokanee at approximately 15 cm total length and Coho Salmon smolts are reported to eat prey as large as 47% of their total length (Pearsons & Fritts, 1999). Therefore, Coho Salmon smolts in Loose Lake are large enough to be predators of Mini Hump kokanee fry which are expected to be less than half the size of the adults. Cutthroat Trout are active predators of Sockeye Salmon fry and are present in Loose Lake and, therefore, could have continued to impact the Mini Hump Creek kokanee after the dams prevented anadromous salmon from entering the system (Cartwright et al., 1998). The emplacement and removal of dams in the Loose Lake system has potentially altered the predation regimes in the lake which may affect the persistence of the Mini Hump Creek kokanee population.

Genetically, the Mini Hump Creek kokanee appear to be most closely related to other coastal kokanee populations including those of Vernon and Powell lakes. The Mini Hump and Connector creek populations had relatively high average differentiation (Fst) values meaning they were not likely closely related to other populations in the analysis. The Mini Hump Creek populations had lower expected than observed heterozygosity, and lower than average among populations, meaning they may be inbreeding at a higher level than other population included in the study. Conversely, the Connector Creek population had a higher observed heterozygosity than expected, which could be explained by immigration from the Mini Hump Creek population. The Mini Hump Creek kokanee population has above average genetic differentiation and lower than average observed heterozygosity, which could be the result of isolation or reduced genetic diversity due to an environmental disturbance or a period of very small population size (i.e. a recent bottle neck event) (Luikart et al., 1998; Nei et al., 1975; Withler et al., 2000) The pairwise differentiation (Fst) and FCA analysis suggest the Loose Lake populations are genetically closer to Powell Lake kokanee (208 km) opposed to the geographically closer Vernon Lake (101 km). The chord distance measure and FCA find Vernon and Powell Lake grouping closer together to each other than to the Loose Lake populations. This would suggest the Loose Lake populations were isolated from the other coastal kokanee populations, which fits with what we know of the recent history of the Loose Lake system.

The microsatellite analysis did not have the power to infer a timeline of genetic isolation or differentiation from other kokanee and Sockeye Salmon populations (Hey & Nielsen, 2004; Tamura et al., 2012; Zhivotovsky, 2001). Divergence time estimates require making large assumptions of population size, migration rates and genetic drift rates (Zhivotovsky, 2001). Without having genetic samples from anadromous Sockeye Salmon from the system it is improbable that an effective estimate of divergence time could be gleaned using strictly molecular genetic methods.

Chord distance measures created phylogenies with unlikely results. Alouette Lake in the Fraser Valley and Lake Saiko in Japan were grouped together, with high bootstrapping values, which is a highly improbable relationship. This relationship is likely the result of long-branch attraction. Long-branch attraction is a problem that affects genetic analysis using microsatellites and chord distances especially when microsatellites are used from similar species, which is the case for microsatellite analysis in salmon (Beacham et al., 2001; Bergsten, 2005; Straub et al., 2014). Long-branch attraction was likely responsible for clustering Grafton Lake and Connector Creek populations. Grafton Lake, Connector Creek and Lake Saiko all had low sample sizes, which may have caused the long-branch attraction in the chord distance analysis (Bergsten, 2005). In microsatellite based population analyses, 25 to 30 individuals is believed to be enough to make accurate assessments of population structures (Hale, Burg, & Steeves, 2012).

The Grafton Lake population offers some interesting comparisons to Mini Hump and Connector Creek populations as the Grafton Lake system currently has no anadromous Sockeye Salmon, there is a manmade barrier to migration on the outflow river of the lake and they are a coastal population. Grafton Lake is small at 10 hectares, Loose Lake is 280 hectares for comparison. The Grafton population appeared to have gone through a major genetic bottleneck as nine of the 14 loci were at fixation in our analysis. The Grafton population had more genetic samples than that of Connector Creek (26 and 10), yet Connector Creek had more genetic variation within my analysis with heterozygosities of 0.40 and 0.12, respectively. The Grafton Lake population is now believed to be extirpated as a spawning population has not been observed since the tissue samples were collected in 2002 (Bell-Irving, 2016).

Logging and road building operations are still taking place around the Loose Lake watershed. Specifically, clear cutting is occurring surrounding the middle reach of Connector Creek and a bridge was built over the same segment. Logging and environmental alteration may have shaped the life history and morphology of the Loose Lake and Connector Creek population yet they remain under threat. Both populations have low spawning abundance, while the Mini Hump population is confirmed to have a very low fecundity. It seems very likely that further alterations to the ecosystem could push the populations past the point of being self-sustaining. The Grafton Lake population offers an example of what can happen to a small isolated population when it reaches below a certain threshold of genetic diversity and potentially low population size. The Mini Hump Creek and Connector Creek population should be offered protection or at the very least considered when land use decisions are being made in the region. While communicating with biologists from Interfor logging company, the logging license holder from the watershed, Mini Hump Creek was labeled fish bearing-high importance while Connector Creek was marked with a fish bearing-low importance designation, indicating logging could encroach on the riparian buffer if the title holders agreed to do so (Iverson, 2016). While completing salmon enumerations in 2016, a temporary logging bridge was built over the middle reaches of Connector Creek to gain access to trees in the upper reach of the Connector Creek watershed.

The Mini Hump Creek kokanee population offers an example of the adaptive potential of Sockeye Salmon. The formation of kokanee populations is usually considered as occurring since the last glacial maxima and the presence of residual Sockeye Salmon are considered an intermediary between Sockeye Salmon and kokanee (Groot & Margolis, 1991b; Ricker, 1938, 1959; Taylor & Volpe, 1997). The Mini Hump population along with those from Grafton and Alouette lakes offer examples of rapid adaptation of Sockeye Salmon populations brought on by human induced disturbances (Godbout et al., 2011). It is likely there are more cases of recently derived kokanee populations throughout the native Sockeye Salmon range. Some Sockeye Salmon populations have the genetic variation and phenotypic plasticity to undertake anadromous or strictly freshwater lifecycles (Groot & Margolis, 1991b; Ricker, 1938, 1959). Ecological disturbances, in this case, dam building, created environmental changes that were still within the capacity of a Sockeye Salmon population to survive. If disturbances occur and create an environment in which a population does not have the plasticity or standing genetic variation from which to adapt and survive then it would likely be extirpated. The strictly anadromous

components of the Grafton Lake and Alouette Lake Sockeye Salmon populations were extirpated when barriers to migration prevented completion of their life cycle (Bell-Irving, 2016; Godbout et al., 2011). The anadromous component of the Mini Hump Creek population was also likely extirpated leaving behind only the component of the population that could feed and reproduce entirely in fresh water. Overall, my results indicate that the diversity of life histories and the adaptive potential of Sockeye Salmon may have allowed the species to persist as kokanee in the Loose Lake system following the installation of a dam that prevented anadromy and changed the environmental conditions of the lake and river system.

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6 Appendix

Appendix 1 summary statistic data from the Molecular Genetics Lab analysis of South Coast, BC, Fraser River, BC and Columbia River, BC kokanee populations. Fourteen Loci and 14 populations were included

Alouette	1b	3dre	i1	oki10	oki16	oki1a	oki1b	oki29	oki6	omy77	one8	ots103	ots2	ots3	Avg
N	104	104	99	102	102	102	102	101	101	102	103	101	99	103	101.79
Alleles	4	10	7	18	6	2	4	13	6	4	4	12	7	3	12
Allelic richness	3.90	6.08	3.36	8.16	4.12	1.67	2.26	6.87	4.36	2.72	3.13	7.22	4.07	2.81	4.34
He	0.73	0.84	0.65	0.90	0.72	0.14	0.51	0.86	0.75	0.39	0.42	0.85	0.59	0.47	0.63
Ho	0.74	0.93	0.65	0.87	0.71	0.13	0.55	0.82	0.74	0.40	0.46	0.84	0.60	0.43	0.63
Anderson	1b	3dre	i1	oki10	oki16	oki1a	oki1b	oki29	oki6	omy77	one8	ots103	ots2	ots3	
N	14	18	10	8	12	31	24	10	18	10	17	18	7	13	15
Alleles	6	10	9	9	6	2	3	6	3	6	6	12	7	4	26
Allelic richness	4.65	6.08	7.67	8.35	5.23	2.00	2.54	4.78	2.39	5.59	3.94	8.07	7.00	3.80	5.15
He	0.68	0.78	0.85	0.84	0.74	0.44	0.32	0.55	0.51	0.79	0.48	0.86	0.81	0.71	0.67
Ho	0.71	0.78	1.00	0.75	0.58	0.58	0.29	0.60	0.61	0.80	0.59	0.83	0.86	0.85	0.70
Chilliwack	1b	3dre	i1	oki10	oki16	oki1a	oki1b	oki29	oki6	omy77	one8	ots103	ots2	ots3	
N	93	94	91	92	95	94	99	93	86	93	88	90	94	93	92.5
Alleles	4	10	10	15	8	3	4	13	4	7	7	18	9	3	26

Allelic richness	**2.23**	**4.86**	**5.00**	**8.49**	**3.74**	**2.75**	**2.11**	**5.73**	**3.34**	**3.01**	**3.20**	**8.69**	**4.04**	**2.98**	**4.30**
He	**0.21**	**0.69**	**0.77**	**0.91**	**0.65**	**0.57**	**0.34**	**0.81**	**0.63**	**0.44**	**0.50**	**0.91**	**0.64**	**0.65**	**0.62**
Ho	**0.20**	**0.71**	**0.77**	**0.95**	**0.67**	**0.56**	**0.28**	**0.83**	**0.64**	**0.44**	**0.49**	**0.89**	**0.67**	**0.68**	**0.63**
Coquitlam	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**59**	**59**	**53**	**55**	**49**	**58**	**56**	**51**	**56**	**57**	**53**	**58**	**56**	**59**	**55.64**
Alleles	**5**	**10**	**9**	**16**	**7**	**4**	**2**	**12**	**5**	**6**	**5**	**15**	**7**	**3**	**7.57**
Allelic richness	**3.77**	**6.88**	**5.93**	**7.57**	**4.70**	**2.58**	**2.00**	**7.81**	**4.17**	**4.76**	**3.31**	**7.16**	**4.54**	**2.75**	**4.85**
He	**0.53**	**0.86**	**0.81**	**0.87**	**0.73**	**0.30**	**0.47**	**0.89**	**0.72**	**0.71**	**0.45**	**0.86**	**0.64**	**0.43**	**0.66**
Ho	**0.42**	**0.83**	**0.79**	**0.89**	**0.65**	**0.33**	**0.43**	**0.86**	**0.71**	**0.72**	**0.47**	**0.90**	**0.68**	**0.44**	**0.65**
Grafton	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**26**	**25**	**26**	**26**	**23**	**26**	**26**	**24**	**24**	**26**	**24**	**24**	**25**	**25**	**25**
Alleles	**2**	**3**	**2**	**8**	**1**	**1**	**1**	**1**	**1**	**3**	**1**	**4**	**1**	**1**	**2.14**
Allelic richness	**1.27**	**2.73**	**1.47**	**4.58**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.54**	**1.00**	**2.94**	**1.00**	**1.00**	**1.61**
He	**0.04**	**0.47**	**0.07**	**0.63**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.07**	**0.00**	**0.53**	**0.00**	**0.00**	**0.13**
Ho	**0.04**	**0.44**	**0.08**	**0.62**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.50**	**0.00**	**0.00**	**0.12**
Horsefly	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**116**	**113**	**108**	**109**	**107**	**114**	**113**	**113**	**112**	**109**	**99**	**118**	**106**	**118**	**111.07**
Alleles	**4**	**11**	**16**	**21**	**20**	**2**	**4**	**14**	**8**	**10**	**11**	**14**	**9**	**8**	**10.86**

Allelic richness	**3.01**	**4.98**	**5.09**	**8.94**	**6.99**	**1.17**	**2.46**	**5.54**	**3.83**	**4.64**	**5.96**	**7.51**	**5.80**	**3.99**	**4.99**
He	**0.60**	**0.76**	**0.65**	**0.92**	**0.85**	**0.03**	**0.31**	**0.66**	**0.68**	**0.74**	**0.82**	**0.87**	**0.79**	**0.55**	**0.66**
Ho	**0.59**	**0.73**	**0.63**	**0.92**	**0.86**	**0.03**	**0.29**	**0.65**	**0.67**	**0.74**	**0.92**	**0.86**	**0.79**	**0.58**	**0.66**
Meadow	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**289**	**282**	**295**	**293**	**292**	**295**	**295**	**291**	**294**	**285**	**290**	**297**	**292**	**294**	**291.71**
Alleles	**5**	**21**	**22**	**29**	**15**	**4**	**6**	**17**	**16**	**13**	**8**	**26**	**16**	**15**	**15.21**
Allelic richness	**2.71**	**8.52**	**8.37**	**9.50**	**4.18**	**2.70**	**2.74**	**5.17**	**4.55**	**5.31**	**3.19**	**9.85**	**7.31**	**4.19**	**5.59**
He	**0.56**	**0.91**	**0.90**	**0.93**	**0.54**	**0.54**	**0.51**	**0.64**	**0.70**	**0.65**	**0.35**	**0.94**	**0.86**	**0.70**	**0.69**
Ho	**0.57**	**0.91**	**0.89**	**0.92**	**0.56**	**0.53**	**0.56**	**0.66**	**0.68**	**0.67**	**0.36**	**0.91**	**0.87**	**0.71**	**0.70**
Powell	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**31**	**31**	**27**	**31**	**30**	**31**	**31**	**31**	**31**	**31**	**31**	**31**	**31**	**31**	**30.64**
Alleles	**8**	**16**	**15**	**21**	**8**	**5**	**5**	**16**	**11**	**7**	**10**	**13**	**14**	**5**	**11**
Allelic richness	**5.52**	**8.45**	**8.51**	**10.01**	**4.81**	**2.57**	**3.40**	**9.16**	**5.72**	**5.19**	**5.54**	**7.62**	**7.24**	**3.48**	**6.23**
He	**0.79**	**0.89**	**0.89**	**0.92**	**0.71**	**0.31**	**0.61**	**0.91**	**0.77**	**0.71**	**0.68**	**0.86**	**0.83**	**0.43**	**0.74**
Ho	**0.81**	**0.94**	**0.85**	**1.00**	**0.83**	**0.35**	**0.55**	**0.90**	**0.84**	**0.65**	**0.61**	**0.87**	**0.81**	**0.39**	**0.74**
Sakinaw	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**67**	**70**	**64**	**67**	**59**	**70**	**67**	**62**	**69**	**69**	**70**	**68**	**68**	**67**	**66.93**

Alleles	**5**	**15**	**12**	**18**	**11**	**3**	**3**	**17**	**5**	**6**	**6**	**17**	**9**	**5**	**9.43**
Allelic richness	**2.9**	**8.5**	**5.6**	**9.1**	**5.4**	**2.2**	**2.5**	**7.4**	**3.0**	**4.6**	**2.2**	**7.0**	**4.3**	**3.3**	**4.8**
He	**0.4**	**0.9**	**0.8**	**0.9**	**0.7**	**0.4**	**0.4**	**0.8**	**0.4**	**0.8**	**0.2**	**0.8**	**0.7**	**0.6**	**0.6**
Ho	**0.4**	**0.9**	**0.8**	**0.9**	**0.8**	**0.4**	**0.4**	**0.9**	**0.3**	**0.7**	**0.2**	**0.8**	**0.7**	**0.6**	**0.6**
Seton	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**33**	**29**	**27**	**29**	**27**	**36**	**35**	**26**	**34**	**24**	**33**	**31**	**28**	**36**	**30.57**
Alleles	**4**	**14**	**16**	**19**	**7**	**3**	**4**	**15**	**6**	**8**	**7**	**15**	**10**	**5**	**9.5**
Allelic richness	**2.22**	**8.87**	**7.93**	**10.21**	**3.40**	**1.99**	**3.25**	**7.10**	**3.69**	**5.52**	**4.21**	**8.81**	**6.22**	**3.35**	**5.48**
He	**0.20**	**0.91**	**0.87**	**0.93**	**0.36**	**0.20**	**0.61**	**0.78**	**0.60**	**0.79**	**0.55**	**0.91**	**0.80**	**0.65**	**0.65**
Ho	**0.21**	**0.83**	**0.89**	**0.97**	**0.33**	**0.22**	**0.69**	**0.77**	**0.71**	**0.83**	**0.61**	**0.77**	**0.82**	**0.61**	**0.66**
Shushwap	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**93**	**86**	**85**	**96**	**72**	**92**	**85**	**66**	**83**	**79**	**95**	**87**	**78**	**85**	**84.43**
Alleles	**5**	**15**	**17**	**21**	**5**	**3**	**3**	**20**	**17**	**10**	**10**	**23**	**11**	**7**	**11.93**
Allelic richness	**3.70**	**8.17**	**8.45**	**9.67**	**1.93**	**2.46**	**2.47**	**6.96**	**6.66**	**5.94**	**5.64**	**9.08**	**6.37**	**3.98**	**5.82**
He	**0.66**	**0.90**	**0.90**	**0.93**	**0.14**	**0.44**	**0.33**	**0.79**	**0.84**	**0.77**	**0.77**	**0.92**	**0.84**	**0.65**	**0.71**
Ho	**0.62**	**0.81**	**0.93**	**0.91**	**0.10**	**0.49**	**0.28**	**0.71**	**0.83**	**0.73**	**0.81**	**0.90**	**0.83**	**0.73**	**0.69**
Vernon	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	

N	**18**	**18**	**17**	**18**	**17**	**18**	**18**	**18**	**14**	**18**	**18**	**18**	**18**	**18**	**17.57**
Alleles	**5**	**10**	**9**	**15**	**7**	**2**	**3**	**11**	**4**	**5**	**7**	**12**	**7**	**5**	**7.29**
Allelic richness	**4.05**	**6.23**	**6.49**	**9.51**	**6.01**	**1.78**	**2.92**	**6.17**	**3.48**	**4.21**	**4.18**	**7.79**	**4.85**	**3.59**	**5.09**
He	**0.63**	**0.77**	**0.74**	**0.91**	**0.80**	**0.15**	**0.52**	**0.67**	**0.62**	**0.65**	**0.59**	**0.86**	**0.62**	**0.45**	**0.64**
Ho	**0.67**	**0.78**	**0.76**	**1.00**	**0.82**	**0.17**	**0.50**	**0.67**	**0.86**	**0.67**	**0.67**	**0.94**	**0.67**	**0.39**	**0.68**
MiniHump	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**89**	**89**	**90**	**91**	**91**	**91**	**91**	**87**	**90**	**88**	**91**	**91**	**90**	**89**	**89.86**
Alleles	**5**	**14**	**10**	**20**	**7**	**3**	**4**	**16**	**6**	**5**	**10**	**14**	**9**	**6**	**9.21**
Allelic richness	**3.57**	**6.50**	**5.17**	**9.25**	**3.07**	**2.19**	**2.87**	**6.54**	**3.16**	**3.74**	**5.84**	**7.76**	**4.73**	**3.74**	**4.87**
He	**0.68**	**0.84**	**0.72**	**0.92**	**0.47**	**0.30**	**0.53**	**0.78**	**0.57**	**0.68**	**0.76**	**0.89**	**0.70**	**0.62**	**0.68**
Ho	**0.70**	**0.85**	**0.72**	**0.95**	**0.44**	**0.24**	**0.54**	**0.74**	**0.62**	**0.72**	**0.77**	**0.87**	**0.63**	**0.65**	**0.67**
connector	**1b**	**3dre**	**i1**	**oki10**	**oki16**	**oki1a**	**oki1b**	**oki29**	**oki6**	**omy77**	**one8**	**ots103**	**ots2**	**ots3**	
N	**10**	**7**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**9.71**
Alleles	**3**	**6**	**3**	**5**	**3**	**1**	**1**	**4**	**3**	**3**	**1**	**2**	**1**	**1**	**2.64**
Allelic richness	**2.68**	**6.00**	**2.98**	**4.97**	**2.70**	**1.00**	**1.00**	**3.62**	**2.84**	**2.98**	**1.00**	**2.00**	**1.00**	**1.00**	**2.56**
He	**0.34**	**0.76**	**0.52**	**0.80**	**0.45**	**0.00**	**0.00**	**0.53**	**0.34**	**0.52**	**0.00**	**0.50**	**0.00**	**0.00**	**0.34**
Ho	**0.40**	**1.00**	**0.50**	**1.00**	**0.30**	**0.00**	**0.00**	**0.60**	**0.40**	**0.60**	**0.00**	**0.80**	**0.00**	**0.00**	**0.40**
average by locus															

N	**74.43**	**73.21**	**71.57**	**73.36**	**70.43**	**76.29**	**75.14**	**70.21**	**73.00**	**71.50**	**73.00**	**74.43**	**71.57**	**74.29**	**73.03**
Alleles	**4.64**	**11.79**	**11.21**	**16.79**	**7.93**	**2.71**	**3.36**	**12.50**	**6.79**	**6.64**	**6.64**	**14.07**	**8.36**	**5.07**	**11.48**
Allelic richness	**3.29**	**6.63**	**5.85**	**8.45**	**4.09**	**2.00**	**2.39**	**5.99**	**3.73**	**4.27**	**3.74**	**7.25**	**4.89**	**3.14**	**4.69**
He	**0.50**	**0.81**	**0.72**	**0.88**	**0.56**	**0.27**	**0.39**	**0.69**	**0.58**	**0.62**	**0.47**	**0.83**	**0.63**	**0.49**	**0.60**
Ho	**0.51**	**0.82**	**0.73**	**0.90**	**0.55**	**0.29**	**0.38**	**0.69**	**0.62**	**0.63**	**0.50**	**0.83**	**0.64**	**0.51**	**0.61**

Appendix 2 summary statistic data from the Taylor Lab analysis of kokanee populations from the South Coast of BC, Fraser River, BC, Columbia River, BC, Alaska and Japan. Nine microsatellite loci and 11 populations were included in the analysis

Chedazuk	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	30	30	30	30	30	30	30	30	30	30
Alleles	20	7	10	9	6	5	22	12	10	11.22
Allelic richness	12.38	5.56	7.59	6.02	5.04	3.37	13.13	6.99	7.21	7.48
He	0.93	0.77	0.81	0.78	0.73	0.43	0.93	0.71	0.84	0.77
Ho	0.90	0.83	0.70	0.73	0.20	0.37	0.97	0.70	0.87	0.70
Davidson	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	31	31	31	31	31	31	31	31	31	31
Alleles	18	5	14	10	5	3	21	11	10	10.78
Allelic richness	11.61	4.88	9.52	7.07	4.44	2.54	12.98	7.68	8.02	7.64

He	0.92	0.77	0.89	0.83	0.70	0.34	0.94	0.82	0.87	0.78
Ho	0.84	0.74	0.71	0.84	0.16	0.23	0.94	0.77	0.74	0.66
Meadow	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	30	30	30	30	29	30	30	30	30	29.89
Alleles	22	10	18	11	14	17	25	16	16	16.56
Allelic richness	13.40	6.39	12.53	6.53	9.04	10.91	12.97	7.68	8.02	9.72
He	0.94	0.65	0.93	0.69	0.84	0.90	0.92	0.92	0.90	0.85
Ho	0.93	0.63	0.77	0.77	0.41	0.80	0.90	0.80	0.90	0.77
Anderson	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	79	80	79	80	77	78	80	80	79	79.11
Alleles	53	12	24	23	18	20	38	16	17	24.56
Allelic richness	15.60	7.02	11.53	8.36	8.48	9.89	14.80	8.56	9.15	10.37
He	0.96	0.84	0.93	0.70	0.81	0.89	0.96	0.87	0.89	0.87
Ho	0.97	0.76	0.86	0.73	0.43	0.83	0.83	0.83	0.89	0.79
Seton	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	47	46	43	47	42	47	46	45	47	45.56
Alleles	36	9	19	16	16	16	32	14	15	19.22
Allelic richness	15.50	5.81	11.20	9.50	10.03	9.33	14.52	7.82	9.70	10.38
He	0.96	0.75	0.91	0.84	0.89	0.86	0.95	0.82	0.89	0.87

Ho	0.91	0.67	0.91	0.87	0.33	0.79	0.93	0.78	0.85	0.78
Hansen	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	20	20	20	19	14	20	18	20	20	19
Alleles	21	5	12	9	7	13	13	11	11	11.33
Allelic richness	14.44	4.25	9.82	7.04	6.76	9.73	10.57	8.97	9.10	8.96
He	0.94	0.62	0.89	0.81	0.81	0.88	0.90	0.87	0.87	0.84
Ho	0.95	0.65	0.90	0.89	0.14	0.70	0.67	0.95	0.95	0.76
Gates	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	40	40	41	41	30	40	41	40	41	39.33
Alleles	23	7	13	12	8	12	16	6	9	11.78
Allelic richness	9.98	5.43	6.63	6.26	5.79	6.86	8.89	5.06	4.98	6.65
He	0.86	0.74	0.69	0.69	0.71	0.83	0.87	0.77	0.73	0.77
Ho	0.78	0.80	0.68	0.59	0.07	0.73	0.83	0.68	0.61	0.64
Portage	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	34	36	36	35	29	36	36	36	36	34.89
Alleles	22	10	13	17	12	12	25	14	18	15.89
Allelic richness	13.14	7.08	9.21	8.11	7.77	7.08	13.40	8.79	11.29	9.54
He	0.94	0.84	0.89	0.70	0.76	0.77	0.94	0.86	0.92	0.84
Ho	1.00	0.81	0.89	0.71	0.24	0.69	0.83	0.78	0.97	0.77

Japan	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	12	12	11	12	12	12	10	12	12	11.67
Alleles	12	4	12	8	5	4	9	10	7	7.89
Allelic richness	11.06	3.83	11.36	7.93	4.98	3.83	9.00	9.12	6.64	7.53
He	0.89	0.67	0.88	0.85	0.77	0.69	0.86	0.84	0.80	0.81
Ho	0.92	0.33	0.91	0.83	0.83	0.67	0.20	0.83	0.75	0.70
MiniHump	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	40	40	40	40	16	40	40	40	40	37.33
Alleles	22	6	11	15	6	10	26	14	14	13.78
Allelic richness	12.12	4.54	8.58	8.46	5.56	5.90	13.70	8.41	7.58	8.31
He	0.92	0.70	0.88	0.80	0.68	0.68	0.94	0.86	0.82	0.81
Ho	0.93	0.60	0.80	0.78	0.06	0.68	0.95	0.85	0.80	0.72
Alouette	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	pop avg.
N	38	38	38	38	32	38	38	38	38	37.33
Alleles	12	6	14	12	7	7	16	15	11	11.11
Allelic richness	8.20	3.69	9.35	8.82	5.73	4.50	9.64	9.27	8.13	7.48
He	0.86	0.47	0.87	0.88	0.78	0.69	0.89	0.87	0.87	0.80
Ho	0.79	0.39	0.92	0.95	0.38	0.82	0.84	0.68	0.89	0.74
locus averages										

N	37.1	37.3	36.9	37.3	31.2	37.2	37	37.2	37.4	36.51
Alleles	24.1	7.4	15	13.3	9.8	11.4	22.1	12.7	12.8	14.29
Allelic richness	12.51	5.29	9.97	7.81	6.86	7.06	12.05	8.13	8.26	8.66
He	0.92	0.70	0.88	0.78	0.78	0.75	0.92	0.85	0.86	0.83
Ho	0.90	0.64	0.83	0.80	0.31	0.69	0.79	0.79	0.84	0.73
	One103	Omy77	Ots103	Oki29	Ots108	Ots100	Oki10	One108	One110	