

**Ancient DNA Analysis of Middle and Late Period
Archaeological Fish Remains from
Kamloops, British Columbia**

by

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Abstract

In this study, ancient DNA (aDNA) analysis was used to assign species identifications to a sample of Middle (7,000 to 4,500 years BP) and Late (4,500 to 200 years BP) Period fish remains from EeRb-144, a large campsite located in the Interior Plateau region of south-central British Columbia, Canada. The results of this analysis indicate that largescale sucker (*Catostomus macrocheilus*) (NISP=12) and northern pikeminnow (*Ptychocheilus oregonensis*) (NISP=8) are the most abundant species in the assemblage of Late Period fish remains from EeRb-144. This suggests these two taxa were the focus of the Late Period fishery at EeRb-144. Smaller quantities of peamouth chub (*Mylocheilus caurinus*) (NISP=3), longnose sucker (*Catostomus catostomus*) (NISP=1), and Chinook salmon (*Oncorhynchus tshawytscha*) (NISP=1) were also identified in the assemblage. Ecological data concerning the seasonal availability of these taxa and limited ethnographic accounts suggest EeRb-144's Late Period fishery likely occurred during the spring and summer. The Middle Period fishery at the site also harvested largescale sucker (NISP=2), peamouth chub (NISP=1), and longnose sucker (NISP=1). These findings indicate locally abundant resident fish species were a potentially significant component of EeRb-144's pre-contact fisheries, corroborating and refining the results of morphological faunal analyses from the area. In addition, the identification of largescale sucker, peamouth chub, and longnose sucker in both assemblages suggests there was some long-term continuity in fishing practices at the site.

This study demonstrates the feasibility of using ancient DNA analysis to identify fish remains from a variety of taxa to the species-level even when they lack taxonomically informative morphological features. The results also highlight that in order to improve aDNA analysis' ability to discriminate between fish species there needs to be continued research into identifying useful DNA markers for species identification.

Keywords: ancient DNA; fisheries; Interior Plateau; species identification; universal primers; zooarchaeology

*To my father, John W. Royle, who introduced
me to history and taught me that there is
always more to discover.*

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List of Acronyms

aDNA	Ancient Deoxyribonucleic Acid (DNA)
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data System
BP	Before Present (1950 CE)
bp	Base Pair
BSA	Bovine Serum Albumin
ca.	Circa
COI	Cytochrome c Oxidase I
ddH ₂ O	Double-Distilled Water
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylenediaminetetraacetic Acid
jPCR	Java Web Tools for Polymerase Chain Reaction, <i>In Silico</i> Polymerase Chain Reaction, Oligonucleotide Assembly and Analysis
KCl	Potassium Chloride
HCl	Hydrochloric Acid
m asl	Meters Above Sea Level
MgCl ₂	Magnesium Chloride
NaOH	Sodium Hydroxide
NMWL	Nominal Molecular Weight Limit
PCR	Polymerase Chain Reaction
rpm	Rotations Per Minute
SDS	Sodium Dodecyl Sulphate
SNP	Single Nucleotide Polymorphism
Tris-HCL	Tris(hydroxymethyl)aminomethane Hydrochloride
tRNA	Transfer Ribonucleic Acid
UV	Ultraviolet

A Note on the Names and Taxonomy of Fish

Throughout this thesis, fish species are referred to by common names followed by their scientific names in brackets. For the most part, these common and scientific names follow the nomenclature for North American fish species recommended by the American Fisheries Society in the 7th edition of the *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* (Page et al. 2013). In cases where non-North American species of fish are discussed, the common and scientific names recommended by FishBase (Froese and Pauly 2011) are used. Common and scientific names for families and information concerning the members of these families are also derived from FishBase. Due to this use of standardized names, some of the common names for fish species used in the archaeological literature concerning the Interior Plateau are not used (e.g., Use of northern squawfish as the common name for *Ptychocheilus oregonensis*). Although these standardized names are used whenever possible, non-standard nomenclature (e.g., trout) is occasionally used, especially when discussing previous studies that used such nomenclature in lieu of standardized terms. Finally, this use of standardized names is not meant to dismiss the value of local folk and indigenous taxonomies, which provide an invaluable source of traditional ecological knowledge. Instead, these standardized names are used in order to facilitate the communication of the results of this research across disciplinary and regional boundaries.

Chapter 1.

Introduction

In many regions, fish remains are routinely recovered from archaeological sites (see Colley 1990 and references therein). These remains represent a rich dataset that can be used to infer the dynamics of past fisheries. At the most basic level, the species identification of archaeological fish remains can be used to document the focus of past fisheries and the range of taxa they harvested (Wake 2004:178). In addition to this basic catch data, past fisheries' seasonality, catchment area, and harvesting techniques can be inferred from the species composition of assemblages of archaeological fish remains (Colley 1990; Cooke and Jimenez 2004; Howarth-Needs and Thomas 1998; Lubinski and Partlow 2012; Wake 2004:178). Moreover, the study of archaeological fish remains can potentially shed light on the historical ecology of fish species. Specifically, the identification of remains from different spatial and temporal contexts can provide information about the historical distribution, abundance, and variability of fish species (McKechnie et al. 2014). Such information concerning the historical ecology of fish species can help establish preindustrial ecological baselines for fish that can be used to inform modern fish conservation efforts (McKechnie et al. 2014).

To obtain the species identifications needed to address these and other questions, archaeologists have historically used morphological analysis to assign species identifications to fish remains. However, it is sometimes difficult to assign species-level identifications to fish remains using this morphology-based approach for a variety of reasons (Wheeler 1978). Since the morphology of some skeletal elements does not vary between related fish species, it is not possible to assign species identifications to these elements through traditional morphological analysis (Wheeler 1978:70-71). Even when *interspecific* morphological differences exist, *intraspecific* variation can obscure the magnitude of these differences, making delineating species

through skeletal morphology problematic (e.g., Gobalet et al. 2004:807). The degradation and fragmentation of fish remains further complicates species-identification as it can result in the loss of taxonomically informative morphological features (Colley 1990:211, 215). In addition to these factors intrinsic to fish remains that confound species identification, researchers' ability to identify fish remains can be also hindered by factors extrinsic to the remains. These factors include the existence of undocumented species, time limitations, a lack of comprehensive comparative collections, the amount of fish biodiversity in the vicinity of their collection location, and individual researchers' unfamiliarity with fish biogeography and anatomy (Colley 1990; Cooke and Jimenez 2004:26; Gobalet 2001:384-385; Wake 2004; Wheeler 1978:69-70).

In order to overcome some of these barriers to morphology-based species identification, researchers are increasingly using ancient DNA (aDNA) analysis to identify fish remains (e.g., Cannon and Yang 2006; Cannon et al. 2011; Ewonus et al. 2011; Grier et al. 2013; Moss et al. 2014; Nicholls et al. 2003; Rodrigues et al. 2014; Royle et al. 2013a, 2013b; Speller et al. 2005; Yang et al. 2004). Since genetic variation exists between fish species, aDNA analysis, like morphological analysis, can be used to identify fish remains to the species-level provided DNA is preserved in the remains (Nicholls et al. 2003; Yang et al. 2004). This interspecific genetic variation that enables the DNA-based species identification of fish remains exists regardless of their gross morphology. As a result, aDNA analysis, unlike morphological analysis, can be used to identify heavily fragmented fish remains and elements lacking species-specific morphological traits (e.g., Nicholls et al. 2003; Yang et al. 2004). Consequently, aDNA analysis can be used to inform the results of traditional zooarchaeological analyses of fish remains by enabling the identification of remains difficult to morphologically identify. By providing an alternative means of species identification, aDNA analysis can also be used to test the reliability of these identifications and techniques used to assign morphological identifications (e.g., Moss et al. 2014; Pagès et al. 2008).

1.1. Research Objectives

In this study, this DNA-based approach to species identification was used to identify a sample of fish remains from the Interior Plateau region of south-central British

Columbia. This sample consisted of Middle (7,000 to 4,500 years BP) and Late Period (4,500 to 200 years BP) fish remains from EeRb-144, a large multicomponent campsite located near Kamloops (Nicholas et al. 2012; Nicholas and Tyron 1999). To facilitate the identification of these remains, this study also sought to develop new universal primers useful for the species identification for archaeological fish remains.

Using the species identifications assigned to this sample of fish remains from EeRb-144, this study sought to address six questions concerning the site's Middle and Late Period fisheries:

1. What species of fish were harvested by the Middle and Late Period fisheries?
2. What was the taxonomic focus of these fisheries?
3. During what season did these fisheries likely occur?
4. How did the diversity, focus, and timing of EeRb-144's pre-contact fishery change during the Middle to Late Period transition?
5. How were the Middle and Late Period fisheries similar?

In addition to these site specific research questions, this study also sought to obtain a better understanding of Late Holocene fishing practices in the Kamloops area. To accomplish this goal, the sample of Late Period fish remains from EeRb-144 was compared to an assemblage of 19th century fish remains from the nearby Thompson River Post site (EeRc-22) (Carlson 2006) that were identified through morphological analysis.

1.2. Organization of Thesis

The remainder of this thesis is organized into five chapters. Chapter 2 reviews the culture and environmental history of the Interior Plateau. In this chapter, special attention is paid to reviewing changes in fish use and fishing technologies in the region, and exploring how environmental changes possibly affected the region's fish populations. This review is followed by a discussion of the archaeology of EeRb-144 and the evidence for fishing at the site.

Chapter 3 reviews the polymerase chain reaction and discusses the design and evaluation of new universal primers for the species-level identification of archaeological fish remains.

Chapter 4 begins by outlining the excavation and recovery techniques used at EeRb-144 and the zooarchaeological analysis of fish remains from the site. The remainder of this chapter details the protocols and contamination controls employed during the aDNA analysis of fish remains from EeRb-144. The strategy used to select the sample of analyzed fish remains and assess the representativeness of the identified remains is also discussed.

Chapter 5 presents the results of the aDNA analysis and discusses the representativeness of the samples of identified Middle and Late Period fish remains.

Chapter 6 discusses the results of this study. The chapter begins by discussing the evidence supporting the authenticity of the aDNA results and the low degree of DNA preservation observed at the site. Subsequently, the species identification obtained through aDNA analysis are used to address the research questions concerning EeRb-144's Middle and Late Period fisheries that were posed in his chapter. This is followed by a discussion of the usefulness of the universal primers developed in this study. The conclusion of the chapter, which serves as the conclusion for the study as a whole, summarizes the results of this study and highlights their significance.

Chapter 2.

The Environmental and Culture History of Fishing in the Interior Plateau

Pre-contact indigenous fishing practices in the Pacific Northwest varied considerably across time and space (e.g., Butler and Campbell 2004). This diversity reflects the fact that the dynamics of fishing and all subsistence activities are contingent on a number of local and synchronic environmental and cultural factors that can influence the nature of fish use (Cannon 1998; Moss 2012). Tastes (Cartledge 2002), beliefs (Campbell and Butler 2010), income or status level (Cartledge 2002; Marcus et al. 1999), available fishing technologies (Orchard 2011), laws (Ignace 1998:206), prevalence of fish pathologies (Palmer 2005:60), and the abundance of fish relative to non-fish species (Kuijt 1989; Moss 2012), among other factors, can influence the nature of fish use. Consequently, in order to understand the nature of pre-contact fishing in the Interior Plateau, one must also understand the environmental and cultural milieu in which this fishing occurred.

This chapter provides that context through a review of the environmental and culture history of the Interior Plateau. Special attention is paid here to how environmental change affected the region's fish communities and how people's fishing technologies, and use of fish changed over time. The chapter concludes with a discussion of the archaeology of EeRb-144, and the evidence for fishing at the site.

2.1. The Interior Plateau Culture Area

British Columbia falls within the boundaries of three culture areas: the Northwest Coast, Plateau, and Subarctic (Muckle 1998:33). The Plateau Culture Area encompasses most of the Columbia, and Fraser River watersheds located east of the

Cascade and Coast Mountains (Hunn 1997:421).¹ This culture area is frequently subdivided into the Interior and Columbia Plateau (Hunn 1997:421). The Interior Plateau, the focus of this study, encompasses a large portion of south-central British Columbia (Pokotylo and Mitchell 1998:81; Rousseau 2004:3). It is bounded on the east by the Rocky and Columbia Mountains, and the Coast Mountains in the west, and extends from approximately the Canadian-American border in the south to the bend of the Fraser River in the north (Pokotylo and Mitchell 1998:81; Rousseau 2004:3). This large area is principally drained by the Fraser and Columbia River systems, which harbor a diverse range of resident freshwater fish species and a few anadromous species (Appendix A and B) (McPhail and Carveth 1993; Pokotylo and Mitchell 1998:81). Portions of the northern Interior Plateau are also drained by the Bella Coola, Dean, Klinaklini, and Homathko Rivers, which flow to the coast rather than into the Fraser or Columbia Rivers (McPhail and Carveth 1993; Pokotylo and Mitchell 1998:81). Due to the post-glacial dispersal of fish from the Fraser River into upper reaches of these rivers, their fish communities are similar to those found in the Fraser River (McPhail and Carveth 1993).

2.2. Environmental History of the Interior Plateau

Since the end of the last glacial period, the Interior Plateau has experienced several climatic changes. These climatic changes and their effects on local vegetation, hydrological systems, and faunal, especially fish, communities are reviewed here. This is done by describing the climatic and ecosystem changes in the Interior Plateau associated with the Late Pleistocene (20,000 to 10,500 years BP), and Early (10,500 to 7,000 years BP), Middle (7,000 to 4,000 years BP), and Late Holocene (4,000 years BP to present).

¹ Although part of the Columbia River watershed, the upper Snake River region is typically classified as belonging to the Great Basin rather than Plateau Culture Area (Hunn 1997:421).

2.2.1. Late Pleistocene (ca. 20,000 to 10,500 years BP)

Glaciation

During the last glacial period, which is referred to locally as the Fraser Glaciation, virtually all of British Columbia was glaciated (Clague and James 2002:72-73). Approximately 20,000 years BP, glacial complexes in the eastern and western Canadian Cordillera expanded into low elevations in central British Columbia and coalesced to form the Cordilleran Ice Sheet (Clague 1981:10-13). Although the growth of this ice sheet was initially slow, it covered much of the Interior Plateau by 17,000 years BP and reached its maximum extent approximately 14,000 years BP (Clague and James 2002:72-76). At its maximum, the Cordilleran Ice Sheet covered most of British Columbia, southern Alaska and Yukon, portions of western Alberta, and extended westward to the fringe of the continental shelf (Clague and James 2002:75-76). Large lobes of the ice sheet also protruded southwards into Washington, Idaho, and Montana (Clague and James 2002:75-76).

In these glaciated areas, fish populations went extinct or survived by dispersing into refugia located in Beringia, and the mid-Columbia, Chehalis, upper Missouri, and upper Mississippi River systems (McPhail 2007:xli; McPhail and Lindsey 1986:616). Minor refugia also possibly existed in the Nahanni River, the eastern slopes of the Rockies, and along the Pacific Coast (Crossman and McAllister 1986:86-88; Foote et al. 1992; McPhail 2007:xlii-xliii; McPhail and Lindsey 1986:624).

Deglaciation

Following the glacial maximum, the deglaciation of the Interior Plateau appears to have occurred relatively rapidly (Clague 1981:18). Pollen records and radiocarbon dates indicate much of the Plateau became ice-free between approximately 13,000 and 11,000 years BP, and by 9,500 years BP glaciers were largely confined to alpine areas (Clague 1981:17-18; Clague and James 2002:76-77; Fulton 1971:16-17; Hebda 1982:176). However, some areas may have been ice-free prior to 13,000 years BP. Salmon fossils recovered along the shoreline of Kamloops Lake have been dated to 18,110 and 15,480 years BP, suggesting there was a glacial lake in Thompson River valley between 18,000 and 15,000 years BP (Carlson and Klein 1996). Carlson and

Klein (1996:277) suggest these fossils' small heads (<11.5 cm in length), and terrestrial carbon-13 isotope signatures indicates they were possibly kokanee (*Oncorhynchus nerka*). This indicates a population of landlocked salmon inhabited these glacial lakes in the Thompson River valley.

The rapid deglaciation of the Interior Plateau primarily occurred through stagnation and downwasting (Clague and James 2002:74; Fulton 1969:1, 1971:16). Due to downwasting's top-down nature, upland areas became ice-free first while ice persisted in valleys and some upland areas (Clague 1981:17; Fulton 1969:1, 1971:16; Johnsen and Brennand 2006). This remnant ice in valleys acted as dams that caused large glacial lakes to form in many of the region's valleys (Fulton 1969:1; Johnsen and Brennand 2004; Johnsen and Brennand 2006). One of the valleys in which a glacial lake formed was the Thompson River Valley, where there was a succession of large glacial lakes (Glacial Lakes Thompson and Deadmen) (Fulton 1969:6; Johnsen and Brennand 2004). Prior to its failure and the resultant drainage of these lakes between 10,210 and 9,740 years BP, an ice dam blocked the flow of these lakes westward into the Fraser River (Fulton 1969; Johnsen and Brennand 2004). As such, they initially drained eastward into Glacial Lake Shuswap, which drained into the Columbia River system via spillways (Fulton 1969; Johnsen and Brennand 2004).

This drainage connection between the Columbia and Thompson River systems was as an important route for the post-glacial dispersal of fish into the Interior Plateau (McPhail 2007:xlix; McPhail and Lindsey 1986:631-632). Using this connection, a variety of fish species dispersed from the mid-Columbia refugia into Thompson River, and subsequently the remainder of the Fraser River system (McPhail 2007:xlvi, xlix; McPhail and Lindsey 1986:631-632). Although this dispersal from the mid-Columbia refugium was the main source of the Fraser River system's fish taxa, a few species from the upper Missouri refugium also colonized the system (McPhail and Lindsey 1986:631-632). This dispersal was facilitated by a drainage connection between the Upper Fraser River, and Glacial Lake Peace, which had been previously colonized by fish from the Missouri refugium (McPhail and Lindsey 1986:632; McPhail 2007:l). Due to these early postglacial fish dispersals into the Fraser River, most of the Interior Plateau's native fish

taxa were likely present in the region by the end of the early Holocene. Consequently, the early inhabitants of the region possibly had access to a wide range of fish taxa.

2.2.2. Early Holocene (ca. 10,500 to 7,000 years BP)

During the early Holocene, northern North America experienced a pronounced period of warming and increased aridity, referred to as the Altithermal. In the Interior Plateau, the warmer and drier than present conditions associated with the Altithermal began around 10,500 years BP and persisted until approximately 7,000 years BP (Hebda 1982, 1995; Pellatt et al. 2000; Smith et al. 1998). This increased temperature and aridity caused forests to contract and become restricted to higher elevations and moister areas (Hebda 1982, 1995). Conversely, xeric grasslands expanded and reached their maximum extent between 10,000 and 8,000 years BP (Hebda 1982, 1995). These early Holocene open vegetation communities likely supported populations of antelope (*Antilocapra americana*), bison (*Bison occidentalis*), bighorn sheep (*Ovis canadensis*), deer (*Odocoileus* sp.), elk (*Cervus canadensis*), and moose (*Alces alces*) (Rousseau 1993:142; Stryd and Rousseau 1996:180).

Effect of the Altithermal on Regional Hydrology and Fish Populations

The climatic and vegetation changes associated with the Altithermal had a profound effect on the hydrology of the Interior Plateau. In response to the increased aridity and temperature, the water level of lakes and rivers decreased, while their temperature increased (Chatters et al. 1995; Hebda 1982, 1995; Mathewes and King 1989; Pellatt et al. 2000; Smith et al. 1998). Furthermore, the reduction in the size of forests, which help reduce soil erosion, caused the sediment load of the Columbia River system, and possibly the Fraser, to increase (Chatters 1998:43, 46-47; Chatters et al. 1995). By decreasing the amount of shade in riparian areas, this reduced forest coverage likely contributed to the increase in water temperatures (Chatters 1998:47).

These changes in the hydrology of the Interior Plateau possibly depressed the region's early Holocene salmon populations through three mechanisms. First, higher water temperatures and sediment loads increase the mortality of salmon at various life stages (Chatters 1998:46; Chatters et al. 1995:492). Second, increased sediment loads

can result in the siltation of the gravel river beds salmon use for spawning (Chatters et al. 1995), which reduces the amount of available spawning habitat. Third, the warmer than present temperatures during the Early Holocene meant the spring freshet was shorter than present, which hinders the successful outmigration of salmon smolts (Chatters 1998:46; Chatters et al. 1995:492). While this increase in the sediment load and temperature of rivers may have depressed salmon populations, it may have had the opposite effect on other taxa. Chatters (1998:47) hypothesizes populations of resident fish tolerant of these conditions, such as suckers, may have boomed.

The increased water temperature also likely altered the structure and timing of early Holocene salmon runs. Since salmon migrate upstream earlier, and spawn later with increasing temperatures, runs likely began earlier and lasted longer than present during the early Holocene (Chatters 1998:46). Extending the length of the runs would have also meant that during the early Holocene fewer individual salmon were running at any given moment than present (Chatters 1998:46).

2.2.3. Middle Holocene (ca. 7,000 to 4,000 years BP)

Towards the end of the Early Holocene, between 8,000 and 7,000 years BP, precipitation began to increase (Hebda 1982, 1995; Pellatt et al. 2000). During the Middle Holocene (ca. 7,000 to 4,000 years BP), the climate of the region continued to moisten and cool, but temperatures remained warmer than present (Hebda 1982, 1995; Pellatt et al. 2000; Smith et al. 1998). These moister conditions caused forests to increase in size and expand into new areas, including mid-elevation areas formerly occupied by grasslands (Hebda 1985, 1995; Pellatt et al. 2000). However, grasslands, which had become mesic in character, continued to cover lower elevations and were still common in some mid-elevation areas (Hebda 1982; Hebda 1995). Bighorn sheep, deer, elk, and possibly antelope (*Antilocapra americana*) were likely some of the common species in these mesic grasslands (Chatters 1998:44; Kuijt 1989; Stryd and Rousseau 1996:186).

In response to the shifting climates of the Middle Holocene, the hydrology of the Interior Plateau continued to fluctuate. With increasing precipitation, the water level of

lakes rose and new lakes formed (Mathewes and King 1989), thereby expanding fish habitats. Conversely, the flow of the Columbia River system, and potentially others, remained at low levels comparable to those that characterized the Early Holocene (Chatters 1998). The temperature of these rivers and lakes continued to be warmer than present, but cooled slightly due to the Middle Holocene reduction in air temperatures (Chatters et al. 1995; Pellatt et al. 2000; Smith et al. 1998). Moreover, this cooling trend meant the freshet gradually lengthened during the Middle Holocene, thereby increasing the opportunity for the outmigration of smolts (Chatters et al. 1995). Like water temperature, the turbidity of rivers also decreased, but remained higher than present, due to the expansion of soil-stabilizing forests (Chatters et al. 1995). This decreased turbidity resulted in the formation of more gravel beds (Chatters et al. 1995) suitable for salmon spawning. These hydrological changes meant water conditions were more favourable for salmon during the Middle Holocene than the Early Holocene, which may have caused salmon populations to grow (Chatters 1998:47). Nonetheless, archaeological data (Chatters et al. 1995; Kuijt 1989) and palaeoecological modelling (Chatters et al. 1995) indicate salmon populations remained below modern levels during the Middle Holocene.

2.2.4. Late Holocene (ca. 4,500 years BP to Present)

In the Interior Plateau, the Late Holocene is characterized by cooler and wetter conditions than during the Middle Holocene, and the establishment of modern climatic and vegetation patterns (Hebda 1982, 1995; Mathewes and King 1989; Pellatt et al. 2000; Smith et al. 1998). Between approximately 4,500 and 3,000 years BP, temperatures in the region were the coldest they had been since the early postglacial period. During this cold interval, grasslands retreated from the mid-elevations areas they formerly dominated and became confined to valley bottoms (Hebda 1982). In these mid-elevation areas, grasslands were replaced by closed forests resembling the region's modern Interior Douglas-fir forest (Hebda 1995). In addition to causing forest expansion, the increased precipitation during the Late Holocene prompted lake levels to rise to modern levels (Mathewes and King 1989).

These environmental changes possibly had a significant effect on the abundance of both ungulates and salmon in the Interior Plateau (Kuijt 1989). The contraction of grasslands during this period possibly caused populations of taxa heavily reliant on grasses or sedges for nutrition, such as deer and elk, to plummet (Chatters 1998:47; Kuijt 1989). However, this expansion and closing of forests at the expense of grasslands likely had the reverse effect on salmon. The closing and continued growth of forests further stabilized soils thereby reducing the turbidity counterproductive to salmon productivity (Chatters and Hoover 1992; Chatters et al. 1995). The cool temperatures also helped reduce the sedimentation of riverbeds by weakening floods' erosive powers by decreasing the number of flood-triggering rain-on-snow events and slowing the spring thaw (Chatters and Hoover 1992). Moreover, the cool temperatures enhanced the outmigration of smolts by prolonging the spring freshet, which was about a month longer during the early Late Holocene than present (Chatters et al. 1995). The cooler temperatures and the increased shading of rivers caused by expanding forests also reduced water temperatures to modern temperatures more conducive to salmon survival (Chatters et al. 1995; Pellatt et al. 2000; Mathewes and King 1989; Smith et al. 1998). As a result of these favourable conditions, populations of salmon possibly boomed during the early late Holocene and were likely larger than present (Chatters 1998:45-46, 48; Chatters et al. 1995). Due to this increase in salmon populations and cooler temperatures, salmon runs during the early late Holocene also likely began and ended earlier, and were thus far denser than present (Chatters 1998:46, 48).

Between 2,800 and 1,800 years BP, temperatures in the Interior Plateau rose slightly and were comparable to present-day temperatures (Chatters 1998:46, 48). In response to this warming, grasslands expanded slightly between 2,000 and 300 years BP, and reached their modern extent (Hebda 1982). This rise in temperature and grassland expansion marks the establishment of the Interior Plateau's modern climatic and vegetation patterns (Chatters 1998:46; Hebda 1982). Despite this general warming, there is no evidence that waters warmed (Chatters et al. 1995; Pellatt et al. 2000; Smith et al. 1998). However, the increased temperature did shorten the length of freshet to its current length (Chatters et al. 1995). Except for an interval of increased turbidity between 2,400 and 1,800 years BP, the sediment load of rivers also remained low (Chatters and Hoover 1992; Chatters et al. 1995). The decreased freshet length, along

with the interval of increased turbidity, may have resulted in a slight decline in salmon abundance from the peak achieved during the early late Holocene (Chatters 1998:46, 48; Chatters et al. 1995).

Following this decline, palaeoecological modelling indicates the size of salmon populations during this period were comparable to historic levels (Chatters et al. 1995). However, the results of diatomic and stable nitrogen isotope analyses of sediments from sockeye nursery lakes in Alaska and Idaho indicate that Pacific Northwest salmon population sizes continued to fluctuate (Finney et al. 2002; Gregory-Eaves et al. 2003; Selbie et al. 2007). These analyses suggest that while the size of salmon populations in the Pacific Northwest was comparable to historic levels around 2,200 years BP, populations collapsed around 2,100 years BP (Finney et al. 2002; Gregory-Eaves et al. 2003). This collapse may be related to a change in oceanic-atmospheric circulation patterns that altered marine conditions (Finney et al. 2002). However, populations began to recover around 1,300 years BP, and continued to increase until around 800 years BP when they peaked, and were much larger than present (Finney et al. 2002; Gregory-Eaves et al. 2003; Selbie et al. 2007). Following this peak, populations declined slightly, but remained larger than present and remained relatively stable until the beginning of commercial fishing in the late 19th century (Finney et al. 2002; Gregory-Eaves et al. 2003; Selbie et al. 2007). With the onset of commercial fishing, salmon populations collapsed and reached unprecedented low levels (Finney et al. 2002; Gregory-Eaves et al. 2003; Selbie et al. 2007). In the Fraser River system, this collapse was exacerbated by the 1913 and 1914 Hell's Gate landslides that blocked the upstream migration of salmon (Thompson 1945).

2.3. Culture History of the Interior Plateau

Archaeological research in the Interior Plateau began in the late 19th century. Between 1877 and 1900, individuals such as George Dawson (1892:10-12), and Harlan I. Smith (1899; 1900) recorded and excavated old habitation and burial sites in the region. However, a cultural historical sequence for the region did not emerge until the 1960s when David Sanger (1967) proposed a sequence for the Lochnore-Nesikep locality. Following the publication of this article, sequences for other localities, including

the Kamloops locality (Wilson and Carlson 1980) and Arrow Lakes-Slocan area (Turnbull 1977), were proposed. Through the synthesis of these local sequences and the analysis of new data, Rousseau and colleagues (Arcas Associates 1985; Richards and Rousseau 1987; Rousseau 2004; Stryd and Rousseau 1996) devised a single sequence for the Interior Plateau. Based on temporal changes in material culture, as well as burial, settlement and subsistence patterns, Rousseau et al. (Rousseau 2004; Richards and Rousseau 1987; Stryd and Rousseau 1996) divide the region's culture history into three periods: the Early (ca. 11,500 to 7,000 years BP), Middle (7,000 to 4,500 years BP), and Late (4,500 years to 200 years BP) periods. Although unified sequences for the entire Plateau Culture Area have been proposed (e.g., Prentiss et al. 2006), recent studies (e.g., Badenhorst 2009; Cybulski et al. 2007; Prentiss and Kuijt 2012; Sakaguchi et al. 2010) continue to use Rousseau et al.'s sequence. Due to its widespread use, this study uses the culture history sequence proposed by Rousseau and colleagues, which is summarized below.

2.3.1. Early Period (ca. 11,500 to 7,000 years BP)

The peopling of the Interior Plateau is hypothesized to have occurred soon after terrestrial ecosystems became established in the region, between approximately 11,500 and 10,000 years BP (Rousseau 2008:221-222; Stryd and Rousseau 1996:179). This marks the beginning of the Early Period, which lasts until the end of the Altithermal, approximately 7,000 years BP (Stryd and Rousseau 1996:179). Only three sites (Drynoch Slide, Gore Creek, and Landels) in the region with components securely dated to this period have been investigated (Stryd and Rousseau 1996:184-185). Consequently, little is known about the lifeways of the region's Early Period inhabitants. However, available data suggest they were highly mobile hunter-gatherers whose subsistence pattern focused on the exploitation of a range of terrestrial resources (Rousseau 1993; Stryd and Rousseau 1996:184-185). Analysis of Early Period faunal assemblages from the Drynoch Slide (Stryd and Rousseau 1996:184-185), and Landels (Rousseau 1991) sites indicate they were heavily reliant on large ungulates, specifically deer and elk. The presence of a bone fragment from a muskrat-sized rodent at Landels (Rousseau 1991) supports Rousseau's (1993) hypothesis that small game was also hunted by Early Period peoples. Although plants were also undoubtedly part of Early

Period peoples' diet, there is no evidence indicating that they were being consumed during this period (Rousseau 1993; Stryd and Rousseau 1996:184).

Archaeological data indicate Early Period peoples supplemented this mainly terrestrial diet with some fish (Rousseau 1993; Stryd and Rousseau 1996:198). During investigations of exposed Early Period deposits at the Drynoch Slide site, an unidentified fishbone, possibly salmon, was recovered (Stryd and Rousseau 1996:185). This bone's presence at Drynoch Slide and the site's location adjacent to the Thompson River suggests that Early Period peoples had a small riverine fishery (Stryd and Rousseau 1996:185). Stable carbon isotope analysis of the Gore Creek Man, a $8,250 \pm 115$ -year-old male skeleton from the South Thompson River valley (Cybulski et al. 1981), confirms fish was a part Early Period peoples' diet (Nelson and Chisholm 1983). This analysis indicated that while this individual's protein intake was mainly from of terrestrial sources, $9\% \pm 10\%$ of their protein intake was anadromous salmon (Nelson and Chisholm 1983). Despite this evidence for small-scale fishing during the Early Period, no materials from this period are clearly associated with fishing. However, Rousseau (2008:229) speculates that salmon, and presumably other fish, and terrestrial game, were processed with the large Old Cordilleran foliate bifaces found in the region.

2.3.2. Middle Period (7,000 to 4,500 years BP)

The Middle Period commences at the end of the Altithermal, approximately 7,000 years BP, and terminates with the emergence of semi-sedentism towards the end of the Lochnore Phase, around 4,500 years BP (Rousseau 2004:13; Stryd and Rousseau 1996:185). Temporally, the period can be subdivided into the Nesikep Tradition (7,000 to 4,500 BP), consisting of the Early Nesikep (7,000 to 6,000 years BP) and Lehman Phases (6,000 to 4,500 years BP), and the Lochnore Phase (5,000 to 3,500 years BP) (Rousseau 2004:3-13; Stryd and Rousseau 1996:187-197). The origin of the Lochnore

Phase and its relationship to the preceding Nesikep Tradition is heavily debated.² However, for the purpose of this study, the Nesikep Tradition and Lochnore Phase are treated as single entity due to their broadly similar subsistence and settlement patterns.

Subsistence Pattern

The lifeways of people during the Middle Period appears to have been similar to those of Early Period peoples. People during this period remained highly mobile hunter-gatherers; except for the Baker site whose cultural affinity with the Interior Plateau is debated (Appendix C), there is no evidence for the use of structures or food storage (Rousseau 2004; Stryd and Rousseau 1996). Likewise, subsistence patterns during the Middle Period remained focused on a range of terrestrial resources. While deer and elk continued to dominate people's diet, bighorn sheep, beaver (*Castor canadensis*), canids (*Canis* sp.), snowshoe hare (*Lepus americanus*), marmot (*Marmota* sp.), muskrat (*Ondatra zibethicus*), porcupine (*Erethizon dorsatum*), small rodents, turtle, and an array of birds were also hunted (Badenhorst 2009; Kuijt 1989; Rousseau 2004:6,10,14; Stryd and Rousseau 1996:187-188,191,196; I.R. Wilson Consultants 1992). In addition to hunting, the presence of eggshell in Middle Period deposits at EdRh-31 (Badenhorst 2009:31) opens up the possibility that people were also collecting bird eggs. Plants were also likely collected during the Middle Period, but as with the preceding period, direct evidence of their use is lacking (Rousseau 2004:6,10,14; Stryd and Rousseau 1996:191,196).

Although terrestrial resources continued to occupy a central place in people's diet, the importance of aquatic resource appears to have increased during the Middle Period. Despite their absence in Early Period contexts, shells from freshwater mussels, primarily western pearlshell (*Margaritifera falcata*) are commonly found in Middle Period

² The Lochnore Phase has variously been argued to have developed *in situ* from the Nesikep Tradition (Chatters and Prentiss 2005; Prentiss and Chatters 2003; Prentiss and Kuijt 2004; Prentiss and Kuijt 2012a:38-39), or represent the migration of Coast Salish groups into the Plateau (Rousseau 2004a:12; Stryd and Rousseau 1996:200). Alternatively, given their contemporaneity, similar artifacts and economies, and the co-occurrence of their diagnostics in some contexts, it has also been argued that the Lehman and Lochnore Phases might be functional variants of a single cultural entity (Prentiss and Kuijt 2012:41; I.R. Wilson Consultants 1992:187-190).

contexts indicating shellfish were now a component of people's diet (Lindsay 2003:85-92). Similarly, stable carbon isotope analysis of three Middle Period skeletons (Chisholm 1986:124; Cybulski et al. 2007) indicates anadromous salmon grew in dietary importance. According to these analyses, the proportion of people's protein intake consisting of anadromous salmon rose from the 9% seen during the Early Period to approximately 24 to 40% (Chisholm 1986:124; Cybulski et al. 2007). In addition to anadromous salmon, Middle Period fisheries also harvested non-anadromous salmonids, including trout and kokanee (*Oncorhynchus nerka*), sucker (*Catostomus* sp.), and possibly cyprinids (Cyprinidae) (I.R. Wilson Consultants 1992:92-93). The presence of Middle Period sites near mid-elevation lakes and in riverine settings indicates these fisheries centered not only on rivers but also lakes (Stryd and Rousseau 1996:191). Rousseau (2004:10) suggests these lacustrine fisheries focused on exploiting the many suckers and non-anadromous salmonids that run from lakes to riverine spawning beds during spring. However, given the lack of species or genus-level identifications available for fishbone from lacustrine Middle Period sites this suggestion is largely speculative.

Fishing Technology

Artifacts possibly associated with fishing appear in the archaeological record for the first time during the Middle Period, which reflects the growing dietary importance of fish. Notched pebbles that possibly functioned as net sinkers are commonly recovered from Middle Period contexts (Arcas Associates 1985:93; Rousseau 2004:13; Stryd and Rousseau 1996:193). If these artifacts are net sinkers, then their presence at Middle Period sites suggests drag or set nets were used to harvest fish during this period (Arcas Associates 1985:93). Splintered bone uni-points that possibly were the barb of a composite fishhook used in hook and line fishing are also found at Middle Period sites (I.R. Wilson Consultants 1992:88; Rousseau 2004:13; Stryd and Rousseau 1996: 193). Rousseau (2008:235) hypothesizes line fishing was also conducted during the Middle Period using cigar-shaped bifaces associated with the Lochnore Phase that could have functioned as fish gorges.

2.3.3. Late Period (4,500 to 200 years BP)

The final pre-contact cultural historic period in the Interior Plateau, the Late Period, begins around, 4,500 years BP, and terminates at the time of European contact, approximately 200 years BP (Richards and Rousseau 1987:21; Rousseau 2004:13). In much of the Interior Plateau, the Late Period is subdivided into three horizons: the Shuswap (3,500 to 2,400 years BP), Plateau (2,400 to 1,200 years BP), and Kamloops Horizon (1,200 to 200 years BP) (Richards and Rousseau 1987; Rousseau 2004:13).³ These three horizons, and the Lochnore Phase, constitute the Plateau Pithouse Tradition, which is ancestral to the historic Interior Salish inhabitants of the region (Rousseau 2004:13; Stryd and Rousseau 1996:198-200).

Plateau Pithouse Tradition

Settlement Pattern

With the advent of the Plateau Pithouse Tradition, settlement patterns in the Plateau shifted from the high mobility that seems to have prevailed during preceding periods to a semi-sedentary settlement pattern (Rousseau 2004:13). This semi-sedentary settlement pattern is reminiscent of the ethnohistoric settlement pattern of the Interior Salish inhabitants of the Interior Plateau (Rousseau 2004:13). During the winter, Plateau Pithouse Tradition families aggregated in pithouse villages in river valleys (Richards and Rousseau 1987:49-50; Rousseau 2004:13-21). These wintertime aggregations were sustained by salmon, terrestrial game and plants stored in storage pits inside pithouses, and later in external pits (Richards and Rousseau 1987:25,32-34,43,50; Rousseau 2004:15,17). Plant foods and salmon were also stored in birch bark containers that were often placed in storage pits (Croft and Mathewes 2014). During the

³ In some areas, the Plateau Pithouse Tradition ended prior to contact. In the northern Interior Plateau, excavations in the Eagle Lake area indicate the Plateau Pithouse Tradition occupation of the area ended around 475 years BP (Matson and Magne 2007). Subsequently, Athapaskan speakers ancestral to the Tsilhqot'in (Chilcotin) migrated into this area around 250 years BP (Matson and Magne 2007). The archaeological manifestation of this prehistoric Athapaskan occupation is the Eagle Lake Phase (300 to 100 years BP) (Matson and Magne 2007:7). Athapaskan speakers ancestral to the Nicola also migrated into the Nicola and Similkameen Valleys in the southern Plateau during the Late Period (Copp 2008). Unfortunately, previous efforts to document and date their occupation of the area using the archaeological record have not been fruitful (e.g., Copp 2008; Wyatt 1971).

warm months, Plateau Pithouse Tradition village groups dispersed into smaller family groups. These smaller groups subsequently travelled to their hunting and plant collection grounds in mid- and high-elevation areas in order to collect resources (Richards and Rousseau 1987:50; Rousseau 2004:15,17,19-20). There they established temporary base camps and facilities, such as plant drying hearths and root roasting pits, near resource collection grounds to process resources (Pokotylo and Froese 1983; Rousseau 2004:15, 17, 19-20; Wollstonecroft 2002)

Subsistence Pattern

This shift to a semi-sedentary settlement pattern was accompanied by change in subsistence patterns. Terrestrial game, especially deer, as well as elk, dog, mountain sheep, snowshoe hare and other small and large animals, and birds remained a part of people's diet (Richards and Rousseau 1987:29,50,92-93). However, fishing intensified and salmon became the predominant source of protein in people's diet and terrestrial game became a supplementary protein source (Kuijt 1989:109-112; Richards and Rousseau 1987:50; Rousseau 2004:13). Stable carbon isotope of human skeletons indicates that in general approximately 50% to 70% of Late Period people's protein intake was salmon (Lovell et al. 1986). Ancient DNA analyses of salmon remains from the Keatley Creek site (Speller et al. 2005) and EeRb-77 (Royle et al. 2013a) indicate sockeye salmon was the most commonly consumed species of salmon. People also consumed significant amounts of Chinook salmon, and lesser amounts of chum salmon, coho salmon and rainbow trout (Speller et al. 2005; Royle et al. 2013a). In addition to salmon, trout, mountain whitefish (*Prosopium williamsoni*), northern pikeminnow (*Ptychocheilus oregonensis*), and sucker were also caught (Matson and Magne 2007:96; Richards and Rousseau 1987:29, 93; Royle et al. 2013b). Archaeobotanical remains and the appearance of root-roasting pits during this period indicates a variety of plant resources, including berries and roots, were also consumed (Lepofsky et al. 1996; Pokotylo and Froese 1983; Wollstonecroft 2002).

Geographic Variation in Salmon Consumption

Although salmon was generally the predominant staple in the diet of Plateau Pithouse Tradition groups, the degree to which different populations relied on salmon

spatially varied (Lovell et al. 1986). Stable carbon isotope analysis of 44 Late Period individuals from 21 sites by Lovell et al. (1986) indicates the amount of salmon that people consumed varied with its local availability. In general, populations located further upstream and inland where salmon is generally less available (Kew 1992:186-192) consumed less salmon than downstream populations (Lovell et al. 1986). For example, 60% of the protein intake of people residing in the lower Thompson River consisted of salmon, while it accounted for 45% of the diet of populations living in the Shuswap Basin at the system's headwaters (Lovell et al. 1986). Populations in the upper Columbia River system that were removed from salmon-bearing watercourses relied on minimal amounts of salmon ($\leq 10\%$) (Lovell et al. 1986).

Differences in the taxonomic composition of fishbone assemblages from sites located in the mid-Fraser region and the Eagle Lake area in the upper Fraser region reflect this spatial variation in salmon consumption. All of the identified fishbone from two pithouse villages in the salmon-rich Mid-Fraser region, Bridge River (Bochart 2005:73; Carlson 2010:92-94; Prentiss et al. 2009:86-112) and Keatley Creek (Lepofsky et al. 1996:43; Muir et al. 2008:44-47) sites are salmon. Conversely, at the Shields site in the Eagle Lake area where salmon is less abundant, salmon accounts for only 7.20% of the site's identified fishbone assemblage (Matson and Magne 2007:96). Instead, sucker appears to have been the primary fish taxa harvested by the Shield site's inhabitants; where it represents 92.8% of the site's identified fishbone assemblage (Matson and Magne 2007:96). This similar spatial patterning observed in the stable carbon isotope values of humans and the faunal record suggests Plateau Pithouse Tradition fisheries were somewhat opportunistic. Although Plateau Pithouse Tradition peoples favoured anadromous salmon, their fisheries only specialized in the harvest of salmon when it was locally available in large numbers. Otherwise, fisheries primarily targeted resident fish.

Use of Non-Local Salmon Fisheries

Recently, Royle et al. (2013a, 2013b) has identified salmon remains from EeRb-77, a pithouse village located on the north shore of the South Thompson River, as chum salmon. This species does not currently, and is not known to have historically, run in the South Thompson River adjacent to the site. To date, single specimens of chum salmon

from the Adams (Welch and Till 1996), and Salmon Rivers (British Columbia Ministry of the Environment 2014) are the only two chum salmon that have been identified in the Thompson River system. The vast majority of chum salmon that spawn in the Fraser River system do so downstream of Hells Gate (Figure 2.1) (McPhail 2007:264-265). Consequently, the presence of chum salmon at EeRb-77 suggests Plateau Pithouse Tradition people were possibly obtaining fish from non-local fisheries located downstream (Royle et al. 2013a, 2013b). Alternatively, the presence of chum salmon at EeRb-77 might indicate that prior to the 20th century this species had a wider distribution (Royle et al. 2013a; 2013b). Nonetheless, isotopic analysis of Late Period human remains indicates people from locales lacking salmon runs were consuming salmon, which further reflects Late Period peoples' use of non-local fisheries (Lovell et al. 1986). These results from Lovell's (1986) study suggests Late Period peoples were counteracting the abovementioned spatial variation in the abundance of salmon by gaining access to non-local salmon.

In addition to varying across space, the abundance of salmon varies across time. Salmon populations in the Interior Plateau frequently experience cyclical and non-cyclical fluctuations in their abundance (Kew 1992:182-185; Ward and Larkin 1964:1). Of particular note is the longstanding pattern of quadrennial cyclic dominance exhibited by Fraser River sockeye that sees one year of large runs followed by three years of much smaller runs (Kew 1992:182-183; Ward and Larkin 1964). These temporal fluctuations in salmon abundance can greatly reduce the size of salmon runs. For example, sockeye salmon are 1000 times more abundant in the Adams River during the peak year in their quadrennial dominance cycle than there are during non-peak years (Ward and Larkin 1964:1). Given the magnitude of these temporal fluctuations they can potentially trigger local salmon fishery collapses.

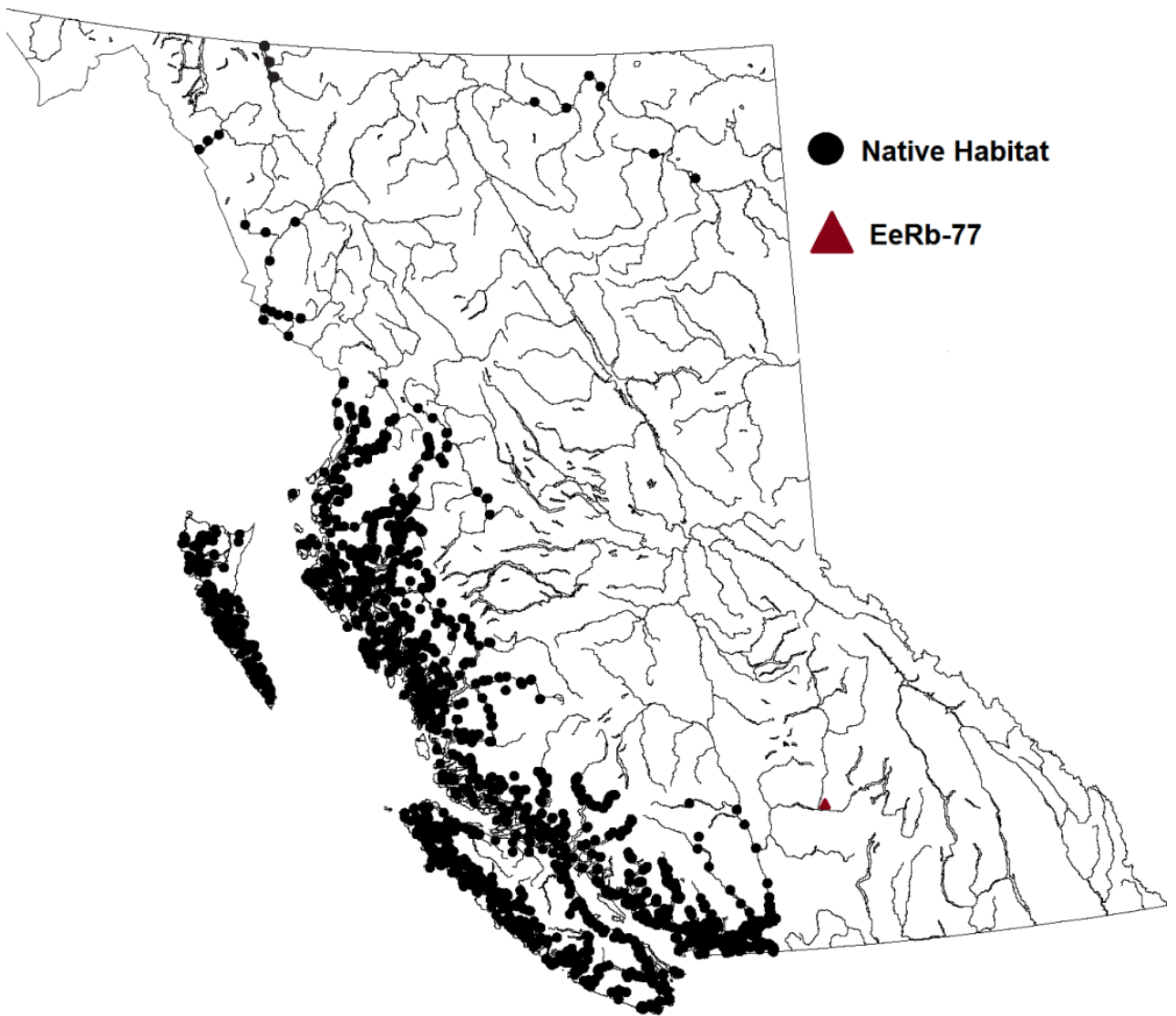


Figure 2.1. Distribution of chum salmon in British Columbia. Base map displaying the distribution data was provided by J. Donald McPhail and is used with permission.

Royle et al. (2013a; 2013b) and Rousseau (2004) hypothesize the aforementioned use of non-local salmon fisheries by Plateau Pithouse Tradition groups served to mitigate these periodic local fishery collapses. Historically, this appears to have been the case among the indigenous peoples of the Interior Plateau (Royle et al. 2013a, 2013b). For example, Teit (1900:259) notes:

A noted resort for trading and fishing was at the "Fountain," near the borders of the Shuswap [Secwepemc] and Lillooet [St'át'imc] territory,

where also the Lower Lillooet came. Here, on Fraser River, salmon were caught in abundance...When fish were scarce in Thompson River, the Spences Bridge and Nicola bands [of the Nlaka'pamux (Thompson) people], Okanagon, and eastern Shuswap came here for salmon.

As Plateau Pithouse Tradition people were heavily dependent on salmon, failure to mitigate salmon fishery collapses through this use of non-local salmon could trigger food shortages (Hayden and Ryder 1991; Royle et al. 2013a, 2013b). Rousseau (2004:18) argues, contrary to Hayden and Schulting (1997), that the Plateau Interaction Sphere was driven not by the inter-elite exchange of prestige goods but this need to obtain non-local salmon to mitigate food shortages. Instead, the exchange of prestige goods was a form of diplomacy aimed at maintaining cordial relationships between groups in order to guarantee the flow of salmon during times of need (Rousseau 2004:18).

Fishing Technology

Fishing technologies appear to have significantly diversified during the Late Period. Composite toggling harpoons, unilaterally and bilaterally barbed points used to arm leisters or fixed point harpoons, small bi-points for leisters, and dip nets all appear during this period (Richards and Rousseau 1987; Stryd 1972:23). Although data concerning the dates of pre-contact fish weirs in the Interior Plateau are scanty, weirs also appear to have come into use during the Late Period. Radiocarbon dates of stakes from a large fish weir complex (EdRa-41) located in the South Thompson River mapped by Carlson and Nicholas indicates it was in use by at least 1,560 years BP (Figure 2.2) (Nicholas 2002; Nicholas et al. forthcoming). Interestingly, this weir complex appears to have been maintained for over a millennium as the most recent date obtained from the complex is 120 ± 60 years BP (Nicholas 2002; Nicholas et al. forthcoming). A second fish weir complex, also, identified by Carlson and Nicholas just upstream of EdRa-41 consists of boulders, with no extant wooden stake remains (George Nicholas, pers. comm. 2014). Another fish weir on the South Thompson River, EdQx-1, is associated with a pithouse village (Mohs 1981:165), which suggests that it too was in use during the Late Period. Although this apparent technological diversification during the Late Period may have actually occurred, it may also be the product of taphonomic processes. As these new fishing technologies are made of perishable materials (bark, bone, wood), earlier examples of these technologies may have simply not preserved.

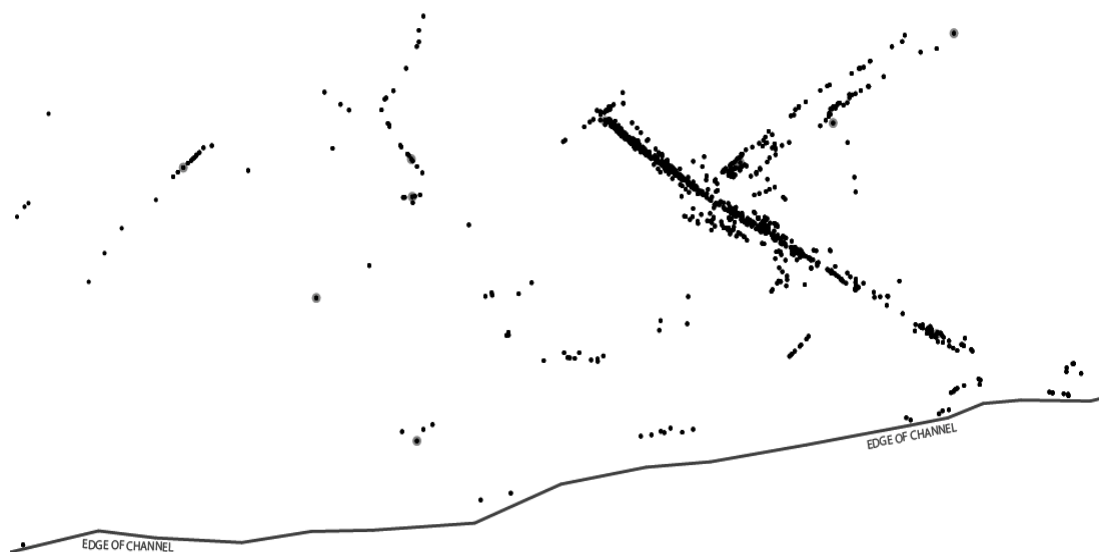


Figure 2.2. Plan view of large Late Period fish weir complex (EdRa-41) located in the South Thompson River. Each dot represents an individual wooden stake. This figure is courtesy of George Nicholas and is used with permission.

Fishing technologies that previously emerged during the Middle Period also continued to be used. Netsinkers and fish hook barbs continue to be present at sites during the Late Period indicating the use set and gill nets, and hook and line fishing persisted into this period (Richards and Rousseau 1987; Pokotylo and Mitchell 1998:87). Moreover, the aforementioned potential stone “fish gorges” identified by Rousseau (2008:235) continued to be used until 2,400 years BP. After 2,400 years BP, this tool type appears to have been infrequently used (Rousseau 2008:235).

2.4. The Archaeology of EeRb-144

EeRb-144 is a large multi-component campsite located in the traditional territory of the Secwepemec, near modern-day Kamloops. The site is situated on a low glaciolacustrine terrace approximately 1 km north of the South Thompson River (Figure 2.3). Radiocarbon dates from EeRb-144 indicates it was first occupied during the Middle Period by approximately 6,000 years BP (Nicholas et al. forthcoming). However, the recovery of a projectile point resembling an Old Cordilleran Tradition point from the eroded front slope of the site suggests its occupation stretches back to the Early Period

(Nicholas and Tyron 1999). Radiocarbon dates and the presence of diagnostic artifacts associated with each of the subdivisions of the Middle (Early Nesikep Tradition, Lehman Phase, and Lochnore Phase), and Late Periods (Shuswap, Plateau, and Kamloops Horizons) indicate the site was subsequently intermittently reoccupied throughout these periods (Table 2.2) (Nicholas and Tyron 1999; Nicholas et al. 2012). However, the occupation of EeRb-144, like the Interior Plateau as a whole (Rousseau 2004:19), appears to have been most intensive during the Plateau Horizon. Projectile points diagnostic of this horizon are the most abundant projectile points at the site, and much of the site's lithic debitage is associated with this horizon (Nicholas and Tyron 1999).

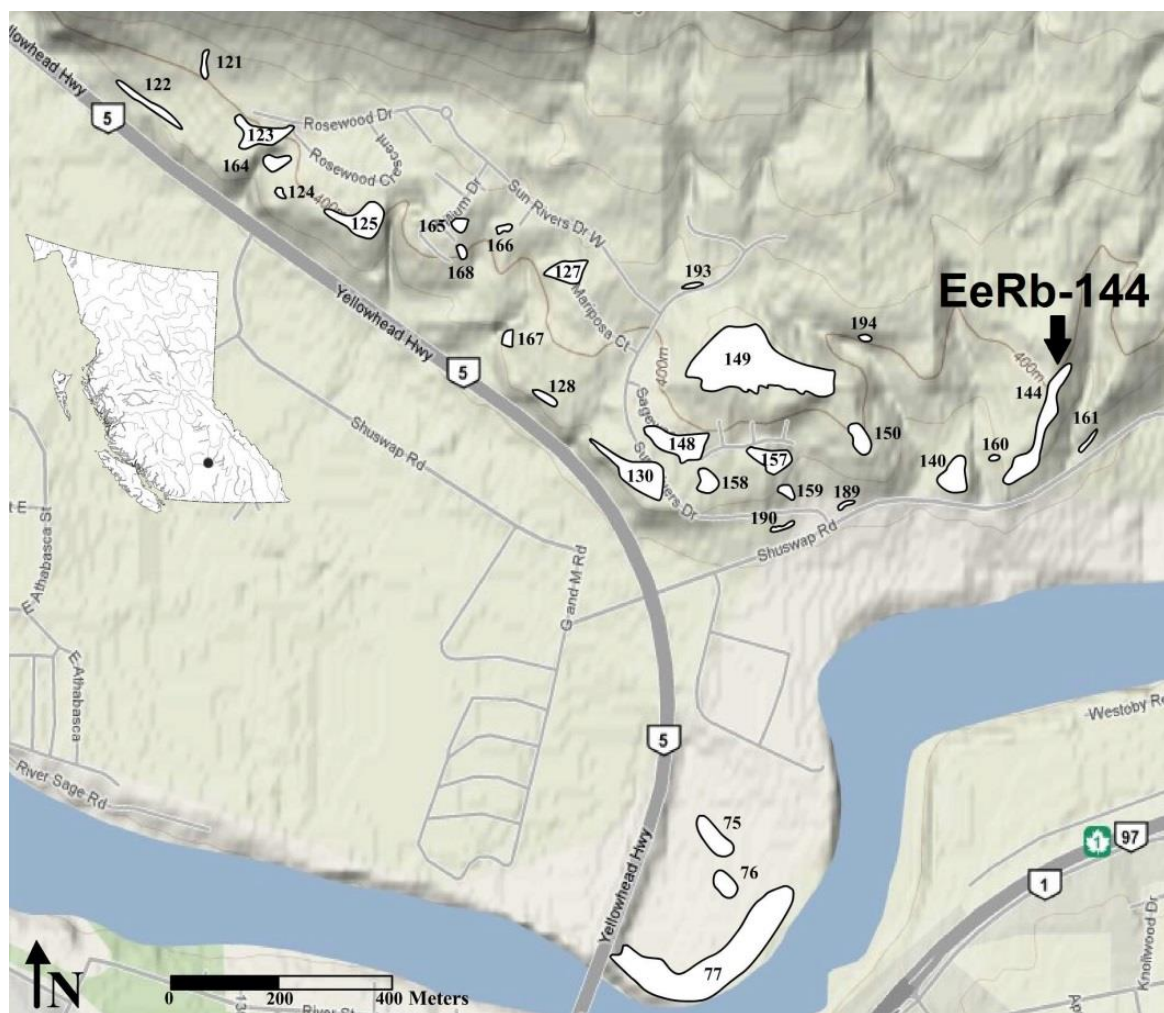


Figure 2.3. Location of EeRb-144 and other archaeological site within the Kamloops area. This figure is courtesy of George Nicholas and is used with permission.

Table 2.1. Radiocarbon dates from EeRb-144. This table is adapted from Nicholas et al. (forthcoming:Table 1).

Sample	Lab Number	Unit	Depth Below Surface (cm)	Radiocarbon Years BP	Material
1	Beta 116172	N12 E8	20 to 30	5,250 ± 50	Charcoal
2	Beta 116173 ¹	N11 E8	60 to 70	5,120 ± 70	Bird bone
3	Beta 149799	N10 E12	15 to 20	2,310 ± 60	Charcoal from hearth
4	Beta 149800	N20 E27	31 to 40	6,140 ± 50	Shell
5	149801	N12 E8 N12 E6	15 to 20	2,140 ± 60	Charcoal from hearth
6	149802	N10 E11	35 to 45	4,080 ± 80	Charcoal from hearth with microblades

¹AMS Date

Preliminary analyses of materials from EeRb-144 have provided some hints about the nature of the occupation of EeRb-144. The presence of a deciduous second molar from a 10- to 12-year-old child (Nicholas et al. forthcoming) at EeRb-144 suggests it was at least occasionally occupied by family groups rather than adult-only task-groups. As the analysis of botanical and faunal materials from the site is incomplete, the seasonality of the various occupations of EeRb-144 by these groups is not entirely clear. Nonetheless, Nicholas et al. (forthcoming) posits the relative thinness of the birch bark debitage recovered from the site sheds some light on the timing of some of these occupations. As the Secwepemec traditionally harvested thin sheets of birch bark during the late spring and early summer, the thinness of this debitage indicates it was possibly harvested during this period (Nicholas et al. forthcoming). Assuming birch bark was processed soon after it harvested, this suggests EeRb-144 was at least occasionally occupied sometime between late spring and early summer (Nicholas et al. forthcoming).

During their occupation(s) of the site, EeRb-144's inhabitants appear to have engaged in a variety of activities. These activities appear to have included processing birch bark, manufacturing and repairing lithic tools, and preparing plant and animal foods (Nicholas and Tyron 1999; Nicholas et al. 2012). Faunal remains from EeRb-144 indicates animals being processed at the site either for food or goods include fish, birds,

including magpie (*Pica Pica*) (Speller et al. 2011), and mammals, including deer, elk and beaver (Nicholas and Tyron 1999). Some of this preparation of animal foods appears to have involved cooking as burnt bone were found associated with hearths at the site (Nicholas and Tyron 1999).

2.4.1. Evidence for Fishing at EeRb-144

The presence of fishbone at EeRb-144 indicates its inhabitants fished, which is not surprising given its proximity to the South Thompson River. To date, 1,285 faunal remains from EeRb-144 have been identified as bony fish (Superclass: Osteichthyes), specifically ray-finned fish (Class: Actinopterygii) (Table 2.3) (David Maxwell, pers. comm. 2014). Due to the heavy fragmentation of these fish remains, only 117 (9.11%) of these remains have been identified to the order level or lower (Table 2.3) (David Maxwell, pers. comm. 2014). The overwhelming majority of these identified specimens are Cypriniformes (Order: Cypriniformes) (n=96), (David Maxwell pers. comm. 2014), which suggests this diverse order of freshwater species was the primary target of the fishing activities of EeRb-144 inhabitants (Table 2.3). The presence of salmonid (Family: Salmonidae) bones, including Pacific salmon (*Oncorhynchus* spp.), and a single percid (Family: Percidae) bone at EeRb-144 demonstrates these taxa were also caught (Table 2.3) (David Maxwell, pers. comm. 2014). However, this percid specimen may be a misidentification as the province's only native percid, walleye (*Sander vitreus*), is confined to northeastern British Columbia, well beyond the borders of the Plateau (Figure 2.4) (McPhail 2007:523, 530-531).

Table 2.2. Number of identified specimens (NISP) of the different fish taxa represented in the faunal assemblage from EeRb-144.

Taxon	Common Name	NISP
Cypriniformes		96
<i>Ptychocheilus oregonensis</i>	Northern Pikeminnow	29
Cyprinidae	Carps and Minnows	2
Unidentified Cypriniformes		65
Perciformes		1
Percidae	Perches	1
Salmoniformes		20
<i>Oncorhynchus</i> spp.	Pacific Salmon	2
Salmonidae	Salmon, Trout, Chars, Whitefishes and Graylings	18
Total Number of Identified Fish Remains		117
Unidentified Actinopterygii		1,168
Total Number of Fish Remains		1,285

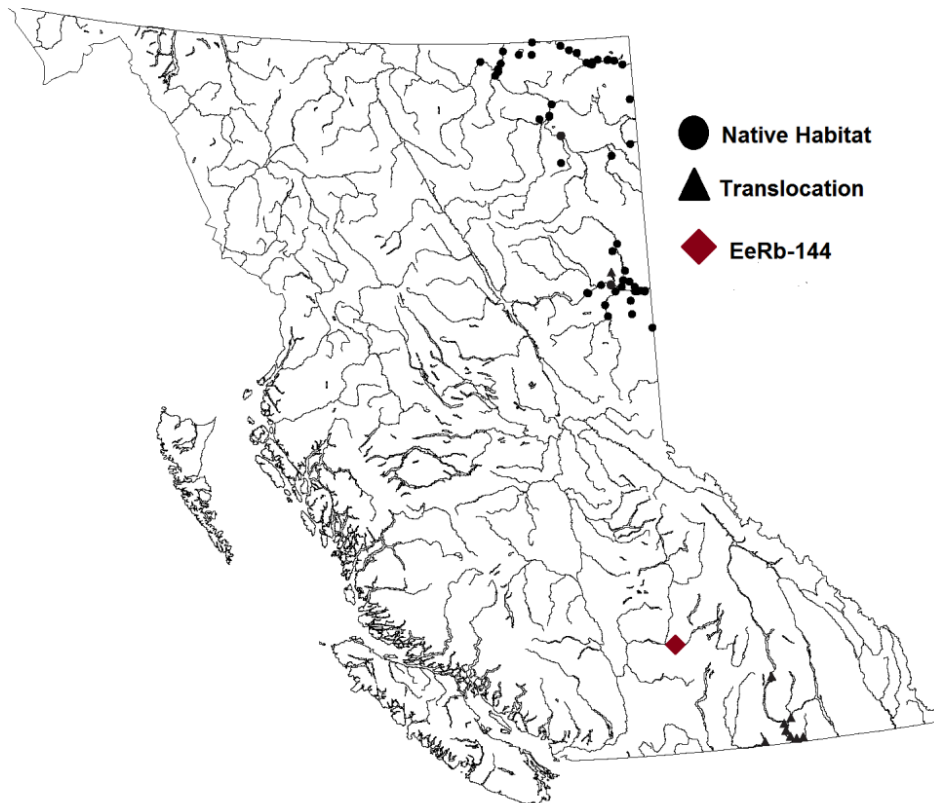


Figure 2.4. Distribution of walleye in British Columbia. Base map displaying the distribution data was provided by J. Donald McPhail and is used with permission.

Fishing Technology

Fishing-related artifacts recovered from EeRb-144 provide some insights into the methods the site's inhabitants were using to capture these fish. Middle Period components at the site have yielded Lochnore Phase notched pebble netsinkers, indicating its Middle Period inhabitants were possibly catching fish with nets (Figure 2.5) (Nicholas and Tyron 1999). Long narrow bone bi-points recovered from EeRb-144 may also be related to fishing, as they may have been used to arm leisters used to spear fish (Figure 2.6) (Nicholas and Tyron 1999).



Figure 2.5. Middle Period notched pebble netsinker from EeRb-144.



Figure 2.6. Bone bi-points from EeRb-144 and other nearby sites that were possibly used to arms fishing leisters. This figure is courtesy of George Nicholas and is used with permission.

2.5. Chapter Summary

Since the end of the last glacial period fish populations and human fishing activities in the Interior Plateau have experienced significant change. During the Early Holocene, salmon populations may have been depressed as a result of high water temperatures and riverine sediment-loads, which are deleterious to salmon. Conversely, fish species tolerant of these conditions may have blossomed. The Early Holocene (Early Period) peoples of the region relied heavily on terrestrial resources for subsistence and consumed limited amounts of fish. Subsequently, salmon populations grew in response to the Middle Holocene's more favourable hydrological conditions for salmon. Although the dietary importance of anadromous salmon and resident fish also increased in the Middle Holocene (Middle Period) people were still focused on terrestrial resources. This growing dietary importance of fish is reflected by the first appearance of fishing technologies during this period. In the Late Holocene (Late Period), people became semi-sedentary and generally relied much more heavily on salmon. In contrast with preceding periods, anadromous salmon, rather than terrestrial resources, was generally people's chief source of protein. However, the exact degree to which people relied on salmon varied and appears to have been dependent on the local abundance of salmon. This intensification of salmon fishing that occurred during the Late Period may have been triggered by the concomitant expansion of salmon populations (Fladmark 1975; Kuijt 1989) and/or the development of new fishing technologies that occurred during this period (Hayden et al. 1985:186)

Chapter 3.

Primer Design and Evaluation

Little is known about the taxonomy of most of the fish remains from EeRb-144. As discussed in Chapter 2, 90.89% of the fish remains from EeRb-144 could only be identified through morphological analysis to the class-level. Twenty-eight fish species belonging to this class are native to the Thompson River system that neighbours the site (McPhail and Carveth 1993) (Appendix A). These 28 species are members of 14 different genera and 6 different families (Froese and Pauly 2011; McPhail and Carveth 1993). Consequently, identifying the majority of the fish remains from EeRb-144 through aDNA analysis requires using primers capable of amplifying DNA from a broad range of fish species. Such primers are termed universal primers (Linacre and Tobe 2013:136).

Unfortunately, few universal primers useful for the species identification of ancient fish remains have been developed. This largely due to the lack of primer annealing sites conserved across fish species that flank short variable fragments likely to preserve in ancient remains (Jordan et al. 2010:225). In addition, the use of the universal primers that have been developed (Jordan et al. 2010) is complicated by their ability to amplify DNA from common contaminant sources (Yang et al. 2003; Leonard et al. 2007), including humans and mice (e.g., Grier et al. 2013; Kemp et al. 2014). Accordingly, this study sought to design universal primers able to amplify a DNA fragment useful for the identification of archaeological fish remains, yet preclude the amplification of contaminant human DNA. The methods and results of this investigation are presented in this chapter. In addition, an overview of the polymerase chain reaction is included in this chapter. This is included in order to provide readers with an understanding of the role of primers in the polymerase chain reaction and this technique's importance to aDNA analysis.

3.1. The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a technique widely used to amplify (copy) a specific fragment of DNA (Bartlett and Stirling 2003:5; Mullis et al 1986:263). As PCR's name suggests, the amplification of a DNA fragment through PCR is accomplished through a series of reactions. First, DNA molecules are denatured through heating (Figure 3.1) (Mullis et al. 1986:265). This denaturation and a subsequent temperature reduction allow a pair of primers (short oligonucleotides) to anneal to complementary regions located on the DNA molecules' two strands (Figure 3.1) (Mullis et al. 1986:265-267; Pääbo et al. 1989:9710). One of these primers—the forward primer—anneals to the Crick strand (3' to 5' strand) at a complementary site that flanks the 5'-end of the DNA fragment that is to be amplified (Figure 3.1) (Pääbo et al. 1989:9710). Conversely, the reverse primer binds to a complementary site on the Watson strand (5' to 3 strand) that flanks the fragment's 3'-end (Figure 3.1) (Pääbo et al. 1989:9710). Subsequently, a temperature increase prompts an enzyme called DNA polymerase to elongate the annealed primers in the 5' to 3' direction using free nucleotides (Figure 3.1) (Mullis et al. 1985:265-267). This results in the amplification of the fragment of interest (Figure 3.1) (Mullis et al. 1985:265-267). With each additional repetition (cycle) of this process, the number of copies of this fragment is exponentially increased (Figure 3.1) (Mullis et al. 1985:263). Through this exponential amplification, it is possible to generate billions of copies of a specific DNA fragment from the exceedingly small amount of DNA molecules preserved in ancient remains (Pääbo et al. 1989:9710; Pääbo et al. 2004:646). These large quantities of a specific aDNA fragment generated through PCR enables the direct sequencing of the fragment (Pääbo et al. 1989:9710). In this study, the direct sequencing of the fragment targeted by the universal primers provides the sequence data necessary to assign taxonomic identifications to fish remains.

Polymerase chain reaction - PCR

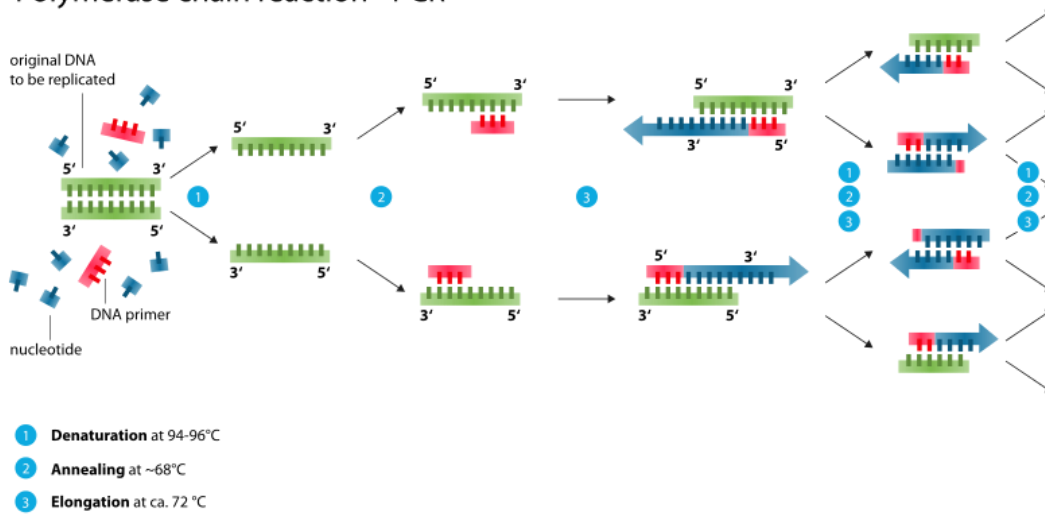


Figure 3.1. Schematic of the polymerase chain reaction (PCR) used to amplify a fragment of DNA. Each PCR cycle consists of a (1) denaturation, (2) annealing, and (3) elongation step. The progression from one step to the next is accomplished through a temperature change. This figure is courtesy of Enzoklop and is licensed under a CC BY-SA 3.0 Unported License.

3.2. DNA Marker Selection

Throughout the years, many different genetic markers have been used to identify fish specimens to the species-level (Teletchea 2009:273-274). However, during the past decade, the cytochrome *c* oxidase I (COI) DNA barcode region has increasingly become the *de facto* standard marker used for DNA-based fish species identification (Hughes and Page 2010:2094). This DNA barcode region consists of an approximately 650 bp fragment of the 5'-end of the mitochondrial COI gene. Among fish, this region typically varies less than 1% within species but generally varies more than 3% between species (Hubert et al. 2008:e2490-e2491; Ward et al. 2009:336-338). Because of this pattern of sequence variation, this region can discriminate between approximately 99% of fish species (Ward et al. 2009:338), which makes it a very useful marker for fish species identification. As a result of this usefulness, COI-based DNA barcodes have been used to assign species identifications to modern fish specimens for a variety of applications. Some of these applications include documenting incidences of mislabelled fish products

(e.g., Carvalho et al. 2011; Cawthorn et al. 2012; Filonzi et al. 2010; Hanner et al. 2011), identifying a case of puffer fish (tetrodotoxin) poisoning (e.g., Cohen et al. 2009), ascertaining the prey of piscivorous species (e.g., Côté et al. 2013; Dunn et al. 2010; Valdez-Moreno et al. 2012), monitoring invasive species (e.g., Valdez-Moreno et al. 2012), and determining the spawning time and grounds of fish species through the identification of juveniles, larva, and roe (e.g., Valdez-Moreno 2010).

Although DNA barcodes have been widely used to identify modern fish specimens, aDNA studies of archaeological fish remains have largely ignored this marker. A review of published aDNA studies identified only one study (Shirak et al. 2013) that used this marker to identify fish remains.⁴ The paucity of aDNA studies utilizing DNA barcodes for fish species identification is largely a product of the large size of standard DNA barcodes (~650 bp). Since amplifiable DNA fragments longer than 500 bp are rarely preserved in ancient remains (Pääbo et al. 2004:647), complete ~650 bp standard barcodes cannot generally be amplified from ancient remains (Hajibabaei et al. 2006; Meusnier et al. 2008). Consequently, complete barcodes cannot be readily used to identify archaeological fish remains.

However, by analyzing short fragments (100-300 bp) of the COI DNA barcode region, so-called mini-barcodes, a barcode-based approach can still be used to identify degraded remains (Hajibabaei et al. 2006; Meusnier et al. 2008). As a result of their significantly smaller size, mini-barcodes are much more likely to be preserved in archaeological fish remains than complete barcodes (Hajibabaei et al. 2006). Moreover, despite their diminutive length, mini-barcodes remain useful for species identification since they retain adequate interspecific variability to enable the genetic delineation of

⁴ Shirak et al. (2013) amplified a 140 bp fragment of the COI barcode region from three fish remains recovered from the 7th to 8th century Tantura F shipwreck located off the coast of Tel Dor, Israel (Shriak et al. 2013). Through the sequence analysis of this fragment, these remains were identified as a possibly extinct tilapiine (Tribe: Tilapiini) species or sub-species (Shirak et al. 2013). Phylogenetic analysis indicates this species or sub-species is closely related to blue (*Oreochromis aureus*) and mango (*Sarotherodon galilaeus*) tilapia (Shriak et al. 2013). Although these findings are intriguing the authors' failure to use a dedicated aDNA laboratory (Shirak et al 2013) during aDNA extraction means they may be the result of contamination.

species (Hajibabaei et al. 2006; Meusnier et al. 2008). In the case of bony fish, previous studies have demonstrated that mini-barcode can be used to discriminate salmonid (Rasmussen et al. 2009) and catfish (Order: Siluriformes) species (Bhattacharjee and Ghosh 2014), and an array of Australian fish taxa (Hajibabaei et al. 2006). Since mini-barcodes can discriminate between species of fish, the COI barcode region was selected as the target for the universal primers.

3.3. Materials and Methods

3.3.1. Primer Design

Primers targeting a 220 bp fragment of the COI gene of bony fish were designed using a sample of bony fish reference sequences collected from GenBank.⁵ This sample consisted of the complete sequences of the COI and tRNA-Tyrosine genes of 33 bony fish species from 12 families (Table 3.1). Following the assembly of this sample, the reference sequences were aligned using the Clustal W (Thompson et al. 1994) function in BioEdit⁶ (Hall 1999). Subsequently, the alignment was examined, and primers complementary to regions relatively conserved among species but flanking a mini-barcode region exhibiting interspecific variability were designed. As no region was completely conserved among all species, degenerate bases were used at variable positions. This process resulted in the creation of a single forward primer, F271 (CYAYCYTACCTGTGGCMAT), and a reverse primer, R271 (ACTATAAAGAARATYATWACRAARGCRTG).⁷ The potential amplification efficiency of these primers was evaluated by using NetPrimer⁸ and Oligoanalyzer⁹ to determine their melting temperatures and potential to form secondary structures.

⁵ <http://www.ncbi.nlm.nih.gov/genbank>

⁶ <http://www.mbio.ncsu.edu/bioedit/bioedit.html>

⁷ M, R, Y, and W are IUPAC degenerate base that indicate positions in the primers where more than one base (A,C,G,T) may be used. The following bases may be substituted for the degenerate bases included in these primers: C or T for M, A or G for R, C or T for Y, and A or T for W.

⁸ <http://www.premierbiosoft.com/netprimer>

⁹ <http://www.idtdna.com/analyzer/applications/oligoanalyzer>

Table 3.1. Sample of bony fish references sequences that were used to design primers.

Common Name	Scientific Name	Family	GenBank Accession Number
White Sturgeon	<i>Acipenser transmontanus</i>	Acipenseridae	NC004743
White Sucker	<i>Catostomus commersonii</i>	Catostomidae	NC008647
Blue Sucker	<i>Cycleptus elongatus</i>	Catostomidae	AB126082
Largemouth Bass	<i>Micropterus salmoides</i>	Centrarchidae	DQ536425
Atlantic Herring	<i>Clupea harengus</i>	Clupeidae	AP009133
Pacific Herring	<i>Clupea pallasii</i>	Clupeidae	NC009578
N/A	<i>Cottus hangiongensis</i>	Cottidae	NC014851
Grass Carp	<i>Ctenopharyngodon idella</i>	Cyprinidae	EU391390
Common Carp	<i>Cyprinus carpio</i>	Cyprinidae	AP009047
Zebrafish	<i>Danio rerio</i>	Cyprinidae	AC024175
Nazas Chub	<i>Gila conspersa</i>	Cyprinidae	NC013761
Roundtail Chub	<i>Gila robusta</i>	Cyprinidae	NC008105
Peamouth Chub	<i>Mylocheilus caurinus</i>	Cyprinidae	NC013763
Golden Shiner	<i>Notemigonus crysoleucas</i>	Cyprinidae	AB127393
Ladyfish	<i>Elops saurus</i>	Elopidae	AP004807
Alaska Pollock	<i>Gadus chalcogrammus</i>	Gadidae	NC004449
Pacific Cod	<i>Gadus macrocephalus</i>	Gadidae	DQ356937
Pacific Tomcod	<i>Microgadus proximus</i>	Gadidae	DQ356944
Burbot	<i>Lota lota</i>	Lotidae	NC04379
Starry Flounder	<i>Platichthys stellatus</i>	Pleuronectidae	EF424428
Lake Whitefish	<i>Coregonus clupeaformis</i>	Salmonidae	NC020762
European Whitefish	<i>Coregonus lavaretus</i>	Salmonidae	NC002646
Cutthroat Trout	<i>Oncorhynchus clarkii</i>	Salmonidae	NC006897
Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Salmonidae	EF455489
Chum Salmon	<i>Oncorhynchus keta</i>	Salmonidae	NC017838
Coho Salmon	<i>Oncorhynchus kisutch</i>	Salmonidae	NC009263
Rainbow Trout	<i>Oncorhynchus mykiss</i>	Salmonidae	DQ288271
Sockeye Salmon	<i>Oncorhynchus nerka</i>	Salmonidae	NC008615
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Salmonidae	NC002980
Mountain Whitefish	<i>Prospium williamsoni</i>	Salmonidae	JQ390061
Arctic Char	<i>Salvelinus alpinus</i>	Salmonidae	NC000861
Brook Trout	<i>Salvelinus fontinalis</i>	Salmonidae	AF154850
Korean Rockfish	<i>Sebastes schlegelii</i>	Sebastidae	NC005450

3.3.2. DNA Extraction, Amplification, and Sequencing

Samples of modern tissue were obtained from 11 species of fish from four families (Table 3.2). DNA was extracted from the samples using a DNeasy Blood and Tissue Kit (QIAgen, Hilden, Germany) following the manufacturer's protocols. PCR amplifications were executed using a Mastercycler Personal Thermal Cycler (Eppendorf, Hamburg, Germany) using a 25 µl reaction mixture that included 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 1.0 mg/mL BSA, 0.3 µM of forward primer F271, 0.3 µM of reverse primer R271, and 0.75 U AmpliTaq Gold (Applied Biosystems, Foster City, California). The amplification conditions consisted of an initial denaturation step at 95 °C for 12 minutes followed by 30 cycles comprised of a denaturation step at 95 °C for 30 seconds, an annealing step at 50 °C for 30 seconds, and an extension step at 72 °C for 40 seconds, and a final extension step at 72 °C for 7 minutes.

Following amplification, 5 µl of PCR product was pre-stained with SYBR Green (Invitrogen, Carlsbad, California) then electrophoresed at 135 V for 20 minutes on a 2% agarose gel, and visualized with a Dark Reader Transilluminator (Clare Chemical Research, Dolores, Colorado). PCR products were cleaned up using ExoSAP-IT (USB Corporation, Cleveland, Ohio) (Bell 2008) and directly sequenced from both directions by Eurofins Genomics (formerly Eurofins MWG Operon) (Louisville, Kentucky, formerly Huntsville, Alabama) using F271 and R271. The resulting sequences were inspected, edited, and assembled using ChromasPro.¹⁰

3.3.3. Species Identification

Edited sequences were compared to reference sequences in the Barcode of Life Data System (BOLD) Species Level Barcode Records database using the BOLD identification engine¹¹ (Ratnasingham and Hebert 2007), and cross-checked against reference sequences in GenBank via a BLAST (Altschul et al. 1990) search.¹² Previous studies of the COI region of fish have found that the amount of sequence divergence

¹⁰ <http://www.technelysium.com.au>

¹¹ http://www.boldsystems.org/index.php/IDS_OpenIdEngine

¹² <http://www.blast.ncbi.nlm.nih.gov/Blast.cgi>

between conspecific individuals generally does not exceed 1% (Hubert et al. 2008:e2490-e2491; Ward et al. 2009:336-338). Accordingly, a species identification was assigned to a sample if the similarity of its sequence to reference sequences from a single species exceeded or was equal to 99% (Ratnasingham and Hebert 2007:362). In cases where reference sequences from more than one congeneric species exceeded this threshold, the sample was assigned to this genus. If no reference sequence surpassed this threshold, the sample was assigned genus-level identification if its divergence from reference sequences from a single genus did not exceed 3% (Ratnasingham and Hebert 2007:363). No taxonomic designation was assigned to a sample if none of these conditions were met.

3.3.4. *In Silico* PCR of Human DNA

An *in silico* PCR of human DNA using the F271 and R271 primer pair was performed with jPCR¹³ (Kalendar et al. 2011) in order to assess their ability to amplify contaminant human DNA. The primer pair was loaded into jPCR and then searched against the revised Cambridge Reference Sequence for human mitochondrial DNA (NC012920) (Andrews et al. 1999) using the degenerate sequence search option.

3.4. Results

DNA was successfully amplified from all eleven fish tissue samples using the F271 and R271 primer pair. However, interspecific variation in the visibility of the amplification bands on the electrophoresis gel indicates the strength of the amplifications varied between species (Figure 3.2). Nonetheless, the sequences obtained from each sample were in general by clear. Through the analysis of these sequences, species identifications were successfully assigned to all six Pacific salmon samples and to the lone sample of lingcod (Table 3.2). Genus-level identifications were successfully assigned to the samples of Arctic char, Pacific herring, and rockfish (Table 3.2). The *in silico* PCR of human DNA using the universal primers failed to yield any PCR products.

¹³ <http://www.primerdigital.com/tools/pcr.html>

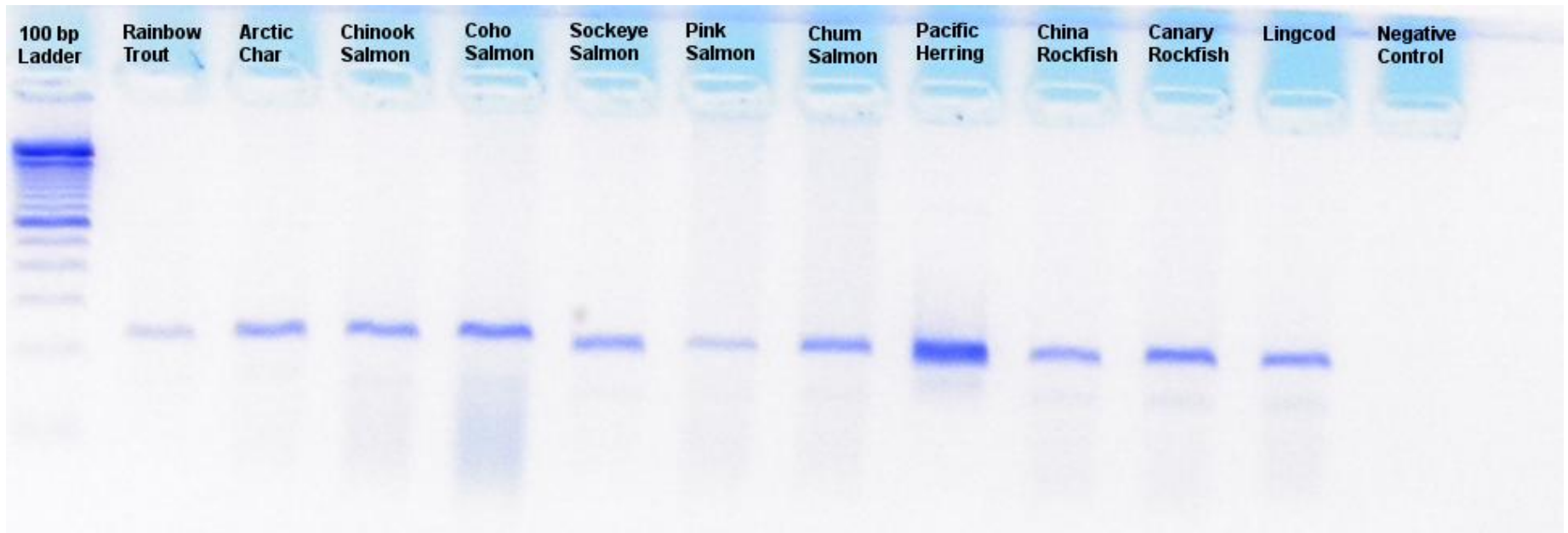


Figure 3.2. Electrophoresis gel of PCR products obtained from modern tissue samples from eleven species of bony fish. The PCR amplifications that generated these products utilized the universal primers, F271 and R271.

Table 3.2. Species identifications assigned to tissues samples from modern fish of known taxonomy through the analysis of the mini-barcode amplified using the universal primers, F271 and R271

Sample Code	Common Name	Scientific Name	Family	Top Matches (>99% sequence similarity)	DNA Species ID
CHM4	Chum Salmon	<i>Oncorhynchus keta</i>	Salmonidae	<i>Oncorhynchus keta</i> (100%, 99.42%, 99.22%)	<i>Oncorhynchus keta</i>
CM70	Pacific Herring	<i>Clupea pallasii</i>	Clupeidae	<i>Clupea pallasii</i> (100%, 99.42%, 99.22%), <i>C. harengus</i> (99.22%)	<i>Clupea</i> sp.
MF1	Rainbow Trout	<i>Oncorhynchus mykiss</i>	Salmonidae	<i>Oncorhynchus mykiss</i> (100%)	<i>Oncorhynchus mykiss</i>
MF2	Arctic Char	<i>Salvelinus alpinus</i>	Salmonidae	<i>Salvelinus alpinus</i> (100%, 99.22%), <i>S. umbla</i> (100%, 99.22%), <i>S. evasus</i> (100%), <i>S. kuznetzovi</i> (99.36%), <i>S. confluentus</i> (99.22%, 99.21%), <i>S. malma</i> (99.22%, 99.21%)	<i>Salvelinus</i> sp.
MF3	Chinook salmon	<i>Oncorhynchus tshawytscha</i>	Salmonidae	<i>O. tshawytscha</i> (100%, 99.42%)	<i>Oncorhynchus tshawytscha</i>
MF4	Coho Salmon	<i>O. kisutch</i>	Salmonidae	<i>O. kisutch</i> (100%)	<i>O. kisutch</i>
MF5	Sockeye Salmon	<i>O. nerka</i>	Salmonidae	<i>O. nerka</i> (100%, 99.22%)	<i>O. nerka</i>
MF6	Lingcod	<i>Ophiodon elongatus</i>	Hexagrammidae	<i>Ophiodon elongatus</i> (100%, 99.26%, 99.22%, 99.06%)	<i>Ophiodon elongatus</i>
PNK2	Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Salmonidae	<i>Oncorhynchus gorbuscha</i> (100%, 99.22%)	<i>Oncorhynchus gorbuscha</i>
RFM1	China Rockfish	<i>Sebastes nebulosus</i>	Sebastidae	<i>Sebastes nebulosus</i> (100%), <i>S. pinniger</i> (100%), <i>Sebastes</i> spp. (99.42%) ¹	<i>Sebastes</i> sp.
RFM2	Canary Rockfish	<i>S. pinniger</i>	Sebastidae	<i>S. pinniger</i> (100%), <i>S. nebulosus</i> (100%), <i>Sebastes</i> spp. (99.42%) ¹	<i>Sebastes</i> sp.

¹ The sequence similarity between RFM1 and RFM2 and reference sequences from 52 other *Sebastes* species was 99.42%: *Sebastes alutus*, *S. atrovirens*, *S. auriculatus*, *S. aurora*, *S. baramenueke*, *S. borealis*, *S. brevispinis*, *S. carnatus*, *S. chrysomelas*, *S. ciliatus*, *S. cortezi*, *S. crameri*, *S. dallii*, *S. diploproa*, *S. emphaeus*, *S. entomelas*, *S. fasciatus*, *S. flavidus*, *S. gilli*, *S. glaucus*, *S. goodei*, *S. iracundus*, *S. levis*, *S. maliger*, *S. melanops*, *S. melanosema*, *S. melanostictus*, *S. melanostomus*, *S. mentella*, *S. miniatus*, *S. minor*, *S. mystinus*, *S. norvegicus*, *S. ovalis*, *S. owstoni*, *S. pachycephalus*, *S. paucispinis*, *S. peduncularis*, *S. phillipsi*, *S. proriger*, *S. rastrelliger*, *S. reedi*, *S. rufinanus*, *S. serranoides*, *S. sinensis*, *S. taczanowskii*, *S. trivittatus*, *S. variabilis*, *S. variegatus*, *S. viviparus*, *S. vulpes*, *S. zacentrus*.

3.5. Discussion

3.5.1. Universality, Utility, and Reliability of Primers

As a result of the sequence diversity fish exhibit at COI, universally conserved primer binding sites flanking a variable region of COI that is of a size suitable for aDNA analysis do not exist (Jordan et al. 2010:225). However, by including degenerate bases at variable positions, it was possible to design primers (F271/R271) that flank a variable mini-barcode, and have degenerate sequences 'conserved' across species. PCR amplifications utilizing these primers successfully amplified a 220 bp fragment of COI from all eleven fish species that were tested, which suggests these primers are universal. Moreover, this fragment displayed sufficient interspecies variability to allow species or genus identifications to be assigned to each sample. The taxonomic identification assigned to each sample matched their known species or genus. This clearly indicates these primers can be used to assign reliable species identifications to fish remains. However, the fact that four of the samples could only be assigned genus identifications demonstrates that assigning a species identification to a sample may require the amplification of a second fragment. Ideally, these secondary amplifications should use genus-specific primers that target more variable fragments of COI or more variable regions.

3.5.2. Susceptibility to Contamination

Ancient DNA is highly susceptible to contamination from modern sources as a result of its highly degraded nature and the hypersensitivity of PCR (Yang et al. 2003; Yang and Watt 2005). To prevent such contamination, primers should not be able to amplify DNA from species that are not the desired target (Speller et al. 2011:16). In particular, since contaminant human DNA is omnipresent in reagents and laboratories (Leonard et al. 2007; Yang et al. 2003), primers, if possible, should preclude the amplification of human DNA (Speller et al. 2011).

With these concerns and recommendations in mind, an effort was made to assess whether the F271 and R271 primer pair can amplify contaminant human DNA.

This was accomplished by using these primers in an *in silico* PCR of human DNA. This *in silico* PCR failed to yield a PCR product when this primer pair was tested against the human mitochondrial genome. This suggests these primers cannot amplify contaminant DNA, which indicates they are well suited for use in aDNA analyses of fish remains. However, *in silico* PCRs are only models of primer specificity. Due to computing limitations, *in silico* PCR can never simulate the effects of all the PCR conditions and events that influence primer specificity *in vitro* (Henriques et al. 2012:637). As such, *in vitro* PCR of human DNA using these primers needs to be attempted in order to confirm their inability to amplify human DNA.

3.5.3. Comparison with Jordan et al.'s Universal Primers for Fish

Jordan et al. (2010) has developed primers capable of amplifying a 148 bp region of the 12S gene from a variety of bony fish species. Although 12S is more conserved than COI, the fragment amplified by these primers, like the mini-barcode amplified by F271 and R271, exhibits interspecific variation (Jordan et al. 2010). As such, species identifications can also be assigned to fish remains through the analysis of this fragment targeted by the primers developed by Jordan et al. (2010). However, unlike F271 and R271, these primers can amplify contaminant human and mouse DNA (Grier et al. 2013:550). As a result of this ability, studies that have utilized these primers often amplify human or mouse DNA, rather than endogenous fish DNA, from at least one fishbone (e.g., Grier et al. 2013; Kemp et al. 2014; Monroe et al. 2013). Due to their apparent inability to amplify human DNA, such contamination could be avoided by utilizing F271 and R271 to identify fishbone. That being said, Jordan's et al. primers are still undeniably useful for species identification. Since they amplify a considerably shorter fragment (148 bp vs. 220 bp), Jordan et al.'s (201) primer will be more useful than F271 and R271 in situations where the DNA in fish remains is extremely degraded (<200 bp in length),

3.6. Chapter Summary

This study developed universal primers capable of amplifying a cytochrome c oxidase I-based 220 bp mini-barcode from a diverse range of bony fish species. Despite

its small size, this mini-barcode was sufficiently variable to allow species or genus identifications to be assigned to all of the modern fish tissue samples that were tested. In each instance, these taxonomic designations matched the samples' known genus or species identification. These results clearly demonstrate that the mini-barcode targeted by these primers can be used to assign reliable species identification to samples of fish. This discriminatory power and these primers' inability to amplify human DNA make them well suited for use in aDNA analyses seeking to identify archaeological fishbone.

Chapter 4.

Materials and Methods

This chapter describes the methods used to recover fish remains from EeRb-144 and analyze these remains through morphological and ancient DNA analysis. The chapter begins with a description of the methods used to excavate EeRb-144 and recover fish remains from the excavated deposits. The methods used to morphologically identify these fish remains and select a sample of these remains for aDNA analysis are then described. This is followed by an in depth description of the aDNA analysis protocols used to analyze these fish remains. Finally, the chapter concludes with a discussion of the method use to assess the representativeness of the samples of identified Middle and Late Period fish remains.

4.1. Excavation Methods and Sample Recovery

EeRb-144 was first identified in 1991 by George Nicholas during a systematic survey and testing program focused on the glaciolacustrine terraces located on Kamloops Indian Reserve Number 1 on the north side of the South Thompson River. Testing of the site was initially limited to eight shovel test pits and three 1-m² units in addition to surface collections. The site was subsequently the focus of full-scale excavations between 1997 and 2001 in order to investigate Middle Period and potentially earlier components at the site (Nicholas et al. forthcoming). During the course of this investigation, 200 m² of EeRb-144, representing approximately 20% of site's total area, was exposed through the excavation of 29 1-m² and 43 2-m² units in 5 cm arbitrary levels (Figure 4.1) (Nicholas et al. forthcoming). Archaeological materials, including fish remains, were recovered from these units by sifting all excavated deposits through a 0.11 mm screen (Nicholas et al. forthcoming).

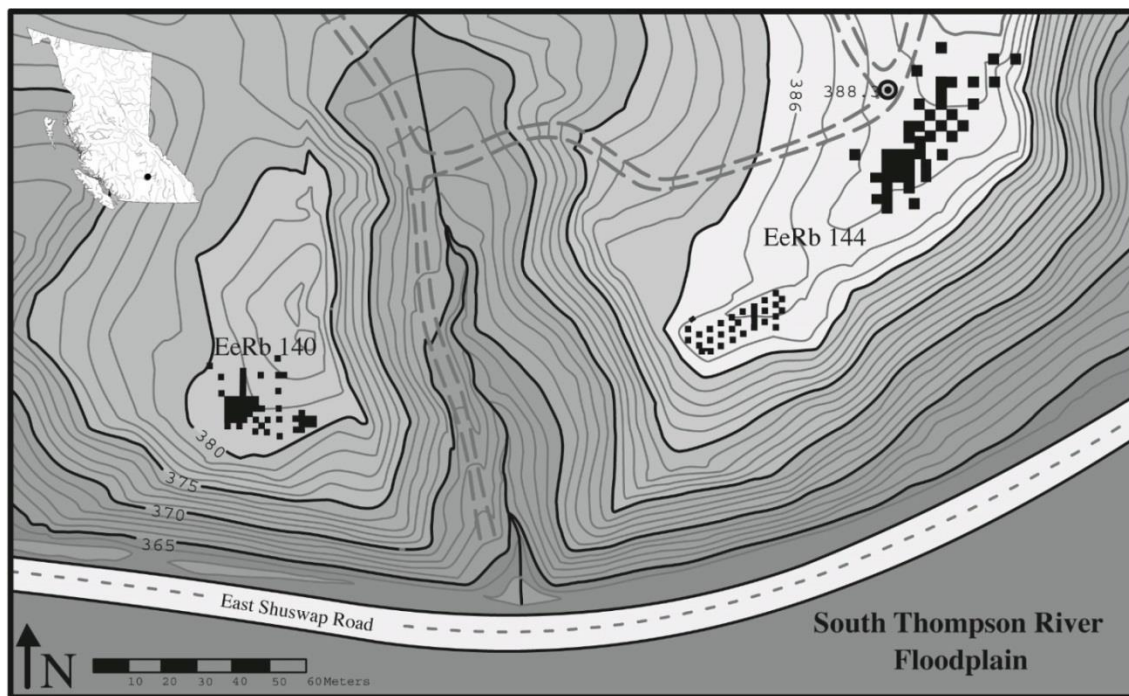


Figure 4.1. Plan view of the excavated portion of EeRb-144 and EeRb-140. This figure is courtesy of George Nicholas and is used with permission.

4.2. Zooarchaeological Analysis

A team directed by David Maxwell (Department of Archaeology, Simon Fraser University) analyzed the fish remains recovered from EeRb-144. Fish remains were identified by comparing them to fish specimens of known taxonomy housed in the comparative faunal collection at the Department of Archaeology, Simon Fraser University. Although attempts were made to identify the remains to the lowest possible taxonomic level, 90.89% were only assigned to the class-level due to sample fragmentation. Any macroscopic taphonomic damage to the remains was recorded and when possible the skeletal elements the remains represented were identified.

4.3. Ancient DNA Analysis

4.3.1. Sample Selection

In total, 86 faunal remains from EeRb-144 that were morphologically identified as fish were selected for aDNA analysis. All of these remains were recovered from units located in the northeastern quadrant of EeRb-144. Sampling was restricted to the northeastern aspect because fish remains from this aspect were easier to assign to a period than those from its southeastern aspect. Dating of remains from the northeastern quadrant was facilitated by its large assemblage of temporally diagnostic artifacts, and the six radiocarbon dates available for this quadrant (Table 2.2). Conversely, the southeastern quadrant lacked radiocarbon dates and had a smaller assemblage of diagnostic artifacts. Appendix D lists the time periods to which each of the analyzed samples were dated.

Initially, the fish remains from EeRb-144's northeastern quadrant that were selected for analysis were chosen through non-blind semi-random sampling. A series of numerical designations were assigned to each context (unit and level) in EeRb-144's northeastern quadrant that yielded fish remains. The number of designations assigned to each context corresponded with the number of faunal remains from the context that were identified as bony fish. Using the random number function in Microsoft Excel, a sample of these numerical designations was randomly selected. A single specimen from the context each numerical designation in this sample corresponded to was then selected for analysis.

As this sampling process progressed it was observed that the contexts being randomly selected for sampling were predominately those with heavily fragmented fish remains. This is not surprising as the chance of randomly selecting a context for sampling was directly proportional to the size of its assemblage—a property fragmentation tends to increase. Consequently, to ensure the sample of analyzed remains represented a range of contexts, remains from contexts with fewer and less fragmented remains were judgementally sampled. Moreover, deliberately selecting samples from a variety of contexts also increases the likelihood that multiple individuals

are represented in the sample (Speller et al 2005:1382). The context of the analyzed samples can be found in Appendix D.

A few samples were also deliberately selected for analysis on the basis of the taxonomic identifications assigned to them through the zooarchaeological analysis. Examples of the different taxa identified during the zooarchaeological analysis as being present in EeRb-144's faunal assemblage (Table 2.3) were purposely selected for analysis. The one exception to this was the single fishbone from EeRb-144 that was, as previously discussed in Section 2.4.1, curiously identified as being a percid. Since this fishbone could not be relocated, it was not selected for aDNA analysis. By purposely including examples of different species in the sample of analyzed remains the likelihood that it reflected the taxonomic diversity of the entire assemblage was maximized.

4.3.2. Decontamination

In order to eliminate contaminant DNA adhering to their surfaces, all of the analyzed samples were decontaminated using a modified version of the decontamination protocol described by Yang et al. (2004). Samples were first immersed in a 100% commercial bleach solution (6 to 7.4% sodium hypochlorite) for 5 to 7 minutes and then rinsed in ddH₂O for approximately 30 seconds to remove any residual bleach. Initially, the samples were then rinsed in HCl for 30 seconds to 1 minute, followed by NaOH for an equal length of time in order to neutralize the HCl. However, after the first batch of samples (FH1, FH2, FH3, FH4, FH5) the use of HCl and NaOH was discontinued, as the HCl appeared to be rapidly dissolving thin elements. Following these chemical washes, the samples were submerged in ddH₂O for 5 to 12 minutes to remove any chemical residues. Each sample was then UV irradiated at 120,000 $\mu\text{J}/\text{cm}^2$ for 15 minutes twice, once on each side, to crosslink any lingering surface contaminant DNA.

4.3.3. DNA Extraction

DNA was extracted from the decontaminated samples using a modified silica-spin column method (Yang et al. 1998). Typically, entire samples were added to 2 to 5

ml of lysis buffer (0.5 M EDTA pH 8.0, 0.5% SDS and 0.5 mg/mL proteinase K), and incubated overnight in a rotating hybridization oven at 50 °C. Due to their large size, a few samples were manually crushed into a coarse powder prior to being added to the lysis buffer. Following incubation, the samples were centrifuged at 4400 rpm until a pellet of undigested material formed. To concentrate the resulting supernatant, 2 to 3 ml of supernatant was transferred to a 10,000 or 30,000 NWML Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) and centrifuged at 4400 rpm until concentrated to less than 100 µl.

Concentrated samples were purified using a QIAquick PCR Purification kit (QIAGEN, Hilden, Germany). 500 µl of PB buffer (QIAGEN, Hilden, Germany) was mixed with the concentrated samples in the centrifugal filters, and transferred to QIAquick silica-spin columns (QIAGEN, Hilden, Germany). The DNA in the samples was then bound to the columns by centrifuging them for 1 minute at 6000 rpm. The collection tube containing the resulting flow-through was discarded and replaced. The samples were then washed twice by adding 400 µl of PE buffer (QIAGEN, Hilden, Germany) to the columns and centrifuging them for 1 minute at 6000 rpm. After each wash the flow-through was discarded, and the collection tube was replaced. Following these washes, DNA was eluted from the column. To elute the DNA from the column, 100 µl of EB buffer (QIAGEN, Hilden, Germany) was added to the columns, which were then incubated for 5 minutes at approximately 65 °C in a dry-block incubator, and centrifuged for 1 minute at 13,000 rpm. Subsequently, the eluted DNA was transferred to a 2-ml tube.

4.3.4. PCR Amplification and Sequencing

All PCR amplifications were executed using a Mastercycler Personal or Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). Initial PCR amplifications were conducted in a 30 µl reaction mixture that included 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 1.0 mg/mL BSA, 0.3 µM of forward universal primer F271 (Table 4.1), 0.3 µM of reverse universal primer R271 (Table 4.1), 0.75-2.25 U AmpliTaq Gold (Applied Biosystems, Foster City, California), and 3.0-6.0 µl of DNA. The thermal conditions of these initial amplifications consisted of an initial denaturation step at 95 °C for 12 minutes followed by 30 cycles comprised of a denaturation step at 95 °C for 30

seconds, an annealing step at 50 °C for 30 seconds, and an extension step at 72 °C for 40 seconds, and a final extension step at 72 °C for 7 minutes. The efficacy of these amplifications was monitored by including a positive control consisting of ancient fish DNA in most PCR setups.

Using the criteria outlined in Section 4.3.5., some samples could only be identified to the genus-level using the fragment generated by this initial amplification. In such instances, a second non-overlapping fragment of COI was amplified using the corresponding genus-specific primers (Table 4.1). The conditions for these secondary amplifications were the same as the initial amplifications except for their annealing temperature, and the replacement of F271 and R271 with 3 µM of F647 and R647 or F311 and R311 (Table 4.1).

Table 4.1. Universal and genus-specific primers used to amplify a fragment of cytochrome c oxidase I.

Primer	Length	Sequence (5'-3')	Annealing Temperature	Amplicon Size
Bony fish (<i>Osteichthyes</i>)				
F271	19 bp	CYAYCYTACCTGTGGCMAT	50 °C	220 bp
R271	29 bp	ACTATAAAGAARATYATWACRAARGCRTG		
Sucker (<i>Catostomus</i> spp.)				
F647	24 bp	GGGTTTGGAAACTGACTTGTACCA	52 °C	233 bp
R647	23 bp	CCTGCCAGGTGAAGAGAAAAGAT		
Pikeminnow (<i>Ptychocheilus</i> spp.)				
F311	24 bp	GCACCTGATATAGCATTCCCACGA	52 °C	173 bp
R311	23 bp	GTTAGATCTACTGATGCCCCCGC		

Following amplification, 5 µl of PCR product was pre-stained with SYBR Green (Invitrogen, Carlsbad, California). The samples were then electrophoresed at 135 V for 20 minutes on a 2% agarose gel and visualized with a Dark Reader Transilluminator (Clare Chemical Research, Dolores, Colorado). Unpurified PCR products were directly sequenced from the forward direction or both directions by Eurofins Genomics (formerly Eurofins MWG Operon) (Louisville, Kentucky, formerly Huntsville, Alabama) using the

same primers used for amplification. ChromasPro¹⁴ was used to inspect, edit, and assemble the resulting sequences.

4.3.5. Species Identification

Edited sequences were compared to reference sequences in the BOLD Species Level Barcode Records database using the BOLD identification engine¹⁵ (Ratnasingham and Hebert 2007), and cross-checked against GenBank¹⁶ through a BLAST (Altschul et al. 1990) search¹⁷. A species identification was assigned to a sample if the similarity of its sequence to reference sequences from a single species exceeded or was equal to 99% (Ratnasingham and Hebert 2007). In cases where reference sequences from more than one congeneric species exceeded this threshold, the sample was assigned to this genus. If no reference sequence surpassed this threshold, then samples were assigned a genus-level identification if its divergence from reference sequences from a single genus did not exceed 3% (Ratnasingham and Hebert 2007). If none of these conditions could be met, then no attempt was made to assign a taxonomic designation to a sample.

4.3.6. Contamination Controls

To decrease the likelihood of contamination, this study employed a series of vigorous contamination controls (Kemp and Smith 2010; Poinar 2003; Yang and Watt 2005). All pre-PCR laboratory work was conducted in the dedicated Ancient DNA Laboratory at Simon Fraser University. This laboratory is physically separated from the post-PCR laboratory and personnel are prohibited from moving from the post-PCR laboratory to the aDNA laboratory without showering and changing clothes. Protective clothing consisting of dedicated scrubs, DuPont Tyvek coveralls, two layers of single-use latex or nitrile gloves, and a surgical mask was worn while working in the aDNA laboratory. The outer layer of gloves was routinely changed in order to avoid the cross-contamination of samples. Moreover, disposable pipette tips with an aerosol barrier were

¹⁴ <http://www.technelysium.com.au>

¹⁵ http://www.boldsystems.org/index.php/IDS_OpenIdEngine

¹⁶ <https://www.ncbi.nlm.nih.gov/genbank>

¹⁷ <http://www.blast.ncbi.nlm.nih.gov/Blast.cgi>

used to transfer reagents and samples and replaced between the handling of each sample. To detect instances of contamination, blank extracts were processed alongside the samples, and negative PCR controls were included in each PCR setup. In addition to these established contamination controls, the author sought to further minimize the risk of contamination by abstaining from eating fish on the days aDNA laboratory work was conducted.

4.4. Assessment of Sample Representativeness

The taxonomic richness of zooarchaeological samples is influenced by the size of the sample in a predictable manner (Lepofsky and Lertzman 2005:176-177). As the size of a sample increases, its richness will increase until it stabilizes at a value that approximates the true richness of the entire assemblage (Lepofsky and Lertzman 2005:176). To assess whether the analyzed samples of identified Middle and Late Period fishbone were sufficiently large to have reached this point, species accumulation curves were constructed in order to examine the relationship between their size and richness (Lepofsky and Lertzman 2005). These curves were constructed by creating a scatterplot in which the cumulative number of taxa identified (y-axis) was plotted against the cumulative number of samples identified to the species-level (x-axis). The curves were then visually examined to determine whether or not they had plateaued.

4.5. Chapter Summary

EeRb-144 was first identified in 1991 and subsequently excavated between 1997 and 2001. A sample of 85 faunal remains recovered during these excavations that were morphologically identified as fish were selected through semi-random and judgemental sampling for aDNA analysis. These fish remains selected for analysis were decontaminated using a combination of chemicals and UV irradiation (Yang et al. 2004). The likelihood of contamination was further reduced by conducting all pre-PCR laboratory work in a dedicated aDNA laboratory, and adhering to strict contaminations controls (Kemp and Smith 2010; Poinar 2003; Yang and Watt 2005). Following decontamination, DNA was extracted from the remains using a modified silica spin

column method (Yang et al. 1998). Subsequently, a fragment of COI was targeted using the universal primers developed in Chapter 3. A genus or species identification was assigned to samples based on this fragment's similarity to reference sequences in BOLD and GenBank (Ratnasingham and Hebert 2007). If a sample could only be identified to the genus-level, a second non-overlapping fragment of COI was targeted using genus-specific primers. Subsequently, this second fragment was compared to reference sequences in an attempt to assign species identification to such samples. Finally, species accumulation curves (Lepofsky and Lertzman 2005) were constructed in order to determine whether or not the samples of identified Middle and Late Period fish remains were representative.

Chapter 5.

Results

This chapter presents the results of the aDNA analysis of fish remains from EeRb-144. The chapter begins by describing the results of the PCR amplifications and the direct sequencing of the products these amplifications generated. The following section reviews the results of the species identification of the analyzed samples, including the relative abundance of the different taxa identified in the samples of Middle and Late Period fish remains. The chapter concludes with an assessment of the representativeness of the samples of identified Middle and Late Period fishbones. The discussion and interpretation of these results is presented in Chapter 6.

5.1. PCR Amplification

A fragment of the appropriate length was amplified from 48¹⁸ of the 86 samples with the universal primers, F271 and R271. The temporal distribution of the samples from which this fragment was amplified is presented in Table 5.1. Subsequent analysis of this fragment (see Section 5.3) indicated that assigning species identification to 40 of these samples required secondary amplification utilizing genus-specific primers. The secondary rounds of amplification needed to identify 11 of these samples were conducted using the pikeminnow-specific primers, F311 and R311. All 11 of these samples yielded a fragment of the expected length. The sucker-specific primers, F647

¹⁸ Figure includes FH48 which yielded a PCR product of the appropriate length but was later found to have a sequence that most closely resembled bacterial, rather than fish, reference sequences.

and R647, were used in the secondary rounds of amplification required to identify the remaining 29 samples. Using these primers, a fragment was successfully amplified from 24 of these samples. DNA could not be amplified from the remaining five samples (FH59, FH64, FH70, FH71, and FH75). The failure to amplify DNA from these samples in spite of the fact that DNA was amplified from them using the universal primers is not unexpected. Since the fragment targeted by the sucker-specific primers is longer than the one targeted by the universal primers it is less likely to be preserved.

Table 5.1. Success rate of the PCR amplifications conducted with the universal primers.

Time Period	Number of Samples Analyzed	Positive Amplifications	Failed Amplifications	Amplification Success Rate
Middle Period	6	5	1	80.33%
Late Period	61	28 ¹	33	45.9%
Unsecure Date	20	14	6	70%
Overall	86	47 ¹	39	54.65%

¹Although FH48 dates to the Late Period and yielded DNA it is excluded from these totals. This is due to the fact that a BLAST search of the sequence of the PCR product obtained from this sample indicates this DNA is bacterial, rather than piscine, in origin.

DNA was not amplified from any of the negative PCR controls and the majority of the blank extracts. On one occasion rainbow trout DNA was successfully amplified using the universal primers from one of the blank extracts. However, a previous PCR setup failed to amplify DNA from this blank as did a subsequent setup.

5.2. Sequencing

In general, the direct sequencing of PCR products yielded clear sequences with well-defined peaks (Figure 5.1-A). However, some sequences had messy beginnings characterized by multiple overlapping and often broad peaks (Figure 5.1-B). Some sequences also exhibited background noise (Figure 5.1-C). Although the strength of such background noise was typically low and did not interfere with base-calling, a few sequences had stronger background noise that resulted in ambiguous base calls. This background noise and the messy beginnings observed in some sequences is likely the

result of the presence of primer-dimers and unused primers in the sequenced PCR products (Wu et al. 2013:110; Yang et al. 2004:624). Since the PCR products were not purified prior to sequencing the presence of unused primers and primer-dimers in the sequenced products and their interference in sequencing was expected. To obtain higher quality sequence data, some of the samples exhibiting messy beginnings and/or background noise were reamplified and resequenced.

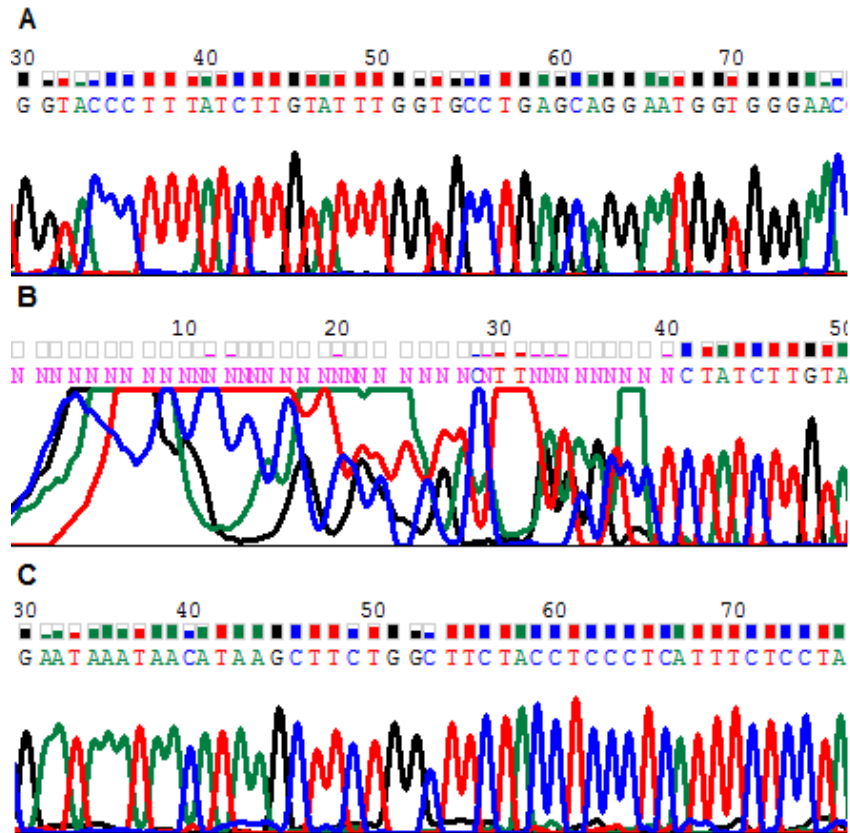


Figure 5.1. Example of a chromatogram of a (A) clear sequence, (B) sequence with a messy beginning, and (C) a sequence with background noise.

A handful of sequences contained strong broad peaks that overlapped with the main sequence. In each instance, these peaks occurred between approximately position 70 and 90 in the raw unedited sequences (Figure 5.2). These broad peaks likely represent dye-blobs, a common type of sequencing artifact caused by the presence of excess unincorporated dyes-terminators (Wu et al. 2013:107, 111). In some instances, these dye-blobs obscured the underlying sequence, which meant the sample had to be re-sequenced in order to obtain a clear sequence (Figure 5.2-A). However, other

samples displaying dye-blobs did not require resequencing since the portion of the sequence overlapping the dye-blob could easily be read (Figure 5.2-B). Consequently, no attempt was made to re-sequence these samples.

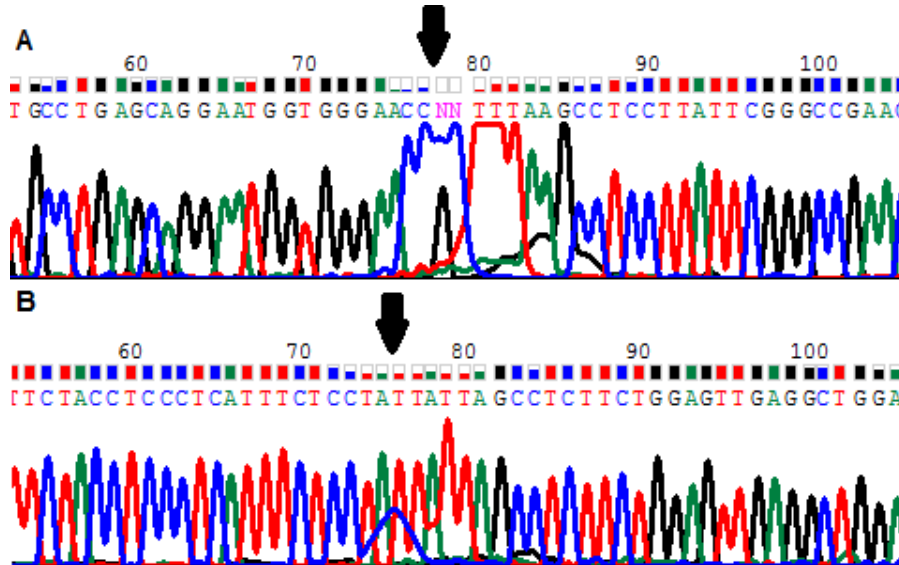


Figure 5.2. Examples of (A) a dye-blob that obscures the main sequence, and (B) a sequence in which the portion overlapping the dye-blob can still be read. The approximate positions of the dye-blobs is indicated by the arrows.

5.3. Species Identification

The sequences of the fragments amplified from all of the samples except FH48 closely matched bony fish reference sequences in BOLD and GenBank. In the case of FH48, the sequence of the fragment generated using the universal primers most closely resembled bacterial reference sequences in GenBank. Given its non-piscine origin, this sequence obtained from FH48 was disregarded, and the sample was not analyzed further, and not included in the succeeding data analyses. This apparent amplification of bacterial DNA from FH48 is likely the result of the non-specific amplification of DNA from environmental bacteria.

The ability to assign species-level identifications to the remaining samples that yielded fish DNA varied. It was possible to identify samples as Chinook salmon (n=1), peamouth chub (n=5), and rainbow trout (n=1) based on the sequence of the fragment

amplified by the universal primers. Nonetheless, the majority of the samples (85.10%, n=40) could only be identified as pikeminnow (*Ptychocheilus* sp.) (n=11) or sucker (*Catostomus* sp.) (n=29) using this fragment. In these instances, multiple congeneric species exceeded the 99% sequence similarity threshold used to delineate species.

Consequently, a second fragment was amplified from these samples' using genus-specific primers and analyzed in order to try and identify them to the species-level. All of the pikeminnow samples were identified as northern pikeminnow based on the sequence of the second fragment. In addition, two of the sucker samples (FH25 and FH61) were identified as longnose sucker (*Catostomus catostomus*) on the basis of the second fragment. Conversely, the remaining sucker samples could not be identified to the species-level. Five of these samples (FH59, FH64, FH70, FH71, and FH75) could not be identified to the species-level due to the aforementioned failure to amplify a second fragment from these samples. However, the remaining sucker samples yielded a second DNA fragment but still could not be identified to the species-level using the criteria outlined in Section 4.3.5.

Inability to Discriminate Between Largescale and Utah Suckers

The inability to assign species-level identifications to these sucker samples was the result of the sequence of the second fragment closely matching reference sequences from two sucker species. In each instance, the sequence similarity of this second fragment to largescale (*Catostomus macrocheilus*) and Utah sucker (*Catostomus ardens*) reference sequences surpassed 99%.¹⁹ Since the similarity between their complete COI barcodes exceeds 99.5%, the inability to discriminate between these sucker species using this fragment is not unexpected.

Although it was not possible to determine whether these samples are largescale or Utah suckers using the criteria outlined in Section 4.3.5, these species' biogeography

¹⁹ Two of these samples (FH8 and FH14) could be identified as largescale sucker using the criteria outlined in Section 4.3.5. This was the result of post-mortem damage (C →T transition) to the sequences obtained from these samples creating artificial increased divergence between their sequences and Utah sucker reference sequences. However, if this damage is ignored, then it is not possible to determine whether they are Utah or largescale sucker.

suggests they more likely represent largescale sucker. Of these two species, only largescale sucker is known to be native to the Interior Plateau and British Columbia (McPhail and Carveth 1993). Conversely, the Utah sucker is endemic to the upper Snake River system above Shoshone Falls in Idaho, closed watersheds in Idaho, Wyoming, and Utah, and the Lake Bonneville basin in Idaho and Utah (Page and Burr 1991:171). Due to Utah suckers' absence from the Interior Plateau and British Columbia, it can be argued these samples are largescale rather than Utah sucker.

For a plethora of reasons, the modern range of fish species does not always reflect their historical distribution (Colley 1990:217). Consequently, making an inference about the species identity of fish remains based solely on the modern biogeography of species is fairly problematic. Consequently, to further examine whether these samples represent largescale sucker a single nucleotide polymorphism (SNP) analysis of the two fragments amplified from these samples was conducted. This analysis entailed comparing the SNPs the samples and largescale and Utah sucker exhibit in these fragment. To conduct this SNP analysis, the sequence of the fragment amplified from these samples was aligned with largescale and Utah sucker COI reference sequences using the Clustal W (Thompson et al. 1994) function in BioEdit. To aid in the visual analysis of the alignment, the aligned sequences were trimmed to the same length.

Segments of the edited alignments are presented in Figure 5.3 and Figure 5.4. As demonstrated in Figure 5.3, Utah suckers have a C at position 59 in the fragment amplified by the universal primers. Conversely, largescale sucker and the samples exhibit a T at this position (Figure 5.3). Similarly, the samples and most of the largescale suckers lack the G at position 68 in the second fragment exhibited by Utah sucker and two largescale sucker specimens (Figure 5.4). Instead, the samples and eight of the largescale suckers have an A at this position (Figure 5.4). The fact that the samples lack the SNPs displayed by Utah sucker and share SNPs with most largescale suckers indicates they have a closer genetic affinity with largescale sucker. This supports the inference made from the biogeographical data that these samples likely represent largescale suckers. Due to this concordance between the genetic and biogeographic data, these samples were identified as largescale suckers.

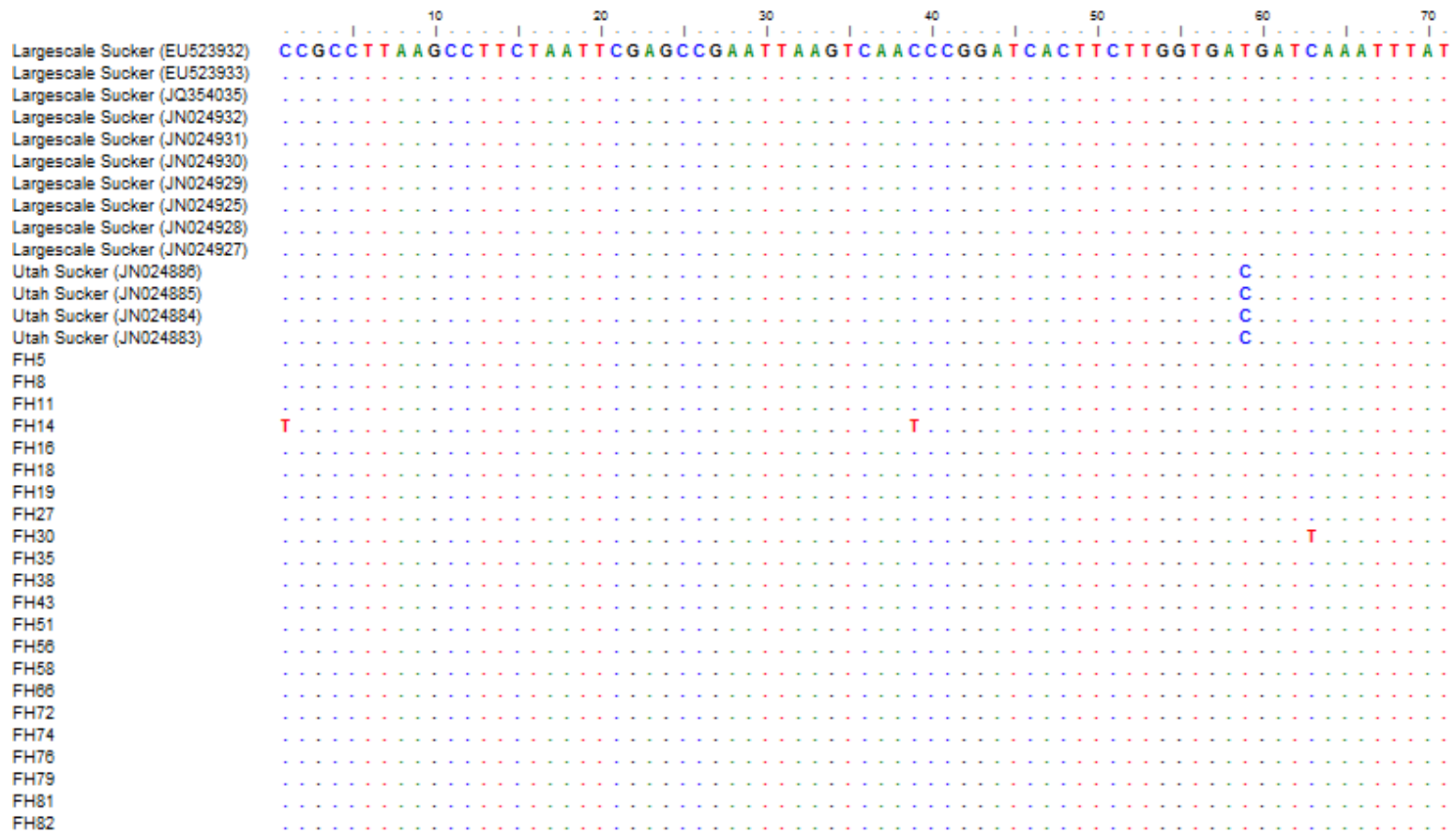


Figure 5.3. Segment of the multiple alignment of the fragment amplified from the samples tentatively identified as largescale sucker using the universal primers, F271 and R271, and the analogous region in largescale and Utah sucker reference sequences. Dots indicate positions where the nucleotide exhibited by the samples or reference sequence do not differ from those exhibited at this position by largescale sucker reference sequence (EU523932) used as the alignment reference. References sequences used for the alignment consisted of all publicly-available largescale and Utah sucker reference sequences published in BOLD.

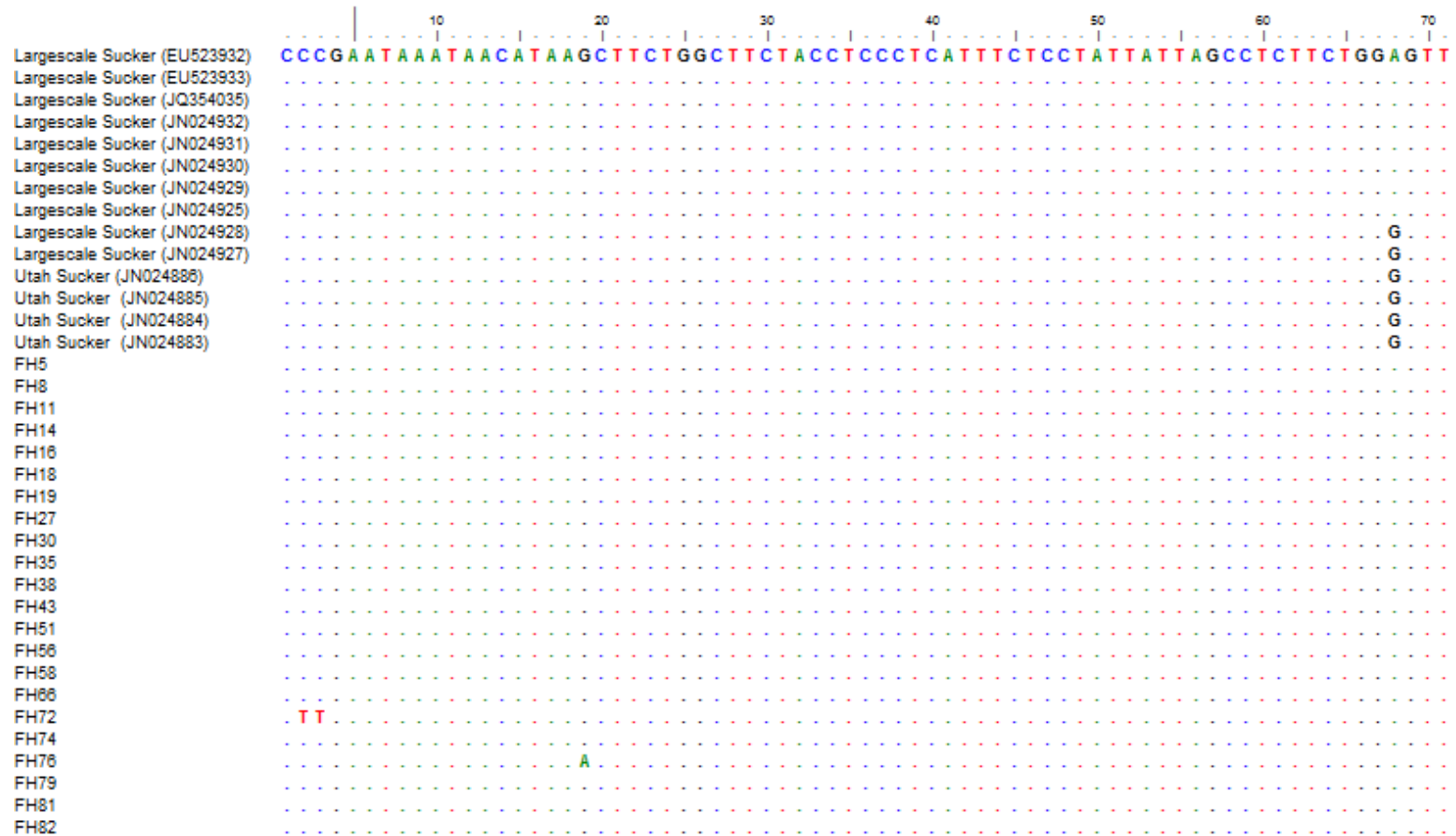


Figure 5.4. Segment of the multiple alignment of the fragment amplified from the samples tentatively identified as largescale sucker using the sucker-specific primers, F647 and R647, and the analogous region in largescale and Utah sucker reference sequences. Dots indicate positions where the nucleotide exhibited by the samples or reference sequence do not differ from those exhibited at this position by largescale sucker reference sequence (EU523932) used for the alignment reference. Reference sequences used in the alignment consisted of all publicly-available largescale and Utah sucker reference sequences published in **BOLD**.

5.3.1. Species Composition of Temporal Samples

Taking into account the identification of the Utah/largescale sucker samples as largescale sucker, species identifications were assigned to 42 of the 47 fish remains from EeRb-144 that yielded fish DNA. As previously discussed, the remaining 5 samples could only be identified as sucker. The DNA-based taxonomic identifications assigned to each of the samples that yielded DNA can be found in Appendix E. In total, six species were identified in this sample of fish remains that were successfully assigned taxonomic identifications. These six species are Chinook salmon, largescale sucker, longnose sucker, northern pikeminnow, peamouth chub, and rainbow/steelhead trout. Figure 5.5 presents the relative abundance of these taxa in the samples of identified Middle and Late Period fish remains, and the collection of remains lacking secure dates.

Middle Period

In spite of its exceedingly small size (n=5), three species of fish were identified in the sample of identified Middle Period fish remains (Figure 5.5). Two of these species, longnose sucker and peamouth chub, each make up 20% of the sample and are represented by single samples (Figure 5.5). The third species, largescale sucker, is the most abundant species in the sample. It accounts for two of the five identified Middle Period remains or 40% of the sample (Figure 5.5). The remaining Middle Period sample was identified as sucker (*Catostomus* sp.), and constitutes 20% of the sample.

Late Period

Slightly more species (n=5) were identified in the sample of Late Period fish remains than the Middle Period sample (Figure 5.5). Although this might indicate EeRb-144's Late Period fishery was more diverse than its Middle Period counterpart, it is more likely a product of the sample's larger size (n=28 versus n=5). As was the case with the Middle Period sample, largescale sucker (42.86%) is the most abundant species in the Late Period sample (Figure 5.5). Northern pikeminnow, although not present in the Middle Period sample, constitutes a large proportion (28.57%) of the identified Late Period remains, and is the second most abundant species in the Late Period sample (Figure 5.5). Peamouth chub, longnose sucker, and Chinook salmon were also identified among the Late Period fish remains, albeit in significantly lesser quantities than

largescale sucker and northern pikeminnow (<11%) (Figure 5.5). Of these three species, only Chinook salmon was not identified in the Middle Period sample. In addition, three Late Period remains or 10.71% of the sample was classified as sucker.

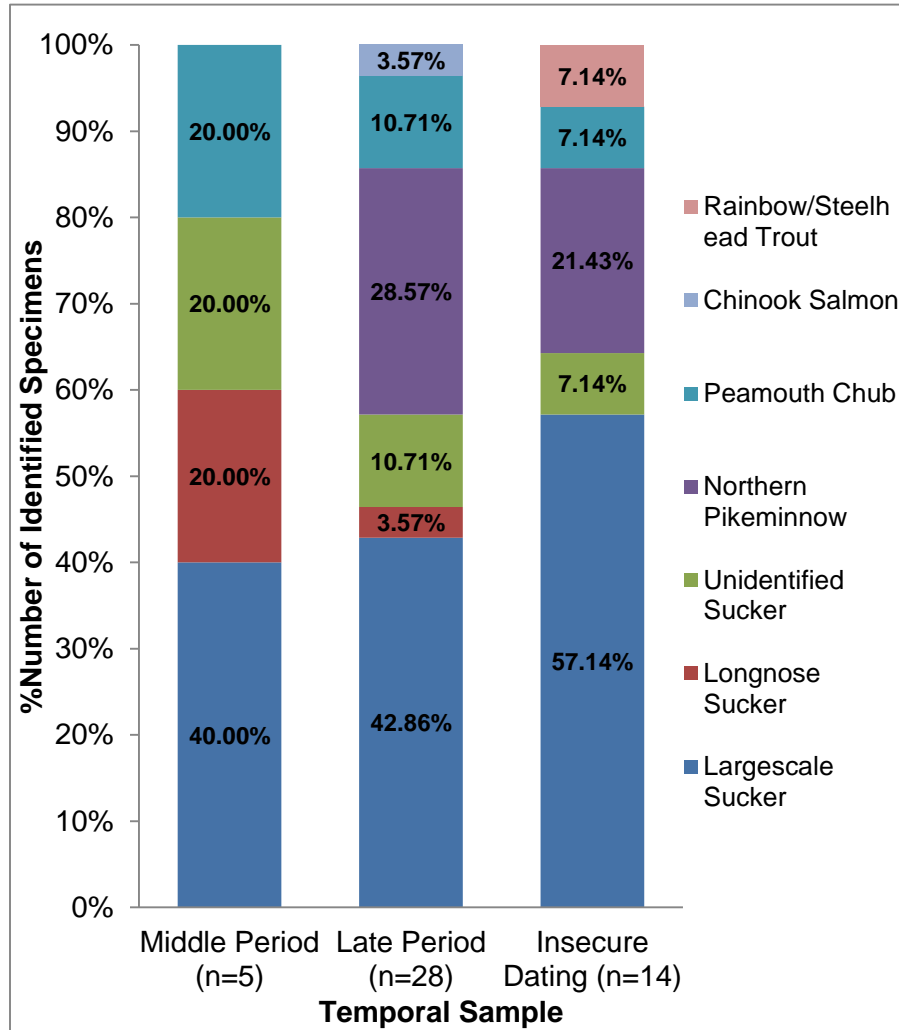


Figure 5.5. Relative abundance of different species in the temporal samples of identified fish remains from EeRb-144.

Samples Lacking Secure Dates

Thirteen of the fish remains that were identified through aDNA analysis could not be securely dated to a particular time period. Four species were identified in this sample of remains (n=4) making it slightly less diverse the sample of identified Late Period remains. However, in general its taxonomic composition is quite similar to the

composition of the Late Period sample. As was the case with the Late Period sample, largescale sucker (57.14%) and northern pikeminnow (21.43%) are the most and second most abundant taxa in this sample (Figure 5.5). Moreover, peamouth chub and a salmonid species were also identified in this collection. However, unlike the Late Period sample, the salmonid identified in this sample was rainbow/steelhead trout rather than Chinook salmon (Figure 5.5). As these identified remains lack a temporal context they are not discussed further.

5.4. Assessment of Sample Representativeness

Figure 5.6 depicts the species-accumulation curves constructed for the samples of identified Middle and Late Period fish remains. The positive slope of these curves indicates both samples, as expected, exhibit a positive relationship between sample size and richness. However, the curve constructed for the Late Period sample indicates its richness following a rapid early increase stabilized at four after 13 fishbones had been identified (Figure 5.6-A). Only after the number of the identified fish remains had been nearly doubled (NISP=24) was another species (Chinook salmon) identified (Figure 5.6-A). This stabilization of the Late Period sample's richness suggests its composition closely approximates the composition of the entire assemblage of Late Period fish remains from EeRb-144. Nonetheless, the late identification of a specimen of Chinook salmon (FH69) indicates some less common species present in the assemblage are likely not represented in the sample.

In contrast, the Middle Period sample's composition does not appear to be representative of the Middle Period assemblage of fish remains from EeRb-144. The steep slope of the species-accumulation curve (Figure 5.6-B) for this sample demonstrates its richness has not stabilized. Instead, a novel species was identified almost every time a Middle Period sample was identified. This suggests the Middle Period assemblage has not been adequately sampled and more Middle Period remains need to be identified in order to obtain a representative sample.

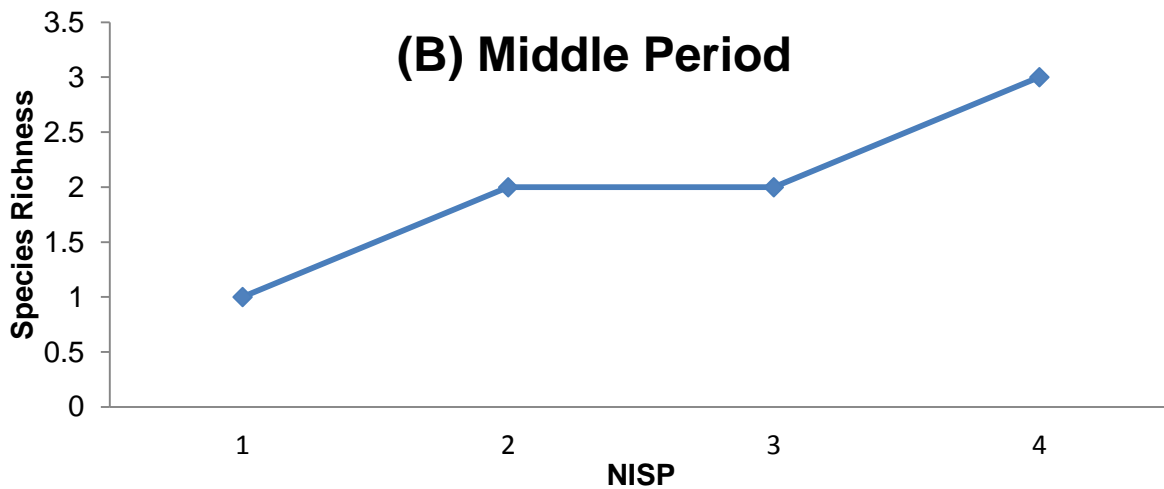
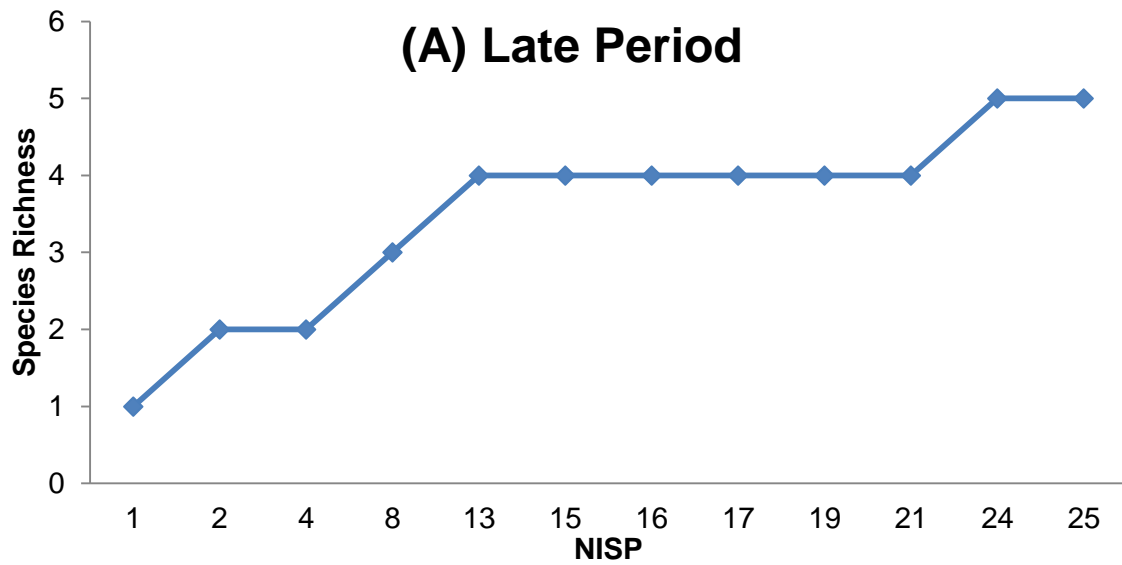


Figure 5.6. Species-accumulation curve documenting the relationship between the number of identified samples (NISP) and the species richness of the sample of identified (A) Late Period and (B) Middle Period fish remains.

5.5. Chapter Summary

DNA was amplified from 48 of the 86 fish remains from EeRb-144 that were analyzed. The sequences obtained from all but one of these remains closely resembled

reference sequences from bony fish. Using a combination of the species identification criteria established in Section 4.3.5, biogeographic data, and SNP analysis, species-level identifications were assigned to 42 of the 47 remains that yielded fish DNA. In some instances, these identifications were based solely on the sequence of the fragment amplified by the universal primers. However, in most cases samples could only be assigned a genus identification using this fragment. In such instances, a second fragment had to be amplified with genus-specific primer in order to identify their species. The remaining 5 samples that yielded fish DNA could only be identified to the genus-level (*Catostomus* sp.) due to the failure of the second round of amplification.

In total, 33 of the fish remains from EeRb-144 that were identified through aDNA analysis could be dated to a time period. Five of these dated to the Middle Period while the remaining the 28 date to the Late Period. In both of these temporal samples largescale sucker was the most abundant species. However, the Late Period sample was slightly more rich (n=5) than the Middle Period sample (n=3). This might indicate the Late Period fishery targeted a wider range of fish than its Middle Period counterpart. However, it is more likely a product of the smaller size of the Middle Period sample. A species accumulation curve constructed for this sample indicates its richness, due to its small size, is not representative of the complete assemblage of Middle Period fish remains from EeRb-144. Conversely, the species accumulation curve constructed for the Late Period sample indicates its richness approximates the richness of the entire Late Period assemblage.

Chapter 6.

Discussion and Conclusion

This chapter discusses the results of this study as they pertain to the research objectives outlined in Chapter 1. Since this study addressed both archaeological and methodological issues, this discussion is divided into two parts that address each of these dimensions individually.

The first part discusses the results of the aDNA analysis of fish remains from EeRb-144. This section begins with a discussion of the authenticity of the aDNA results, and the degree of DNA preservation exhibited the analyzed fish remains. It concludes with an interpretation of the nature of (e.g., range of fish species harvested, focus of fishery, seasonality of fishery) EeRb-144's Middle and Late Period fisheries based on the DNA-based species identifications that were generated. Limited evidence for continuity in fish practices between EeRb-144's Middle and Late Period fisheries is also presented. In this section, the results of this study are compared to the results of Carlson's (2006) analysis for fish remains from the nearby Thompson River Post site (EeRc-22)

In the second part, the methodological aspects of this study are discussed. This discussion largely focuses on the universality and utility of the universal primers designed as part of this study. Although this topic was touched on in Section 3.4.1, the results of the aDNA analysis are used to expand upon this previous discussion and identify the primers' limitations. Subsequently, the discriminatory power of COI-based mini-barcodes and their usefulness in the DNA-based species identification of fish is discussed.

The chapter ends with a summary of the thesis and highlights the significance of the research methods and results.

6.1. Ancient DNA Analysis

6.1.1. Authenticity of Ancient DNA

As a result of a variety of post-mortem chemical and biological processes, aDNA is highly degraded (Pääbo et al. 2004:646-647). The few aDNA molecules that are preserved in ancient remains are highly fragmented, and frequently contain crosslinks and base lesions (Pääbo et al. 2004:647-649). This damage makes aDNA highly susceptible to contamination with modern DNA (Yang and Watt 2005:332). Since PCR preferentially amplifies undamaged DNA molecules, aDNA molecules are readily out-competed during PCR by contaminant modern DNA, which is typically less damaged (Yang and Watt 2005:332). Due to aDNA's low copy number and PCR's hypersensitivity, only a few contaminant modern DNA molecules need be present for the out-competition of aDNA during PCR to occur (Yang and Watt 2005:332). Moreover, in cases where DNA is not preserved in ancient remains the presence of contaminant DNA can lead to false positive amplifications (Pääbo et al. 2004:654).

To reduce the likelihood of the occurrence of such contamination, all of the analyzed fish remains were decontaminated. Samples were decontaminated by submersing them in bleach and irradiating them with UV light. Controlled studies by Kemp et al. (Barta et al. 2013; Kemp and Smith 2005) have demonstrated that submersing samples in bleach will destroy most of the contaminant DNA present on their surfaces. Likewise, the UV irradiation of the samples should prevent the amplification of contaminant surface DNA by creating polymerase-blocking crosslinks within the contaminant DNA (O'Rourke et al. 1996:562). Consequently, these decontamination measures should have been sufficient to eliminate most contaminant DNA adhering to the surfaces of the analyzed fish remains.

The risk of contamination was further minimized by employing strict contamination controls aimed at limiting cross-sample contamination and samples' contact with modern DNA and PCR products. These controls included wearing protective clothing, changing gloves between the handling of different samples, and using disposable pipette tips with aerosol barriers throughout the pre-PCR laboratory

work. Moreover, all pre-PCR laboratory work was conducted in a positively-pressured dedicated aDNA laboratory that is physically separated from the post-PCR laboratory, and stocked with dedicated reagents and equipment. By segregating pre-PCR and post-PCR activities, the likelihood of samples becoming contaminated with high-copy number PCR products is greatly reduced (Willerselv and Cooper 2005:6).

The failure to amplify from any of the negative PCR controls and all but one of the blank extract indicates these precautionary measures were generally effective at preventing contamination. Nonetheless, the amplification of rainbow trout DNA from one the blank extract indicates contamination possibly occurred. However, the failure to repeatedly amplify DNA from this extract indicates this possible contamination is sporadic rather than systematic (Yang et al. 2003:359). Alternatively, this possible contamination might be the result of one-off human error rather than contamination (e.g., accidentally loading a sample rather than the blank extract into the tube containing the PCR reaction mixture for the blank extract). As this one possible case of contamination appears to have been sporadic contamination or the result of one-off human error it likely did not greatly affect the results of the aDNA analysis. The lack of evidence for systematic contamination suggests the sequences this study obtained from the analyzed fish remains from EeRb-144 are likely authentic.

The authenticity of the DNA sequences obtained from these fish remains is further supported by the samples' exhibition of molecular behaviour appropriate for aDNA (Cooper and Poinar 2000; Pääbo et al. 2004:656; Poinar 2003:577). Specifically, the inverse relationship between fragment size and amplification success that is characteristic of aDNA was observed (Handt et al. 1994:524-525). This relationship is exemplified by the failure to amplify a 238 bp fragment from five sucker samples from which it was possible to amplify a shorter 220 bp fragment. Since the post-mortem fragmentation of aDNA results in the destruction of longer fragments, this inverse relationship between amplification success and fragment length is expected to be exhibited by aDNA (Cooper and Poinar 2000; Handt et al. 1994: 524-525; Pääbo et al. 2004:656; Poinar 2003:577).

Five other lines of evidence provide further support for the authenticity for the DNA recovered from the analyzed fish remains. First, in each instance where two non-overlapping fragments of COI were amplified from a sample, analysis of these fragments yielded concordant taxonomic assignments (Appendix E), indicating the results are reproducible. Second, multiple species (n=6) were identified in the sample of analyzed fish remains, which is indicative of a lack of cross-sample contamination (cf. Speller et al. 2012: e51122). Furthermore, the identification of six species makes contamination a less likely explanation for the aDNA results, as it would have to have originated from multiple sources (Yang et al. 2004:627). Third, the presence of preserved birch bark (Nicholas et al. forthcoming) and a feather (Speller et al. 2011) at EeRb-144 demonstrates conditions at the site are conducive to the preservation of biomolecules, such as DNA. Fourth, the amplification of DNA from a 200-year-old feather from EeRb-144 by Speller et al. (2011) provides independent support for the preservation of DNA in materials from the site. Finally, the taxa that were identified have been identified in assemblages from other nearby sites (e.g., Carlson 2006; Royle et al. 2013a, 2013b) and/or are ethnographically known to have been harvested by the local Secwepemc people (Bouchard and Kennedy 1975). Consequently, the results of the aDNA analysis are consistent with those previous studies and make cultural sense.

6.1.2. Low Degree of DNA Preservation

In general, environmental conditions in the Pacific Northwest are conducive to the long-term survival of DNA (Cannon and Yang 2006:128; Speller et al. 2012: e51122). Consequently, DNA in faunal remains from the Pacific Northwest is generally well preserved (Speller et al. 2012: e51122). This high degree of DNA preservation is reflected by the high amplification success rates obtained by previous aDNA studies of mammal and fish remains from the region (Speller et al. 2012:e51122). In the case of fish, previous aDNA analyses of archaeological fishbone from the Pacific Northwest have generally amplified DNA from more than 60% of the analyzed samples (Cannon and Yang 2006; Ewonus et al. 2011; Moss et al. 2014; Speller et al. 2012; Speller et al. 2005; Kemp et al. 2014). Only one previous study (Grier et al. 2013) failed to successfully amplify DNA from more than 60% of the fish remains it analyzed. In comparison to these generally high amplification success rates, the 54.65% amplification

success rate obtained in this study is fairly low. This indicates DNA is relatively poorly preserved at EeRb-144.

The relatively low degree of DNA preservation observed at EeRb-144 might in part relate to the exposure of some of the site's fish remains to heat. Some fishbones from the site, including four that were analyzed in this study (FH23, FH45, FH46, and FH73), are blackened, indicating they were possibly burned through direct exposure to fire. High temperatures, such as those these bones would have experienced in a fire, increase the rate at which abasic sites form (Mitchell et al. 2005:268). At these abasic sites, the phosphodiester backbone of DNA rapidly cleaves via β -elimination, thereby resulting in the fragmentation of DNA (Mitchell et al. 2005:268). This heat induced DNA degradation likely explains why DNA was not amplified from any of the blackened samples. In addition to this thermal degradation of some samples' DNA, unknown site specific environmental conditions also undoubtedly contributed to the poor DNA preservation observed at EeRb-144.

6.1.3. The Pre-Contact Fisheries at EeRb-144

Composition

Late Period Fishery

The identification of Chinook salmon, largescale sucker, longnose sucker, northern pikeminnow, and peamouth chub in the Late Period sample indicates EeRb-144's Late Period fishery harvested a variety of species. All of these species are native to the Thompson River system (Appendix A) (McPhail and Carveth 1993), and are among the most abundant species in the Interior Plateau (McPhail 1999:Table 2). This suggests the Late Period fishery was orientated towards the exploitation of locally abundant resources.

Although the Late Period fishery exploited a range of locally abundant taxa, differences in the relative abundance of these taxa in the Late Period sample indicate their importance varied. The two most abundant taxa in the Late Period sample, largescale sucker and northern pikeminnow, collectively represent 71.43% of identified remains. In contrast, the three remaining species each represent 3.57% (longnose

sucker and Chinook salmon) or 10.71% (peamouth chub) of the identified remains. This high relative abundance of largescale scale and northern pikeminnow suggests EeRb-144's Late Period fishery was largely focused on exploiting these two taxa. Nonetheless, peamouth chub, given its intermediate relative abundance, may have been an important supplementary catch. Conversely, the markedly lower relative abundance of Chinook salmon and longnose sucker suggests their importance to EeRb-144's Late Period fishery was minimal. However, as discussed below, the results of the aDNA underemphasize the importance of salmon.

Underrepresentation of Salmonids in the Late Period Sample

Six of the analyzed Late Period samples (FH7, FH22, FH45, FH62, FH69, FH86) could be morphologically identified as salmonids. However, DNA was only amplified from one of these samples, FH69, which was identified as Chinook salmon. Due to this relatively high amplification failure rate (83.33%), salmonids are undoubtedly underrepresented in the sample of identified Late Period fish remains. If these salmonid samples that did not yield DNA were included in the sample, the relative abundance of salmonids would increase from 3.57% to 18.18%. This figure is consistent with the results of the zooarchaeological analysis, which found that salmonids account for 17.09% of the faunal remains from EeRb-144 classified to at least the order-level (Table 2.3). This indicates salmon made a more significant contribution to EeRb-144's Late Period fishery than the results of the aDNA analysis suggests. Nonetheless, the inclusion of these samples does not contradict the notion that the Late Period fishery at EeRb-144 was focused on catching largescale sucker and northern pikeminnow. These taxa would remain the most abundant taxa in the Late Period sample, and comprise 60.60% of the sample. However, the reduction of the relative abundance of these taxa from 71.43% indicates the Late Period fishery was less focused on these two taxa.

Middle Period Fishery

Although only a handful of Middle Period fish remains from EeRb-144 were successfully identified, the results of this study provide some insights into the site's Middle Period fishery. The presence of largescale sucker, longnose sucker, and peamouth chub in the Middle Period sample indicates these taxa were caught by EeRb-

144's Middle Period fishery. Even though no other species were identified in this sample, the Middle Period fishery was in all likelihood more diverse. This is supported by steepness of the species accumulation curve constructed for the Middle Period sample, which indicates it does not encapsulate the taxonomic diversity of EeRb-144's Middle Period fishbone assemblage. Since the Middle Period sample is not representative, the relative importance of the three taxa that were identified in the sample cannot be determined. Consequently, the focus of the Middle Period fishery and the range of species it exploited cannot be determined at this time.

Temporal Trends in Fish Use at EeRb-144

Since the Middle Period sample is unrepresentative, a meaningful comparison of the taxonomic composition of the samples of identified Middle and Late Period fish remains cannot be made. Consequently, temporal changes in the range of fish species harvested by EeRb-144's fishery and the relative importance of different taxa cannot be discerned. Nonetheless, the identification of peamouth chub, largescale sucker, and longnose sucker in both samples demonstrates EeRb-144's Middle and Late Period fisheries both harvested these taxa. This use of some of the same taxa indicates there is some continuity between the Middle and Late Period in fishing practices at EeRb-144.

Seasonality

Except for Chinook salmon, all of the species represented in the sample of identified Late Period fish remains are resident fish (McPhail 2007). Consequently, they are present in the Interior Plateau year round, and thus could have potentially been caught throughout the year. However, the density of resident taxa and their proximity to the shoreline changes throughout the year, which alters their accessibility to humans (cf. Needs-Howarth and Thomas 1998:111, 113). Documenting such seasonal changes in the accessibility of the resident fish taxa identified in the Late Period sample can provide insights into the timing of the Late Period fishery (cf. Needs-Howarth and Thomas 1998:111, 113), as outlined here.

In the Interior Plateau, largescale suckers are common in both lakes and large rivers (McPhail 2007:185). Conversely, the other sucker species represented in the sample, the longnose sucker, is typically only found in the deep waters of lakes (McPhail

2007:165). Despite these differences in habitat preferences, both of these species typically migrate in large numbers up tributary streams in order to aggregate at spawning sites (McPhail 2007:164-165, 183-184). The stream spawning sites utilized by largescale sucker are typically located in water between 0.6 and 2 meters deep, while the depth of those used by longnose sucker is usually between 0.1 and 0.6 meters (Roberge et al. 2002:37, 43). Some populations of these species also spawn in shallow areas of lakes that are less than 2 meters in depth (McPhail 2007:164, 183). Among southern interior populations, these spawning migrations, and the associated spawning, typically occur between late May and June in the case of largescale sucker, and mid-April and mid-May in the case of longnose sucker (McPhail 2007:183; Roberge et al. 2002:37; Scott and Crossman 1998:532).

Northern pikeminnow has a life history pattern similar to that exhibited by the largescale and longnose suckers. In order to spawn, most northern pikeminnow migrate up streams, and form large dense aggregations along their shorelines (McPhail 2007:122; Roberge et al. 2002:29). As with suckers, some populations instead aggregate in the shallow littoral zone of lakes or in areas of lakes located near the mouth of inlet streams (Roberge et al. 2002:29). In British Columbia, northern pikeminnow typically form spawning aggregations between May and July, but may begin spawning in late April and continue until August depending on local water temperatures (McPhail 2007:122; Scott and Crossman 1998:489; Roberge et al. 2002:29). Following spawning, northern pikeminnow disperse but continue to inhabit the littoral zone until the fall when they migrate to their winter habitats in deeper offshore waters (McPhail 2007:123-124; Scott and Crossman 1998:489).

The spring to mid-summer spawning migrations and aggregations of largescale suckers, longnose suckers, and northern pikeminnows, represent an optimal time to catch these species. As these migrations and aggregations may involve hundreds or thousands of individuals, they provide an excellent opportunity to catch these species en masse. Moreover, since these spawning aggregations occur in extremely shallow waters not typically occupied by these species, they are more accessible to humans during this spawning period. The ethnographic record supports the notion that these three species were caught during their spring to mid-summer spawning periods. Kennedy and

Bouchard (1992:279), for example, note that both sucker and northern pikeminnow were traditionally caught by the Stal'atl'imx during the spring. Taken together this data suggests these three taxa represented in the Late Period sample were caught by EeRb-144's Late Period inhabitants during the spring to mid-summer.

Given their apparent spring to mid-summer harvest date, the presence of largescale sucker, longnose sucker, and northern pikeminnow in the Late Period sample suggests EeRb-144's Late Period fishery occurred during this period. However, since Chinook salmon runs in the nearby South Thompson River from August to late October (Brown et al. 1979:200), its presence in the sample suggests this fishery continued into at least the late-summer. The Late Period fishery's estimated spring to late summer timing is consistent with other seasonal indicators from EeRb-144, which suggest it was occupied sometime between the spring and summer (Nicholas et al. *forthcoming*). Although the data from the Middle Period are sparse, the presence of multiple sucker samples in the Middle Period sample *possibly* suggests a similar spring to summer timing for that period's fishery. However, this is only a tentative hypothesis since pinpointing the timing of the fishery with any confidence is not possible due to the Middle Period sample's unrepresentative nature.

6.1.4. Comparison with the Thompson River Post's (EeRc-22) Indigenous Fishery

The Thompson River Post (EeRc-22) site is a 19th century Euro-Canadian fur trading post and a Secwepemc winter pithouse village located just west of EeRb-144 along the shoreline of the North Thompson River. Excavations of two of the pithouses at site recovered 243 fish remains that could be identified to the genus or species-level through morphological analysis (Carlson 2006:Table 2). The majority of these remains were identified as Pacific salmon, which account for 71.60% of the assemblage (Carlson 2006:Table 2). The high relative abundance of salmon in this assemblage sets it apart from the sample of identified Late Period fish remains from EeRb-144, which has a markedly lower relative abundance of salmon (3.57% or 18.18% if samples morphologically identified as salmonids are included) (Figure 6.1). This large difference in the relative abundance of salmon in these two assemblages indicates the importance

of salmon to EeRb-144's Late Period fishery and the Thompson River Post's indigenous fishery greatly differed.

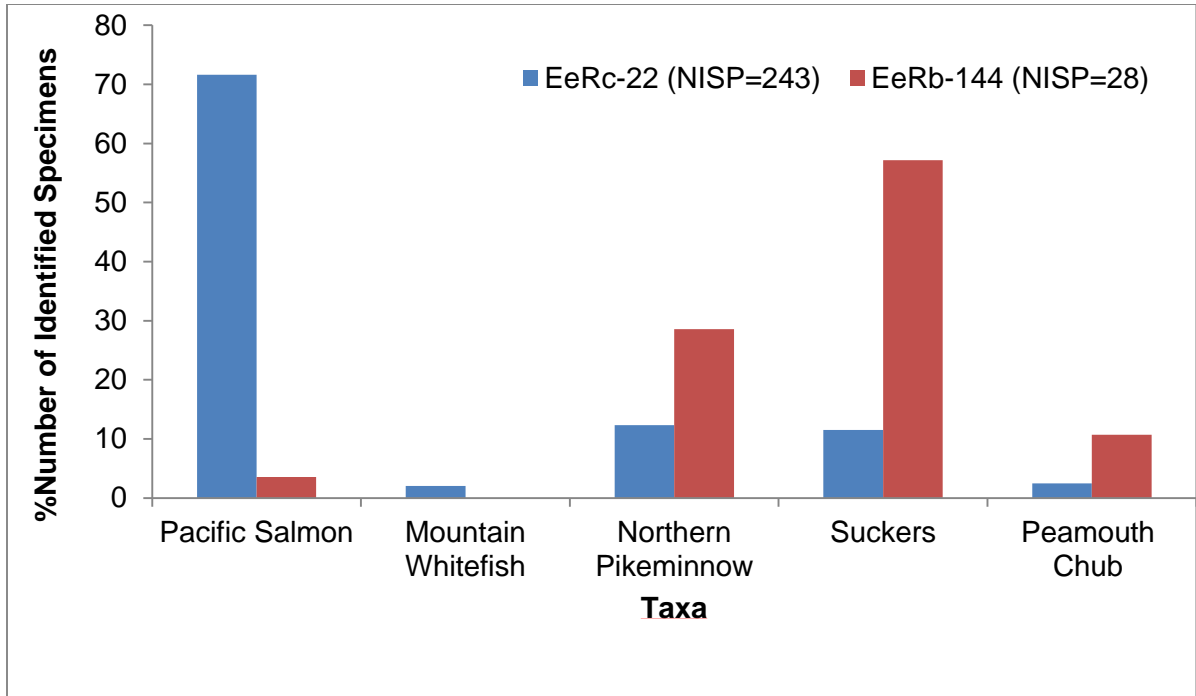


Figure 6.1. Relative of abundance of different fish taxa in the assemblage of fish remains from the Thompson River Post (EeRc-22) and the sample of identified Late Period remains from EeRb-144.

The differing importance of salmon to these two indigenous fisheries might reflect seasonal variation in the abundance of salmon in the Thompson River system. During the fall, salmon runs throughout much of eastern Thompson River system are at their peak (Brown et al. 1979). Throughout the Plateau it was during these periods of peak salmon abundance that indigenous peoples historically caught much of the fish that was stored to provision winter pithouse villages (Alexander 1992:160-161). Consequently, the indigenous fishery that supplied the pithouse village at the Thompson River Post likely had access to these large salmon stocks. Conversely, during the spring to late summer when EeRb-144's Late Period fishery is hypothesized to have occurred much fewer salmon are present in the Thompson River system (Brown et al. 1979). As such the Late Period fishery at EeRb-144 possibly had more restricted access to salmon than the Thompson River Post's fishery, which possibly explains why it harvested salmon to a lesser degree. Instead, it focused to a greater extent than Thompson River Post's fishery on two resident taxa—sucker and northern pikeminnow—that were easily accessible

during the spring and summer. This is reflected by the higher relative abundance of these taxa at EeRb-144 (Figure 6.1). Collectively, these inferences suggest the importance of salmon to Late Holocene indigenous fisheries in the Kamloops area seasonally shifted as its abundance and accessibility ebbed and flowed. To further test this hypothesis, more data concerning the species composition of the assemblage of fish remains from Late Holocene sites occupied during different seasons is needed.

6.2. Utility of Universal Primers and Mini-Barcodes in Species Identification of Archaeological Fish Remains

6.2.1. Utility of Universal Primers

To facilitate the DNA-based identification of fish remains from EeRb-144 that could only be identified morphologically as bony fish, this study sought to develop universal primers targeting a 220 bp fragment of COI. Between the ancient and modern DNA analyses, this fragment was amplified from 15 bony fish species using the primers (F271 and R271) that were developed in response to this goal. These 15 species belong to 8 genera and 6 families that in many cases diverged hundreds of millions of years ago (Peng et al. 2009).²⁰ Although these primers were tested against a limited number of species, their ability to amplify DNA from widely divergent taxa suggests they exhibit a high degree of universality.

Through the analysis of the fragment targeted by these primers, it was possible to assign species or genus-level identification to each ancient and modern sample that yielded DNA. These identifications, as discussed in Chapter 3, appear to be reliable, as the identifications assigned to the modern specimens matched their known genus or species. Collectively, these observations indicate these primers are useful for the reliable identification of fish remains to at least the genus-level. Moreover, these primers' universality means nothing needs to be known about the taxonomy of these remains

²⁰ For example, the divergence between the Euteleostei clade, which includes the Salmonidae family, and the Otocephala clade, which includes the Catostomidae and Cyprinidae families, is estimated to have occurred between 230 to 307 million years ago (Peng et al. 2009:336).

other than that they are bony fish in order to obtain these identifications. This makes these primers, as intended, particularly useful for the identification of archaeological or palaeontological fish remains that cannot be morphologically identified to lower taxonomic levels.

Limitations of Primers

While the results of this study demonstrate that these universal primers can be used to identify archaeological fish remains, the results also highlight their limitations. The relatively low success rate of the PCR amplifications of aDNA conducted with these primers indicates their ability to amplify DNA from specimens with poor DNA preservation is low. This finding is likely a function of the relatively large size of the fragment targeted by these primers. Due to increased DNA fragmentation, relatively large fragments, like the one targeted by these primers, may not be preserved in remains with poorly preserved DNA (cf. Pääbo et al. 2004:647-648). Consequently, identifying remains from which DNA could not be amplified with the F271/R271 primer pair or are suspected to have extensive DNA degradation will require the use of primers targeting shorter fragments. Since they amplify a considerably shorter fragment and can discriminate between species, Jordan et al's (2010) universal primers targeting the 12S gene could be used to identify such remains. However, as previously discussed, these primers' ability to amplify contaminant mouse and human DNA (Grier et al. 2013:550) complicates their use in aDNA analysis. Hopefully, future studies will address this issue by developing new universal primers that target very short fragments of DNA, but exclude the amplification of non-fish DNA.

6.2.2. Discriminatory Power of Mini-Barcodes

In this study, species-level identifications were successfully assigned to modern and archaeological fish samples through the analysis of mini-barcodes. The successful identification of these samples to the species-level demonstrates that mini-barcodes can be used to discriminate between species of fish. This finding is consistent with the results of previous studies, which have found that salmonids (Rasmussen et al. 2009), catfishes (Bhattacharjee and Ghosh 2014), and an array of Australian fish taxa (Hajibabaei et al. 2006) can be differentiated with mini-barcodes. The discrimination of

fish species in this study and previous studies through the analysis of mini-barcodes indicates they are useful markers for species identification of fish specimens, including archaeological remains. That being said, the results of this study also demonstrate that mini-barcodes cannot discriminate all species of fish. As previously noted, largescale and Utah sucker could not be readily differentiated using mini-barcodes due to the low degree of genetic divergence between these taxa in the COI barcode region.

Ward et al. (2009) suggests a lack of interspecific divergence in the COI barcode region may be the result of the erroneous classification of conspecific individuals as different species, hybridization, or incomplete lineage sorting. In the case of largescale and Utah sucker, the lack of divergence between these taxa is likely a product of incomplete lineage sorting. Utah and largescale sucker are closely related sister species (Smith 1978:36). Consequently, they may have not had enough time to accumulate sufficient genetic differences to undergo lineage sorting. This suspected incomplete lineage sorting is evidenced by the A→G transition Utah sucker and some largescale suckers share at position 68 in the mini-barcode targeted by the sucker-specific primers.

The inability to discriminate between largescale and Utah sucker due to this suspected incomplete lineage that is a product of their close relatedness has significant implications. It indicates COI-based mini-barcodes alone cannot be used to differentiate recently diverged species. Previous studies have made a similar observation. April et al. (2011) and Hubert et al. (2008) both found that discriminating between recently diverged (read: closely related) North American freshwater fish species using complete COI-based barcodes is oftentimes not possible.

Two-Marker Approach to Fish Species Identification

This study's and previous studies' failure to discriminate closely related species using mini-barcodes indicates the differentiation of sister taxa may require the analysis of other more variable markers. This indicates the universal primers designed herein may need to be used in conjunction with primers targeting more variable regions in order to assign species-level identifications to fish remains. Potentially, the mini-barcode targeted by the universal primers could be used to obtain an initial species or genus-

level identification. A fragment of a more variable marker could then be amplified using genus-specific primers and used to confirm these initial species-level identifications or refine genus-level identifications to the species-level.

Markers that could potentially be used to refine or confirm initial identifications assigned to fish remains through mini-barcode analysis include ATPase and D-loop. In general, both of these markers are more variable than COI (Page and Hughes 2010:2215). Thus, they may be able to discriminate closely related species that cannot be differentiated using COI. However, it is likely no single marker will be useful for confirming or refining the initial identifications assigned to all fish remains irrespective of their taxonomy. Since the degree of interspecific variability exhibited by different markers is not uniform across taxonomic groups (Page and Hughes 2010) different groups will likely require the use of different secondary markers. Consequently, improving the discriminatory power of DNA-based species identification will require further research into identifying markers suitable for the identification of species belonging to different taxonomic groups.

In addition to aiding in the identification of remains, this proposed two-marker approach potentially has an added benefit. Yang and Speller (2006:607) argue that a two-marker approach to species identification is useful for detecting contamination. Disagreement between the taxonomic identifications suggested by the two markers is a clear sign that contamination has occurred (Yang and speller 2006:607). Since contamination is a major concern in aDNA research (Pääbo et al. 2004:654-655), the two-marker approach's ability to potentially aid in the detection of contamination is not an insignificant benefit.

6.3. Summary and Conclusion

This study attempted to use ancient DNA analysis to identify Middle and Late Period fish remains from EeRb-144, a multicomponent campsite located in British Columbia's Interior Plateau Region. In total, 47 of the 86 fish remains from EeRb-144 that were subjected to aDNA analysis were identified to species or genus-level. The successful identification of these remains demonstrates the feasibility of using aDNA

analysis to assign species and other taxonomic identifications to archaeological fish remains. Moreover, the universal primers developed in this study enable the application of this species identification approach to fragmented fish remains that can only be morphologically identified as bony fish.

Although this study demonstrates the feasibility of using aDNA analysis to identify a range of fish remains, this study also highlights one of the major drawbacks of this approach. Specifically, the large number of remains that could not be identified due to PCR failure indicates the usefulness of DNA-based species identification is reduced when DNA preservation is poor. In such situations, the morphological analysis of remains lacking preserved DNA may provide additional taxonomic identifications that can supplement those obtained through aDNA analysis. For example, in this study five samples that could not be identified through aDNA analysis due to poor DNA preservation were identified as salmonids through morphological analysis. Likewise, the results of this and other studies (e.g., Nicholls et al. 2003; Yang et al. 2004) indicate aDNA analysis can be used to assign species identification to remains that cannot be identified through morphological analysis. Consequently, an approach to the taxonomic identification of fish remains that integrates these analyses can potentially maximize the number of fish remains that can be identified.

The species identification of the fish remains from EeRb-144 that yielded DNA was accomplished through the analysis of cytochrome *c* oxidase I-based mini-barcodes. This indicates COI-based mini-barcodes are useful markers for the species identification of fish remains. This is consistent with the results of previous studies (Bhattacharjee and Gosh 2014; Hajibabaei et al. 2005; Rasmussen et al. 2009), which found mini-barcodes can be used to discriminate between species of fish. Nonetheless, this study's failure to discriminate between two sister taxa (largescale and Utah sucker) using mini-barcodes indicates COI may not be useful as a marker for species identification in every instance. As such, other more variable markers may need to be analyzed in conjunction with COI in order to identify fish remains from sister taxa. Consequently, improving aDNA analysis' ability to discriminate will require continued research aimed at identifying more variable markers useful for the discrimination of closely related species. Once these markers have been identified new primers targeting them will have to be developed.

In addition to these methodological insights, the aDNA analysis of fish remains from EeRb-144 has also shed light on the nature of the Middle (7,000 to 4,500 years BP) and Late (4,500 to 200 years BP) Period fishery at the site. The results of this analysis indicated EeRb-144's Late Period fishery harvested a range of fish species that were locally-available abundant. However, this fishery was primarily focused on largescale sucker and northern pikeminnow. In addition, the life history of the species represent in the sample of identified Late Period remains that were suggests this fishery occurred sometime between spring and late-summer. Comparatively less can be said about Middle Period fishery due to the small number of Middle Period remains that were identified. However, the species identifications that were obtained indicate it too harvested a range of species, all of which were also harvested by the Late Period fishery. The fact that both of these fisheries harvested the same size indicates that there was some long-term continuity in fishing practices at the site. Continued identification of fish remains from EeRb-144 will ultimately provide a more detailed understanding of the temporal trends in fish use at the EeRb-144 and the site's Middle Period fishery.

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Appendix A.

Fish Communities of the Fraser River System

Table A.1. Composition of the fish communities in the Middle and Upper Fraser and Thompson River systems. Table compiled using data from Bouchard and Kennedy (1992), British Columbia Ministry of the Environment (2013), McPhail and Carveth (1993), Speller et al. (2005), and Welch and Till (1996).

Common Name	Scientific name	Middle Fraser	Upper Fraser	Thompson
Pacific Lamprey	<i>Entosphenus tridentatus</i>	+	—	+
White Sturgeon	<i>Acipenser transmontanus</i>	+	+	+
Chiselmouth	<i>Acrocheilus alutaceus</i>	+	—	+
Goldfish	<i>Carassius auratus</i>	—	—	
Lake Chub	<i>Couesius plumbeus</i>	+	+	+
Common Carp	<i>Cyprinus carpio</i>		—	
Brassy Minnow	<i>Hybognathus hankinsoni</i>	+	—	—
Peamouth	<i>Mylocheilus caurinus</i>	+	+	+
Northern Pikeminnow	<i>Ptychocheilus oregonensis</i>	+	+	+
Longnose Dace	<i>Rhinichthys cataractae</i>	+	+	+
Leopard Dace	<i>R. falcatus</i>	+	—	+
Redside Shiner	<i>Richardsonius balteatus</i>	+	+	+
Longnose Sucker	<i>Catostomus catostomus</i>	+	+	+
Bridgelip Sucker	<i>C. columbianus</i>	+	—	+
White Sucker	<i>C. commersoni</i>	+	—	+
Largescale Sucker	<i>C. macrocheilus</i>	+	+	+
Mountain Sucker	<i>C. platyrhynchus</i>	—	—	+
Coastal Cutthroat Trout	<i>Oncorhynchus clarki clarki</i>	R	—	—
Westslope Cutthroat Trout	<i>O. clarki lewisi</i>	—	—	+
Pink Salmon	<i>O. gorbuscha</i>	+, H?	—	+, H?
Chum Salmon	<i>O. keta</i>	+	—	R
Coho Salmon	<i>O. kisutch</i>	+	—	+
Rainbow Trout	<i>O. mykiss</i>	+	+	+
Sockeye Salmon	<i>O. nerka</i>	+	+	+
Chinook Salmon	<i>O. tshawytscha</i>	+	+	+
Bull Trout	<i>Salvelinus confluentus</i>	+	+	+

Table A.1. Composition of the fish communities in the Middle and Upper Fraser and Thompson River systems. Table compiled using data from Bouchard and Kennedy (1992), British Columbia Ministry of the Environment (2013), McPhail and Carveth (1993), Speller et al. (2005), and Welch and Till (1996). (continued)

Common Name	Scientific name	Middle Fraser	Upper Fraser	Thompson
Brook Trout	<i>S. fontinalis</i>		—	
Lake Trout	<i>S. namaycush</i>	+	+	+
Lake Whitefish	<i>Coregonus clupeaformis</i>	+	+	+
Pygmy Whitefish	<i>Prosopium coulteri</i>	+	+	+
Mountain Whitefish	<i>P. williamsoni</i>	+	+	+
Burbot	<i>Lota lota</i>	+	+	+
Coastrange Sculpin	<i>Cottus aleuticus</i>	+	—	—
Prickly Sculpin	<i>C. asper</i>	+	—	+
Slimy Sculpin	<i>C. cognatus</i>	+	+	+
Torrent Sculpin	<i>C. rhotheus</i>	—	—	+

Legend: +=native and present, —= absent, |=introduced and present, R=rare/strays, H?=possibly historically absent

Appendix B.

Fish Communities of the Columbia River System

Table B.1. Composition of the fish communities in the Upper and Lower Columbia, Flathead, Kettle, Upper and Lower Kootenay, Okanagan, and Similkameen River Systems. Table compiled using data from McPhail and Carveth (1993), McPhail (2007), and Rae (2005).

Common Name	Scientific Name	Upper Columbia	Lower Columbia	Flathead	Kettle	Upper Kootenay	Lower Kootenay	Okanagan	Similkameen
White Sturgeon	<i>Acipenser transmontanus</i>	—	+	—	—	—	+	E, ?	—
Chiselmouth	<i>Acrocheilus alutaceus</i>	+	+, B	—	+	—	+, B	+, B	+, B
Goldfish	<i>Carassius auratus</i>	—		—	—	—	—		, ?
Lake Chub	<i>Couesius plumbeus</i>	—	+	—	+	—	+	+	+, B
Common Carp	<i>Cyprinus carpio</i>	—		—		—			—
Peamouth Chub	<i>Mylocheilus caurinus</i>	+	+	—	+	+	+	+	+, B
Northern Pikeminnow	<i>Ptchocheilus oregonesis</i>	+	+	—	+	+	+	+	+, B
Longnose Dace	<i>Rhinichthys cataractae</i>	+	+	—	+, B	+	+	+	+
Leopard Dace	<i>R. falcatus</i>	—	+, B	—	+, B	—	+	+	+, B
Speckled Dace	<i>R. osculus</i>	—	+, B	—	+	—	—	—	—
Umatilla Dace	<i>R. umatilla</i>	—	+, B	—	+, B	—	+	—	+, B
Redside Shiner	<i>Richardsonius balteatus</i>	+	+	—	+	+	+	+	+, B
Tench	<i>Tinca tinca</i>	—		—	, B	—	—	, B	—
Longnose Sucker	<i>Catostomus catostomus</i>	+	+	+	+	+	+	+	+, B
Bridgelip Sucker	<i>C. columbianus</i>	—	+	—	—	—	+	+, B	+, B

Table B.1. Composition of the fish communities in the Upper and Lower Columbia, Flathead, Kettle, Upper and Lower Kootenay, Okanagan, and Similkameen River Systems. Table compiled using data from McPhail and Carveth (1993), McPhail (2007), and Rae (2005). (continued)

Common Name	Scientific Name	Upper Columbia	Lower Columbia	Flathead	Kettle	Upper Kootenay	Lower Kootenay	Okanagan	Similkameen
Largescale Sucker	<i>C. macrocheilus</i>	+	+	—	+	+	+	+	+, B
Mountain Sucker	<i>C. platyrhynchus</i>	—	—	—	—	—	—	—	+, B
Black Bullhead	<i>Ameiurus melas</i>	—		—	—	—		, B	—
Brown Bullhead	<i>A. nebulosus</i>	—	?	—	—	—	—	?	—
Westslope Cutthroat Trout	<i>Oncorhynchus clarki lewisi</i>	+	+	+	-	+	+	—	
Pink Salmon	<i>O. gorbuscha</i>	—	—	—	—	—	—	E?	—
Chum Salmon	<i>O. keta</i>	—	—	—	—	—	—	E?	—
Coho Salmon	<i>O. kisutch</i>	—	—	—	—	—	—	E?	—
Rainbow Trout	<i>O. mykiss</i>	+	+	+	+	+	+	+	+
Sockeye Salmon	<i>O. nerka</i>	—	+	—		—	+	+	+, B
Chinook Salmon	<i>O. tshawytscha</i>	E	E,	—	—	—	—	+, B	—
Brown Trout	<i>Salmo trutta</i>	—	—	—		—	—	—	
Bull Trout	<i>Salvelinus confluentus</i>	+	+	+	—	+	+	—	—
Brook Trout	<i>S. fontinalis</i>	—		—		—			
Lake Trout	<i>S. namaycush</i>	—		—	—	—			—
Arctic Grayling	<i>Thymallus arcticus</i>	—	—		—	—	—	-	—
Lake Whitefish	<i>Coregonus clupeaformis</i>	—		—	—	—			—
Pygmy Whitefish	<i>Prosopium coulteri</i>	+	+	—	—	+	+	+	—

Table B.1. Composition of the fish communities in the Upper and Lower Columbia, Flathead, Kettle, Upper and Lower Kootenay, Okanagan, and Similkameen River Systems. Table compiled using data from McPhail and Carveth (1993), McPhail (2007), and Rae (2005). (continued)

Common Name	Scientific Name	Upper Columbia	Lower Columbia	Flathead	Kettle	Upper Kootenay	Lower Kootenay	Okanagan	Similkameen
Mountain Whitefish	<i>P. williamsoni</i>	+	+	+	+	+	+	+	+, B
Burbot	<i>Lota lota</i>	+	+	—	—	+	+	+	+, B
Rocky Mountain Sculpin	<i>Cottus sp.</i>	—	—	+	—	—	—	—	—
Prickly Sculpin	<i>Cottus asper</i>	—	+, B	—	+, B	—	+, B	+	+, B
Columbia Sculpin	<i>C. hubbsi</i>	—	+, B	—	+, B	—	+, B	—	+, B
Slimy Sculpin	<i>C. cognatus</i>	+	+	+	+	+	+	+	+, B
Shorthead Sculpin	<i>C. confusus</i>	—	+, B	—	+, B	—	+, B	—	—
Torrent Sculpin	<i>C. rhotheus</i>	+	+	—	B	+	+	B	B
Pumpkinseed	<i>Lepomis gibbosus</i>	—	I	—	I,B	—	I	I,B	—
Bluegill	<i>Lepomis macrochirus</i>	—	—	—	—	—	—	I	—
Smallmouth bass	<i>Micropterus dolomieu</i>	—	I	—	I,B	—	—	I	—
Largemouth Bass	<i>M. salmoides</i>	—	I	—	I,B	—	I	I	—
Black Crappie	<i>Pomoxis nigromaculatus</i>	—	—	—	—	—	—	I,B	—
Yellow Perch	<i>Perca flavescens</i>	—	I	—	—	I	I	I	I
Walleye	<i>Stizostedion vitreum</i>	—	I,B	—	I,B	I	—	—	—

Legend: +=native and present, —= absent, I=introduced and present, I?=possibly introduced but sightings unconfirmed, B=present below barriers to upstream dispersal, E=extirpated, E?=possibly historically present, ?= unconfirmed sightings

Appendix C.

The Baker Site (EdQx-43)

To date, the only Middle Period site that has produced evidence for structures and food storage facilities is the Baker site (EdQx-43). Excavations of Zone 3 at the site by I.R. Wilson Consultants (1992) uncovered three pithouses associated with internal and external food storage pits. These pithouses were occupied between fall and spring, indicating their inhabitants, unlike their contemporaries, were semi-sedentary (I.R. Wilson Consultants 1992). The presence of food storage pits indicates their semi-sedentary lifestyle was supported by a delayed-return economy unique during the Middle Period to the Baker site. The presence of salmon remains in food storage pits at the site indicates suggest salmonids were being stored as part of this delay-return economy (I.R. Wilson Consultants 1992). In addition, the recovery of salmonid bones from an exterior pit that resembles historic Interior Salish smudge pits suggests some of this stored salmon may have been smoked (I.R. Wilson Consultants 1992).

Radiocarbon dating places the occupation of this component at the Baker site towards the end of the Lochnore Phase, between 4,400 and 4,200 years BP (I.R. Wilson Consultants 1992). However, due to its uniqueness, the nature of the relationship between Zone 3 at the Baker site and the Lochnore Phase has been a topic of considerable discussion. Some suggest the housepits at the Baker site reflects a shift by Lochnore Phase groups to semi-sedentism (e.g., Rousseau 2004; Stryd and Rousseau 1996). Others, citing the absence of diagnostic Lochnore artifacts in Zone 3, contend it reflects a temporary intrusion into the region by semi-sedentary people from the Columbia Plateau or southern British Columbia coast (e.g., Prentiss and Kuijt 2012:62-63; Prentiss and Kuijt 2004; I.R. Wilson Consultants 1992).

Subsistence Pattern

The inhabitants of the pithouses at the Baker site had a fairly broad spectrum diet. Like their contemporaries, they hunted a range of terrestrial animals, including deer, as well as lesser amounts of beaver, bear, elk, marmot, muskrat, porcupine, and rabbit (I.R. Wilson Consultants 1992:178). They also took turtles and a variety of birds, and

gathered freshwater mussels (I.R. Wilson Consultants 1992). However, unlike their contemporaries, their main source of protein appears to have been fish, rather than terrestrial resources (I.R. Wilson Consultants 1992). Fish consumed by the Baker site's inhabitants include, in order of decreasing importance, medium salmonids (likely sockeye salmon), small salmonids (likely trout and kokanee), sucker, whitefish, burbot, and potentially cyprinids (I.R. Wilson Consultants 1992:179). To the best of my knowledge, burbot and whitefish have not been identified in other Middle Period assemblages. As such, their presence at the Baker site seemingly suggests its fishery was more diverse than its contemporaries.

The apparent increased importance and taxonomic diversity of fish at the Baker site might reflect differences in recovery techniques rather than behavioural differences between its occupants and their contemporaries. In the past, researchers have tended to sift Middle Period deposits using a 6.35 mm mesh screen (Huculak 2004). In contrast, Zone 3 deposits at the Baker site were screened using a 3.18 mm mesh screen (I.R. Wilson Consultants 1992:29-30). Use of a smaller screen size has repeatedly been found to increase both the size, and taxonomic diversity of archaeological fishbone assemblage (Gordon 1990; Nagaoka 1994, 2005; Partlow 2006; Zohar and Belmaker 2005). This is because the use of a smaller screen size results in the increased recovery of remains from small-bodied taxa and individuals, and small or narrow elements (Gordon 1990; Nagaoka 1994, 2005). Consequently, the larger amount and the increased range of fish at the Baker site could be a reflection of the use of a smaller mesh screen at the site. To resolve this issue, future excavations of Middle Period sites, as previously recommended by Huculak (2004:124), should utilize 3.18 mm mesh screens to sieve deposits.

Fishing Technology

The fishing technology used by the inhabitants of the Baker site differed from that employed by other Middle Period groups. The net sinkers commonly found at other Middle Period sites are absent from this component (I.R. Wilson Consultants 1992:115-116). Instead, a fishing technology that utilizes bone appears to have played a more prominent role in the site's fishery. Six of the bone uni-points that possibly represent fish

hooks barbs present at other Middle Period sites are also present at the Baker site (I.R. Wilson Consultants 1992). This suggest the site's inhabitants, like their contemporaries, caught fish using hook and line. However, unlike their contemporaries, they also appear to have utilized bone spear technology to capture fish, as examples of such technology were found at the Baker site. Fish spear technology recovered from the site includes a bone unipoint potentially used to arm a leister, and a unilaterally barbed antler point that resemble historic fish spears (I.R. Wilson Consultants 1992).

Appendix D.

Provenience of the Analyzed Samples

Table D.1. Provenience and estimated age of the analyzed fish remains from EeRb-144.

Sample	Period	Unit	Level
FH1	Late	N20 E16	3
FH2	Middle	N12 E5	6
FH3	Late	N26 E21	6
FH4	Late	N16 E13	3
FH5	Middle/Late	N11 E9	8
FH6	Late	N15 E13	4
FH7	Late	N15 E13	4
FH8	Late	N20 E17	4
FH9	Late	N22 E20	3
FH10	Late	N20 E17	4
FH11	Late	N11 E9	4
FH12	Late	N29 E24	4
FH13	Middle/Late	N12 E11	4
FH14	Late	N20 E17	3
FH15	Late	N30 E24	5
FH16	Middle	N13 E11	6
FH17	Late	N21 E21	6
FH18	Late	N19 E16	4
FH19	Late	N30 E24	4
FH20	Middle/Late	N13 E15	9
FH21	Late	N4 E2	7
FH22	Late	N8 W0	3
FH23	Middle/Late	N12 E11	3
FH24	Late	N30 E25	5
FH25	Late	N20 E17	5
FH26	Late	N12 E14	4
FH27	Late	N20 E17	4
FH28	Late	N26 E24	4

Table D.1. Provenience and estimated age of the analyzed fish remains from EeRb-144. (continued)

Sample	Period	Unit	Level
FH29	Late	N9 W1	3
FH30	Late	N20E16	4
FH31	Late	N30 E24	5
FH32	Late	N13 E8	3
FH33	Middle	N18 E18	8
FH34	Middle?	N17 E18	5
FH35	Late	N26 E21	5
FH36	Late	N22 E14	4
FH37	Late?	N26 E24	6
FH38	Middle/Late	N13 E14	4
FH39	Late	N8 E2	6
FH40	Late	N26 E24	3
FH41	Late	N9 E11	2
FH42	Late	N12 E15	5
FH43	Late	N20 E17	4
FH44	Late	N29 E27	5
FH45	Late	N8 E9	5
FH46	Late	N29 E27	5
FH47	Late	N29 E25	5
FH48	Late	N26 E24	4
FH49	Late	N30 E24	4
FH50	Late	N22 E21	5
FH51	Late	N22 E21	3
FH52	Late	N20 E17	4
FH53	Late	N29 E27	5
FH54	Late	N12 E9	3
FH55	Late	N30 E24	4
FH56	Late	N26 E24	2 to 5
FH57	Late	N5 E3	5
FH58	Middle	N10 E9	8
FH59	Middle/Late	N17 E18	4
FH60	Late	N30 E25	5

Table D.1. Provenience and estimated age of the analyzed fish remains from EeRb-144. (continued)

Sample	Period	Unit	Level
FH61	Middle	N11 E7	10
FH62	Late	N8 W0	3
FH63	Late?	N26 E23	5
FH64	Late	N10 E9	3
FH65	Late	N12 E8	3
FH66	Middle/Late	N13 E14	4
FH67	Late	N12 E15	4
FH68	Late	N13 E8	4
FH69	Late	N13 E11	4
FH70	Middle	N9 E3	7
FH71	Late	N20 E17	4
FH72	Late	N26 E20	4
FH73	Late	N9 E11	2
FH74	Middle/Late	N11 E9	9
FH75	Late	N20 E17	5
FH76	Late?	N20 E16	4
FH77	Middle/Late	N18 E18	5
FH78	Middle?	N13 E8	6
FH79	Middle?	N13 E15	8
FH80	Late	N26 E24	2 to 5
FH81	Middle/Late	N22 E14	8
FH82	Late?	N10 E11	6
FH83	Late?	N7 E4	8
FH84	Late	N29 E24	4
FH85	Late?	N26 E24	6
FH86	Late	N15 E13	4

Appendix E.

DNA and Morphology-based Taxonomic Identifications Assigned to the Analyzed Samples

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis.

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH1	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH2	Bone fragment	Actinopterygii	No DNA	No DNA	No DNA
FH3	Quadrate	<i>Ptychocheilus oregonensis</i> cf.	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH4	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH5	Bone fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH6	Maxilla fragment	<i>Ptychocheilus oregonensis</i>	No DNA	No DNA	No DNA
FH7	Vertebrae fragment	Salmonidae	No DNA	No DNA	No DNA
FH8	Radial	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i> (with damage) ¹	<i>Catostomus macrocheilus</i>
FH9	Bone fragment	Actinopterygii	No DNA	No DNA	No DNA

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological analysis and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH10	Vertebrae	Actinopterygii cf.	No DNA	No DNA	No DNA
FH11	Bone fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH12	Precaudal Vertebrae	Gadidae cf.	No DNA	No DNA	No DNA
FH13	Vertebrae	Ptychocheilus oregonensis cf.	No DNA	No DNA	No DNA
FH14	Vertebrae fragment	Actinopterygii cf.	<i>Catostomus</i> sp. (with damage) ¹	<i>Catostomus macrocheilus</i> (with damage) ²	<i>Catostomus macrocheilus</i>
FH15	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH16	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH17	Unidentified fragment	Actinopterygii cf.	<i>Mylocheilus caurinus</i>	—	<i>Mylocheilus caurinus</i>
FH18	Radial	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH19	Radial	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH20	Premaxilla	Actinopterygii cf.	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH21	Quadrate	<i>Ptychocheilus oregonensis</i> cf.	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH22	Tooth	Salmonidae	No DNA	No DNA	No DNA
FH23	Radial	Actinopterygii	No DNA	No DNA	No DNA
FH24	Vertebrae	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH25	Caudal vertebrae	<i>Ptychocheilus oregonensis</i>	<i>Catostomus</i> sp.	<i>Catostomus catostomus</i> (with damage) ¹	<i>Catostomus catostomus</i>

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH26	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH27	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH28	Vertebrae	Cypriniformes	No DNA	No DNA	No DNA
FH29	Unidentified fragment	Actinopterygii	<i>Mylocheilus caurinus</i>	—	<i>Mylocheilus caurinus</i>
FH30	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp. (with damage) ¹	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH31	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH32	Vertebrae	Cypriniformes	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH33	Unidentified fragment	Actinopterygii	<i>Mylocheilus caurinus</i>	—	<i>Mylocheilus caurinus</i>
FH34	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH35	Ultimate vertebrae	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH36	Unidentified fragment	Actinopterygii cf.	No DNA	No DNA	No DNA
FH37	Vertebrae fragment	Salmonidae	<i>Oncorhynchus mykiss</i>	—	<i>Oncorhynchus mykiss</i>
FH38	Vertebrae	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH39	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH40	Vertebrae	Cypriniformes	No DNA	No DNA	No DNA
FH41	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH42	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH43	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH44	Vertebrae	Cypriniformes	No DNA	No DNA	No DNA
FH45	Vertebrae fragment	Salmonidae	No DNA	No DNA	No DNA
FH46	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH47	Vertebrae	Gadidae cf.	No DNA	No DNA	No DNA
FH48	Vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH49	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH50	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH51	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH52	Tooth	Actinopterygii cf.	No DNA	No DNA	No DNA
FH53	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH54	Vertebrae	Cypriniformes	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH55	Vertebrae	Cypriniformes	No DNA	No DNA	No DNA
FH56	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH57	Vertebrae	Cypriniformes	No DNA	No DNA	No DNA
FH58	Unidentified fragment	Cypriniformes	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH59	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	No DNA	<i>Catostomus</i> sp.
FH60	Pharyngeal	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH61	Spine fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus catostomus</i>	<i>Catostomus catostomus</i>
FH62	Tooth	Salmonidae	No DNA	No DNA	No DNA
FH63	Vertebrae (atlas?)	Actinopterygii	<i>Mylocheilus caurinus</i>	—	<i>Mylocheilus caurinus</i>
FH64	Maxilla fragment	<i>Ptychocheilus oregonensis</i> cf.	<i>Catostomus</i> sp.	No DNA	<i>Catostomus</i> sp.
FH65	Vertebrae	Cypriniformes	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH66	Vertebrae (atlas?)	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH67	Unidentified fragment	Actinopterygii cf.	No DNA	No DNA	No DNA
FH68	Unidentified fragment	Actinopterygii	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH69	Vertebrae fragment	Salmonidae	<i>Oncorhynchus tshawytscha</i>	—	<i>Oncorhynchus tshawytscha</i>
FH70	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	No DNA	<i>Catostomus</i> sp.
FH71	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	No DNA	<i>Catostomus</i> sp.
FH72	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i> (with damage) ¹	<i>Catostomus macrocheilus</i>
FH73	Vertebrae fragment	Actinopterygii	No DNA	No DNA	No DNA
FH74	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH75	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	No DNA	<i>Catostomus</i> sp.
FH76	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i> (with damage) ²	<i>Catostomus macrocheilus</i>
FH77	Vertebrae fragment	Actinopterygii	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH78	Vertebrae fragment	Actinopterygii	<i>Ptychocheilus</i> sp.	<i>Ptychocheilus oregonensis</i>	<i>Ptychocheilus oregonensis</i>
FH79	Vertebrae fragment	Cypriniformes	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH80	Vertebrae fragment	Actinopterygii	<i>Mylocheilus caurinus</i>	—	<i>Mylocheilus caurinus</i>

Table E.1. Taxonomic identifications assigned to the analyzed fish remains through morphological and ancient DNA analysis. (continued)

Sample	Element	Morphological Taxonomic ID	DNA Taxonomic ID (universal primers)	DNA Taxonomic ID (genus-specific primers)	DNA Consensus Taxonomic ID
FH81	Spine	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH82	Unidentified fragment	Actinopterygii	<i>Catostomus</i> sp.	<i>Catostomus macrocheilus</i>	<i>Catostomus macrocheilus</i>
FH83	Unidentified fragment	Actinopterygii	No DNA	No DNA	No DNA
FH84	Vertebrae	Gadidae cf.	No DNA	No DNA	No DNA
FH85	Unidentified vertebrae	Actinopterygii	No DNA	No DNA	No DNA
FH86	Vertebrae fragment	Salmonidae	No DNA	No DNA	No DNA

¹C→T Transition, ²G→A Transition