Understanding Traditional Salmon Fishery of Ancestral Lake Babine Nation People through the Ancient DNA Analysis of Archaeological Salmon Remains (*Oncorhynchus spp.*)

by

Ellery T. Procter

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Declaration of Committee

Name:	**Ellery Procter**
Degree:	**Master of Arts**
Title:	**Understanding Traditional Salmon Fishery of Ancestral Lake Babine Nation People through the Ancient DNA Analysis of Archaeological Salmon Remains (*Oncorhynchus* spp.)**
Committee:	**Chair: Ross Jamieson** Associate Professor, Archaeology
	Dongya Yang Supervisor Professor, Archaeology
	Farid Rahemtulla Committee Member Senior Instructor, Anthropology University of Northern British Columbia
	Hua (Grace) Zhang Committee Member Adjunct Professor, Archaeology
	Michael Price Committee Member Adjunct Professor, Biology
	Rudy Reimer Examiner Associate Professor, Archaeology

Abstract

This thesis aimed to reconstruct traditional Nedut'en (Lake Babine Nation, LBN) fishery practices through ancient DNA (aDNA) analysis of 87 archaeological salmon remains from Smokehouse Island (GiSp-001), occupied ~1,000 BP within the asserted traditional territory of LBN in northcentral British Columbia. Abundant salmon remains and ancestral knowledge demonstrated salmons' importance, while aDNA revealed salmon sex and species to identify selective fishery practices. With high rates of successful amplification, mitochondrial DNA (mtDNA) identified sockeye (*Oncorhynchus nerka*) as the predominant species, consistent with regional ecology and LBN oral histories. Chinook (*O. tshawytscha*) and coho (*O. kisutch*) were also identified. Nuclear DNA indicated that sex biases were location-specific, suggesting sex-selective harvests. In total, 5 mtDNA haplotypes were observed in ancient sockeye remains, indicating high genetic diversity of sockeye ~1,000 years ago. These findings enhance understandings of human-salmon interactions in the understudied northcentral BC and may influence future LBN fishery and conservation practices.

Keywords: ancient DNA; Pacific salmon; Indigenous fisheries; conservation; resource management

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Table of Contents

List of Tables

List of Figures

List of Acronyms

aDNA	Ancient Deoxyribonucleic Acid
BC	British Columbia
BLAST	Basic Local Alignment Search Tool
BP	Before Present
cytB	Cytochrome B gene
DFO	Department of Fisheries and Oceans
D-Loop	Control Region of mtDNA
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleoside triphosphates
EB	Elution Buffer
EDTA	Ethylenediaminetetraacetic Acid
HBC	Hudson's Bay Company
ID	Identification
LBN	Lake Babine Nation
mtDNA	Mitochondrial Deoxyribonucleic Acid
PB	Phosphate Buffer
PCR	Polymerase Chain Reaction
PE	Phosphate-Ethanol Buffer
SDS	Sodium Dodecyl Sulfate
sdY	Sexually Dimorphic on the Y gene
SFU	Simon Fraser University
SNP	Single Nucleotide Polymorphism
Spp	Species pluralis, multiple species within a genus

Chapter 1. Introduction

For thousands of years, Pacific salmon (*Oncorhynchus* spp.) have played an important role in the subsistence practices and sociocultural development of Indigenous Peoples in northwestern North America (Atlas et al., 2021; Cannon & Yang, 2006; Steel et al., 2021). From Alaska to California, Indigenous communities often consider themselves as "salmon people", with salmon acting as a central pillar not only to their diets, but to their cultures, economies, and lifeways as well (Reid, 2020; Efford, 2023). The salmon resource management systems developed by traditional Indigenous communities are based upon aspects of cultural processes, traditions, and beliefs (Atlas et al., 2021).

Salmon, as an anadromous species, move predictably, with known spawning seasons where they migrate upstream from the ocean to freshwater spawning grounds (Reid, 2020). This migration allows the ready exploitation of the salmon, which can be captured in great abundances as they make their journey upstream (Reid, 2020). Despite this, knowledges, technologies, and management by Indigenous communities maintained the abundance of salmon from year-to-year, allowing their perpetuation as a foundation species within their ecosystem (Kaeriyama, 2022).

Resource management strategies can reflect human choice, preference, knowledge, and values. In understanding methods and strategies used to procure resources, it may be possible to then understand some of the human behaviours involved in the decision-making processes. To expand this further, in investigating the remains of salmon at an archaeological site, in consort with oral histories, it may be possible to infer resource management strategies applied to the harvest of the salmon. In uncovering the resource management strategy used archaeologically, the human-behaviours, preferences, and decision-making processes may be reflected and understood. Thus, through understanding the faunal, or salmon, assemblage at an archaeological site, it is possible to understand, or infer, human behaviour and interactions with the environment through the investigation of the assemblage as a proxy.

Previous studies in northwestern North America have explored the species and sex composition of Indigenous fisheries through ancient DNA (aDNA) analysis of archaeological salmon remains (e.g., Cannon & Yang, 2006; Morin et al., 2021a; Morin

et al., 2021b; Royle et al., 2018; Royle et al., 2020; Speller et al., 2005; Yang et al., 2004). These aDNA techniques have provided valuable insights previously inaccessible through morphological analysis alone. In using aDNA analysis to examine archaeological salmon remains, the importance of salmon can be better understood in terms of human-salmon interactions and relationships. Despite these advances, human-salmon interactions in northcentral British Columbia (BC) remain understudied.

In addition to investigating resource management and human-salmon interactions, aDNA analysis on archaeological salmon remains may also provide a deep-historical perspective of the regional ecology during the occupation of the archaeological site (Price et al., 2019). Due to the current decline in modern salmon populations, stemming from factors such as overfishing and climate change (Kaeriyama, 2022; Efford et al., 2023; Reid, 2020), understanding past diversity and abundancies may allow historical and archaeological species baselines to be understood. Furthermore, with the application of genetic investigations, baselines for genetic diversity of a certain species in a region can also be known. Through making historical and archaeological baselines available to conservationists, comparisons may be made to modern data to understand declines that are occurring within certain populations, and where conservation efforts need to be directed (Price et al., 2019).

1.1. Research Objectives

This project aims to reconstruct traditional fishery practices in north central BC through the aDNA analysis of archaeological salmon remains from Smokehouse Island (GiSp-001) (Fig. 3.1), an archaeological site in the north central region of BC on the asserted traditional territory of the Nedut'en, or Lake Babine Nation (LBN). To investigate fishery practices, such as species and sex selection, 87 salmon vertebrae samples across 5 excavation units on Smokehouse Island are analyzed using mitochondrial and nuclear DNA to identify sex and species composition of the assemblage. Genetic diversity across the samples are also explored.

This aDNA analysis was conducted to address the following objectives:

- Obtain a sample that is representative of the assemblage on Smokehouse Island. Effectively demonstrate that the sample is representative and that one individual is not over represented in the data.

- Identify the sex and species of selected vertebrae samples to determine if a selective harvesting strategy was employed by LBN on Smokehouse Island.
- Observe genetic sequences to determine if genetic change is occurring over time.

It is hypothesized that sockeye salmon (*Oncorhynchus nerka*) was the species most-harvested at the Smokehouse Island site, so it is predicted that sockeye will be the most predominant species represented in the data at the Smokehouse Island site. This is supported by LBN oral histories and regional salmon ecology (Rahemtulla, 2019; Department of Fisheries and Oceans, 2024). It is also hypothesized that seasonal occupation of the site, and thus fishery, occurred throughout the summer, because if sockeye is the species most commonly harvested at the Smokehouse Island site, then the occupation and fishery on the site likely occurred predominantly during the sockeye spawning season, which peaks in mid-August in the Babine region (Department of Fisheries and Oceans, 2024). Despite the fact that sex-selective fishery strategies are not uncommon in BC (Morin et al., 2021a; Royle et al., 2020), there is currently no regional data or oral histories regarding sex-selective fishery at the Smokehouse Island site to formulate a hypothesis on the basis of. It is hypothesized that in applying a low-resolution snapshot of diversity, some ancient haplotypes may be identified. Diversity may decrease overtime due to modern overexploitation and climate change.

The results from my thesis research will enhance the understanding of human-salmon interactions in the north central region of BC. It is our hope that these results will also provide information about traditional fishery practices that benefits the Lake Babine Nation.

1.2. Thesis Structure

This thesis will begin by providing a thorough background on important topics that will facilitate a deeper understanding of the conclusions drawn through my research, as well as the implications of the research, found in Chapter 2. The background includes a discussion of the Lake Babine Nation, starting with a presentation of the social and trade networks and an overview of diet, also including a brief discussion of LBN in historical times. Following, the ecosystem surrounding Smokehouse Island and the LBN will be discussed, with implications of the ecosystem on the data presented here noted. A description of the salmon species present in the region will also be provided, noting

specific biologies of the different species such as spawning time, appearance, and fecundity. Finally, the background will close with a description of traditional Indigenous salmon harvest and preservation methodologies. This section will also include discussions of sex-selective harvests. Following, Chapter 3 will detail the materials and methods utilized in this thesis. This section starts with an overview of the materials used and the archaeological site (Smokehouse Island) from which they were obtained. Next, the procedures followed for sample selection, DNA extraction, PCR amplification, and sequence analysis are all discussed. Chapter 4 presents the results of the analyses conducted in my thesis. These results include the success rate of DNA amplification, sex and species composition, and genetic diversity identified across the samples. Chapter 5 provides an interpretation of the results within the context of the provided background as the discussion. Here, the authenticity of the DNA research is first discussed, followed by the implications of the identified sex and species compositions of the hypothesized fishery methods, and an integration of modern biological data with the ancient data from Smokehouse Island. Finally, Chapter 6 presents the conclusions obtained from this research, and identifies several areas for continued and future research.

Chapter 2. Background

This chapter provides an in-depth discussion of the Lake Babine Nation peoples and practices, as well as a discussion of the regional ecology, including the behaviour of the salmon species present in the waters. This chapter can facilitate a deeper understanding of the implications of this thesis research and may clarify conclusions presented in the discussion. Passing over this chapter, however, should not impinge on the understanding of the aDNA research presented here.

2.1. The Lake Babine Nation

The asserted traditional territory of the Lake Babine Nation (LBN), or Nedut'en Nation (Fiske & Patrick, 2000), surrounds Babine Lake on the Skeena River watershed of northcentral British Columbia.

The ancestral LBN shared intricate trade and social networks with neighboring communities, including the Gitksan, and most strongly, the Wet'suwet'en (Bouchard, 2012). Kinship ties were built and maintained with these neighboring communities through clan and house systems (Bouchard, 2012). The matrilineal clans among the LBN had direct counterparts in the Wet'suwet'en and Gitksan communities, and also some similarities to Carrier clans (Bouchard, 2012). Houses, within the clans and often family units, held ownership over certain territories and fishing locations (Bouchard, 2012; Fiske & Patrick, 2000). The four clans among the Babine were "Likhc'ibu (bear), Jilhtsehyu (Frog), Gilantin (Caribou), and Likhtsemisyu (Beaver)" (Bouchard, 2012; Kantakis, 2017; Fiske & Patrick, 2000). Clan affiliations with the Wet'suwet'en and Gitksan provided connections and diplomacy between the communities and facilitated negotiations pertaining to socio-cultural aspects such as trade and marriage (Bouchard, 2012). Clan representatives from Babine, Wet'suwet'en, and Gitksan would attend feasts held by a community for a variety of purposes, often to settle disputes or for funerals (Bouchard, 2012).

The Babine-Wet'suwet'en language is considered to be an Athabaskan language, a family of languages sharing similar characteristics (Bouchard, 2012). The Babine-Wet'suwet'en language is composed of 4 different dialects, based on location

across the territory (Bouchard, 2012). Speakers of this language were also often familiar with the Gitksan language (Bouchard, 2012).

Berries, barks, and furs were important interior trade items, which was often exchanged with coastal groups for goods such as oolichan grease and shellfish (Bouchard, 2012). The steep difference in resource availability between the interior and the coast created a strong basis for trade between communities (Bouchard, 2012). LBN men and women both actively participated in resource harvesting and trade (Fiske & Patrick, 2000).

Sockeye salmon were central to the diets of many communities in the region, with some dependent on the Skeena watershed stock, like LBN, and others reliant on the Fraser watershed stock, like most other Carrier groups (Bouchard, 2012). Additionally, the drier environment of the interior, and salmon fat-loss due to migration, made the interior region of BC better suited for the drying and preservation of fish than the coastal region (Bouchard, 2012). Given the abundance of salmon at Babine, and the reliance by regional communities on these stocks, and differential access to salmon based on return cycles, salmon were a key pillar of the LBN economy (Bouchard, 2012).

The Lake Babine Nation had interactions with the Hudson's Bay Company (HBC) with the arrival of the fur trade to the Skeena around the 1780s and later establishment of Fort Kilmaurs on Babine Lake in 1822 (Bouchard, 2012). Despite the introduction of European goods through the HBC, Babine trade networks were not significantly impacted (Bouchard, 2012). Later in the historical record, Lake Babine Nation were forced to remove their fishing weirs in 1904, despite millennia of successful management, which was met with resistance by LBN (Atlas et al., 2021). Following, in 1946 the DFO installed a count fence to monitor the salmon in the region (Atlas et al., 2021), which was comanaged by the DFO and LBN starting in 1993 (Stiff et al., 2015) and later fully managed by LBN fisheries since 2008 (Atlas et al., 2021; Stiff et al., 2015).

2.2. Babine Lake Region Ecosystem

Babine Lake is a part of the Skeena River watershed, located 380 km from the Pacific Ocean (Burgner, 1991). The lake is at an elevation of 708 m above sea level, with a maximum depth of 186 m and a mean depth of 55 m (Burgner, 1991; BC Ministry of the

Environment, 2008). The surrounding region of the watershed is a sub-boreal spruce biogeoclimatic zone (BC Ministry of the Environment, 2008; Stiff et al., 2015; Meidinger et al.,1991), dominated by spruce, fir, pine, and aspen trees (BC Ministry of the Environment, 2008; Meidinger et al., 1991). The region is somewhat hilly, and generally lacks glaciation (BC Ministry of the Environment, 2008). The bedrock is largely formed of sedimentary, volcanic, and plutonic rock (BC Ministry of the Environment, 2008). The soil types in the biogeoclimatic zone are predominantly Luvisolic, Podzolic, and Brunisolic (Meidinger et al., 1991), and may be acidic in the region near Babine (BC Ministry of Agriculture, 1991). As part of the central interior of British Columbia, the region central to Babine Lake (Topley Landing) experiences cold winters, with average annual minimum temperatures around -35˚C, and warm summers, with average annual maximum temperatures around 30.1˚C (BC Ministry of the Environment, 2008; Meidinger et al., 1991). This area near the central portion of the lake typically sees 53.4 cm of precipitation on average and an average snowfall of 2.3 m (BC Ministry of the Environment, 2008). The region typically develops an ice cover from December to January, with this beginning to disappear in April to May (BC Ministry of the Environment, 2008).

Babine Lake is considered a dimictic lake (BC Ministry of the Environment, 2008), meaning with seasonal temperature changes, the upper and lower lake strata will circulate. The lake is described as dystrophic and oligotrophic, meaning the lake water is dark in colour with low-clarity due to dissolved organic matter and has a low nutrient level (BC Ministry of the Environment, 2008; Stiff et al., 2015). The pH of the water is slightly basic (BC Ministry of the Environment, 2008). The water temperature during salmon migration season at the Babine River Counting Fence was found to be ~14.5 ± 2.6˚C, based on data obtained from 2003-2010 and 2012-2014 (Stiff et al., 2015).

Through developing an understanding of the ecosystem near Smokehouse Island and in the territory of LBN, it is possible to understand patterns of preservation of regional archaeological sites. For example, the extreme range in temperatures, noted to be from -35˚C to 30˚C may not be conducive to high degrees of preservation. Additionally, archaeological and organic materials on land sites in the region may not have high preservation due to the acidic soils causing degradation (Nicholson, 1996). However, because the pH of the water is basic, as noted above, this may rescue the archaeological materials on Smokehouse Island from suffering the proposed fate of

nearby land sites, based on the understanding that the basic water will have a neutralizing effect on the acidic soil.

2.3. Pacific Salmon Regional Ecology

Based on previous chapters, it is clear that Pacific salmon are culturally and economically important to Indigenous communities across the North Pacific rim. What is particularly interesting about Pacific salmon is the fact they are an anadromous species of fish, that will typically return to their river of origin to spawn and die after spending years in the ocean (Groot and Margolis,1991). This tendency to return to the river of origin creates a reliable and abundant food source for Indigenous communities, that can be expected at specific times, without the need for animal husbandry practices such as feeding and breeding.

The Skeena River watershed sees five different species of Pacific salmon. These include Chinook (*Oncorhynchus tshawytscha*), chum (*Oncorhynchus keta*), coho (*Oncorhynchus kisutch*), pink (*Oncorhynchus gorbuscha*), and sockeye (*Oncorhynchus nerka*) (Groot & Margolis, 1991). Based on modern data obtained from the Babine River count fence, only four of these species (Chinook, coho, pink, and sockeye) reach as far up in the watershed as Babine (Fisheries and Oceans Canada, 2024). While chum salmon are strong swimmers, they are not strong leapers (Salo, 1991), which along with spawning location preference, could indicate why chum do not reach the Babine region. The following paragraphs will explore the salmon present in the Babine region.

2.3.1. Sockeye Salmon (*Oncorhynchus nerka*)

Within British Columbia, the Skeena River is the second most important production area for sockeye salmon, and within the Skeena, the Babine Lake tributaries are the most important (Burgner, 1991).

Sockeye salmon start as an egg. Based on the climate in British Columbia, the sockeye will incubate for around 47-65 days prior to emerging as alevin, depending on the environment and temperature (Burgner, 1991). For sockeye, emergence typically occurs from April – June (Burgner, 1991). Once the yolk has been absorbed the fish will become fry and will pursue food (Burgner, 1991). In the Babine River, the sockeye fry

diet is dominated by lake plankton, and also includes copepods and chironomids (Burgner, 1991; McCart, 1967).

The fry will become parr as they continue to grow, and may spend 1-2 years in freshwater prior to smoltification and preparation for ocean life (Burgner, 1991; McCart, 1967). Around 1 year of age, the juvenile sockeye are about 79mm and weigh around 4.9g (Burgner, 1991). During this time, the fry and smolts are subject to predation by birds and larger fish, and are also subject to infection by parasites (Burgner, 1991; McCart, 1967). The juvenile sockeye will exit the freshwater environment and enter the marine environment in the Spring to Summer, and will typically spend 2-3 years in the ocean, although they may return as “jack” or “jill” salmon after one winter at sea (Burgner, 1991). Most of the sockeye at Babine Lake spend 1 winter in fresh water and 2 to 3 winters in the ocean (Burgner, 1991).

The ocean-dwelling sockeye are sleek and silver, with a white underbelly (Burgner, 1991). By the second winter at sea, the male sockeye are larger than the female sockeye (Burgner, 1991). During the return to their natal freshwater grounds, more marked sexual dimorphism occurs (Burgner, 1991). The abdomen of the female sockeye will swell and there will be slight elongation of the snout (Burgner, 1991). The head of the female will become olive green, with a red back, grey sides, and a white underbelly (Burgner, 1991). The male sockeye will develop a hump in-front of the dorsal fin on its back and will develop a long, hooked upper jaw (Burgner, 1991). The male will also have a green head, red sides, and a white underbelly (Burgner, 1991). When entering the fresh water environments, salmon typically stop eating and rely on their body reserves to fuel the rest of their life journey (Burgner, 1991). Spawning sockeye sexual dimorphism is evident in Figure 2.1.

Figure 2.1 Spawning male and female sockeye salmon (*O. nerka*).
Note: Image from Pacific salmon/steelhead identification and lifecycle (2023).

Male salmon will arrive to the spawning grounds earlier than female salmon, however, spawning is initiated with the preparation of a nest, or redd, by the female (Burgner, 1991). The female salmon will lay her eggs in the redd, which will be fertilized by one, or more, male salmon, and buried (Burgner, 1991). The female may repeat this process, while the male may move on to fertilize additional redds (Burgner, 1991). The female will continue to protect her redds until her subsequent death (Burgner, 1991). The male salmon will survive longer than the female salmon but will also die shortly after spawning (Burgner, 1991).

Because salmon deteriorate after spawning and eventually die, the quality and flavour of the meat will also begin to deteriorate (Reid, 1991). The flesh of the fish, once red, will become less vivid and whiter, and the meat will lose its firmness and become watery (Reid, 1991). Spawning and preparation for spawning also results in a loss of protein and fat (Reid, 1991). Fish may then be ideal for capture and consumption prior to spawning.

2.3.2. Pink Salmon (*Oncorhynchus gorbuscha*)

Pink salmon hatch from their eggs in gravel beds 5 to 8 months after fertilization (Heard, 1991). The newly hatched alevin have a low survival rate, and the time to reach the migrant fry stage is largely dependent on the temperature (Heard, 1991). During the fry stage, the pink salmon will feed infrequently, mainly on dipteran insects, and will be preyed on by other fishes (Heard, 1991). Pink salmon will typically spend less time in

freshwater than other species of Pacific salmon, and will migrate downstream towards the sea around April to May in British Columbia (Heard, 1991).

Once at sea, the pink salmon will practice opportunistic feeding, and will grow more rapidly than the other species of Pacific salmon (Heard, 1991). The salmon will begin their migration back to freshwater from May to July of the year following their hatching, and will return to their spawning region 14 to 16 months from the time they originally entered sea water (Heard, 1991). Their survival on this journey may be impeded by low water flow, or predation by birds, lampreys, and bears (Heard, 1991). At the time of maturity, the pink salmon will be around 45 to 55 cm and will weigh around 1.0 to 2.5. kg, making them the smallest Oncorhynchus species (Heard, 1991). At this time, in preparation for spawning, sexual dimorphism will become apparent between the male and female pink salmon. The male salmon will develop a large back hump, an enlarged head, and a hooked kype jaw (Heard, 1991). The skin colouring of both the male and the female salmon will darken from the original silver to a brown-grey colour, with white underbellies and spots on the back (Heard, 1991). This sexual dimorphism in spawning pink salmon is visible in Figure 2.2.

Figure 2.2 Spawning male and female pink salmon (*O. gorbuscha*).
Note: Image from Pacific salmon/steelhead identification and lifecycle (2023).

Similar to other Pacific salmon species, upon reaching the spawning ground, the female salmon will prepare a redd site for egg deposition (Heard, 1991). The males will mate with females based on a size-determined hierarchy (Heard, 1991). On average, female pink salmon have from 1200 to 1900 eggs (Heard, 1991). Spawning will occur for

1 – 8 days (Smirnov, 1976), and the female will remain at the site to defend the redd for several days following (Smirnov, 1976; Heard, 1991). The pink salmon will eventually weaken and as with other Oncorhynchus species, will die soon after spawning.

Most unique about pink salmon is their fixed two-year life span. This not only results in a typical two-year cycle of dominance between even- and odd-numbered years, but also genetic distinction between the even and odd lines due to reproductive isolation (Heard, 1991).

2.3.3. Chinook Salmon (*Oncorhynchus tshawytscha*)

Chinook salmon are the largest species of the *Oncorhynchus* genus, reaching up to 45kg, however, they are generally the least abundant of the Eastern Pacific salmon species (Healey, 1991). Healey (1991) describes two distinct races of Chinook: the stream-type, or spring run, and the ocean-type, or fall run. The spring run Chinook will spend at least one year in freshwater prior to sea migration and will typically return to their spawning grounds from February to July (Healey 1991). The ocean-type Chinook will begin their migration to sea around three months after they emerge as fry and will return to spawn from July to December (Healey, 1991). The Skeena watershed is composed of 48% stream-type Chinook and 52% ocean type Chinook (Healey, 1983).

Chinook will hatch from an egg and will reach the fry stage of life with a relatively high survival rate (Healey, 1991). After emergence, there will be downstream migration either to a river estuary or stream environment, occurring most predominantly from February to May (Healey, 1991). The stream-type Chinook will remain in this area for at least one year, while the ocean-type Chinook will continue their journey to the sea (Healey, 1991). During the freshwater portion of their life, the Chinook will have a diet similar to those of other *Oncorhynchus* species (Healey, 1991).

Chinook may spend less than 1 to 5 years in the ocean (Healey, 1991). The mean age for stream-type males and females on the Skeena was 4.13 and 4.56 years respectively (Godfrey, 1968; Healey, 1991). The mean age for ocean-type male and female Chinook on the Skeena were 3.47 and 4.19, respectively (Godfrey, 1968; Healey, 1991). During preparation for spawning Chinook will experience slight changes in appearance, with less marked sexual dimorphism than other *Oncorhynchus* species

(Smirnov, 1976; Merz & Merz, 2004). These changes include a longer snout and larger teeth in the male salmon, and a fuller appearance to the female salmon (Smirnov, 1976). The back and head of the female salmon will be very dark, and the sides will be “wine-red”, will a violet-grey underbelly (Smirnov, 1976). The male salmon will have a more vibrant colouring, with the sides appearing to be “raspberry-red” (Smirnov, 1976). This sexual dimorphism is presented in Figure 2.3.

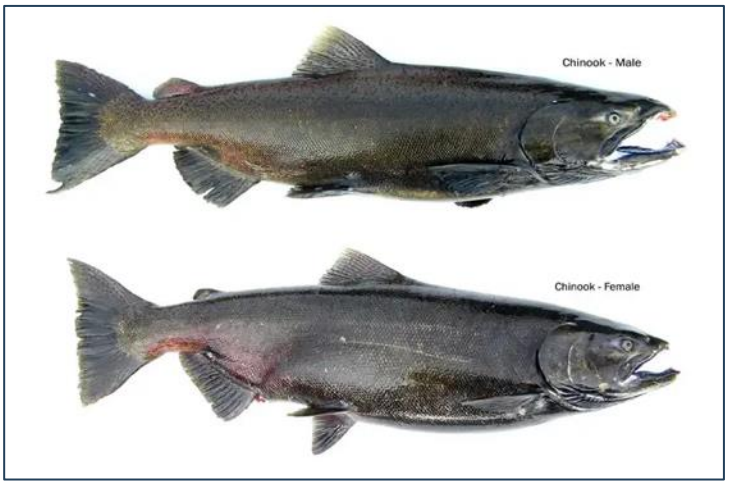

Figure 2.3 Spawning male and female Chinook (*O. tshawytscha*)
Note: Image from Pacific salmon/steelhead identification and lifecycle (2023).

Spawning will proceed similar to that of other Pacific salmon and will occur at a gravel bed redd site. Dependent on the size of the female Chinook, the salmon may have less than 2,000 eggs to over 17,000 eggs (Healey, 1975). On the Skeena, spawning typically occurred between August 15 and September 25 (Healey, 1975). Here, females were found to defend their nest for several days to over two weeks prior to their death (Healey, 1975).

2.3.4. Coho Salmon (*Oncorhynchus kisutch*)

Coho start as eggs in gravel beds, and will incubate for various amounts of time, largely dependent on temperature (Sandercock, 1991). To survive in the sea, the coho fry will typically need to be over 7-8cm, so migration to the ocean in coho that are less than one year old are not typical (Sandercock, 1991). Most coho will spend one winter in freshwater prior to migrating to sea, however some may spend up to 4 winters in freshwater (Sandercock, 1991). During this time, the coho fry will mainly feed on insects,

and may start to feed on smaller salmon fry as they become larger (Sandercock, 1991). The coho begin migrating towards the sea in the Spring, about 15 months after their initial emergence (Sandercock, 1991).

Once at sea, the salmon will spend 16 months growing, and will typically begin to reach maturity in their third year, although some males may mature precociously and return as “jacks” (Sandercock, 1991). Upon reaching maturity, before spawning in the natal streams, the male coho will typically be larger than the females (Sandercock, 1991). The salmon will weigh, on average, between 3 to 5 kg, and will not usually reach over 9kg (Sandercock, 1991). Additionally, sexual dimorphic characteristics will develop with the onset of maturity (Sandercock, 1991). The male coho will develop a long, hooked snout with enlarged teeth and the lower jaw will become hooked or knobbed (Sandercock, 1991). The colouration will change from the oceanic silver to blue-green with a red streak (Sandercock, 1991). The female will have somewhat elongated jaws, and will have similar changes in colour that appear more muted (Sandercock, 1991). The sexual dimorphism of spawning coho is visible in Figure 2.4.

Figure 2.4 Spawning male and female coho salmon (*O. kisutch*).
Note: Image from Pacific salmon/steelhead identification and lifecycle (2023).

Spawning will take place between November and January, and begins with the preparation of the redd by the female (Sandercock, 1991). On the Skeena, the average female coho will have around 3700 eggs (Beacham, 1982). Once spawning has been completed, the male and female salmon will degrade and die shortly thereafter (Sandercock, 1991).

2.4. Traditional Harvest and Preservation of Salmon

Traditional harvest and preservation methods are an aspect of resource management strategies. Different communities will utilize different strategies based on environment, resource availability, and preference. These practices can also reflect cultural teachings as they are used from generation to generation. With an understanding of different traditional harvest and preservation strategies, with particular emphasis on those used by Lake Babine Nation, we can begin to apply this understanding to the archaeological assemblage. This may help us understand how and why certain species were selected, and why the harvest of others was avoided. An understanding of the variation of harvesting strategies present across communities within close proximity, applied to the archaeological assemblage, can allow hypotheses on strategies used to be formed.

2.4.1. Traditional Salmon Harvests

Evidence of the use of fishing weirs at Smokehouse Island is demonstrated through traditional knowledge and the presence of wooden stakes preserved at various locations throughout the island, dating to almost 1000 years before present (Rahemtulla, 2019). Historical photographs and accounts support the use of weirs by Lake Babine Nation around 100 years ago (Kantakis, 2017; Harris, 2001). Fishing weirs are an Indigenous technology (Prince, 2014), that allow the effective capture of an abundance of fish, thereby increasing food security for peoples dependent on fish to meet their nutritional requirements (Kantakis, 2017; Prince, 2014).

Overuse of this weir technology, without consideration of the ecosystem it is employed within, can easily decimate fish stocks, as the weirs can block access to spawning grounds (Kantakis, 2017). Yet, these weirs were employed at various sites around northwest North America, and stocks of salmon and other fish remained abundant. This demonstrates that the employment of weir technology, seen at Smokehouse Island, required an effective resource management strategy to ensure the proliferation of salmon stocks year after year.

Some management strategies pertaining to the use of weirs involved erecting the weirs several weeks after the salmon runs had begun to pass through the region, to ensure the passage of enough salmon to the spawning grounds was allowed to preserve

the breeding population (Kantakis, 2017; Lepofsky & Caldwell, 2013). Additionally, meshes may have been included in the design of certain weirs to prevent the passage of larger fish, while still allowing smaller fish to pass through the weir (Kantakis, 2017; Lepofsky & Caldwell, 2013).

The weir led to spatial-temporal intensification of a resource, as the weir would lead to the concentration of salmon in a specific area during the short salmon spawning season (Kantakis, 2017). This would then require increased labor efforts during that period to harvest and process the salmon (Kantakis, 2017; Hackett, 2017). As salmon would only be present in such abundances for a short period of time, it would also be necessary to preserve a high amount of the fish, to ensure that nutritional needs were also met outside of the seasonal runs and throughout the winter season (Kantakis, 2017; Romanoff, 1992; Hackett, 2017). Additionally, the weirs would require continued maintenance throughout the fishing season, perhaps requiring the construction of maintenance tools (Lawrence, 2023).

2.4.2. Traditional Preservation Techniques

Food preservation techniques across the Northwest Coast and interior plateau can vary due to different environmental conditions however, common goals across most preservation techniques are to reduce the moisture content, prevent fat rancidity, and effectively store the food resource for an extended period of time (Romanoff, 1992; Kitts et al., 2022). In the case of salmon, a moisture content of less than 12% can be considered stable (Romanoff, 1992). Some ways to achieve this include dehydrating, freezing, or drying the salmon (Romanoff, 1992). Due to the smokehouses present at the Smokehouse Island site (Rahemtulla, 2019), it is likely that smoking was a method employed by Lake Babine Nation to achieve a decrease in moisture content. The smoking of large salmon for preservation usually requires eviscerating the fish and removing the head, allowing the smoke better access to the flesh (Doe et al., 1998). Small fish, such as pink salmon, may have been smoked whole, with the viscera intact but bled, to prevent over-drying of the fish, and reduce labour costs (Doe et al., 1998, Kennedy & Bouchard, 1992). After smoking, the total yield may be 20-30% less than before smoking, however the fish will now have an increased resistance to spoilage (Doe et al., 1998). The heads of the salmon may have also been used to create an oil, or

could have been dried and boiled at a later date (Kantakis, 2017; Kennedy & Bouchard, 1992).

2.4.3. Selective Salmon Harvests

Babine Lake, located just upstream of Smokehouse Island, is the largest sockeye salmon nursery lake in Canada, and is the spawning location for most of the sockeye salmon on the Skeena River Watershed (Barouillet et al. 2024; Gottesfeld & Rabnett, 2008; Rahemtulla, 2019). It is clear that salmon were, and continue to be, a dietary staple to peoples located near salmon runs, however pacific salmon also serve as keystone species in their ecosystems (Kaeriyama, 2022). Many species rely on salmon to feed themselves, however upon the death of the salmon after spawning, as per their semelparous nature, the nutrients from their decomposing bodies also feed plants, fungi, and insects (Barouillet, 2024; Reid, 2020). With the importance of salmon not only to human communities, but also to entire ecosystems, the harvest of salmon and their subsequent removal from the watershed must be done in a way that preserves the integrity of the salmon runs.

Management strategies can be in place to ensure the stability of local resources, particularly salmon, to maintain a resilient population of fish returning to spawn year to year (Morin et al., 2021a). Often management strategies are key to preserving the health of communities while also upholding the health and integrity of the populations within the greater foodweb through minimizing the depletion of resources (Efford et al., 2023). Management choices, such as sex and species selectivity, can contribute to maintaining the productivity of the salmon runs (Efford et al., 2023, Royle et al., 2020).

In northwestern North America, a male-preferential harvest of Pacific salmon may have been a wide-spread practice, made possible through the use of Indigenous technologies such as weirs, allowing the harvest of certain salmon and release of others (Royle et al., 2020; Dale & Natcher 2015; Morin et al., 2021a; Atlas et al., 2021). This selective salmon fishery may have been conducted with the intention of maintaining the salmon stocks year after year (Langdon, 2006), however, certain salmon may have been preferred over others for many possible reasons, such as taste or availability (Royle et al., 2020). Whether the act of a sustainable harvest was intentional or not, it should be noted that Indigenous knowledges and knowledge holders likely had a high degree of

awareness as to the influence of their actions within an ecosystem (Lepofsky & Caldwell, 2013). Further, the use of the fishing technology, rather than the technology itself, is what creates the resource management strategy (Lepofsky & Caldwell, 2013). The decisions on how and when to use the technology are created within a framework of Indigenous knowledges and human-environment interactions (Lepofsky & Caldwell, 2013). Decisions on what salmon individuals (sex and species) to harvest was based on these knowledges (Dale & Natcher, 2015).

2.4.4. Selective Salmon Harvests in the Archaeological Record

A recent study (Morin et al. 2021a) applies aDNA analysis to archaeological salmon remains from four different archaeological sites in the Burrard Inlet, BC to explore pre-contact Indigenous fishery management in the region. The analyses are conducted to identify sex and species of the salmon remains (Morin et al. 2021a). Morin et al. (2021a) find that chum salmon were preferentially harvested at the four sites, likely due to their fat content at that location, which is well suited for preservation. A male-preferential harvest, Morin et al. (2021a) explains, could increase the maximum sustainable yield at a fishery as one male salmon can fertilize the eggs from many female salmon. Morin et al. (2021a) hypothesize that a male-preferential harvesting strategy would have been utilized at the four sites. aDNA analysis, however, reveals that at only two of the sites is a biased sex ratio towards male salmon seen, and at the other two sites a sex ratio close to 1:1 is observed (Morin et al., 2021a).

Morin et al. (2021a) demonstrate that there is a high degree of diversity seen in pre-contact Indigenous fishery management, even within the same region. Based on the abundance of fish harvested from the salmon runs, Morin et al (2021a) elucidate that Indigenous terminal fisheries could have destroyed the salmon runs, however there is no evidence to suggest that this occurred. Rather, Morin et al. (2021a) suggests that the cultural teachings and protocols promoted fishery that would preserve the runs. Morin et al. (2021a) concludes by encouraging government fishery management to collaborate with Indigenous fisheries to develop fishery practices rooted in cultural stewardship and enhancing sustainability, as the Indigenous fisheries have been doing for thousands of years.

This study suggests that a male preferential harvest may have been a widespread practice. Additionally, it demonstrates that generalizations about Indigenous fishery practices, even within the same region, cannot be made, as there is a great amount of diversity.

2.5. Conclusion

This chapter has demonstrated the interconnectedness of regional Indigenous communities and the importance of regional resources, such as salmon, for economy, through trade, and subsistence. The ecosystem is highly diverse, with many species of plants and animals represented, and has allowed for excellent preservation of the Smokehouse Island site. With note of the salmon species present and traditional harvest and preservation techniques, an understanding of preferences can begin to be formed and used to facilitate understanding of the salmon assemblage found on Smokehouse Island.

Chapter 3. Materials and Methods

This chapter begins with a discussion of the archaeological context from which the archaeological salmon vertebrae samples were obtained. Following, there is a discussion of the samples themselves, and the strategies applied to ensure analyzed samples were representative of the greater assemblage. An overview of all the methods applied in this thesis is then provided. These methods include decontamination and sample preparation, extraction, PCR setup, gel electrophoresis, and sequence analysis.

3.1. Smokehouse Island (GiSp-001)

In 2010, the Lake Babine Nation Treaty Office initiated the Babine Archaeology Project with Dr. Farid Rahemtulla from the University of Northern British Columbia. One of the many project goals was to learn more about ancestral fishery practices and occupation of the land (Rahemtulla, 2019). Beginning in 2014 the Babine Archaeology Project began excavations on Smokehouse Island (GiSp-001), a 150m x 150m island that has a large wet site component (Rahemtulla, 2019). This site is situated on the Babine River, where it widens into Nilkitkwa Lake (Fig. 3.1).

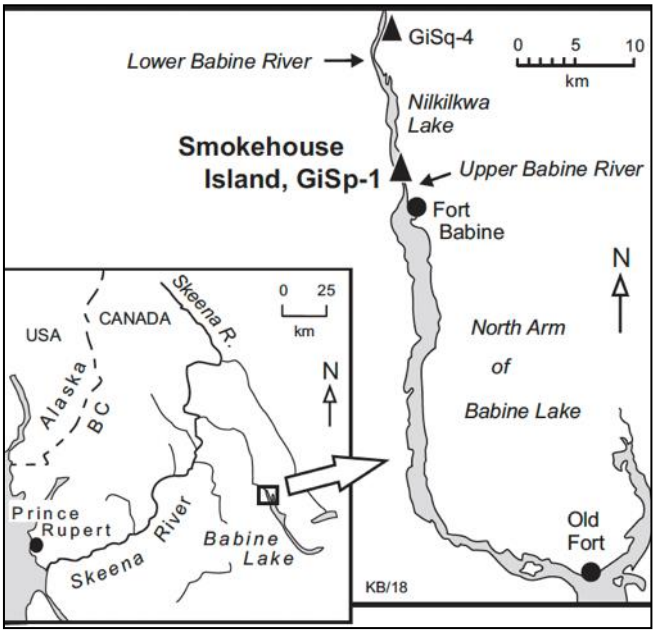

Figure 3.1 Smokehouse Island (GiSp-001), situated on the Skeena River watershed at the confluence of the Babine River and Nilkitkwa Lake.
Note: Image from Rahemtulla (2019).

Nearby village sites occupied by the ancestral LBN had occupation histories dating from 1,300 BP and into the historic period (Rahemtulla, 2012). These village site excavations were the first to occur in the Babine region (Rahemtulla, 2019). The excavation of Smokehouse Island shortly after yielded well-preserved wooden-fishing weirs, which were subject to radiocarbon analysis along with charcoal and bone, indicating site occupancy and weir usage at least 1,000 years BP (Rahemtulla, 2019). Weir remnants were not only found within the site, but also in the waters surrounding the island (Rahemtulla, 2019). The weirs at Smokehouse Island were reported to extend bank-to-bank with possible fish impoundment areas and trap attachments (Kantakis, 2017). The initial excavations also revealed an abundance of archaeological materials including chipped stones, tools, and fire-cracked rocks (Rahemtulla, 2019).

Rahemtulla (2019) highlights that wood materials are rarely found to be preserved in Interior BC; the fact that such a high degree of preservation is found at Smokehouse Island marks it as an especially unique, important site for understanding archaeology in the region. Waterlogged sites, like Smokehouse Island, provide favourable conditions for preservation of organic material, like wood and bone (Werz & Seemann, 1993), which were found to be highly preserved at the Smokehouse Island site (Rahemtulla, 2019). Due to the nature of the site, other well preserved organic elements were also identified, including a piece of a wooden basket trap and a birch bark container (Rahemtulla, 2019).

Salmon remains were found in great abundance at the Smokehouse Island site, with at least eight excavated units yielding such remains. As previously mentioned, because different species of salmon have different spawning seasons, determining what the species composition of the vertebral remains, in consult with oral histories, could generate information about the seasonal use of the site (Morin et al., 2021b; Yang et al., 2004). Furthermore, aDNA applications can also reveal the sex identification of salmon remains, which may be useful in reconstructing the sex ratio of the harvested salmon to examine if a sex-selective strategy was employed on the island (Morin et al., 2021a; Royle et al., 2020). Applying newly generated knowledge about the sex and species composition at Smokehouse Island can reveal insights about the traditional resource management strategies employed by the ancestral Lake Babine Nation people during the site’s occupation.

The high preservation of organic materials makes this site not only unique to the region, but also an invaluable trove of information. The acidic soils and basic waters, as discussed in Chapter 2.2, have allowed excellent preservation of the faunal record, something that cannot typically be achieved in this region. In understanding the faunal assemblage at this site, in this thesis with a focus on fish, a unique and rare perspective on the past is gained. This information will provide information to the Lake Babine Nation about their ancestral use of the land and will also add information to the archaeological record of northcentral British Columbia.

3.2. Selection of Archaeological Salmon Remains

Fish remains, particularly those of salmon, have been shown to yield well-preserved mitochondrial and nuclear DNA fragments, often with a high rate of success (e.g., Yang et al., 2004; Speller et al., 2005; Speller et al., 2012; Royle et al., 2020; Morin et al., 2021). While archaeological DNA samples are subject to processes of degradation over time (Dabney et al., 2013), the waterlogged nature of the Smokehouse Island archaeological site (Werz & Seemann, 1993) may have increased the likelihood of well-preserved samples. Salmon remains from this site are of particular interest as the preservation of bone samples may be low due to the acidic soils (BC Ministry of Agriculture, 1991; Nicholson, 1996). The waterlogged nature of the site, in the somewhat basic waters (BC Ministry of Environment, 2008), may have spared the Smokehouse Island salmon samples from extensive degradation, and allowed a unique glance at the past faunal record.

Salmon vertebral remains from five different units at the Smokehouse Island site were subjected to aDNA analysis (Fig. 3.2, Table 3.1). As discussed in Chapter 2.3, 5 different species of salmon are found within the Skeena River Watershed: sockeye (*Oncorhynchus nerka*), coho (*Oncorhynchus kisutch*), Chinook (*Oncorhynchus tshawytscha*), chum (*Onchorhynchus keta*), and pink (*Oncorhynchus gorbuscha*). The sex and species of salmon cannot be distinguished based on the morphology of the vertebrae alone (Cannon & Yang, 2006; Morin et al., 2021b), thus aDNA analysis is required to obtain this information. One unit selected for examination was located centrally on the site, while the remaining four selected units were located towards the southeast area of the site, towards the perimeter of the island (Fig. 3.2, Table 3.1) While there were no landscape or climate differences on the island to suggest different

assemblages across the units, samples from each of the five units were included in this analysis to generate a representative sample and ensure that one individual was not overrepresented in the data. The texture of the layers and radiocarbon dates indicate that the site is likely composed of one continuous stratigraphic layer and no clear division was observed to compare different layers/levels.

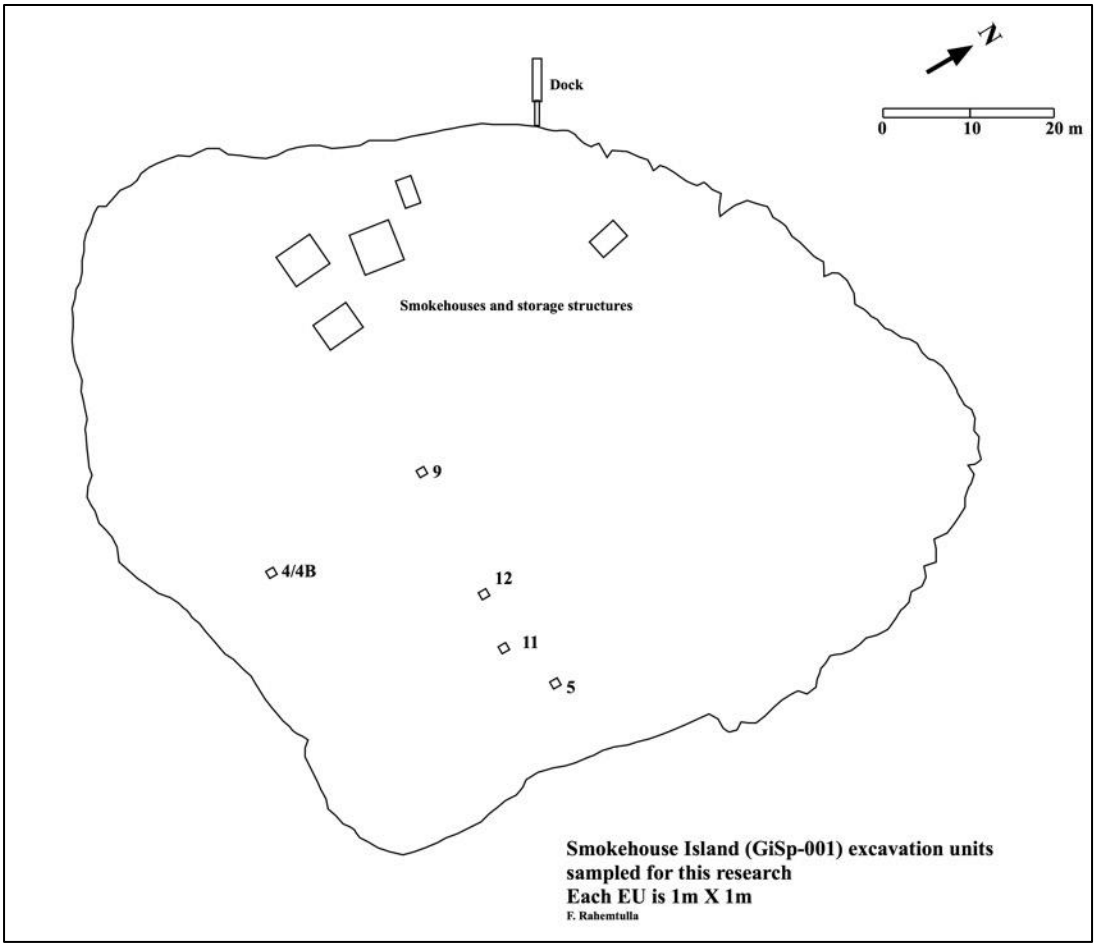

Figure 3.2 Smokehouse Island (GiSp-001) excavation units sampled for this research.

Note: Each excavation units is 1m X 1m, and at least 5m of space exists between each unit. Unit 4 and 4B are the same unit, with the unit 4 expanded to include a 4B at a later date. Figure prepared by Farid Rahemtulla.

Table 3.1 Examined excavation units from Smokehouse Island and weight of faunal assemblage per unit

Year of Excavation	Unit	Level	Layer	Weight (g) of faunal assemblage
2015	4B	12	D/E	161.5
2015	5	8	B/C	85.5
2017	9	7	A	1070.0
2019	11	11	D	150.0
2019	12	10	B	56.5

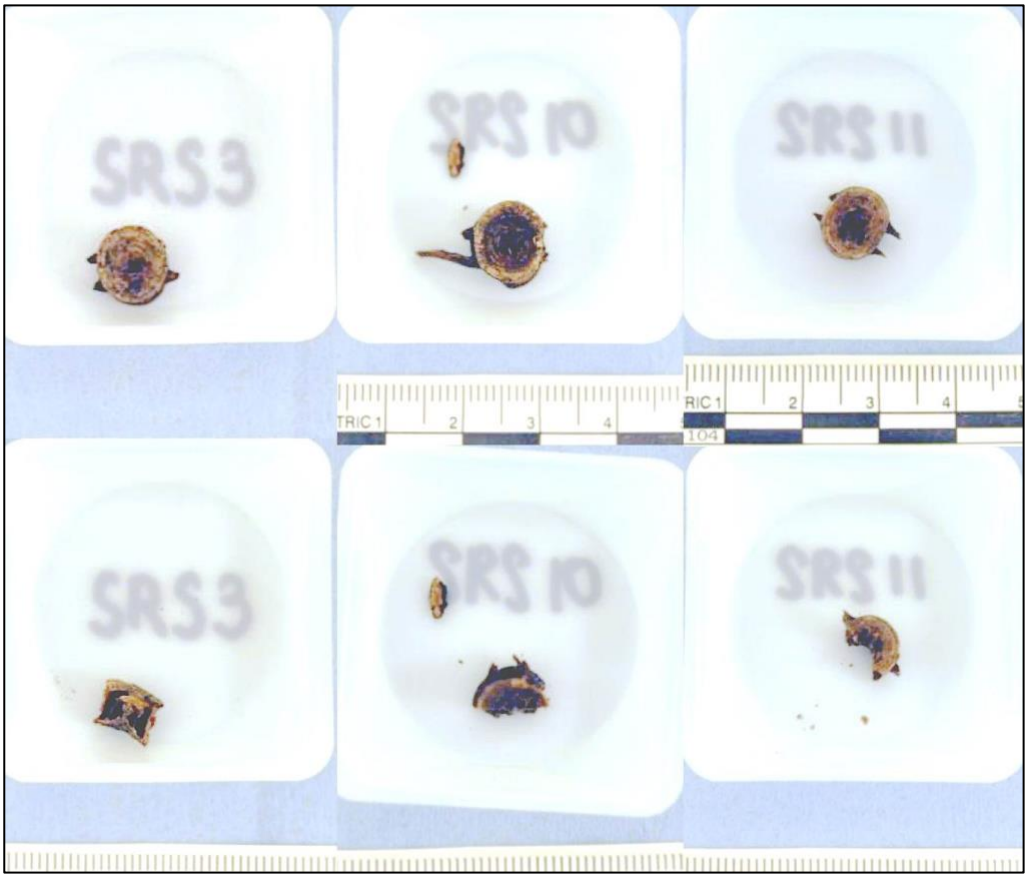

Figure 3.3 Selected salmon vertebrae samples from Smokehouse Island, before and after obtaining one half for analysis.

Note: The whole vertebrae at the top of the figure were first weighed, the bottom image shows one half of the vertebrae which was used for analysis, while the other half was preserved.

Two sampling strategies were applied in this study: simple random sampling and a size specific selection. Through employing simple random sampling, without replacement, each vertebral specimen in the unit has an equal chance of selection at each draw (Latpate et al., 2021). To select the samples in this way, the bag containing

the faunal remains for a specific unit was emptied and spread out onto a table, with a protective sheet laying between the table and the faunal remains. From this, 40 vertebrae were haphazardly selected by spreading out the vertebrae from the top, middle, and bottom of the unit faunal bag and looking away. Following, the selected vertebrae were placed in 4 rows of 10. Here, a random number generator was used to select a vertebra for processing, which was subsequently measured, placed in a sample bag, and labeled for later use. After each vertebra was selected for processing, its position in the rows would be replaced with another vertebrae from the faunal bag, using the haphazard selection method detailed above. This process was repeated for each examined unit. The size specific selection was conducted separately from the random sampling by selecting vertebrae across the 5 examined excavation units with a diameter less than 5mm to test for the presence of pink salmon. This additional test was conducted after initial results suggested an absence of pink on the island despite their abundance in the region. The size specific test was conducted to confirm this finding.

Initially, 30 randomly selected vertebrae samples were processed from Unit 9 as a pilot test to determine if the salmon vertebrae had viable DNA. 30 additional samples from this centrally located unit (Unit 9) were selected for processing to serve as a comparison to the initial 30 results and to confirm observed trends. At this stage, sampling from four additional units (4B, 5, 11, 12) was conducted. Here, 5 vertebrae samples were selected for processing from each unit. Only 5 were selected from each unit as this was sufficient to compare to the patterns observed in the central unit (Unit 9) and would increase the overall sampling size and confidence that one individual was not over-represented in the sample. Across each of the five units a size specific selection was conducted where 1-2 small salmon vertebrae (<5mm in diameter) were selected from each unit, when present, for processing to specifically assess for the presence of pink salmon. In total, 87 vertebrae samples were selected for aDNA analysis. To ensure that the size distribution of the selected specimens was representative of the greater Smokehouse Island assemblage prior to processing, 500 samples were randomly selected for measurement following the methods detailed above, 300 from the central unit (Unit 9), and 50 each from the 4 additional units (Unit 4B, 5, 11, 12) located towards the surrounding area on the island. Normal distributions of diameter size were compared between the samples selected for processing and the samples selected for measurement to ensure that the processed samples were representative of the greater

population (Cannon & Yang, 2006) (Fig. 3.4). Kolmogorov-Smirnov tests performed in R confirmed that the size differences between the groups for measurement and processing were insignificant. The 7 samples selected for processing based on their small size were not included in this assessment of representativeness. Sample information, including weight, size, and name, is denoted in Table 3.2.

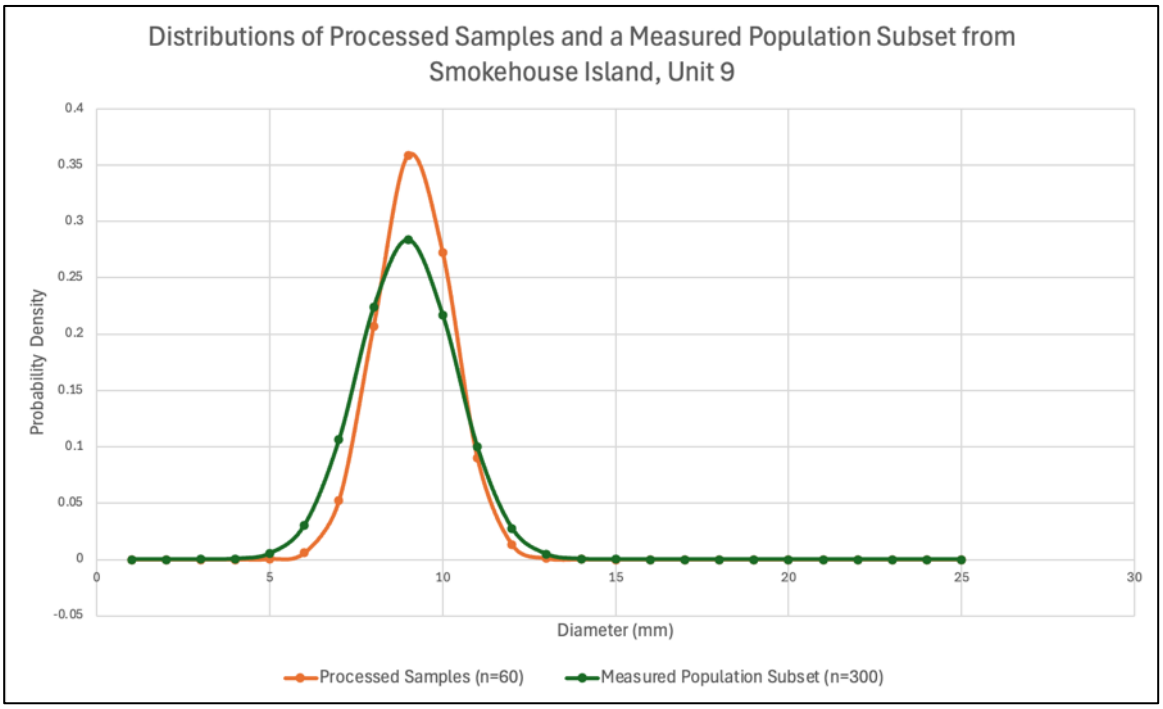

Figure 3.4 Overlayed distributions of diameter measurements for samples selected for processing and a measured population subset from excavation Unit 9 at Smokehouse Island.

Note: The distributions can be seen to display a high degree of similarity. The data points on the plot represent probabilities of selecting a vertebra at a certain diameter (mm).

Table 3.2 Summary table for ancient salmon vertebrae samples processed from Smokehouse Island.

Lab Code	Height (mm)	Length (mm)	Diameter (mm)	D-Loop amp.	CytB amp.	sdY amp.	Species ID	D-Loop Haplotype	Sex ID	Unit #	Initial Weight (g)	Weight Used (g)	Replicate Weight Used
SRS 1	9.0	7.0	10.0	3/5	4/5	7/8	Sockeye	Hap_5	♂ M	9	0.229	.075	.083
SRS 2	8.5	6.5	9.5	2/5	3/3	7/7	Sockeye	Hap_1	♂ M	9	0.232	.105	.096
SRS 3	8.5	6.5	9.5	2/5	3/3	5/7	Sockeye	Hap_4	♂ M	9	0.212	.073	.075
SRS 4	8.0	6.5	9.0	2/5	3/5	2/8	Sockeye	N/A	♂ M	9	0.138	.048	.045

SRS 5	7.0	6.0	8.5	2/6	5/6	5/8	Sockeye	Hap_1	♂ M	9	0.185	.097	.094
SRS 6	5.5	4.5	6.5	3/4	5/5	0/8	Sockeye	Hap_5	♀ F	9	.074	.034	.021
SRS 7	8.5	6.0	10.0	2/3	2/3	2/4	Coho	Hap_6	♂ M	9	.226	.075	.049
SRS 8	6.5	5.5	7.5	3/4	4/5	0/6	Sockeye	Hap_5	♀ F	9	.119	.039	.038
SRS 9	8.0	6.0	8.5	2/3	2/3	0/5	Sockeye	Hap_5	♀ F	9	.222	.075	.083
SRS 10	9.0	7.5	10.5	2/5	2/4	4/7	Sockeye	Hap_5	♂ M	9	.274	.101	.104
SRS 11	7.5	6.5	9.5	2/4	3/3	2/6	Sockeye	Hap_5	♂ M	9	.179	.074	.073
SRS 12	9.0	7.0	10.0	2/4	2/3	3/5	Sockeye	Hap_5	♂ M	9	.278	.093	.083
SRS 13	6.0	5.0	8.0	2/4	2/4	2/5	Sockeye	Hap_5	♂ M	9	.150	.063	.061
SRS 14	8.5	7.0	10.0	2/4	4/5	2/5	Sockeye	Hap_5	♂ M	9	.186	.089	.081
SRS 15	11.0	7.5	11.5	2/4	3/5	0/7	Chinook	N/A	♀ F	9	.447	.122	.107
SRS 16	8.0	6.5	8.5	2/4	4/4	2/4	Sockeye	Hap_1	♂ M	9	.233	.111	.095
SRS 17	8.0	6.0	9.0	2/4	4/4	2/4	Sockeye	Hap_5	♂ M	9	.201	.100	.094
SRS 18	8.0	6.5	9.5	4/5	4/4	0/4	Sockeye	Hap_5	♀ F	9	.221	.103	.100
SRS 19	7.0	6.0	8.5	2/4	3/4	0/4	Sockeye	Hap_1	♀ F	9	.180	.083	.083
SRS 20	11.5	8.0	13.5	3/5	4/5	0/10	Chinook	Hap_7	♀ F	9	.461	.191	.190
SRS 21	8.5	7.0	9.5	2/4	4/4	5/5	Sockeye	Hap_1	♂ M	9	.277	.138	.108
SRS 22	8.0	7.0	9.5	2/4	3/3	0/5	Sockeye	Hap_5	♀ F	9	.298	.131	.120
SRS 23	8.0	6.5	8.0	3/3	2/2	5/5	Sockeye	Hap_1	♂ M	9	.164	.078	.066
SRS 24	7.5	5.0	9.0	2/2	2/2	4/4	Sockeye	Hap_5	♂ M	9	.128	.052	.055
SRS 25	7.0	6.5	9.0	2/2	2/2	0/4	Sockeye	Hap_1	♀ F	9	.201	.092	.066
SRS 26	8.0	6.0	9.0	1/2	1/2	4/4	Sockeye	Hap_1	♂ M	9	.249	.108	.101
SRS 27	9.0	7.0	10.0	2/3	2/2	0/5	Coho	Hap_6	♀ F	9	.223	.105	.089

SRS 28	7.0	5.0	8.5	2/3	2/2	4/4	Sockeye	Hap_5	♂ M	9	.179	.096	.061
SRS 29	9.5	8.0	10.5	1/3	3/4	4/6	Sockeye	Hap_1	♂ M	9	.498	.243	.170
SRS 30	9.0	7.0	10.0	1/2	3/3	4/4	Sockeye	Hap_4	♂ M	9	.192	.084	.047
SRS 31	7.0	5.5	9.0	2/2	2/2	0/2	Sockeye	Hap_1	♀ F	9	.198	.102	
SRS 32	7.0	5.5	7.5	2/3	2/2	0/2	Sockeye	Hap_5	♀ F	9	.140	.070	
SRS 33	6.5	5.0	8.0	2/3	2/2	1/2	Sockeye	N/A	♂ M	9	.135	.068	
SRS 34	6.5	4.5	8.5	2/3	2/2	2/2	Sockeye	Hap_5	♂ M	9	.147	.065	.078
SRS 35	6.0	5.0	8.0	2/3	2/2	2/2	Sockeye	N/A	♂ M	9	.134	.072	
SRS 36	7.0	4.5	8.0	2/3	2/2	2/2	Sockeye	Hap_5	♂ M	9	.142	.079	
SRS 37	8.0	6.0	10.0	2/3	2/2	0/2	Sockeye	Hap_5	♀ F	9	.283	.147	
SRS 38	7.0	5.5	8.0	1/2	2/2	2/2	Sockeye	Hap_1	♂ M	9	.165	.078	
SRS 39	7.5	5.5	9.0	2/2	2/2	2/2	Sockeye	Hap_1	♂ M	9	.182	.089	
SRS 40	6.0	5.0	7.0	2/2	2/2	0/2	Sockeye	Hap_1	♀ F	9	.118	.056	
SRS 41	7.5	5.5	9.0	2/3	2/2	0/2	Sockeye	Hap_1	♀ F	9	.204	.107	.085
SRS 42	9.0	7.0	10.0	3/3	1/2	0/2	Sockeye	Hap_5	♀ F	9	.323	.141	
SRS 43	8.0	7.0	10.0	2/3	2/2	0/2	Sockeye	Hap_1	♀ F	9	.212	.096	
SRS 44	8.0	7.0	9.0	2/3	2/2	2/2	Sockeye	Hap_4	♂ M	9	.238	.106	
SRS 45	8.0	5.0	9.0	2/2	2/2	2/2	Chinook	Hap_7	♂ M	9	.128	.058	
SRS 46	7.0	6.0	9.0	2/2	2/2	2/2	Sockeye	Hap_1	♂ M	9	.184	.090	
SRS 47	8.5	6.5	10.0	2/3	2/2	3/3	Sockeye	Hap_1	♂ M	9	.268	.111	
SRS 48	8.0	6.5	10.0	2/3	2/2	3/3	Coho	Hap_6	♂ M	9	.185	.092	
SRS 49	8.0	5.5	10.0	2/2	2/2	2/2	Sockeye	Hap_1	♂ M	9	.292	.105	
SRS 50	7.0	7.0	9.5	2/3	2/2	0/2	Sockeye	Hap_5	♀ F	9	.258	.109	

SRS 51	8.0	6.0	10.0	2/3	2/2	3/3	Sockeye	Hap_5	♂ M	9	.224	.112	
SRS 52	7.5	5.5	8.5	2/3	2/2	3/3	Sockeye	Hap_1	♂ M	9	.183	.095	
SRS 53	9.5	7.5	10.5	2/3	2/2	3/3	Sockeye	Hap_1	♂ M	9	.411	.130	
SRS 54	7.0	6.0	8.5	2/2	2/2	2/2	Sockeye	Hap_5	♂ M	9	.200	.098	
SRS 55	7.5	6.0	9.0	2/2	2/2	1/2	Sockeye	Hap_4	♂ M	9	.184	.090	
SRS 56	7.0	6.0	9.0	2/2	2/2	2/2	Sockeye	Hap_5	♂ M	9	.244	.104	
SRS 57	8.0	6.0	9.0	2/3	2/2	2/2	Sockeye	Hap_5	♂ M	9	.167	.074	
SRS 58	7.0	6.0	8.0	2/3	2/2	0/2	Sockeye	Hap_1	♀ F	9	.210	.103	
SRS 59	9.0	7.0	10.0	3/3	2/2	0/2	Sockeye	N/A	♀ F	9	.282	.120	
SRS 60	7.0	6.0	8.5	2/3	2/2	2/2	Sockeye	Hap_5	♂ M	9	.182	.085	
SRS 61	12.5	9.0	15.0	2/2	2/2	0/4	Chinook	N/A	♀ F	4B	.697	.113	.224
SRS 62	8.0	6.5	9.0	2/3	2/2	0/4	Sockeye	Hap_1	♀ F	4B	.173	.063	.105
SRS 63	7.0	5.5	9.0	2/3	2/2	1/4	Sockeye	Hap_5	♂ M	4B	.188	.108	.074
SRS 64	7.0	5.5	8.0	3/3	2/2	0/5	Sockeye	N/A	♀ F	4B	.113	.047	.053
SRS 65	7.0	6.0	8.5	2/3	2/3	1/4	Sockeye	Hap_1	♂ M	4B	.147	.082	.060
SRS 71	8.0	7.0	10.5	0/2	0/3	0/5	N/A	N/A	N/A	5	.289	.115	.166
SRS 72	8.0	6.5	9.0	0/2	0/3	0/5	N/A	N/A	N/A	5	.240	.101	.136
SRS 73	7.5	6.5	8.5	1/4	2/3	0/5	Sockeye	N/A	N/A	5	.235	.107	.115
SRS 74	11.0	8.5	12.0	0/3	1/4	0/6	Chinook*	N/A	N/A	5	.551	.136	.217
SRS 75	7.5	6.0	8.5	2/5	3/3	0/5	Sockeye	Hap_2	N/A	5	.146	.056	.078
SRS 81	6.5	6.0	7.0	2/3	2/2	0/2	Sockeye	Hap_3	♀ F	11	.167	.088	
SRS 82	14.5	10.5	17.0	1/2	3/3	0/4	Chinook	Hap_7	♀ F	11	1.019	.145	
SRS 83	8.0	6.5	9.5	2/3	2/2	0/2	Sockeye	N/A	♀ F	11	.292	.154	

SRS 84	14.5	10.0	16.0	2/3	2/2	1/3	Chinook	Hap_7	♂ M	11	.997	.159	
SRS 85	12.0	9.0	14.5	2/2	2/2	0/2	Chinook	Hap_7	♀ F	11	.887	.143	
SRS 91	8.0	7.0	9.0	2/3	2/2	0/4	Sockeye	Hap_1	♀ F	12	.263	.110	
SRS 92	9.0	6.5	9.5	2/2	2/2	2/2	Chinook	Hap_7	♂ M	12	.305	.113	
SRS 93	8.0	7.0	10.0	2/2	2/2	0/2	Sockeye	Hap_5	♀ F	12	.333	.128	
SRS 94	7.0	6.0	7.5	2/2	2/2	0/2	Sockeye	Hap_1	♀ F	12	.195	.118	
SRS 95	7.0	6.0	8.5	2/2	2/2	0/2	Sockeye	Hap_1	♀ F	12	.261	.119	
SRS 101	3.0	2.5	3.5	2/2	2/2	1/2	Sockeye	Hap_5	♂ M	12	.016	.016	
SRS 102	3.5	3.0	4.0	0/2	0/2	0/4	Sockeye*	N/A	N/A	5	.042	.042	
SRS 103	3.5	3.5	3.5	0/3	1/2	0/4	N/A	N/A	N/A	5	.011	.011	
SRS 107	4.0	3.0	5.0	2/2	2/2	0/3	Sockeye	Hap_5	♀ F	4B	.026	.026	
SRS 108	4.0	3.0	4.5	2/2	2/2	0/3	Sockeye	Hap_1	♀ F	4B	.022	.022	
SRS 110	3.0	4.0	3.5	0/4	2/2	0/3	N/A	N/A	N/A	9	.027	.027	
SRS 111	3.0	3.5	3.0	0/2	0/2	0/4	N/A	N/A	N/A	9	.019	.019	

Note: SRS is a labcode and refers to “Skeena River Salmon”. D-Loop, CytB, and sdY amp refer to successful versus attempted PCR amplifications. The high level of amplifications and attempts in SRS1 to 30 are due to student training.
*Species identities from samples SRS 102 and SRS 74 were confirmed using short D-Loop primers.

3.3. Modern DNA Samples

Modern sockeye DNA was obtained from 7 salmon, previously extracted by Thomas Royle in the aDNA laboratory at SFU (Royle et al., 2018), to assess the D-Loop genetic diversity to compare to that of the ancient sockeye samples. These samples were previously obtained by Dongya Yang with Lake Babine Nation at the Babine River counting fence in 2016 over a period of one day, and were collected in keeping with the Canadian Council for Animal Care Guidelines, according to Royle (2018). Royle (2018) extracted the DNA using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) and associated protocol. The samples were collected during the seasonal sockeye run on the same date.

3.4. Decontamination and DNA Extraction

Decontamination, DNA extraction and PCR setup procedures for ancient samples were conducted in a dedicated aDNA laboratory in the Department of Archaeology at Simon Fraser University (Burnaby, BC, Canada), which is physically separated from the post-PCR and modern DNA laboratories and on a different ventilation system. Contamination preventions also included a positive air pressure in the aDNA laboratory, limiting the entrance of external contaminants into the lab space. Physical protections, such as coveralls, masks, hairnets, and gloves were used when working in the lab space. Additionally, all surfaces and equipment within the lab were wiped with 100% bleach prior to and post usage.

Prior to extraction, the salmon vertebrae samples underwent chemical decontamination and ultraviolet irradiation. The samples were first weighed and cut in half. When possible one half of the sample was used for analysis, while the other was reserved for replicates and future analyses. The portions of vertebrae used for aDNA analyses were decontaminated through submersion in 100% bleach followed by 30 minutes of ultraviolet irradiation in a crosslinker. To assess the validity of the results, 42 samples were replicated, thus the remaining portion of these samples also underwent analysis.

The Yang et al. (1998) DNA extraction protocol was followed to extract DNA from the samples. Accordingly, the samples were incubated at 50˚C overnight in a lysis buffer containing 0.5M EDTA, 0.5% SDS, and 0.5 mg/mL proteinase K. Blank controls were incorporated during the extraction procedure to assess for contamination at this stage. Figure 3.5 depicts samples post-incubation. Some samples display a darker colour, which may be indicative of higher levels of inhibition, possibly due to humic substances. The samples were transferred to Amicon Ultra-4 centrifugal filter tubes (Millipore, Billerica, MA) and were centrifuged for ~99 minutes. Subsequently, the samples were transferred to QIAGEN Qiaquick spin columns (QIAGEN, Hilden, Germany) in which the DNA was bound to a silica membrane within the tube, washed, and eluted using Buffer PB (QIAGEN, Hilden, Germany), Buffer PE (QIAGEN, Hilden, Germany), and Buffer EB (QIAGEN, Hilden, Germany), respectively. First and second DNA elutions were collected for each sample, to account for the possibility of high levels of PCR inhibitors within the first elution. To collect the second elute DNA, additional Buffer EB is added. This results

in DNA that is more diluted than the first elution, as well as more diluted PCR inhibitors. The second elution DNA will be tested after the failure of the first elution DNA on the suspicion of inhibition. The second elution DNA requires more DNA solution to be used due to the diluted nature.

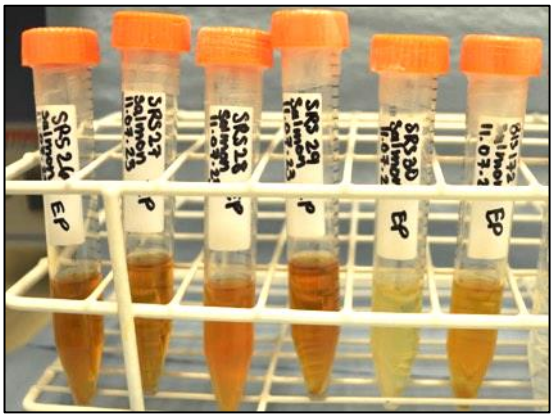

Figure 3.5 DNA samples extracted from Smokehouse Island salmon vertebrae in a dedicated aDNA laboratory at SFU.
Note: Colour variation be indicative of high or low levels of inhibition via humic substances. Inhibition can be overcome by extraction and analyzing a second elution of DNA.

3.5. PCR Amplification and Analysis

Prior to PCR amplification, three separate reactions were prepared using four sets of previously published primers (Table 3.3). The primers for the *cytochrome b* and D-loop (control region) loci were used to amplify species specific regions of mitochondrial DNA (mtDNA). The *sdY* primers amplify a region on the Y chromosome within the nuclear genome, and thus informs about sex. The *sdY* primers were coamplified with D-loop primers (mitochondrial), and alternatively with *clock1a* primers (nuclear), which served as internal positive controls (Royle et al., 2020). It was necessary that *sdY* be co-amplified with control primers, and that the sex determination assay be repeated, as sex is determined visually depending on the banding that is present. Additionally, allelic dropout may occur leading to failed amplification and erroneous results, requiring multiple tests to confirm a sex identification.

Accordingly, each ancient sample underwent three different amplification reactions: a co-amplification with *sdY* and D-loop primers (F19/R20, F800/R1048) (Fig. 3.6), a co-amplification using *sdY* and *clock1a* primers (F19/R20, F50/R60) (Fig. 3.7), and amplification of only *cytochrome b* fragments using *cytochrome b* primers

Cytb5/Cytb6 (Fig. 3.8). Modern samples only underwent amplification of the D-Loop region using primers F800/R1048 to identify D-Loop haplotype, as species and sex were already previously identified by Royle et al. (2018).

Later, 3 additional sets of primers were incorporated as an attempt to rescue failed samples (Fig. 3.9). These primers all belonged to the D-Loop region and were targeting smaller fragments of DNA than F800/R1048. This was conducted with the prediction that samples were failing due to DNA degradation. Targeting smaller fragments could allow for some successful DNA data to be examined, while not necessarily requiring the entire original fragment to be spanned undegraded.

PCR amplification occurred in a 30µL reaction, with 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.0 mg/ml Bovine Serum Albumin, and 0.75–1.5U AmpliTaq Gold (Applied Biosystems), 0.3uM of the forward and reverse primers (except for 0.1uM D-loop and 0.6uM sdY for sex ID), and 3.0uL DNA sample. A negative PCR control was included at this stage. The samples underwent PCR amplification in a 96-well Mastercycler thermocycler starting with 12 minutes of denaturation at 95˚C, followed by 60 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 54˚C, extension for 40 s at 70˚C, and a final extension step at 72˚C for 7 minutes (Eppendorf, Hamburg, Germany).

In the post-PCR laboratory in the Department of Archaeology at Simon Fraser University (Burnaby, BC, Canada) the PCR products underwent agarose gel electrophoresis analysis. Five µL of the products were mixed with SYBR Green DNA stain. Products amplified with *sdY/clock1a* primers were run in a 3% gel at 100V for 60 minutes. Products amplified with *cytochrome b* and *sdY*/D-loop primers were run in a 2% gel at 100V for 30 minutes. Successful samples, displaying strong banding, with species specific primers (*cytochrome b* and D-loop) were sequenced through Eurofins Genomics (Toronto, ON, Canada) in both forward and reverse directions when possible. Sequences sent out in either direction from the same sample belonged to different PCR setups or were from different extractions to enhance the validity of the results and effectively monitor contamination possibilities. Samples exhibiting *sdY* fragment amplification were visually identified as male. This was confirmed across two different assays, one assay using a mitochondrial fragment (D-Loop) as an internal positive control, the other using a nuclear fragment (*clock1a*) as an internal positive control.

Table 3.3 Salmon primers used in PCR amplification.

Locus	Primer	Sequence (5'-3')	Size (bp)	Source
cytochrome b	CytB5 (F)	AAAATCGCTAATGACGCACTAGTCGA	168	Yang et al., 2004
	CytB6 (R)	GCAGACAGAGGAAAAAGCTGTTGA	168	Yang et al., 2004
clock1a	Clk1a-F50	TAGCCATGTCTGTGTGTTTACTTGC	108	Royle et al., 2018
	Clk1a-R60	GCAGCCAGCTAATTKGATTTG	108	Royle et al., 2018
D-loop	F800	AACCCCTAAACCAGGAAGTCTCAA	248 (with R1048)	Yang et al., 2004
	R955	CGAAAACTTTATTAATGTATACTTTAWTTAT	156 (with F800)	Prepared by Dongya Yang (unpublished)
	F926	TAAATAAAGTATACATTAATAAACTTTYCG	123 (with R1048)	Prepared by Dongya Yang (unpublished)
	R1048	CGTCTTAACAGCTTCAGTGTTATGCT	248 (with F800)	Yang et al., 2004
	*Smc1*n	GCTTAATGTAGTAAGAACCGACCAAC	117	Prepared by Dongya Yang (unpublished)
	Smc2	TAGGAACCAAATGCCAGGAAT	117	Prepared by Dongya Yang (unpublished)
sdY	*sdY*-F19	CCCAACACCCTTCCTATCTCC	95	Royle et al., 2018
	sdY-R20	CCTTCCTCCCTAGAGCTTAAAAC	95	Royle et al., 2018

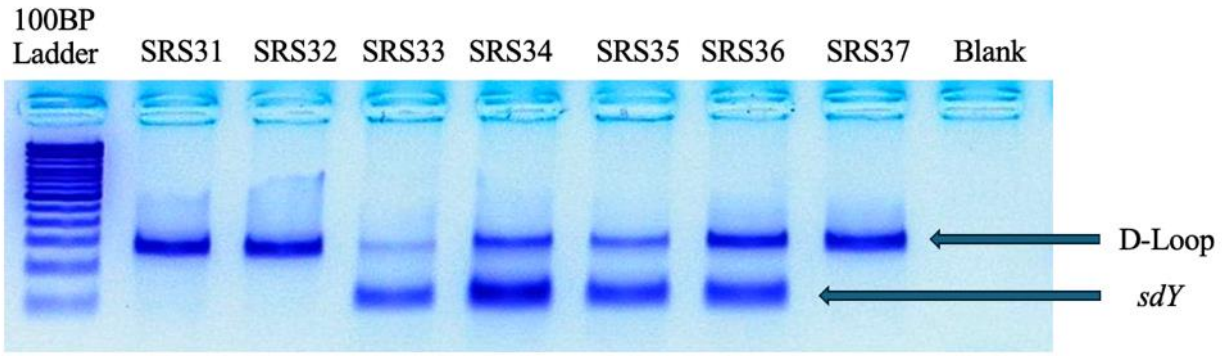

Figure 3.6 Amplification of D-Loop and *sdY* DNA fragments.
Note: Blue banding depicts successful amplification of DNA fragments. The upper band is the D-Loop, a species-specific region in the salmon mitochondrial genome. The lower band is *sdY,* found on the Y chromosome, which is specific to male salmon nuclear DNA.

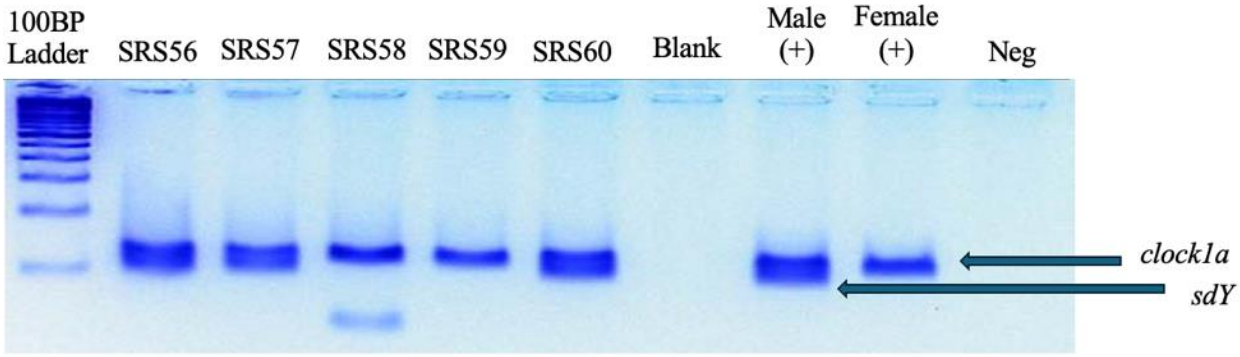

Figure 3.7 Amplification of *clock1a* and *sdY* fragments.
Note: Blue banding depicts successful amplification of DNA fragments. The upper band is clock1a, a nuclear DNA fragment acting as an internal control. The lower band is sdY, which is specific to male salmon.

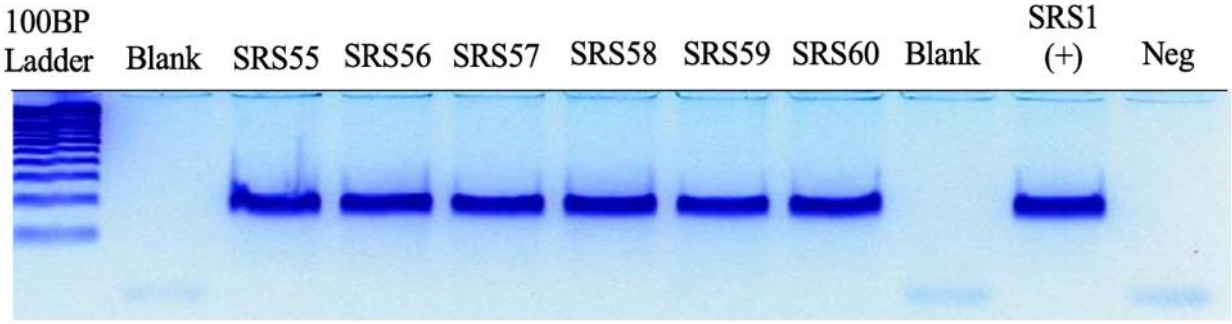

Figure 3.8 Amplification of *cytochrome B* fragments.
Note: Blue banding depicts successful amplification of DNA fragments. The *cytochrome B* fragment is of the mitochondrial genome and is species specific, allowing identification of different salmonid species.

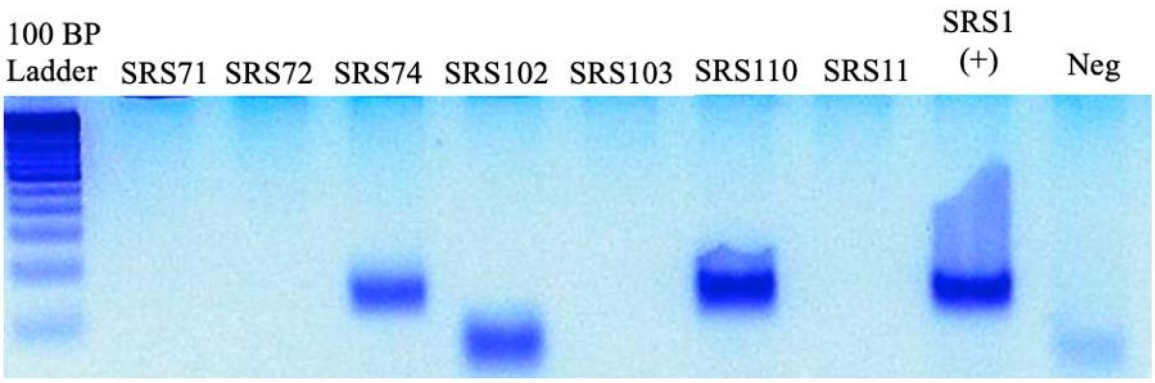

Figure 3.9 Amplification of D-Loop Short fragments (*Smc1n*/*Smc2*).
Note: Blue banding depicts amplification of target fragments. Samples without amplification (banding) were not able to be rescued.

3.6. Sequence Analysis

Upon the return of the sequence data from Eurofins Genomics, the sequences were manually edited using 4peaks software (https://nucleobytes.com/4peaks/). When possible, contiguous sequences were generated using Bioedit (https://www.nucleics.com/), where the forward and reverse strand were aligned to create an overlapping sequence. This edited sequence was run through the GenBank BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine if any uploaded sequences were a match to the query sample sequence. Following, several reference sequences for the five Pacific salmon species represented in North American waters, as well as outgroups, such as Atlantic salmon (*Salmo salar*), were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to serve as a comparison to the sequences from the Smokehouse Island samples. The sequences from Smokehouse Island and the reference samples were input into Bioedit, where the samples were aligned and trimmed. A phylogenetic analysis was then conducted through the construction of a maximum-likelihood phylogenetic tree with MEGA11 software (https://www.megasoftware.net/) with 2000 bootstraps. This process was conducted separately for each species indicating loci (*cytochrome b* or D-loop), leading to the creation of two phylogenetic trees (Fig. 4.2 and 4.3).

3.7. Conclusion

This chapter has provided an in-depth overview of the archaeological context and samples analyzed in this thesis. There is a detailed discussion of the ancient DNA

analysis methods applied here, with descriptions of specific primers, reagents, and software used. The following chapter will discuss the results obtained from the application of these methods.

Chapter 4. Results

In this chapter the results, based on the methods described in the previous chapter, are presented. The success rate of sex and species identification is discussed. The results are presented in terms of sex and species composition across the different excavation units and sex distribution across the different species in the assemblage. The genetic diversity across the analyzed remains is also presented and compared to modern samples. Unexpected amplifications and contamination events are then discussed.

4.1. DNA Amplification

Of the 87 ancient samples analyzed, it was not possible to obtain the sex and species identification from 5 samples, yielding a ~94.25 rate of success to at least one level of identification (sex or species). Amplification of at least one DNA fragment was visualized in all but 3 samples, however, not all of these amplification events led to conclusive results, and may have been erroneous, and are thus not considered as an indicator of success. The high rate of successful identifications (>90%) can be attributed to the relatively recent antiquity of the samples (~1000 years BP) and the temperate climate of North-Central BC leading to increased instances of preserved DNA in ancient faunal remains (Cannon & Yang, 2006). While it is possible that the DNA from the unsuccessful samples did not have sufficient levels of preservation, it is also possible that these samples belonged to a species unrelated to salmon, as the species identification primers used in this study are specific to salmonids and should thus only lead to successful amplification of salmonid DNA (Yang et al, 2004; Yang & Speller, 2006).

4.2. Sex Identification

As previously stated, sex was determined visually through the amplification of *SdY* and an internal positive control on an agarose gel. Two tests were used to determine and confirm sex identification: *sdY* co-amplified with *clock1a* and *sdY* co-amplified with D-Loop. For some samples these two tests did not yield results that were in agreement, which may be due to allelic dropout, where for some reason the *sdY* fragment is failing to amplify, leading to misidentification as female. The possibility of this called for multiple

sex-identification assays to confirm results. When *sdY* was seen to be successfully amplified, the sample was identified as male.

Out of 78 samples with a successful sex determination, 45 were found to be male (~58%) and 33 were found to be female (~42%). Based on the results from only the randomly selected samples, excluding those selected based on size due to bias, 44 were male (~59%) and 31 were female (~41%). The female to male sex ratio of these results was 1:1.42. A two-tailed binomial exact test was conducted to assess for potential male bias in the sex composition of the randomly selected samples, demonstrating a significant value (p=<0.05), suggesting the sex composition of the assemblage at Smokehouse Island is biased towards males (Morin et al., 2021a).

Per unit, the sex ratio can be seen to differ (Fig. 4.1). Unit 4B, 11, and 12 represent more female salmon than male salmon, while Unit 9 represents more male than female salmon. The binomial exact tests per unit suggested that of all the units examined, only unit 9 demonstrated a significant sex bias, specifically towards male salmon (Table 4.1).

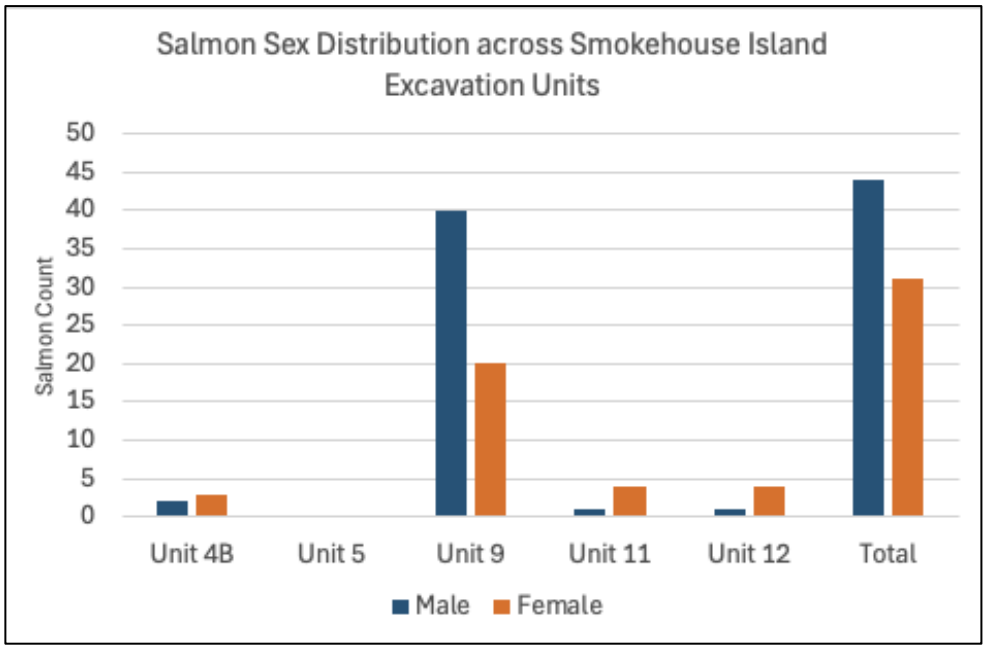

Figure 4.1 Salmon sex composition across analyzed Smokehouse Island excavation units.

Note: The graph visually demonstrates the percent of male (blue) and female (orange) salmon present in an excavation unit. Unit 5 is not represented in the graph as no data pertaining to sex was yielded from this unit. The exact number of samples generating these percentages are available in the table below. This data excludes results from samples selected based on size.

Table 4.1 Salmon sex distribution across all analyzed Smokehouse Island salmon vertebrae samples.

Sex	Unit 4B	Unit 5	Unit 9	Unit 11	Unit 12	Total
Male	2	0	40	1	1	44
Female	3	0	20	4	4	31
Total	5	0	60	5	5	75
Binomial Distribution	0.313	N/A	0.004	0.156	0.156	0.030

Note: Binomial distributions are considered significant when less than 0.05. This data excludes results from samples selected based on size.

4.3. Species Identification

Inputting the generated sequences as queries into the BLAST search engine led to identifications with high degrees of similarity to modern sockeye, Chinook, and coho salmon. Sockeye was the most predominant species seen in the data, at ~85% (n=70), followed by Chinook (~11%, n=9) and coho (~4%, n=3). No chum or pink salmon were represented in the data, despite performing a size-specific selection meant to identify the presence of pink salmon, where samples with a diameter less than 5mm were selected for processing. Species identification was confirmed, when possible, by comparing sequence results from *CytB* and D-Loop fragments sequenced in both forward and reverse directions. Relationships could then be visualized through the phylogenetic trees, where sequences can be seen to cluster with references downloaded from GenBank (Fig. 4.2 and 4.3).

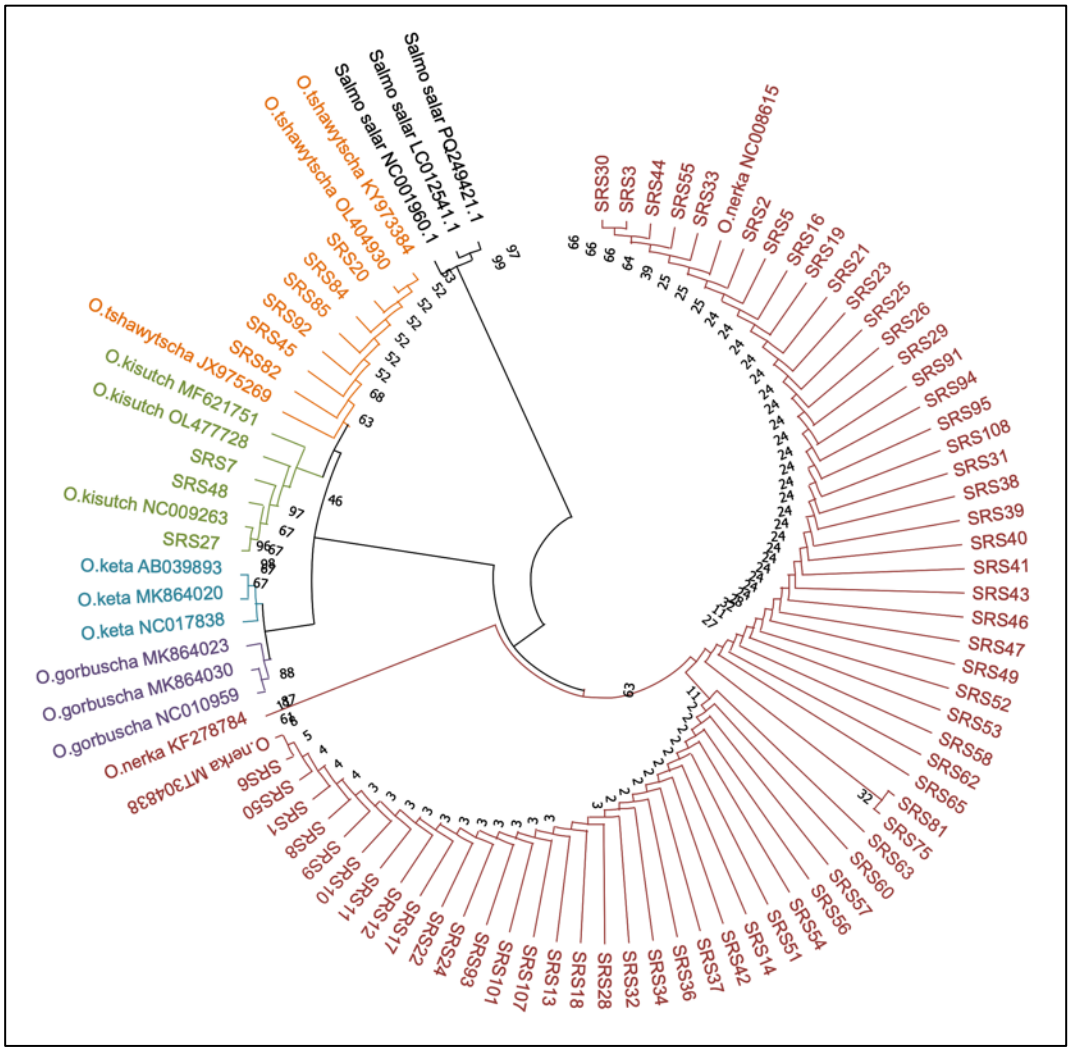

Figure 4.2 **Phylogenetic tree of D-Loop sequences from Smokehouse Island samples compared to three reference sequences per species obtained from Genbank.**

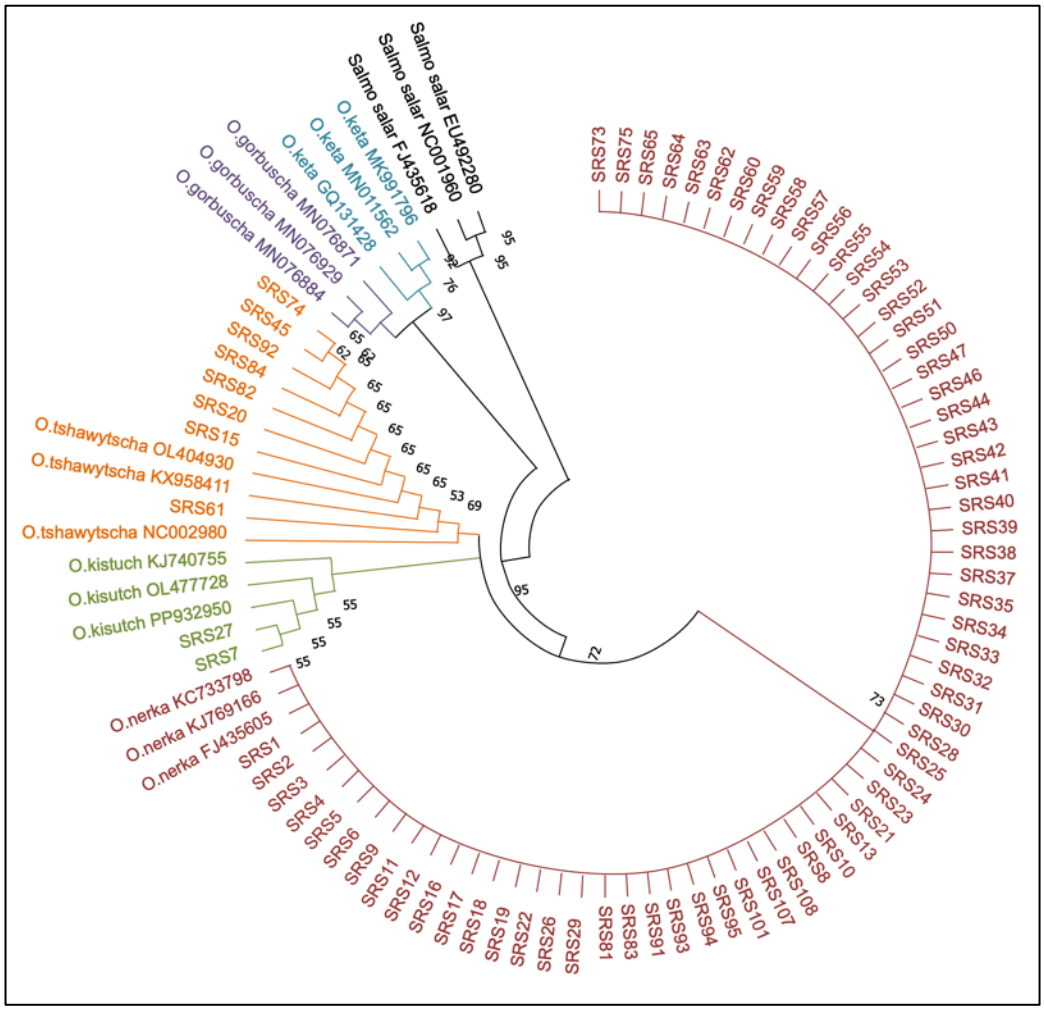

Figure 4.3 Phylogenetic tree of *cytochrome b* sequences from Smokehouse Island samples compared to three reference sequences per species obtained from Genbank.

As visualized in Figure 4.4, sockeye is the dominant species in all units examined, except for Unit 11. The second most dominant species across the units is Chinook, which is the most dominant in Unit 11. The species composition within Unit 9 is dominated by sockeye, a trend that is not as marked in the other units. More specifically, the species composition in Unit 9 is 90% sockeye, 5% Chinook, and 5% coho. The composition of Unit 4B is 80% sockeye and 20% Chinook. In Unit 5 the composition is 66% sockeye, 34% Chinook, with a sample size of only 3. Unit 11 is 40% sockeye, 60% Chinook, and Unit 12 is 80% sockeye, 20% Chinook. What is most apparent in the units other than Unit 9 is the greater representation of Chinook in the assemblage, a clear

increase across all of the units, despite the small sample size. Species compositions are determined with the exclusion of the results from the size specific selection.

Species can also be considered in terms of sex identification. Table 4.3 demonstrates that sockeye and coho are both male dominated species in the assemblage, where Chinook is female dominated. Based on the binomial distribution tests, only the total sex distribution and that of sockeye are considered to be significant, both of these biased towards male. In future analyses, a greater sample size of the salmon assemblage on Smokehouse Island may allow additional examination of this line of evidence. Possible sex-selection may have been occurring among only certain species. The data presented here may suggest that a sex-selective fishery was being applied specifically to sockeye salmon, with the sample sizes of Chinook and coho too small to identify possible sex-selectivity.

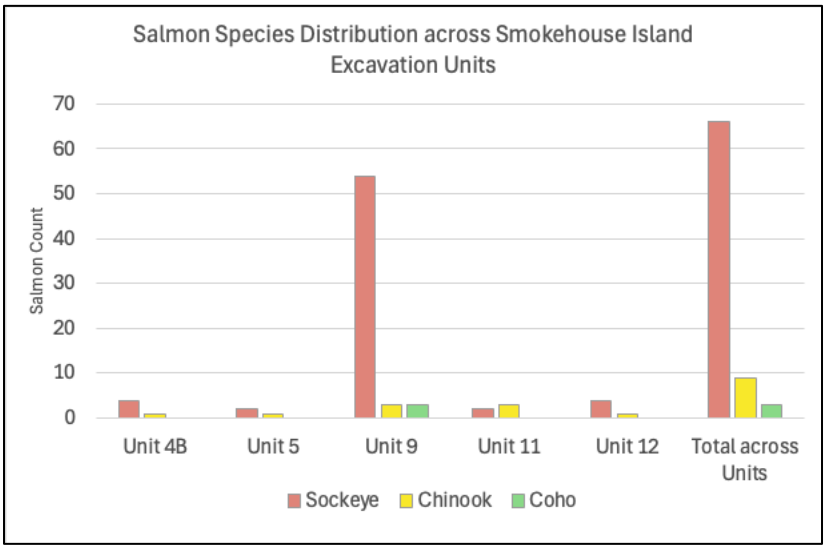

Figure 4.4 Salmon species composition across analyzed Smokehouse Island excavation units.

Note: The graph visually demonstrates the percent of a species of salmon that compose the processed samples from a given unit. The exact number of samples generating these percentages are available in the table 4.2. This data does not include results from the size selected samples.

Table 4.2 Salmon Species Distribution across Analyzed Smokehouse Island Excavation Units.

Salmon Species	Unit 4B	Unit 5	Unit 9	Unit 11	Unit 12	Total across Units
Sockeye	4	2	54	2	4	66
Chinook	1	1	3	3	1	9
Coho	0	0	3	0	0	3
Total	5	3	60	5	5	78

Note: These results do not include data from samples selected based on size.

Table 4.3 Sex distribution across the different species represented at Smokehouse Island.

	Species			
Sex	Sockeye	Chinook	Coho	Total
Male	39	3	2	44
Female	25	5	1	31
Total	64	8	3	75
Binomial Distribution	0.022	0.219	0.375	.030

Note: Binomial distributions are considered significant when less than 0.05. These results do not include data from size selected samples.

4.4. Salmon Population Diversity

Analysis of the D-Loop sequence alignment yielded unexpected results. Based on 1-2 single nucleotide polymorphisms (SNPs) shared between several sockeye sequences, 5 unique ancient sockeye haplotypes were identified. These ancient haplotypes, denoted as Hap_1 to Hap_5 were characterized by 3 SNPs, a C or T at position 32, an A or C at position 44, and an A or G at position 128 (Fig. 4.5, Table 4.4). All positions are relative to the sequences generated from this project. The phylogenetic relationships between the identified ancient haplotypes can be visualized in Figure 4.7, compared to reference sequences, which highlights the genetic distinctiveness of these haplotypes. The reference samples used in the phylogenetic tree were selected by creating a

phylogenetic tree of 1,000 references in Genbank, and selecting one from the top, middle, and bottom region of the tree to reflect increased diversity. Through selecting references with a high-degree of diversity, this also increases confidence in the species identities of the sequences aligning with the references.

An additional SNP was identified as unique to a particular sample. This sample then underwent rigorous examination through multiple PCR setups, extraction replicates, and forward and reverse sequencing, to test if the SNP was in fact present, or was a degradation marker. Examination revealed that the “SNP” was likely a degradation marker, and this possible haplotype was ruled out. This change may have been due to possible cytosine deamination (Hofreiter et al., 2001; Briggs et al., 2007).

Degradation through deamination can be seen to manifest through C to T and G to A changes in the DNA sequence (Hofreiter et al., 2001; Briggs et al., 2007). While these changes are present in 2 of the confirmed SNPs here, Hofreiter et al. (2001) finds that there are not “hotspots” of degradation, which suggests that because these changes are found to occur in multiple samples at the same location, these changes are not due to degradation and are rather SNPs from an earlier, inherited mutation.

Sequences from 7 modern sockeye samples revealed that all 7 samples were within one haplotype, which corresponded to the ancient Hap_1, the second most prevalent haplotype among the ancient sockeye samples (Table 4.4). All of the Chinook and coho D-Loop sequences belonged to one haplotype within each of the species (Fig. 4.5, Fig. 4.6). Additionally, *cytochrome b* sequences did not reveal any unique haplotypes (Fig. 4.7).

Table 4.4 Haplotype composition of Smokehouse Island salmon.

D-Loop Haplotype	SNPs (base at position)	Count
Hap_1	A @ 128	27
Hap_2	C @ 32 G @ 128	1
Hap_3	C @ 32 A @ 128	1
Hap_4	A @ 44 A @ 128	4
Hap_5	G @ 128	29
Hap_6	All coho	3
Hap_7	All Chinook	6

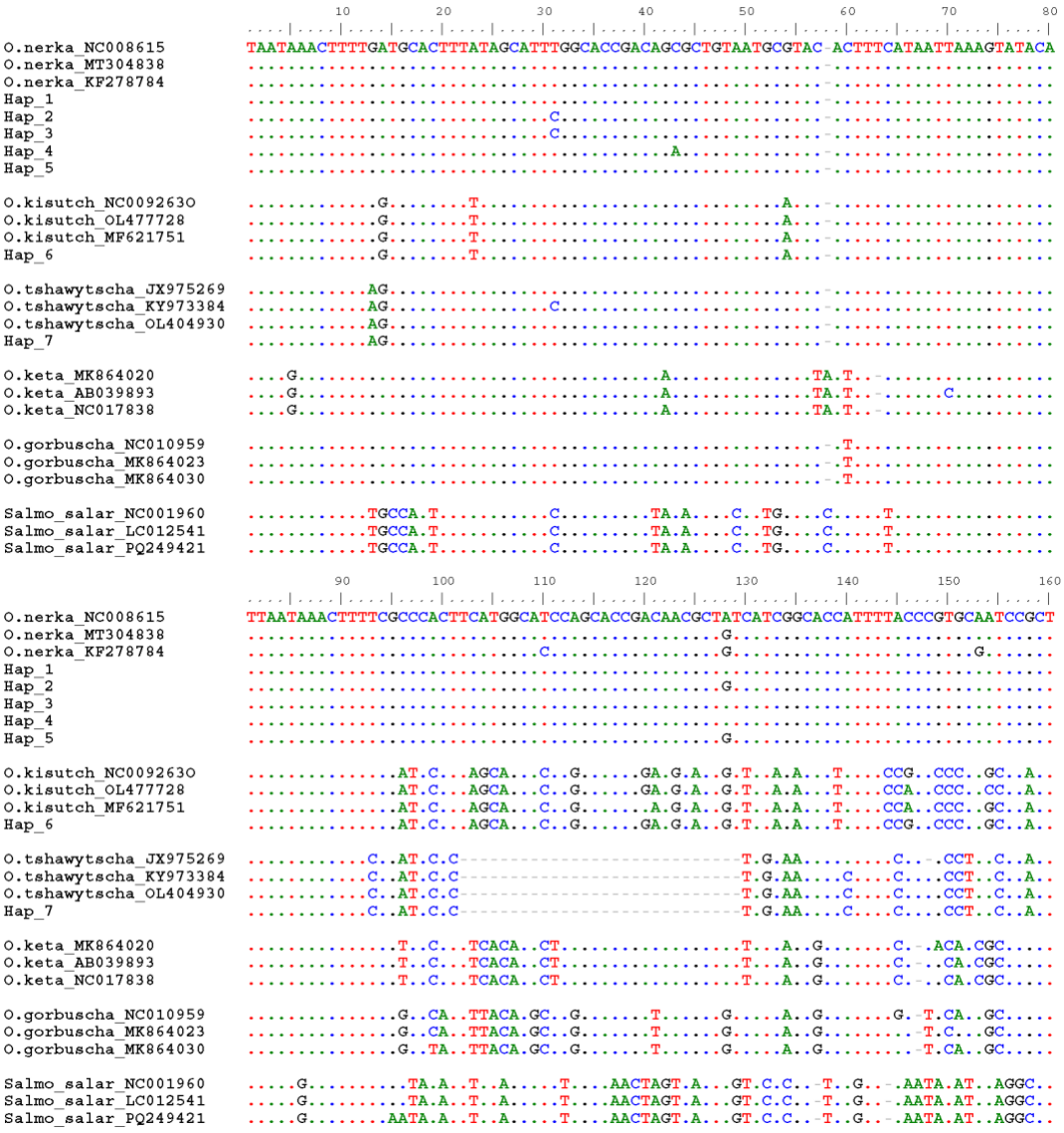

Figure 4.5 Sequence alignment of samples representing 7 haplotypes identified in salmon assemblage at Smokehouse Island.

Note: One sequence from each haplotype identified within the assemblage is included in the alignment and is compared to three reference sequences obtained from Genbank.

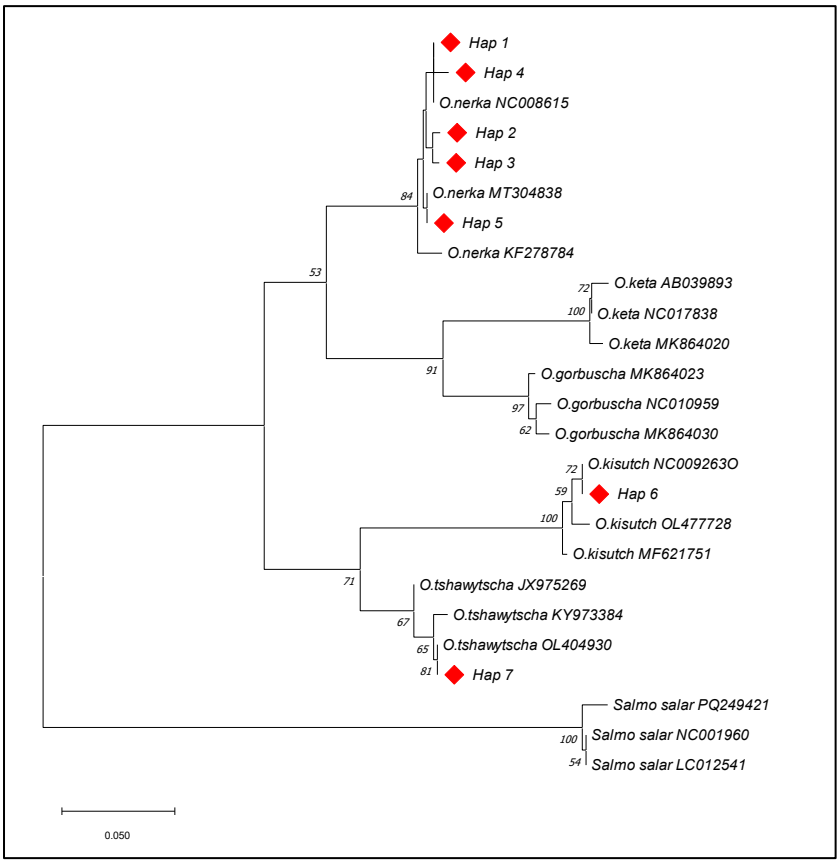

Figure 4.6 Phylogenetic tree of ancient salmon haplotypes identified in the Smokehouse Island assemblage.

Note: This tree also confirms species identification of salmon samples within a certain haplotype.

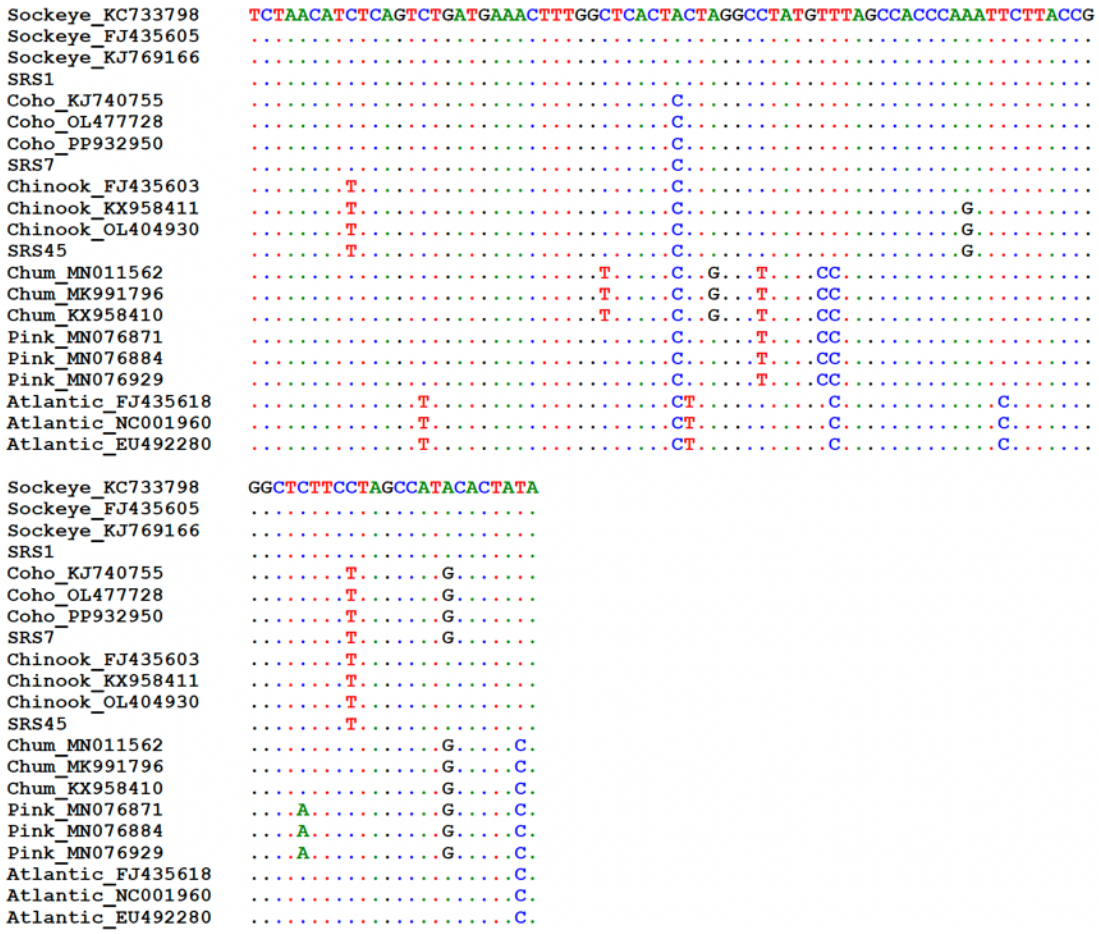

Figure 4.7 Sequence alignment of cytochrome B sequences from Smokehouse Island assemblage

Note: One sequence from each haplotype within the assemblage is included in the alignment and compared to three reference sequences from Genbank. No unique haplotypes are identified within a species group.

4.5. Unexpected Amplifications and Sporadic Contamination

It must be noted that two instances of contamination were observed post-sequencing in samples SRS110 and SRS103. SRS110 yielded inconclusive species identities to multiple Pacific and Atlantic salmonid species, while SRS103 provided a clear species identification of *Salmo trutta* (brown trout). The multiple-species identification of SRS110 was discounted as contamination as the results could not be replicated, despite several attempts and the application of the D-Loop short primers. It is unclear what led to multiple species identities as contamination with these identities was not observed within the other samples or controls tested within the same series as SRS110. Brown trout was

only identified once throughout the testing of SRS103, and the identification could not be replicated. The identification of brown trout was likely contamination as the species is not native to Canada, but was introduced to Newfoundland in 1884, and New Brunswick in 1921 (Ryan, 1988). Regardless, the species identity could not be replicated through further testing and was discounted. It is possible that SRS110 and SRS103 experienced a contamination event, with even one molecule of DNA, which was successfully amplified, rather than silenced, due to the lack of DNA in the samples themselves. However, it remains unclear what the contamination could have stemmed from and why the negative and blank controls remained free of contamination.

4.6. Conclusion

The results presented here indicate that sockeye is the most abundant species in the Smokehouse Island assemblage, followed by Chinook, then coho. No chum or pink salmon are present in the assemblage. Overall, the sex-distribution is biased towards male salmon, however this pattern varies across the excavation units. 5 ancient sockeye haplotypes were also identified, with only one haplotype each for Chinook and coho. The modern sockeye samples examined, obtained at the same time on the same day, represented only one haplotype.

Chapter 5. Discussion

In this chapter, the findings presented in the background and results are combined to generate meaningful interpretations of the data. Here, the authenticity of the results are first discussed in terms of contamination and representativeness. Following, the implications of the sex composition and species composition on the understanding of LBN fishery are explored, and later combined to use a holistic approach in the understanding of the data. The findings are later compared with modern regional data to assess differences in composition and investigate possible change. Lastly, the data is explored regarding its implications on the conservation of salmon in the region.

5.1. Authenticity of Results

Due to the rigorous contamination preventions and controls it is unlikely that accepted results were cross contaminated or were contaminated with modern DNA. The chemical and ultraviolet decontamination measures in place appeared to reduce contamination potential, as evidenced by a lack of contamination in controls, and still allowed the preservation of the ancient DNA, evidenced by electrophoresis and sequencing results. Reproducibility tests, where sex and species were identified using two different assays, allowed data to be crosschecked and validated. The high level of preservation seen across the samples also enhances the validity.

When contamination was apparent in an individual assay, through visible DNA amplification in the extraction blank or negative controls after gel electrophoresis, the results were discarded, and additional testing was required. When replicates of results were not in agreement with the original results, further analysis was conducted to ensure accurate identification. As noted in Chapter 4.5, 2 instances of post-sequencing possible contamination events were identified. While it is currently unclear what led to these likely erroneous identifications, protocols were in place to ensure that events like these would not be falsely included in our findings. In working with aDNA, it is important to understand that while the risk of contamination can be minimized, there is always the possibility that contamination is present. Thus, it is important to have safeguards in place to catch instances of contamination.

In addition to minimizing contamination instances and misidentifications, it is also important to ensure an effective sampling strategy when conducting aDNA research. Our random sampling strategy incorporated two levels of randomness to enhance representativeness (initial haphazard selection, random number generator), and representativeness was later assessed following the Cannon & Yang (2006) method. However, because Pacific salmon can have from around 55-75 vertebrae, and without the atlas vertebra, there may be doubt that the samples are in fact representative of the greater population as one individual may be overrepresented in the data. The identification of 5 unique sockeye haplotypes in the data, in consort with the sex identification data, has allowed effective demonstration that consecutively selected samples can be from different individuals (Table 3.2). Thus, in addition to the highly random sampling strategy and representativeness test, the effectiveness of the random sampling can also be demonstrated in this study.

5.2. Implications of Sex Composition on LBN Traditional Fishery Practices

Due to the bias towards male salmon seen in the data, based on a theoretical sex ratio of 1:1, it is likely that male salmon were harvested at a higher rate than female salmon. Use of a selective harvesting strategy is possible, evidenced by the remnants of weirs surrounding Smokehouse Island (Rahemtulla, 2019; Kantakis, 2017), which allow for the harvest of certain fish and the release of others (Dale & Natcher, 2014; Royle et al., 2020). A male preferential harvest may be considered a sustainable fishery method, as one male salmon is capable of fertilizing eggs from multiple females. In the case of sockeye salmon, one experimental study suggests that a male to female ratio of 1:15 would only decrease the rate of egg fertilization by less than 5% (Morin et al., 2021b; Reed, 1982).

While male salmon may have been preferentially harvested as part of a sustainability effort (Dale and Natcher, 2014; Langdon, 2006; Morin et al., 2021a; Royle et al., 2020), it is also possible that male salmon were harvested at a higher rate due to their larger size (Langdon, 2006; Morin et al., 2021a; Royle et al., 2020) or possible greater relative abundance (Langdon, 2006; Royle et al., 2020). Based on the sexual dimorphism of spawning salmon, it is possible to discern sex based on visual observation, allowing for the possibility of a sex-specific harvest (Dale & Natcher, 2014;

Langdon, 2006; Royle et al., 2020). Thus, it is very possible that this was a practice in place at Smokehouse Island. Further, it is possible that a harvest dominated by male salmon was utilized as a responsive resource management strategy. This could be in response to changes in relative abundance, preference, or fishery strategy, such as an introduction of weir technology allowing increased selection. Male sockeye are the most abundant in the data, which could suggest that a sex-specific harvesting strategy was employed selectively on sockeye.

However, the sex ratios appear to vary per unit, where the central unit depicts a harvest dominated by male salmon, the surrounding units appear to have more female salmon represented in the data, however the data from these surrounding units is quite limited, and binomial distributions indicate that these data are not significant. Considering the 5 examined units as part of two groups, “central” and “surrounding”, based on their location on the island (Fig. 3.2), may allow a more fulsome understanding of the usage of the site, as well as salmon preference. Here, the central region consists of Unit 9, whereas the surrounding region consists of Unit 4B, Unit 5, Unit 11, and Unit 12. Through combining the data from all of the surrounding units to compare to the central Unit 9, a more significant comparison is generated (Fig. 5.1). The binomial distributions for both the central and surrounding units are considered significant ($p=<0.05$), where the central unit is biased towards male, the surrounding region is biased towards female (Table 5.1).

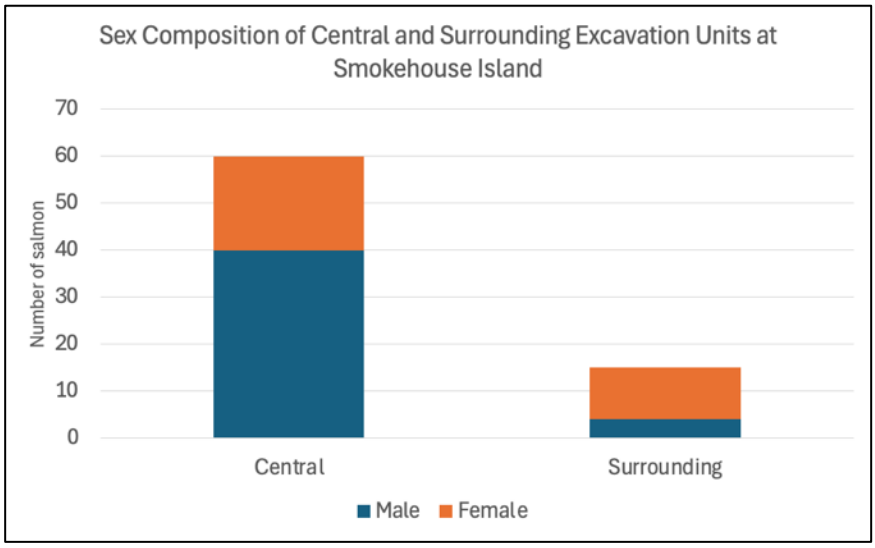

Figure 5.1 Sex distribution of central and surrounding units.
Note: Central unit is composed of the Unit 9 vertebrae samples, while the surrounding units combine the samples from units 4B, 5, 11, and 12. Units are denoted as "central" or "surrounding" based on their location on Smokehouse Island. Specific data regarding sex composition of central versus surrounding units is viewable in Table 5.1 below.

Table 5.1 Sex distribution of central and surrounding units.

Sex	Location of Units		
	Central	Surrounding	Total
Male	40	4	44
Female	20	11	31
Total	60	15	75
Binomial Distribution	0.004	0.042	0.030

Note: Binomial distributions are considered significant when less than 0.05.

This data then suggests that while the total sex distribution suggests a male dominated harvest, the distribution varies per region, and may reflect some different harvest strategy or use of the site. Perhaps egg harvest from female salmon occurred in the surrounding region on the island, where male salmon were prepared for preservation is the central area, a concept discussed further in section 5.4. An additional possible explanation could be the potential for protandry, where male salmon arrive to spawning grounds earlier than female salmon. This has been noted to occur among chum salmon early in the spawning season, with the sex ratio reaching 1:1 during the peak spawning season (Salo, 1991). Some oral histories indicate this may occur near the Babine River

count fence, where male salmon return early, followed by female salmon. Thus, another possible explanation could be the male dominated early harvest is processed centrally on the site, and throughout the harvest season, as more females arrive, the processing locations radiate outwards as the central area fills.

5.3. Implications of Species Composition on LBN Traditional Fishery Practices

Sockeye are very abundant at Babine Lake. As previously stated, it is where 90% of all the sockeye from the Skeena River watershed spawn (Barouillet et al., 2024; Beacham et al, 2014; Gottesfeld & Rabnett, 2008; Rahemtulla, 2019). Sockeye spawn during the summer, and by the time they reach the Smokehouse Island site, they have an ideal amount of fat for smoking and drying (Gottesfeld & Rabnett, 2008). During migration to spawning grounds, Pacific salmon will not eat, and die shortly after spawning (Thurston & Newman, 1962). As a result, upon their arrival at spawning grounds, the oil and fat content of Pacific salmon have decreased markedly (Thurston & Newman, 1962). Modern sockeye activity at the Babine River counting fence peaks from mid- to late-August (Department of Fisheries and Oceans, 2024). Coho, another fatty species of salmon (Gottesfeld & Rabnett, 2008), peak in activity at the Babine River counting fence around September (Department of Fisheries and Oceans, 2024). Chinook is present at the modern Babine River, peaking in abundance in September (Department of Fisheries and Oceans, 2024). Interestingly, the sequence data does not support the harvest of pink salmon at Smokehouse Island, which are known to run at a time between the sockeye and coho runs (Gottesfeld & Rabnett, 2008). Modernly, at the Babine River counting fence, pink salmon are most abundant from mid- to late- August (Department of Fisheries and Oceans, 2024). While they have been known to be abundant at the Babine Counting Fence located near Smokehouse Island, due to their small size and fat content, the capture of pink salmon by the ancestral Lake Babine Nation may have been avoided. A weir mesh, possibly employed as part of a management strategy, may have allowed this smaller salmon species to pass through the weir, while blocking the passage of the larger species (Kantakis, 2017; Lepofsky & Caldwell 2013). Ethnographic studies have suggested that in Northwest North America certain species of salmon have been favoured for harvest based on factors such as taste or ability to be preserved (Speller, 2005; Romanoff, 1992).

It is unlikely that live fish were brought off the island, as the nearest village site is relatively distant, and this would have been extremely inconvenient. Rather, individuals may have worked extreme hours processing the fish, evidenced by the dilapidated smokehouses presently on the island (Rahemtulla, 2019), and may have slept on the island during the harvest season. Based on this knowledge, it appears that all salmon harvested at the Smokehouse Island site, if processed and preserved, would be present in the archaeological record on the site. Some ethnographic reports, however, suggest that smaller fish may have been smoked without processing, which could possibly account for the lack of pink skeletal remains on the island (Doe et al., 1998; Kennedy & Bouchard, 1992). Additionally, because 3 samples yielded no DNA amplification using salmonid primers, it is possible that these samples were non-salmonid species, and with further testing, may provide additional evidence that non-salmonid regional species were also harvested by the community.

These findings are consistent with the LBN oral histories, which indicate that sockeye was the main species harvested at Smokehouse Island, although other species were taken on occasion (Rahemtulla, 2019).

Similar to the sex distribution analysis in Section 5.2, the excavation units can be grouped in terms of their location, central and surrounding, which can be useful in understanding the species distribution across the site. Through this combination it becomes apparent that Chinook make up a greater proportion of the population in the surrounding units than it does in the central unit, additionally, sockeye remains the most predominant species across the entire assemblage (Fig. 5.2, Table 5.2). More specifically, despite the smaller sample size of the surrounding units, the amount of Chinook present sees a 100% increase from the amount seen in the central unit, and makes up 33% of the population in the surrounding unit compared to only 5% in the central unit. This could provide further evidence for a difference in area usage across the site. This finding may also support the surrounding area of the island as a place for egg harvest, and the central area as a place for smoking, as proposed in Section 5.2, and further elaborated on in Section 5.4.

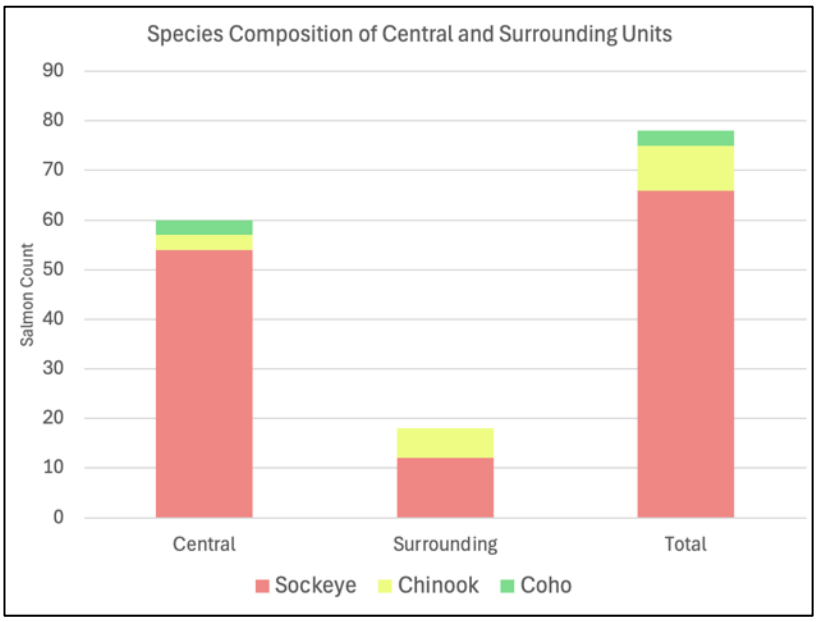

Figure 5.2 Species distribution across central and surrounding units on Smokehouse Island.

Note: Central unit is composed on the Unit 9 vertebrae samples, while the surrounding units combine the samples from units 4B, 5, 11, and 12. Units are denoted as "central" or "surrounding" based on their location on Smokehouse Island. Specific data regarding species composition of central versus surrounding units is viewable in Table 5.2 below. These results exclude data from samples selected based on size.

Table 5.2 Species distribution of central and surrounding units on Smokehouse Island.

Species	Location of Units		
	Central	Surrounding	Total
Sockeye	54	12	66
Chinook	3	6	9
Coho	3	0	3
Total	60	18	78

Note: These results exclude data from samples selected based on size.

The archaeological salmon assemblages from Keatley Creek, along the Fraser River watershed, may serve as an interesting point of comparison to the Smokehouse Island assemblage, situated along the Skeena River watershed. Keatley Creek is an archaeological pithouse village site occupied from 3500 to 1100 BP (Speller et al., 2005). The salmon assemblage recovered from the site, likely dating closer to 1200 BP revealed that sockeye was the dominant species of salmon, followed by Chinook, then

coho, with no pink or chum present based on aDNA analysis of 60 salmon samples (Speller et al., 2005). This is the same composition seen within the Smokehouse Island assemblage. Interestingly, the Keatley Creek D-Loop sequences revealed that all sockeye samples fell within one haplotype, as did the coho, however the Chinook sequences displayed more diversity, with up to 3 haplotypes represented in the assemblage (Speller, 2005). Despite the highly similar assemblages, the patterns of diversity at Keatley Creek are opposite to those findings presented here, where sockeye sequences show high diversity with 5 haplotypes within the Smokehouse Island assemblage, while Chinook sequences all fall under the same haplotype.

With the salmon assemblages dating to similar periods (1000 to 1200 BP), this could suggest some event affecting the Skeena Chinook and the Fraser sockeye, causing a bottleneck in these populations. This comparative dataset tells us that there was high diversity among both of the species at the same time, however not within the same watershed. Perhaps different species were affected by disease or competition within different watersheds.

5.4. Integrating Sex and Species Data

To generate a fulsome understanding of the assemblage at Smokehouse Island, the salmon sex and species data must be integrated. Through this, it may be possible to better understand the harvest strategies used on Smokehouse Island, and the preferences behind the strategies. Table 4.3 demonstrates samples with positive sex and species identifications. It shows that the sockeye assemblage is predominantly male, while the Chinook assemblage is more female dominated. The coho assemblage represents more male salmon, however only three samples are present. Binomial distribution tests indicate that the Chinook and coho sex distributions are not significant. Despite this, the difference in pattern between sockeye and Chinook could suggest some different harvest strategy, or preference on the basis of sex and species, however more data is required to further investigate this.

While some Indigenous traditional harvests have been demonstrated to utilize male-preferential harvesting strategies (eg. Morin 2021a), other harvests were dominated by female salmon based on the method used to capture the fish (Kennedy & Bouchard, 1992). The Smokehouse Island assemblage may then reflect different

harvesting strategies, where sockeye salmon may have been captured using weir technology allowing the larger, brighter-coloured male salmon to be selected, Chinook may have been captured using dip-netting in back-eddies, which are areas that are typically female-dominated (Kennedy & Bouchard, 1992). The use of both weirs and nets by Lake Babine Nation prior to European arrival is noted by Fiske and Patrick (2000).

Another possible explanation for the pattern within the assemblage is that specific salmon were harvested to meet specific needs and preferences. Kantakis (2017) notes that based on Lake Babine Nation oral histories, sockeye were a preferred species of salmon because of their taste and fat content. As previously discussed, ideal salmon must have low enough fat to prevent rancidity during preservation, without sacrificing taste (Romanoff, 1992). Likely by the time the sockeye had reached Smokehouse Island, they were ideal for smoking. Because at maturity male sockeye are larger than female, (Burgner, 1991), it would follow that male sockeye were preferred for harvest due to the higher yield in one salmon, requiring less energy expenditure to reach harvest goals.

Salmon eggs were likely consumed by the ancestral Lake Babine Nation, as with many other communities living in close proximity to salmon runs. According to oral histories from the Stal'àtl'imx community on the Fraser River, eggs collected from the salmon harvest were preserved for the winter by placing the eggs in a birch bark basket and burying them several feet underground (Kennedy & Bouchard, 1992). A well-preserved birch bark container was identified within the assemblage on Smokehouse Island (Rahemtulla, 2019), and while many other uses for these containers exist, this may indicate that egg preservation could have been occurring on the island. Sockeye, however, may only have 2,000 – 5,000 eggs per female, depending on the size of the fish (Burgner, 1991). Chinook, on the other hand, may have from 2,000 eggs to over 17,000 eggs per female (Healey, 1991). Female Chinook may have been a preferred species to harvest high amounts of salmon eggs across less salmon.

With reference to the previous sections, 5.2 and 5.3, Chinook salmon are more prevalent in the surrounding excavation units on the island, which are also female-dominated, whereas the central unit is male-dominated with Chinook only making up a small portion of the species composition. This could suggest some difference in site usage between the central area of the island, and the surrounding area. One explanation

could be that salmon were prepared for smoking centrally, and salmon eggs were harvested in the surrounding area.

5.5. Integrating Modern and Ancient Findings

The Babine River Count fence, located downstream of Smokehouse Island, has publicly available current and archived count data for salmon passing through the fence from 1990 to present (Department of Fisheries and Oceans, 2024). This data not only details the salmon present in the region modernly, but also in what quantities. Based on averages of the data from 1990-2023 (Table 5.3) sockeye is the most dominant species in the region, followed be pink, then coho. Chinook are the least abundant in the region.

Table 5.3 Average modern count data for salmon species at the Babine River Count Fence.

Species	Annual Modern Average (1990-2023)
Sockeye	1,405,352
Pink	116,460
Coho	14,376
Chinook	2,594

Note: Data obtained from Department of Fisheries and Oceans (2024).

While historical and modern salmon populations have been noted to have been affected by overfishing, in addition to climate change and landslides (Kaeriyama, 2022), a comparison between ancient and modern populations may be useful in understanding changes in populations and understanding possible population compositions. Compared to the ancient assemblage at Smokehouse Island (Table 4.2), there is a notable difference in the species composition present. While sockeye remains the predominant species across both the modern and ancient data, no pink is present in the Smokehouse Island assemblage, despite it being the second most dominant species at the modern fence. Additionally, Chinook makes up a greater portion of the Smokehouse Island assemblage than coho, whereas Chinook is the least present in the modern data.

Ratios of the various Pacific salmon species present in the region, compared to sockeye salmon can be used to understand the changes in composition compared to the dominant sockeye, and possibly inform about species preferences (Table 5.4). For example, there is a marked shift in the Chinook ratio between the modern and ancient

data. This data suggests that modernly, for every one Chinook that passes through the fence, 542 sockeye do as well. However, the ancient ratio indicates that for every 1 Chinook in the Smokehouse Island assemblage, there are only 7 sockeye. Should the modern population composition be somewhat representative of the ancient population, this data could then suggest that Chinook were a highly sought after species.

Table 5.4 Modern and ancient ratios of various species of Pacific salmon to sockeye.

Species	Ratio of Species:Sockeye	
	Modern (~)	Ancient (~)
Pink:Sockeye	1:12	0
Coho:Sockeye	1:98	1:23
Chinook:Sockeye	1:542	1:7

Note: Data obtained from Department of Fisheries and Oceans (2024).

The modern data can also be used to visualize the abundance of the salmon return at particular times throughout the spawning season. Figure 5.3 depicts the average salmon return over time at the Babine River count fence from 1990-2018. Sockeye can be seen to be the most dominant species followed by pink, both peaking in abundance around the same time in August. Applying this data to the assemblage on Smokehouse Island, it is understandable that no pink salmon are present on the island, as the larger, fattier sockeye salmon were present in much more abundant quantities. This could also suggest that throughout the occupation of Smokehouse Island, sockeye was consistently abundant, with no need for the harvest of pink salmon to make up for the lack of sockeye. Chinook and coho are both seen in much smaller quantities, with both species appearing to peak in abundance during Autumn. Because of the presence of sockeye, Chinook, and coho on Smokehouse Island, occupation of the island was likely occurring during a period when all of these species were present. Based on the modern return data, this may have been from August to September, or late-Summer early-Fall.

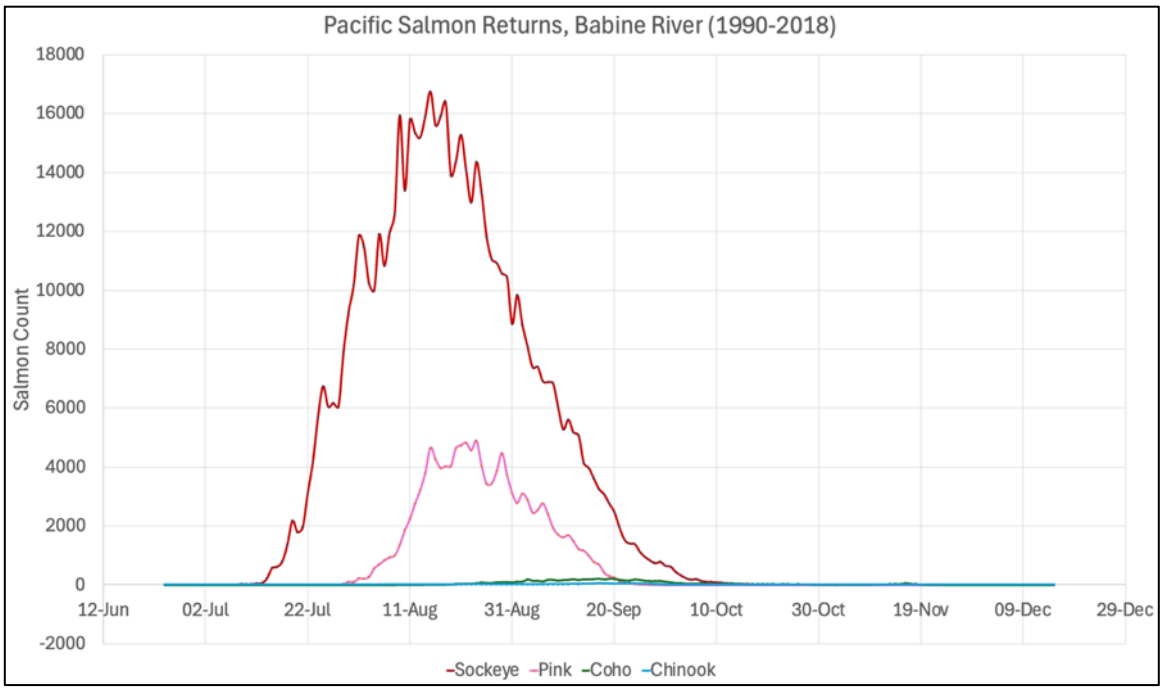

Figure 5.3 Average modern return data for Pacific salmon species at the Babine River Count Fence.

Note: Data obtained from Department of Fisheries and Oceans (2024).

As mentioned above the modern salmon data may not necessarily be reflective of the ancient population. In modern and historical periods, populations of salmon have been subject to overfishing, climate change, and obstructions such as landslides (Kaeriyama, 2022). The continued presence of sockeye as the dominant species in both the modern and the ancient data could indicate that the general population composition has remained similar, however it is also possible that some species, such as Chinook, were more dominant in the ancient past. It is of interest that only one sockeye haplotype was found in the modern data, compared to 5 found in the ancient data. While this could be suggestive of some factor causing a bottleneck in modern populations, more modern samples are required throughout the spawning season to understand modern sockeye diversity, especially when noting that the modern samples were obtained on the same day at the same time.

5.6. Reflections of Human Activities and Interactions

This thesis allows for the study of human activities, interactions with the environment, and preference through the analysis of salmon remains as a proxy. The data presented here is not reflective of the relative abundancies suspected in the region 1,000 years

ago, which in turn suggests selection by people. Selection can also be thought of as choice. This thesis allows us to begin to understand what choices ancestral Lake Babine Nation people were making on Smokehouse Island. This also allows reasons behind certain choices to be investigated.

The data presented here reveals that a high proportion of sockeye and Chinook were captured and processed, while the capture of pink salmon on the island was avoided. Earlier it was proposed that male sockeye may have been preferred for smoking, while female Chinook may have been preferred for egg harvest. It was also suggested that based on the differing sex distributions in the central area of the island compared to the surrounding area, male and female salmon may have been receiving different treatments, possibly egg harvest and smoking occurring in different locations. These data patterns can demonstrate the activities of people involved in the capture of salmon and may even indicate areas that were preferred by people for certain processes.

Further, the absence of pink salmon is a strong indicator of preference of the ancestral LBN people within the data. The complete absence of the species may suggest that salmon were not simply captured and processed, but specific types of salmon were chosen for harvest because of certain attributes that the ancestral LBN people preferred. The lack of preference shown towards pink salmon is likely due to the small amount of eggs and meat associated with the fish, in addition to the undesirable texture and flavour of the meat. If pink were captured, perhaps in a trap or a basket, there may have been a deliberate decision made to toss the fish back into the water, as there would have been a deliberate decision to keep a certain sockeye or Chinook. This then also reflects interactions between people and salmon within the archaeological record.

Additionally, prior to the inclusion of salmon investigation, choices were also reflected in the area chosen for fishing and processing to occur. There were also decisions made to place weirs in the water and capture fish via this method. The practice of salmon harvest is deeply reflective of human behaviour, choice, and interactions with the environment. Choices were made at almost every step throughout the process, of which many steps and decisions were then preserved.

Through excavations of a house pit at Nass Glee (GiSq-4), a Babine village site, Hackett (2017) found that the Babine people were a complex hunter-gather society with evidence of social complexity that predated the arrival of Europeans to the region. More specifically, Hackett (2017) found that the society was socially stratified and specialized in fishery, with radiocarbon dates indicating this to be occurring at least 1,300 years BP. High value coastal items were found in the house pit, such as an urchin spine and oyster shells, which Hackett (2017) suggested would have belonged to high-status individuals. Further on this, Babine oral histories indicated that salmon surpluses occurring at these village sites made it possible to facilitate trade for exotic goods.

The assemblage at Smokehouse Island may provide further evidence for this surplus of salmon among the Babine community, existing at least 1,000 years BP and prior to the arrival of Europeans to the region. Additionally, the sheer volume of salmon that would have been processed at Smokehouse Island based on the abundance of vertebrae present, along with the operation of weirs, may also be suggestive of social complexity and organization at the site and among the Babine community.

More detailed studies of social complexity among the Lake Babine Nation may be possible should sites that span alternative periods of occupation be identified. The salmon assemblage at these sites could be investigated using aDNA analysis to determine sex and species patterns indicative of selection. Identification of dramatic shifts in resource management strategies at a particular site may be examined in the context of social complexity and may reveal possible changes in social organization or differential access to resources.

5.7. Implications for Conservation

Salmon remains to play a vital role in the lives of many Indigenous peoples today. Today, stakeholders in the fishery occurring along the Skeena watershed and on the Babine salmon stocks include Indigenous fisheries, Canadian and US commercial fisheries, as well as recreational fisheries (Barouillet et al., 2024). This is despite a decline in the production of fisheries, occurring since the early 2000s (Barouillet et al., 2024). While these declines are likely due to a multitude of cumulative factors, such as warming temperatures leading to changes in algal production (Barouillet et al., 2024), as

well as historical and modern over-exploitation (Atlas et al., 2021), it is clear that conservation efforts are necessary.

Salmon fishery management in British Columbia strive to conserve the different species of salmon returning to the region (Beacham et al., 2014). These conservation efforts may be at a short coming due to a lack of historical data, which is often necessary to understand abundance baselines (Price et al., 2019). A misunderstanding of these baselines can lead to inaccurate risk characterization, or conservation efforts applied in the wrong places (Price et al., 2019). In conducting this aDNA research, we are able to accurately identify what species were present over 1000 years ago, prior to over-exploitations and extreme changes in climate, as well as the genetic diversity of those species. This allows us to begin to generate a deep historical perspective (Price et al., 2019), which can be applied to conservation efforts in the region, thereby providing the critical missing information (Price et al., 2019). Additionally, in generating an understanding of the traditional Indigenous fishery practices that were used for millennia, allowing for the capture of an abundance of salmon while still upholding the integrity of the regional salmon runs, this knowledge can be applied and integrated to modern fishery and encourage the revitalization of Indigenous fishery management (Atlas et al., 2021).

Based on the data uncovered in this thesis, it can be known that sockeye were present in high abundances and were extremely diverse at least 1,000 years ago. As the investigated modern samples from the Babine River Count Fence all aligned within the same haplotype, this could suggest that there has been a decrease in the genetic diversity of sockeye salmon seen at Babine Lake. While this conclusion requires more modern data to substantiate, a genetic bottle neck could be affecting this population of sockeye, possibly due to modern overfishing and climate change. An additional possible explanation could be the enhancement of certain stocks of salmon, leading to an uptick in certain haplotypes. A reduction in the genetic variation within a population can make that population more susceptible to factors such as disease and environment changes, as there is a decreased possibility of adaptation within a smaller gene pool. Because a possible bottleneck has been identified, this research may encourage conservationists to identify possible factors affecting these populations, and work to facilitate an environment that will maximize biodiversity and genetic diversity to create a sustainable population of salmon.

The assemblage at Smokehouse Island is composed of around 1/8 Chinook salmon. While one presented reason for this increase in the population composition is selection of Chinook by LBN, it is also possible that Chinook made up a higher percentage of the population 1,000 years ago than they do modernly. This deep-historical perspective on population composition can encourage conservationists to identify factors that may lead to the decimation of Chinook populations, and create habitats and environments with the best possibility of supporting the survival of Chinook.

A final implication of conservation could be the fact that no pink salmon are present on Smokehouse Island, despite their high abundance in the modern data. The explanation posed above is that the harvest of pink salmon was not undertaken due to preference of other species. Another possible explanation is that over 1,000 years, pink salmon have increased in abundance, and are currently out-competing other salmonid species, such as sockeye and Chinook, which could be an explanation for the lack of diversity seen in sockeye, and the lack of abundance seen in Chinook. While this implication requires further research, it is possible that the success of pink salmon should be monitored.

Chapter 6. Conclusions

The aDNA techniques applied in this paper have developed a more thorough understanding of the traditional fishery practices employed by Lake Babine Nation at least 1000 years before present.

6.1. Main Findings and New Insights

The main findings outlined in this paper are:

- Sex-distributions varied based on central or surrounding locations on the Island. This may suggest different site usage and preferences on the basis of sex.
- Sockeye was the dominant species identified on the island, followed by Chinook, then coho. Chinook made up around 1/8 of the salmon on the island, a marked increase from the population composition of the modern data. This suggests Chinook were likely highly sought after.
- 5 ancient sockeye haplotypes were identified in the Smokehouse Island assemblage. This suggests that sockeye in the region were highly diverse ~1,000 years ago. Modern data may suggest a decrease in diversity over time.

In determining the sex and species identification of salmon remains recovered from Smokehouse Island, on the asserted traditional territory of Lake Babine Nation, it was revealed that sockeye was the most predominant salmon species seen in the data, with Chinook and coho salmon also represented in small numbers. It was apparent that the resource management strategy employed by LBN involved some species and sex selectivity, demonstrated through differing sex-distributions in certain areas of the site, an absence of pink salmon, and an abundance of Chinook, compared to the modern data.

Interestingly, the fact that the salmon sex ratio on the island varied by location could suggest that different areas of the island were used for different activities, such as egg harvest or smoking. The fishery and occupation of Smokehouse Island likely occurred into the Summer and early Fall seasons, coinciding with the salmon runs of those present in the assemblage. Processing and preservation of an abundance of salmon

likely took place on the island in a short period of time, allowing salmon to remain a diet staple throughout the winter season.

In total, 5 ancient sockeye haplotypes were identified within the Smokehouse Island assemblage, which indicates a high degree of diversity among ancient sockeye. The 7 analyzed modern samples were identified to all fall within one of the five ancient haplotypes. This may suggest a decrease in diversity over time, however additional modern samples across a greater timespan are required to effectively sample from multiple stocks and interpret diversity. The data generated here, regarding genetic diversity and species abundancies, may inform conservation efforts through providing deep-historical baselines allowing efforts to be applied in the right places.

The results from this project have further developed understanding of fishery practices in the understudied region of Northcentral British Columbia. It is also our hope that the results from this project will benefit the Lake Babine Nation, with the potential of influencing fishery practices in the future.

6.2. Areas for Future Research

This thesis has identified possible changes in diversity from ancient and modern populations within the Babine region, with initial results suggesting a decrease in sockeye diversity over time. To form conclusions on this, further modern data is required. As different stocks of salmon return to spawn at varying times, with three distinct and overlapping modern runs returning early, mid-way, and late in the season at Babine Lake (Cox-Rogers & Spilsted, 2012), collecting modern specimens over the entire season would allow for a greater snapshot of diversity to be obtained. To add an additional layer to diversity change overtime, a historical element would be beneficial in spanning the middle ground between the ancient and modern samples. Currently, historical samples exist in the form of previously extracted DNA samples, obtained from scales collected yearly throughout the historic period by the DFO from regions along the Skeena watershed. With the expansion of the modern samples and inclusion of the historical context, it may be possible to achieve a greater understanding of when declines in sockeye occurred and to what magnitude. To understand general trends in diversity, first exploring trends using a low-resolution snapshot method, such as viewing genetic differences via D-Loop fragments on the mitochondrial genome, should be

sufficient, as was done in this thesis. Should unexpected trends be uncovered, or further information be sought, it may then be beneficial to conduct additional studies with a higher resolution, such as next-generation sequencing. Further information regarding changes in genetic diversity over time in the Babine region may be useful to conservationists through providing ancient and historical baselines to understand where efforts should be directed.

Another interesting avenue to explore would be the sex and species composition of nearby LBN village sites to understand what fish were being consumed rather than processed. This may be a difficult topic to explore as noted previously, the DNA and organic preservation in dry sites within the northcentral region is much lower than was seen at Smokehouse Island. Additionally, based on the assemblage seen on Smokehouse Island, much of the skeletal elements were removed from the salmon during processing. Because of this it may be difficult to accurately assess the salmon assemblage at the village sites, however, should a well-preserved faunal assemblage be identified at these sites, it may hold information about non-salmon fish species that could inform additional resource management strategies and preferences employed by LBN.

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