ONE FISH, TWO FISH, OLD FISH, NEW FISH: INVESTIGATING DIFFERENTIAL DISTRIBUTION OF SALMON RESOURCES IN THE PACIFIC NORTHWEST THROUGH ANCIENT DNA ANALYSIS

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Abstract

DNA analysis was applied to approximately 60 ancient salmon remains (1200BP) from the archaeological site of Keatley Creek in British Columbia to examine the distribution of Pacific salmon species between housepits. The success rate of DNA extraction was over 90%, yielding three species of Pacific salmon: Chinook, Sockeye and Coho. Accurate salmon species identification using mitochondrial DNA refined theories of economic stratification and differential access to salmon resources at Keatley Creek. Additionally, the unique information made available by ancient DNA analysis offered insight into prehistoric salmon ecology and spawning behaviour in the region.

Reader's Summary

This study applied ancient DNA techniques to achieve accurate species identifications for the archaeological salmon remains recovered from a prehistoric pithouse village of Keatley Creek in British Columbia, Canada. Previous archaeological studies indicate that economic stratification within the community might have resulted in differential access to some preferred salmon species, such as sockeye and chinook (Berry 2000; Hayden 2000b). Unambiguous ancient DNA species identification now makes it possible to more accurately address the issue of early salmon resource utilization in the region. This study analyzed 60 salmon remains from two specialized structures and two residential structures in order to identify any species differences among bony salmon remains found within the structure. Although high success rates (over 90%) were obtained for ancient DNA tests, only three species (chinook, sockeye and coho salmon) were identified from the remains. Pink salmon was not identified among the tested sample, despite the fact that it was originally assumed to be a staple species for the site's native inhabitants. The absence of pink salmon in our sample significantly altered the picture of early salmon fishing activities in the region. As a result, the effects of economic stratification on differential access to the remaining so-called preferred species of sockeye and chinook within the four structures studied were not as dramatic as previously thought, although access to different sized salmon may still characterize some structures.

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Introduction

Differential access to resources and unequal distribution of wealth are critical factors in the development of complex societies (Hayden 1996; Wiessner 1996). Salmon has been a primary subsistence resource on the Northwest Coast and Plateau for millennia, and concepts of social stratification in this area have often revolved around access to this staple (Hayden 1992; 1997a; 2000b). Ethnographic studies of groups on the Northwest Plateau have suggested that certain species of salmon are preferred over others due to their varying attributes such as taste, oil content, and preservation abilities (Romanoff 1992; Teit 1900). Five species of Pacific salmon were generally available to groups on the Northwest Coast and Plateau, including chinook or spring salmon (*Oncorhynchus tshawytscha*), sockeye (*Oncorhynchus nerka*), coho (*Oncorhynchus kisutch*), pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*). Species such as chinook and sockeye have the highest oil content, and ethnographically, these species were generally preferred over pink and chum because of their rich taste (Teit 1906). The ethnographies also indicate that distribution of preferred species within communities may be related to aspects of economic stratification due to the ownership of certain prime fishing locations (Hayden 1997a; Romanoff 1992).

Archaeological investigations into the relationship between stratification and salmon species distribution in prehistoric societies has been hampered by the fact that accurate identification of salmon remains could not be made to the species level using morphological analysis alone (Berry 2000; Cannon 1987; Hayden 2000b). Ancient DNA

analysis, which focuses on species specific genetic markers, has the ability to accurately identify faunal remains at the species level, allowing for a more refined investigation of dietary practices (Brown, *et al.* 1993; Brown, *et al.* 1994; Newman, *et al.* 2002; Nicholls, *et al.* 2003; Poinar, *et al.* 2001; Yang, *et al.* 2005). Moreover, in recent years, DNA techniques for species identification of Pacific salmon remains from the Northwest Coast of North America have been developed and improved (Butler and Bowers 1998; Yang, *et al.* 2004).

This study employed DNA techniques to obtain accurate species identification of ancient salmon remains to investigate differential access to resources and economic stratification at Keatley Creek (EeR17), an archaeological site located in the Interior Plateau of southwest British Columbia. This site has already undergone extensive archaeological investigation, revealing social inequalities and complexity in the architecture of the site, and in the differential distribution of material culture and subsistence remains (Hayden 2000b; Hayden, *et al.* 1996; Lepofsky, *et al.* 1996). Moreover, the vertebral salmon remains at this site had been subjected to radiographic analysis in order to investigate species composition; the results of the study indicate that there were dramatic differences in terms of the distribution of preferred salmon species among residential structures of varying sizes (Berry 2000).

It was expected that the distribution of salmon remains at Keatley Creek would follow the same pattern as other subsistence remains and material culture at the site. For example, it was assumed that larger residential housepits and specialized structures would have the greatest diversity of salmon species, while the small housepits would demonstrate restriction in salmon species diversity. By examining the diversity of two specialized structures and two residential structures of differing sizes in terms of salmon species abundance and composition, it would be possible to identify any underlying

environmental and cultural factors that affected the differential distribution of preferred species within the community. The following three objectives were outlined for this study:

Research Objectives

1. To determine which salmon species are present in the remains recovered at Keatley Creek.

2. To determine the distribution of salmon species between two specialized structures and two residential housepits of different sizes.

3. To investigate how environmental and cultural factors influenced the distribution of salmon species between structures.

Archaeological Background

Keatley Creek

The Keatley Creek site was a winter pithouse village located near the modern town of Lillooet, BC (Figure 1). It is one of the largest prehistoric village sites in western Canada, extending over 800 m of dry terrace above the Fraser River. The eastern edge of the site is bordered by the Clear Range Mountains, and to the west by the erosional gorge of the Fraser River. Paleoethnobotanical evidence suggests that during the period of site occupation, the environment at Keatley Creek was quite similar to today (Lepofsky 2000). The mountainous area to the east is covered with Douglas fir, spruce and Ponderosa pine, while the terrace itself is covered by small cacti, shrubs, grasses and sagebrush (Mathewes 1978) (although Lepofsky (2000:127) notes that the presence of sagebrush may be a primarily historical introduction to the area).

Keatley Creek was inhabited by the Lillooet, a group linguistically classified as part of the Salishan family, occupying areas in the Southwest Interior of British Columbia (Kennedy and Bouchard 1978). Traditionally, the Lillooet have been divided into two cultural groups: The Upper Lillooet living along the Fraser River (from 25-Mile Village to Leon Creek) and in the Seton Anderson lakes area; and the Lower Lillooet, living along the Lillooet River system (Kennedy and Bouchard 1978). The Upper Lillooet, or "St'átl'imx" have usually been grouped with the Plateau culture area, while the Lower Lillooet "Lílwat" were classified as a part of the Northwest Coast culture area.

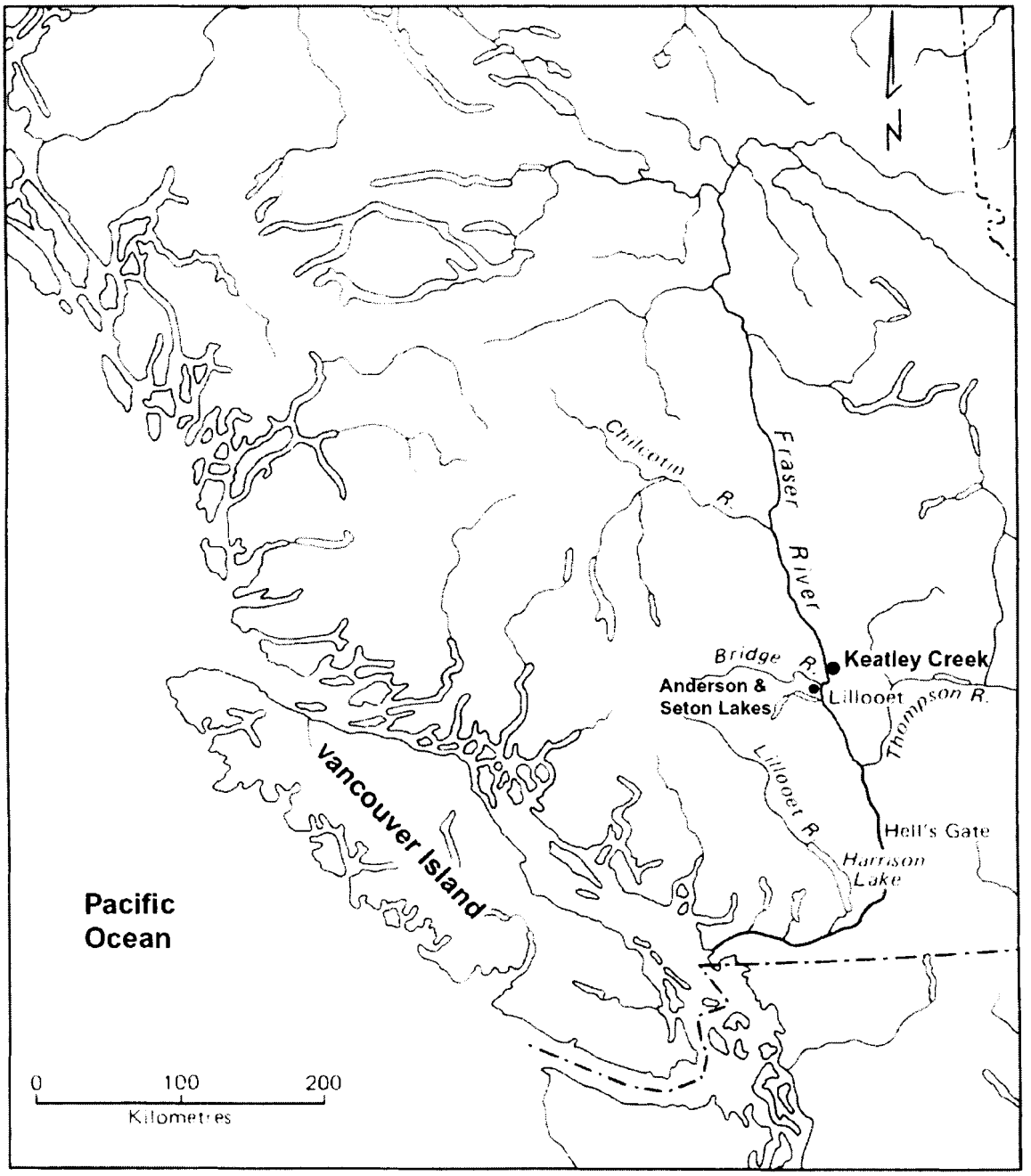

Figure 1 **Location of the Keatley Creek site (after Hayden and Ryder (1991))**

Canadian Plateau Culture Area

The Canadian Plateau, encompassing the Upper Lillooet site of Keatley Creek, is an archaeological culture area located in Southern British Columbia, bounded to the east by the Rocky Mountains and to the west the Coast Mountains. The southern and northern borders of this area lie approximately 50 km north of the International Boundary and around the great bend of the Fraser River, respectively. The Canadian Plateau encompasses an area more than 232,500 square kilometres and in addition to the Lillooet, includes such ethno-linguistic groups as the Thompson, Chilcotin, Shuswap, Okanagan, Lakes and Kutenai (Richards and Rousseau 1987:2). More specifically, Keatley Creek lies within the Mid-Fraser River region, an area of the Fraser River and valley ranging from Big Bar to the north to around Lytton to the South.

The site of Keatley Creek was occupied continuously from around 3,500 BP by Interior Salish people until its sudden abandonment around 1,100 BP --a time period encompassed by the Plateau Pithouse tradition. Although variation occurred between regions and cultural horizons, some attributes within the Plateau Pithouse tradition were maintained for more than 3000 years including: 1) a semi-sedentary settlement pattern involving the use of pithouses for winter dwellings; 2) the use of subterranean storage facilities; 3) a diet including salmon, small and large mammals, birds and plant resources such as roots and berries; 4) cooking technology including stone boiling and earth ovens; 5) tool kits including bone and antler fishing technology, adze, wedge, and maul woodworking technology, and a predominantly chipped stone tool lithic technology; and 6) a trade network ranging from the coast to interior exchanging nephrite and steatite for marine shells (Richards and Rousseau 1987:49-50).

The Plateau Pithouse tradition can be basically divided into three cultural horizons: the Shuswap Horizon (4,000/3,500-2,400 BP); the Plateau Horizon (2,400-

1,200 BP) and the Kamloops Horizon (1,200-200 BP). The information regarding the culture history within the Canadian Plateau from ca. 4,500 to 200 BP is based on the characteristic of the Plateau Pithouse tradition as outlined by Richards and Rousseau (1987) and Stryd and Rousseau (1996).

Shuswap Horizon (4,000/3,500-2,400 BP)

The Shuswap horizon is the earliest of the three Plateau Pithouse horizons, and marks the beginning of semi-subterranean pithouses for winter use on the Canadian Plateau. Typical pithouses were around 10.7 m in diameter and approximately 1.2m in depth (Kennedy and Bouchard 1978:36). Pithouses were constructed from a framework of timbers layered with pine and Douglas fir, and covered with earth (Kennedy and Bouchard 1978:36). Pithouses during this time period typically had earth roofs, flat-bottoms, and steep walls, and either roof or side entrances. They also commonly contained internal storage pits and cooking hearths. Evidence concerning subsistence in the Shuswap horizon is limited, but it appears that groups utilized: small and large land mammals including elk, deer, big-horned sheep, beaver, and muskrat; aquatic resources such as mussels, anadromous salmon and freshwater fish; as well as birds and plant resources.

Lithic assemblages in the Shuswap are relatively simple in composition and workmanship compared to later horizons. However, the 'crude' appearance of the tools may be the result of poor flaking quality of raw material, which included locally available chert, quartzite, argillite, rhyolite and basalts. Bone and antler technology are also present at some sites as well as very limited amounts of artwork such as sculpture or decorated artifacts. Trade and exchange inter-action seems to be limited to nephrite artifacts within the Mid-Fraser region, and the trade of Northwest Coast shells does not appear to be significant during this early horizon.

Plateau Horizon (2,400-1,200 BP)

The next cultural horizon is the Plateau horizon, coinciding with a climatic shift from the cool moist conditions of the Shuswap horizon to a drier and warmer climate. Pithouse size decreases to an average diameter of 6.14 m, except for within the Mid-Fraser region where pithouses remain relatively the same size. Within this time period, the first communities composed of contemporaneous small, medium and large pithouses are seen in the Mid-Fraser Region. Generally, pithouses seem to differ from the earlier horizon not only in terms of size, but also due to their basin-shaped floors, central hearth features, peripheral benches and lack of side entrances. Subsistence practices are similar to the Shuswap horizon, but with an increased reliance on salmon and root resources.

Technological advances are indicated by the use of increased bone, teeth and antler artifacts (*e.g.* bone and antler harpoons, bone and tooth beads, bone gaming pieces), native copper artifacts, and possibly the use of bark containers. There is an improvement in the quality of chipped stone tools, probably due to the shift to higher quality lithic materials such as vitreous basalt, cryptocrystalline silicates, and some obsidian. Coastal trade networks appear to have expanded to include the exchange of nephrite and coastal shells, as well as high-quality lithic materials.

Kamloops Horizon (1,200-200 BP)

Kamloops is the last of the horizons associated with the Plateau Pithouse tradition. The pithouses of this final horizon are quite variable in size, ranging from 5 m to over 20 m in diameter. They are oval, circular, rectangular or square in plan and unlike the previous horizons, demonstrate the presence of a raised earth rim and thin or absent roof insulation. Side entrances are fairly common, and passageways leading to stream banks or other pithouses, are present in some regions, especially in villages

susceptible to frequent raids (Kennedy and Bouchard 1978:37). Multiple storage pits measuring around 2 m in size are often associated with housepits from this horizon. Ground stone tool use seems to have increased, along with the carving of zoomorphic or anthropomorphic forms. The variety and frequency of bone, tooth and antler artifacts also increased, and incised decorations consisting of geometric patterns are common on antler and bone artifacts. There also seems to be an increase in the sophistication of fishing technology and intensification in the use of bark containers and mats. Inter-regional trade is significant within this horizon, including the exchange of vitreous basalts, nephrite celts, ground slate, steatite carvings, whalebone and coastal shells (Richards and Rousseau 1987).

Structures at Keatley Creek

There are more than 100 housepit depressions at Keatley Creek ranging in size from over 20 m to approximately 5 m in diameter. At its peak, the site may have had a population of over 1,000 individuals (Hayden, *et al.* 1996). Paleoethnobotanical evidence as well as ethnographies suggest that the pithouses would have been used primarily in the winter months (Alexander 2000; Lepofsky 2000:128; Teit 1906:212, 224) . In the spring and early summer, most groups would have left their winter village to hunt and gather in the mountains, and fish in the mountain lakes. In the fall, large stores of spawning salmon were caught in the Fraser River and dried for winter food. Once the cold weather set in, groups moved back to the pithouses of the winter village (Alexander 2000:224; Teit 1906).

Residential Structures

Large pithouses of over 20 m in diameter are unusual in the Canadian Plateau, and current archaeological evidence suggests that many of the large pithouses were

constructed during the Plateau Horizon and occupied continuously until the eventual abandonment of the site in the early Kamloops Horizon (ca. 1,100 BP), (although some controversy surrounds the occupational period of one of the large excavated residential housepits (Hayden 2005; Prentiss, *et al.* 2005; Prentiss, *et al.* 2003)).

Early in the 1970s, Stryd (1971) proposed that residential pithouse size within Plateau communities may have been related to wealth and political power. Subsequent excavations revealed that storage facilities, floral, faunal, and lithic remains all vary between housepits of different sizes (Hayden 2000b). Although the sample size of fully excavated housepits is small, in almost all cases, the larger housepits show the greatest diversity of material and subsistence remains. In particular, the density and diversity of faunal and archaeobotanical remains are consistently higher in larger residential housepits than in smaller ones (Lepofsky, *et al.* 1996), indicating a "difference in the economic foundation of the large versus the smaller housepits" (Hayden 1997b:104).

Hayden (1992) has proposed that members of certain large residential households, referred to as 'residential corporate groups' (Hayden and Cannon 1982), would have wielded more economic and political power within the community by controlling access to desirable resources and prime hunting and fishing locations. As mentioned above, archaeological evidence of restricted food resources and a reduction in prestige or non-locally accessible resources suggests that poorer families may have occupied smaller housepits at the site. Economic divisions of space within the larger housepits indicates that poorer individuals may also have acted as labourers within the residences of the corporate groups (Hayden 2000b; Kusmer 2000). In addition to the archaeological evidence, ethnographies also relate the presence of slavery among the Lillooet (Ray 1939:33; Teit 1900:269). Although all individuals at the site would have access to sufficient food resources and materials, non-corporate group members may have had

secondary access to prime harvesting, fishing or hunting locations, and maybe only for a fee (Romanoff 1985; 1992).

An earlier study conducted by Berry (2000) attempted to assess the distribution of salmon species in select residential housepits at Keatley Creek using radiographic analysis of salmon vertebrae. The radiographic method is based on the assumption that growth rates of salmon differ seasonally, creating annual rings on the vertebrae which may be identified through x-ray (Cannon 1988). Age determination, based on annual growth rings, can be correlated with known spawning ages of salmon as a first step in species identification.

Berry's study suggested that the influences of economic stratification were extremely pronounced in the distribution of certain salmon species; a strong correlation was evident between the size of the pit house and the diversity of spawning ages contained within. Smaller, economically low-status housepits contained only two-year-old salmon, assumed to be the least desirable species, pink salmon, the only species to spawn at two-years of age. Although the larger housepits also contained two-year-old salmon, they included a greater proportion of vertebrae with three, four and five year growth rings, assumed to be the preferred salmon species such as chinook or sockeye. In addition, Berry identified differences in the relative distribution of salmon remains within the larger housepits, especially in regards to storage areas. The preponderance of two-year-old salmon in the study suggested that pink salmon was the staple for the majority of the Lillooet individuals, however the radiographic data was not able to identify which additional species were differentially accessed by members of the larger households.

Specialized Structures

As well as residential housepits of varying sizes, the site also contains what appear to be small specialized structures on the periphery of the site that may have been used for ritual purposes. The lack of evidence for some domestic activities and indications of specialized activities are traits that differentiate these specialized structures from other residential housepits at Keatley Creek (Hayden and Adams 2004). The larger residential housepits normally display several hearths around the perimeter of the house and artifacts representing a wide range of functions including food preparation, food consumption and lithic processing. Artifacts recovered in residential housepits included fire cracked rocks, projectile points, pressure flaked knives, utilized flakes, debitage, anvils, and scrapers, indicating a wide variety of domestic tasks (Hayden 1997a). The specialized structures on the other hand, display less evidence of domestic activities and often contain a greater diversity of material remains and increased prestige goods compared to small structures that are clearly residential (Hayden and Adams 2004).

Most transegalitarian hunter-gatherer societies are typified by feasting, which as mentioned earlier, may be a key element in the emergence of social complexity (Hayden 2000b). There is often a link between feasting activities and secret societies, both within the Pacific Northwest and throughout the world (Hayden and Adams 2004). Special structures with restricted access to certain members may be used to house ritual activities or secret society meetings. Two pairs of structures at Keatley Creek are proposed to be ritual structures of this nature (Hayden and Adams 2004).

Hayden and Adams (2004) have outlined several general characteristics that typify ritual structures in transegalitarian societies, including a location away from the core of the site to facilitate secrecy and restricted access, as well as unusual assemblage characteristics. The assemblages recovered will obviously depend on the nature of the

rituals conducted within the structure, however ritual areas usually lack signs of domestic use and may even contain few to no artifacts. Although prestige or ritual items are often removed from structures before abandonment, certain areas may indicate the manufacture of prestige or ritual items. Evidence of use by wealthy or high status individuals may be displayed more often through the recovery of costume elements or musical instruments then by the recovery of cult paraphernalia.

Additionally, structures designed for feasting may exhibit unusually large food preparation or food storage areas, with evidence for consumption of unusually large quantities of food or special food items (Hayden and Adams 2004). Ritual structures may also display unusually large or elaborate hearths, often located within the center or at one end of the structure and a floor space designed to focus attention to the center or one end of the building. Structure design may display an effort to increase floor room by placing supports closer to the wall, and by the addition of benches, or floor coverings such as mats or carpets; occasionally special roof and floor building materials may also be used (Hayden and Adams 2004). Evidence of ritual structures being deliberately destroyed or filled in prior to abandonment is common, and moreover, evidence of ritual structures being rebuilt in the same manner or location has also been noted.

Archaeological excavations of the four specialized structures at Keatley Creek have uncovered evidence of several of these characteristics, indicating their use as probable ritual structures. If so, these specialized structures, like the large residential corporate group housepits, would likely have been used by individuals exerting greater economic and political power, rendering them an essential component of investigations into the effect of economic stratification on resources distribution at Keatley Creek.

Stratification and Resource Access

Resources and the Development of Stratification

Resource exploitation and food choice involve a number of environmental and cultural factors which reflect both the desirability and availability of a food (Jochim 1981). The environmental factors that affect food choice may include the overall abundance of the resource, its seasonal and long-term availability, and its nutritional content (Netting 1986; Wiessner 1996). In addition to the constraints that the environment may have on food access, there are a number of cultural variables that also affect a food's value, desirability and accessibility. These cultural factors can include palatability, the technology required for acquisition, access to procurement locations, and the energetic costs required for processing and preservation (Bettinger 1991; Wiessner 1996).

Economically stratified societies can be defined as those that demonstrate "differential relationships between the members of society and its subsistence means – some of the members of society have unimpeded access to its strategic resources while others have various impediments in their access to the same fundamental resources" (Fried 1968:255). Hayden (1992) has proposed that economic stratification within some transegalitarian communities may have developed through the manipulation of surplus resources and the control of prime resource procurement locations. Briefly summarized, in societies where surplus resources may be accumulated and stored, certain individuals

may have used surpluses to gain advantages in terms of social influence or material profits; these individuals have been termed 'accumulators' (Hayden 1992) or 'aggrandizers' (Clark and Blake 1994). In several publications, Hayden (1996; Hayden 2001; Hayden and Gargett 1990) has outlined the mechanisms by which accumulators may gain power through competitive feasting, a process in which individuals give away valued food items with the expectation that they will be returned in kind, or with interest at a future date. It is often the expenditure of surplus labour involved in the accumulation of resources which creates debts within or between communities, debts which are later used to acquire social influence, material gains, prestige items, or recognition of ownership claims (Hayden 1992:550).

Salmon Subsistence of Lillooet People

Salmon was a staple food for the Lillooet, and ethnographic records suggest that there were prime fishing locations on the Fraser River in the vicinity of Keatley Creek (Teit 1906:228). Dip-nets, woven of bark twine with a cedar or fir handle, were used primarily in muddy, or swiftly moving rivers, while larger drag-nets were used for fishing in lakes or river pools (Teit 1900:250-251; 1906:227-228). Salmon could also be speared from either the shore, or from a canoe (Teit 1900:251-252). Ethnographies relate that several species of Pacific salmon and trout were exploited by the Lillooet in this manner (Kennedy and Bouchard 1978:39-40). The salmon caught in this area was preferred as it was less oily than those caught downstream, but less lean than those caught upstream (Hayden, *et al.* 1996). Salmon caught after August was either wind-dried or smoke-dried for winter storage, and the dry climate of the area was conducive to preserving the salmon and ensuring that it would not go rancid later in the year (Kennedy and Bouchard 1992:291-296; Romanoff 1992:257-258). Groups from the coast, from the Thompson River area, and further south in Washington came to the Lillooet area to trade for dry and

surplus fresh fish, as well as for salmon oil (Kennedy and Bouchard 1992; Teit 1900; 1906).

Salmon and the Development of Lillooet Stratification

Due to several of its unusual attributes, salmon was likely a key resource in the development of social inequalities in the Lillooet area (Hayden 1992). One of its most important characteristics is that in the Mid-Fraser area, salmon is a particularly abundant food source with very little vulnerability to over-exploitation (if traditional fishing technologies are considered). Historical data suggest that perhaps 20 million sockeye and 800,000 chinook per year could have migrated through the Lillooet area (Kew 1992); even years with poor sockeye and chinook runs probably could have yielded hundreds of thousand fish (Kew 1992; Palmer 1975).

Lillooet ethnographies have proposed that the major constraint in salmon accumulation was the labour required for processing and preservation; during the peaks of runs many more fish could be caught in an hour than could be processed in the same amount of time (Romanoff 1992:235). Technological advances just prior to the Shuswap horizon appear to have improved fishing technology and allowed for the storage of surplus salmon beyond immediate use (Stryd and Rousseau 1996). With salmon available in large quantities, all individuals could theoretically obtain and store the minimum amounts of resources required for basic subsistence. The ability of individuals to store enough resources for lean winter times would have decreased the need for food sharing typically observed in egalitarian hunter-gatherer communities. Therefore, accumulators could expend additional energy and labour amassing salmon surpluses without the expectation of relinquishing those resources, without reward, to members of the communities at a future date.

Once all members have access to sufficient quantities of food from a variety of procurement areas, 'ownership' or control may be exerted by certain individuals or groups at prime resource procurement locations. Investment of labour to enhance resource acquisition or perhaps specialized knowledge of resource procurement technology may have been the methods by which initial control was asserted over resource procurement areas (Hayden 1992). Within the Mid-Fraser region, 'ownership' of prime salmon fishing locations may have occurred through specialized knowledge of fish behaviour and through the investment of additional labour and materials required for building fishing platforms.

More importantly, in addition to its abundance, salmon is also a desirable food resource, as it has a high fat and oil content. Fatty fish, fatty portions of fish, and fish oil extracted and bottled in skin containers, were ethnographically valuable in supplementing the lipid contents in the diet, as well as being significant trade items (Teit 1906:232). The surplus accumulation of desirable salmon or salmon parts, and their eventual distribution through feasting or trading for material or prestige goods, is one method by which accumulators or accumulating households could have increased their economic and social standing within the community.

Once economic stratification was present within the community, the ownership or control over resource procurement areas would allow wealthy or powerful individuals to restrict the distribution of certain preferred food resources. It is for this reason that analyzing the distribution of preferred or desirable salmon is an important component of archaeological investigations in the Mid-Fraser region, as it may potentially provide insights into the timing or development of economic stratification at Keatley Creek.

Pacific Salmon

Salmon are part of the family Salmonidae which includes freshwater and anadromous salmon, trout, char, whitefish, and grayling. Pacific salmon specifically belong to the genus *Oncorhynchus*, and include chinook, sockeye, coho, pink, chum, as well as the rainbow (steelhead) trout and cutthroat trout. Detailed information concerning the life histories of each of the Pacific salmon species under investigation in this study are presented in Appendix A.

Although there are some differences in the timing of events in the lifecycles of Pacific salmon species, the lifecycle of all anadromous salmon begins when eggs are deposited in gravel nests called 'redds', typically located in freshwater rivers and lakes. The salmon embryos or 'alevins' remains buried beneath the gravel until they exhaust their supply of yolk, usually emerging from the gravel as fry during spring months. Depending on the species or spawning population, fry may remain in freshwater streams and lakes for as little as a few hours or as long as one to two years before migrating out to sea. Salmon will remain in the ocean for one to eight years before returning once again to their natal rivers, lakes or streams to spawn. Typically, Pacific salmon are semelparous (dying after spawning once), succumbing to the exhaustion of up-river travel and nest protection soon after spawning.

Salmon Species Preference and Distribution

Pacific salmon species differ from each other in several ways, and their attributes would have affected both their desirability and their availability to the Lillooet. The three most important environmental variables affecting salmon access and preference are the availability of the species, the size of the salmon, and its oil content. In addition to the environmental constraints, there are certain cultural factors that will also affect salmon access, including palatability, access to fishing locations and the labour required for preservation. Within Lillooet communities, overall salmon species preference and access would have been affected by these environmental and cultural factors.

As mentioned, one key environmental factor affecting Lillooet subsistence would be the overall availability of the salmon. As seen in Table 1, salmon spawn at varying months throughout the year, and the abundance of salmon in each run may also vary. For example, although sockeye spawn from June to November, salmon availability reaches its peak in July. Current and historical data suggests that there has been a great deal of variability in salmon spawning behaviour, and all five species of salmon may not have been available to the Lillooet (Berry 2000; Kew 1992). For examples, chum salmon usually only spawn in the Fraser River below Hope, B.C. and therefore are not currently available in the Lillooet area (Fraser River Action Plan 1996).

Salmon species also vary in their size, which is in part a reflection of the age at which they spawn. Chinook, sockeye and chum are the largest species, while coho and pink are somewhat smaller (Groot and Margolis 1991). Smaller salmon species tend to run along the sides of the river, taking advantage of slower water and eddies along stream banks in an effort to conserve energy while spawning (Burgner 1991: 15). Larger salmon species normally swim within the deeper central portions, and can migrate in schools close to the river bottom where the current is slower (Burgner 1991:15). Areas where the

river was restricted, or where rocks extended into the waterway, would have been the most productive fishing locations (Kennedy and Bouchard 1978; 1992). Platforms were built in these areas in order to access greater quantities of large salmon. Chinook especially swim close to the bottom of the river and platforms would allow fishers to extend their dip-nets several feet below the surface to catch the chinook (Romanoff 1992:232). Ethnographically these platforms were owned by families (Teit 1900:250; 1906:256).

Table 1 Environmental and cultural attributes related to salmon distribution

Species	Cultural Preference	Taste	Drying Difficulty	Size	Spawning Age	Spawning season
Chinook	High	Rich	High	Large (7-9 kg)	3-8 years	March/April & Aug/Sept
Sockeye	High	Rich	High	Large (5.5 kg)	3-5 years	June-Nov, peak July
Coho	Medium	Average	Medium	Average (4.5 kg)	2-4 years	Nov-Dec
Chum	Low	Minimal	Low	Large (3.5-5.5 kg)	2-7 years	Oct-Nov
Pink	Low	Minimal	Low	Small (1.5-2.5kg)	2 years	Sept-Oct

Note: Summarized from published references (Fraser River Action Plan 1995a; 1995b; 1996; Groot and Margolis 1991)

Oil content also varies between salmon species, and is another key factor in salmon desirability. Species such as chinook and sockeye have the highest oil content, and ethnographically, these species were preferred not only for their rich taste, and value as a trade item, but also because their greater caloric content was valuable for keeping warm in the winter months (Hayden 1997b; Teit 1900). However, the high oil content of the chinook and sockeye, especially in the early runs, makes them much more difficult to dry. Early runs of sockeye and chinook may have been processed for oil, and the flesh rendered into powder (Romanoff 1992:240). The later summer runs of these species

were air-dried or smoked for winter storage, but even so, the oil content of the fish can increase the chances that the flesh will rot or become fly-blown (Romanoff 1992:259). Pink and chum salmon, species with a lower oil content, are much easier to preserve.

The varying attributes of the salmon would affect how these species were preferentially accessed and distributed. However, in addition to environmental constraints of size, oil content, and overall abundance and availability, cultural variables will also affect access to certain species. The first of these cultural variables relates to harvesting locations; ethnographically individuals or families owning fishing platforms had primary access to large species. 'Ownership' or control of prime fishing locations has ethnographically been observed as priority access to certain fishing areas (Kennedy and Bouchard 1978:40; Teit 1906:256) or through the collection of fees from community members wishing to use the areas (Romanoff 1985).

The second cultural variable relates to processing and storage. Oily species such as sockeye and chinook require a greater labour investment for preservation and oil extraction. Ethnographical records suggest that Lillooet people with access to greater labour forces would have had more opportunities exploit these species (Hayden 1997b). Individuals lacking the time or labour required for their preservation may have been restricted to less oily species such as pink and chum. As discussed previously, preferential access to prime harvesting locations and the availability of labour would have generated inequalities within the Lillooet group (Hayden 1997b).

Consequently, the environmental and cultural variables visible ethnographically can be correlated with the distribution of species displayed archaeologically, in order to make predictions concerning the factors that affected the distribution of preferred salmon species in the past. Additionally, since no two salmon have exactly the same qualities or

behaviours, their attributes can be compared to identify which specific environmental or cultural variables were most important in the distribution of species. However, in order to compare these variables, archaeological salmon remains must be accurately identified to the species level.

Modern Salmon Species Identification

Morphological identification of live or recently deceased salmon is rather straightforward, as the five species differ from each with respect to their appearance and colouration. Scale analysis may also be used to distinguish salmon species, as well as certain racial populations within species (Henry 1961). The anterior portions of salmon scales are marked with circuli, or concentric series of ridges, which are most obvious on the protected portion of the scale (McMurrich 1912:12-13). The circuli differ in width and number based on the age of the salmon and the years spent in fresh and salt-water. Since the different species of Pacific salmon undergo varying environmental conditions during the first few years after emergence and during their lifecyle, the identification of smoltification timing, years spent at sea and spawning ages through circuli analysis is useful for distinguishing salmon species (Henry 1961:84-85; Salo 1991).

The circuli formed during freshwater life (*i.e.*, after fry emergence but prior to seaward migration) are located within the inner nuclear zone of the scales (McMurrich 1912:12); they are normally "finer in structure, of lesser height and closer together than those circuli formed in the sea" (Henry 1961:6). Marine circuli begin to form after smoltification, forming in concentric circles in the marine zone, beyond the inner nuclear zone (Henry 1961). The rapid growth characterizing a salmon's first summer at sea is marked by a broad zone of widely spaced bands immediately succeeding the inner nuclear zone. These widely spaced bands, if followed outwards, will form another zone

of narrow lines, representing the decrease in growth during winter months. One year of widely spaced lines followed by a zone of narrow bands indicates the first year spent at sea after migration (McMurrich 1912:12). The zone of narrow bands is designated as the 'annual ring' (Henry 1961; Salo 1991) and is probably formed between the months of November and January, suggesting that fairly constant growth is occurring for most of the year (Burgner 1991:85).

However, scale markings can also display 'checks' in summer growth, areas where bands become narrower resembling those formed during winter months. These 'checks' are likely caused by a decrease in summer growth instigated by temporarily insufficient nutrient levels, and may cause significant confusion in the determination of salmon ages (McMurrich 1912:19). Although scale analysis can be quite accurate at determining salmon species through age and lifeways analysis, an obvious drawback of the method in terms of archaeological investigations is that salmon scales are rarely recovered from archaeological sites.

Archaeological Salmon Species Identification

Since they are so closely related, distinguishing species of Pacific salmon from each other using only osteological elements can be extremely challenging (Butler and Bowers 1998), and usually only possible if all skeletal elements are present (Cannon 1987). Species of *Oncorhynchus* can be distinguished morphologically by their chondrocranium; these head bones are especially useful at distinguishing the genus *Oncorhynchus* from their Atlantic counterparts, S*almo* (Gorshkov, *et al.* 1981). The chinook salmon chondrocranium is the most divergent of the genus, which may facilitate that species identification in archaeological sites (Gorshkov, *et al.* 1981:3). However, salmon cranial elements are very fragile, and due to their reduced density, may be the

least likely elements of the salmon skeleton to preserve in the archaeological record (Butler and Chatters 1994).

Otolith Analysis

Otoliths, like scales, can be useful at providing information about salmon populations, and because they are bony elements they may be preserved in archaeological sites. Otoliths are small white structures found in head of salmon and other fish, which provide a sense of balance and aid in hearing (Weber, *et al.* 2002). Otoliths may be used to identify species of salmon in two ways: first by examining the gross morphological differences between the otoliths of different species (Cannon 1987; Casteel 1974) and secondly by examining patterns of growth visible through a microscope (McMurrich 1912). Otoliths are composed of both organic and inorganic material, and growth takes place by the addition of material in deposited in concentric rings (McMurrich 1912). Restricted winter growth will therefore be visible as areas where the inorganic component is greater than the organic component, appearing as lighter rings when viewed under a microscope using transmitted light (McMurrich 1912:15). The concentric rings can be counted in the same way as the annuli on the scales to determine both age at seaward migration and spawning ages. Using the same principles as scale analysis, determination of smoltification and spawning ages are used to differentiate salmon species. However, otoliths in pink salmon do not always form in concentric rings, but in zones, making age identification very difficult in this species (McMurrich 1912:25-26). Other disadvantages include the fact that clear and complete growth rings can occasionally be difficult to identify, and most importantly, due to their small size, the recovery of otoliths from archaeological sites is quite unusual.

Radiographic Analysis

Both scale and otolith analyses are problematic for archaeological studies because these elements are rarely preserved in archaeological site. However, dense skeletal elements such as the salmon vertebrae are sometimes found ubiquitously in Northwest Coast and Plateau sites (Butler and Chatters 1994). The radiographic method of salmon species identification was developed by Cannon (1988) as a potential solution to the obstacles precluding salmon species identification. Like scale and otolith analysis, the radiographic method is based on variability in salmon seasonal growth rates, which can be observed as annual rings on the vertebrae visible through x-ray (Cannon 1988).

Radiographs of salmon vertebrae are characterized by narrow radio-opaque bands alternating with wider radiolucent bands. The narrow radio-opaque bands are thought to correspond to the dense bone laid down during slowed winter growth, while the wider radiolucent bands relate to periods of regular growth throughout the rest of the year (Cannon 1988). Age identification, based on annual growth rings, can be correlated with known spawning ages of salmon as a first step in species identification. This method can be very useful at distinguishing pink from all other species, as it is the only species that spawns consistently at two years of age. However, the other four species of Pacific salmon species (chinook, sockeye, coho and chum) have overlapping spawning age ranges and therefore cannot be distinguished from each other based on age identification alone.

Although the radiographic method allows for the analysis of many hundreds of vertebrae in a relatively short amount of time, generating age-profiles for a substantial amount of salmon remains, there are some drawbacks to the method. There can occasionally be difficulty in accurately determining how many radio-opaque bands are present on the vertebrae, due to inter-observer subjectivity. Furthermore, the possibility

exists that rings visible radiographically may not always correspond directly to years of life (perhaps due to seasonal growth checks), creating additional ambiguity in the identification of spawning ages.

All the traditional methods of salmon species identification have obvious disadvantages for the archaeologist aspiring to a detailed investigation of salmon resources. In the past, since salmon identification could not reliably be made to the species level, many subsistence studies focused only on the overall abundance of salmon remains, and their distribution at the site. The advent of ancient DNA analysis, however, has recently provided many new opportunities for archaeologists, as the technique allows for accurate and unambiguous species identification of closely-related and morphologically indistinguishable taxa.

Ancient DNA and Archaeology

The Advent of Ancient DNA Studies

Archaeologists and physical anthropologists have always taken advantage of advances in the field of molecular biology to answer questions about past populations. In 1984, a new door was opened with the first successful ancient DNA study, as Higuchi and his colleagues (1984) extracted mitochondrial DNA from a 140 year-old quagga, an extinct species related to the zebra.

This first experiment was followed a year later by the extraction of human DNA from an Egyptian mummy (Paabo 1985), and later by a whole host of experiments involving human specimens (Doran, *et al.* 1986; Hagelberg, *et al.* 1989; Paabo, *et al.* 1988). However, the poor efficiency of ancient DNA cloning resulted in the inability to reproduce results (Hoss, *et al.* 1994). The innovation of the polymerase chain reaction (PCR) by Kary Mullis (1990) made huge advances in the field of ancient DNA, as it allowed for the multiplication of even a few severely degraded DNA molecule into billions of copies (Paabo 1993; Saiki, *et al.* 1985).

Ancient DNA Extraction

Since the advent of ancient DNA analysis, many studies investigated the feasibility of extracting DNA from a variety of tissues (Bonnichsen, *et al.* 2001; Foo 1991; Hagelberg, *et al.* 1991; Rollo, *et al.* 2002). It was noted that skeletal material compared to other ancient tissues, yields a greater amount of amplified product and

requires less purification (Lassen, *et al.* 1994). Soft tissue, such as mummified remains, seems to contain more PCR inhibiting products (Cooper 1992), while hard and dry tissues such as bone and teeth protect the DNA and offer a barrier against chemical reactants and microorganisms. Minerals within bone, such as hydroxyapatite, can also help to stabilize DNA (Turross 1994).

There are various methods that can be used to first extract DNA from tissue and subsequently to purify the samples prior to amplification. These methods will vary according to the type of organic remains being studied, whether it be soft tissue, tooth or bone. Before the extraction procedure can begin, potential surface contamination should be removed either through physical and/or chemical means, followed by brief UV irradiation. Physical decontamination may include the removal of the surface with a scalpel or through abrasion with a dremel, drill, or sandpaper (O'Rourke, *et al.* 2000). Chemical decontamination may include brief submersion of the sample in a commercial bleach solution, 1 N HCl and1 N NaOH (O'Rourke, *et al.* 2000; Yang, *et al.* 2004). Ultraviolet irradiation is used to crosslink nucleotides of DNA on the bone surface, effectively annulling its ability to amplify during PCR (Hummel and Herrmann 1994).

An extraction buffer is normally employed to extract the DNA housed within ancient tissue and hard tissue samples are typically reduced to powder in order to increase the surface area exposed to the solution. The extraction buffer normally includes EDTA for decalcification, an enzyme such as proteinase K to digest the protein fraction, sodium or potassium salt to create an isotonic milieu and often a nonionic detergent to stabilize the buffer (Hummel and Herrmann 1994). The DNA is then isolated from the cell debris produced by the extraction buffer, usually by phenol/chloroform or silica based methods, and concentrated and purified in preparation for PCR amplification (MacHugh, *et al.* 2000).

DNA Amplification

Prior to the discovery of PCR, DNA analysis was conducted through the use of cloning. Since the development of PCR, a variety of publications have defined protocols for ancient DNA amplification (Hagelberg, *et al.* 1991; Hummel and Herrmann 1994; Paabo 1989). Usually, a PCR reaction is set up incorporating the ancient DNA, the selected primers, dNTP, $MgCl_2$, buffer, and a polymerase enzyme such as Taq or Taq Gold. Primers are designed for specific areas of mitochondrial DNA (mtDNA) or nuclear DNA, depending on the type of study being conducted. In ancient DNA studies, the primers usually code for fragments less than 300 bp due to the DNA degradation over time. However, the shortcomings caused by short fragments may be overcome to a certain extent by applying overlapping primers in order to fit together longer sequences (Hoss 1995; Paabo 1993). PCR reactions are placed in a thermocycler and exposed to several cycles of varying temperatures designed to denature the DNA, anneal the primers, and extend the synthesis of sequences, creating billions of copies of the selected ancient DNA sequence.

DNA Degradation

There are two major hurdles to overcome in the analysis of ancient DNA: first, the degraded nature of the nucleotides, and second, the contamination of ancient DNA by modern sources. (Barnes, *et al.* 1998; O'Rourke, *et al.* 2000). All DNA is affected by destructive processes; while DNA damage incurred in a living organism is usually constantly repaired, after death, repair ceases while destructive processes continue (Handt, *et al.* 1994). Unfortunately, it is often difficult to assess the condition of DNA within an ancient specimen. Samples that show good macroscopic preservation are often found to yield greater amounts of amplifiable DNA (MacHugh, *et al.* 2000). Cortical bone is often found to generate greater yields of amplifiable DNA than cancellous bone,

due to its superior structural integrity (MacHugh, *et al.* 2000). Histological preservation also seems to be a suitable predictor for the presence of amplifiable DNA, while bone size is not necessarily correlated to the measure of DNA preserved within (Haynes, *et al.* 2002).

Several publications have discussed the environmental conditions that affect the preservation of ancient DNA in diverse tissue types (Briggs 1999; Burger, *et al.* 1999; Child 1995; Colson 1997; Eglinton and Logan 1991; Haynes, *et al.* 2002; Hoss, *et al.* 1996b; Poinar, *et al.* 1996). In terms of amplifiable DNA yield, various publications have suggested that the environmental conditions surrounding the burial material is more important than the age of the specimen (Fernandez-Jalvo, *et al.* 2002; Hagelberg, *et al.* 1991; Loreille, *et al.* 2001). Samples buried in cold areas often preserve better than those buried in hot dry areas, as the cold temperature reduces the presence of microorganisms. Sites with lower humidity may preserve DNA more successfully, due to a decrease in humic and fulvic acids, which are able to penetrate into hard tissue in the presence of high humidity (Burger, *et al.* 1999). Neutral or slightly alkaline pH is preferred as acidic soils may result in the total decomposition of skeletal matter (Burger, *et al.* 1999). Furthermore, in terms of DNA preservation, the maintenance of constant temperature and humidity is superior to a constantly fluctuating environment. However, it is important to note that even within the most optimal burial conditions, amplifiable DNA does not seem to survive for longer than approximately 100,000 years (Lindahl 1993).

There are two major chemical processes that degrade DNA over time: hydrolysis and oxidation (Eglinton and Logan 1991; Lindahl 1993). Hydrolysis is the process by which the N-gycosyl bond between the base and sugar breaks down in the presence of water, resulting in deaminization of bases and in depurination and depyrimidination

(Lindahl 1993). Oxidation distorts the helix through the interaction of free radicals and ionizing radiation with the DNA, which can result in modified bases (Lindahl 1993).

As well as making DNA more difficult to retrieve, DNA deterioration can also cause miscoding and base modifications in sequencing (Hoss, *et al.* 1996b; Paabo 1989). This is especially problematic if there are only very few molecules available for amplification, as miscoded DNA strands will multiply exponentially, resulting in an erroneous sequence (Handt, *et al.* 1996). Mis-coded DNA may also be confused with heteroplasmy (*i.e.* the occurrence of more than one mitochondrial type within an individual). If heteroplasmy is suspected, further testing such as cloning, can be pursued in order to rule out the possibility of contamination, degradation and PCR errors (Handt, *et al.* 1996)

Besides the damage incurred by chemical reactions, analysis is rendered more difficult by the co-extraction of PCR inhibitors during DNA studies. PCR inhibitors can include heavy metals, organic compounds found in soils, tannins, humic acid, by-products of diagensis, Maillard products and fulvic acid (O'Rourke, *et al.* 1996; O'Rourke, *et al.* 2000; Paabo, *et al.* 1989). However, PCR inhibition may be overcome through the dilution of the sample, or through various additional purification steps (Hoss and Paabo 1993; Hummel, *et al.* 1999; Hummel, *et al.* 1992; O'Rourke, *et al.* 1996).

Contamination Control and DNA Authentication

In addition to the degraded nature of ancient DNA, the second major hurdle in ancient DNA analysis is controlling contamination of ancient samples from modern sources. The issue of contamination is of vital concern in ancient DNA analysis, and the degradation and damage to ancient DNA means that even minuscule amounts of modern DNA can overwhelm the ancient template during PCR (Richards, *et al.* 1995).

Comprehensive contamination controls, therefore, must be taken during every step of the extraction and amplification procedure. Contamination may be caused by exogenous DNA from bacteria or fungi, by human handling, or by exposure to modern collections of the same species. Although the physical and chemical decontamination methods mentioned may be able to sufficiently remove surface contamination, care must be taken to avoid exposing ancient samples to modern sources of contamination prior to DNA analysis. In order to ensure that the DNA sequences recovered from ancient samples are authentic, protocols such as careful in-lab contamination controls, independent replication and phylogenetic analysis should be included in every ancient DNA study.

If possible, archaeologists should take precautions when recovering remains that may potentially be used for DNA analysis (Scholtz, *et al.* 2000; Yang and Watt 2005). Before archaeological recovery is commenced, appropriate field methodology such as the use of protective clothing should be considered (Evison 2001; Yang and Watt 2005). In addition, washing, bone glue treatment and unnecessary handling should be avoided at all costs if ancient DNA studies are to be conducted (Hagelberg 1997; Nicholson, *et al.* 2002; Scholtz, *et al.* 2000; Yang and Watt 2005).

Ancient DNA and Species Identification

Although there are many challenges involved in extracting and analyzing DNA from ancient remains, the success of ancient DNA studies in recent years has demonstrated both the feasibility and suitability of ancient DNA analysis for archaeological studies. Since the extraction of DNA from the quagga, there have been myriad studies exploring DNA from extinct animals and their phylogenetic relationships to extant species including: the marsupial wolf (Thomas, *et al.* 1989); the saber-tooth cat (Janczewski, *et al.* 1992); flightless ratites (Cooper, *et al.* 1992); mammoth (Hagelberg,

et al. 1994; Hoss, *et al.* 1994); Wooly rhino (Orlando, *et al.* 2003); ground sloth (Hoss, *et al.* 1996a) and; extinct cave bear (Kuhn, *et al.* 2001).

However, ancient faunal DNA analysis can offer more to the archaeologist than mere phylogenetic applications. Unambiguous species identification can be practical in a variety of investigations including the reconstruction of diet and paleoenvironments (Barnes, *et al.* 1998; Nicholls, *et al.* 2003; Yang, *et al.* 2004) and ancient DNA is particularly well suited this task. Species identification of archaeological faunal remains using ancient DNA analysis has become more common in the world of archaeology, and studies have been performed on numerous animals including: equine remains at Pompeii (Sica 2002); rat (Matisoo-Smith and Allen 2001) and fish (Nicholls, *et al.* 2003) remains from the Southern Cook Islands; sheep and goat in Nigeria and Northern Cameroon (Newman, *et al.* 2002); rabbit species in the American southwest (Yang, *et al.* 2005); as well as in the study of European rabbit species on Mediterranean islands (Hardy, *et al.* 1994).

Archaeological ancient DNA analysis usually focuses on organellar DNA, from mitochondria and chloroplasts, rather than from nuclear DNA. Where each cell usually only contains two copies of nuclear DNA, there are hundreds of mitochondria in each cell, each containing multiple copies of mtDNA. Therefore, there is a greater likelihood of extracting mtDNA from ancient samples (Kaestle and Horsburgh 2002). In addition, the control region (D-loop) of the mitochondrial DNA does not code for proteins, therefore mutations may occur at an elevated rate compared to nuclear DNA (Avise 2000:13-15). Since the control region of mtDNA is often species or even population specific, it is especially useful for studying phylogenetic relationships between members of a taxa (Jones 2001:53). Alternatively, unambiguous species identification for regionally or temporally distinct populations may require the use of a highly conserved

portion of DNA, and gene-coding regions of mtDNA, such as the Cytochrome B gene, are particularly well suited for such studies (Yang, *et al.* 2004). Although species-specific markers can also be found in nuclear DNA, the reduced likelihood of amplifying ancient nuclear DNA has encouraged the use of mtDNA in archaeological species identification studies (Matisoo-Smith and Allen 2001; Nicholls, *et al.* 2003; Yang, *et al.* 2004).

DNA Analysis of Salmonid Species

During the 1990s, researchers began to take advantage of the information present in salmonid mitochondrial and nuclear DNA in order to understand the evolutionary histories of the species (Churikov and Gharrett 2002; Polyakova N 1999; Shedlock, *et al.* 1992; Takasaki 1996). The genomic maps of salmonid DNA, as well as the published reference sequences produced by these studies realized the potential for salmonid genetic species identification. Efficient methods of salmon species identification based on restriction fragment length polymorphism (RLFPs) were first developed particularly for quality control in the food industry (Carrera, *et al.* 2000; Carrera, *et al.* 1999; Carrera, *et al.* 1998; Hold, *et al.* 2001; Rehbein, *et al.* 1997; Russell, *et al.* 2000).

However, the DNA sequence analysis methods of species identification were soon introduced to ancient salmon remains. A preliminary study of archaeological salmon remains from the Pacific Northwest coast dating from 9000-500 BP determined that the recovery of DNA from archaeological salmon remains was indeed a possibility (Butler and Bowers 1998). A more effective ancient DNA technique for salmon species identification was developed by Yang *et al.*(2004) and it has been tested on 7000-2000 year-old ancient salmon remains from the Namu site on the Central Coast of British Columbia. The study at Namu confirmed that mitochondrial DNA analysis could successfully identify individual species of Pacific salmon. Based on the success of these

two studies, archaeologists now have the opportunity to assess prehistoric salmon subsistence on a far more detailed level than ever before.

Materials and Methods

Structures Characteristics

In this study, salmon remains from two residential housepits (#12 and #3) and two specialized structures (#107 and #9) at Keatley Creek were selected for DNA analysis. The characteristics and contents of these structures are summarized in Table 2. Housepits #12 and #3 are small and medium residential housepits, respectively. Housepit #12 contains the remains of a small hearth, a small storage pit containing salmon vertebrae (often still articulated) as well as living floor remains, consisting mostly of mammal bones and approximately 30 fish bones (Hayden 2000b; Kusmer 2000). Housepit #3 contained an overwhelmingly greater density of fish and mammal bones compared to #12, as well as a greater occurrence of prestige lithic items and specialized fauna (Hayden 2000b).

Structures #9 and #107 constitute a pair of small structures located approximately 100 m from the site core, and each displays a variety of characteristics that suggest that they are specialized and perhaps ritual in nature (Hayden and Adams 2004). Structure #9 is replete with artifacts and food items, including an unusually large number of prestige goods, articulated salmon vertebrae, fins, tails, and head elements, and the only loon bones at the site - ethnographically worn by shamans (Teit 1900:381). It also contains two central superimposed hearths, one of which is rock lined, a special loam floor and an unusually large storage pit for salmon.

Table 2 Characteristics of the four studied structures

Structure	Type	Size in diameter	Salmon Remains	Other Faunal remains	Artifacts & features
#12	Residential	Small (6 m)	~ 30 salmon remains on floor, ~200 from storage pits	Some unidentified mammal remains	Ephemeral hearth
#3	Residential	Medium (12 m)	~300 salmon remains on floor and ~ 1700 from storage pits	Floor remains contain dog, artiodactyla, and unidentified mammal	Freshwater shells, prestige lithic items, one main hearth and ephemeral hearths
#9	Specialized	Small (7.9m)	~2000 salmon remain on floor, often with articulated head and fin elements,	Big horn sheep, elk, loon and bald eagle bones, and beaver incisors	Dentalium shells and shell beads, prestige lithic items, pipe fragments, worked elk and deer antler, a variety of lithics, stone-lined hearth and a large storage pit.
#107	Specialized	Small (8m)	~325 very fragments salmon remains, no complete vertebrae on floor surface	Very few non-salmon faunal remains found	Rock lined hearth, some unusual lithics remains (fan-tail bifaces), two large storage pits

Note: the information in this table is based on published references (Hayden 2000b; Hayden and Adams 2004; Kusmer 2000)

Structure #9's counterpart, structure #107, is almost devoid of artifacts, containing only a small number of cultural and faunal remains, two uncharacteristically large storage pits partly filled with cobbles before abandonment, some unusual lithics and a large cobble circle in the center of the structure (Hayden 2000b).

Salmon Sample Selection

A total of 60 salmon remains recovered from the four structures were selected for ancient DNA analysis. When available, the salmon remains analyzed in this study were recovered from the living floor context, which during excavation had been successfully differentiated from roof, rim and other deposits on the basis of sediment, faunal, botanical and artifact analysis (Hayden and Spafford 1993:114; Lepofsky, *et al.* 1996:35-36). However if few floor samples were available, as in housepit #3 and #12, samples were also chosen from storage pits dating to the same occupation as the living floor. Since Keatley Creek was primarily a winter village, it is assumed that the salmon samples represent the remains of either bones thrown away from meals or unconsumed dried fish stored for winter food.

All of the living floor and storage pit samples used in the study were inferred to have been deposited between 1,500 and 1,100 BP (Late Plateau to Early Kamloops Horizon) – a period of relative cultural stability. Dates for the living floor context and associated storage pits are based on relative dating techniques associated with projectile point styles and artifact types as well extensive radiocarbon dating at the site completed by Simon Fraser University radiocarbon lab and Beta Analytic (Hayden 2000a). The provenience and element type of the salmonid samples analyzed from each housepit are listed in Appendix B.

In an effort to decrease the chance of analyzing multiple bones from the same animal, samples were selected from as many archaeological units as possible within each structure. Initially, all salmon bones were selected randomly from the remains of each unit, except two vertebrae from housepit #12, which were judgmentally sampled due to their unusually large size. Salmon vertebrae were usually selected preferentially over other elements due to the fact that radiographic analysis for age identification could be completed in addition to DNA analysis. Once radiographic analysis was complete, some age-dependent selection was conducted to ensure that a variety of salmon ages were included for DNA analysis. Non-vertebral elements from housepit #12 and #3 were included in the sample to help determine whether differing butchering techniques was a factor in species representation. Structure #107 did not contain any vertebral remains and therefore only head, fin and rib elements were available for DNA analysis. Due to the fragmentary nature of the non-vertebral remains, very few specific elements could be identified. One non-salmon sample from the remains was included in this study as a control to monitor possible contamination.

Radiographic Analysis

All vertebrae analyzed in this study were x-rayed prior to DNA analysis in order to make the samples more comparable to the results of Berry's (2000) radiographic study. Salmon vertebrae were x-rayed using the H.G Fischer model FP200 portable x-ray unit at SFU using a radiographic output of 80kvp at 15 ma at a distance of approximately 60 cm for 1.5 seconds. Spawning age of the salmon was identified by examining the radiographic annuli on the vertebrae using the method set out by Cannon (1988). Although DNA can be damaged by long-term exposure to irradiation, it was assumed that the effects of a single acute low-dose x-ray on the ancient DNA housed within the matrix

of the bone likely would not significantly affect the amount of DNA recovered or the quality of the sequences obtained.

Bone Preparation, Decontamination and DNA Extraction

One half of larger vertebra, whole small vertebra or a fragment of head, rib or fin element were prepared for DNA extraction based on the method developed by Yang et al. (Yang, *et al.* 2004; Yang, *et al.* 1998). Samples were kept whole for the decontamination procedure and examples of typical samples are displayed in Figure 2. Samples were placed in 15 or 50 ml sterile tubes and submerged in a 50-100% commercial bleach solution for 2-5 min followed by a wash in Ultra-pure water for 2-5 min to remove any bleach residue. Selected samples were submerged in 2 ml of 1N HCl for approximately 20 seconds, drained, and immediately covered with an equal amount of 1N NaOH for the same amount of time. Samples were then rinsed twice with Ultra-pure water. All samples were subjected to UV irradiation in a cross-linker at 260 nm for 30 min on each side and left to dry completely before they were crushed into bone powder.

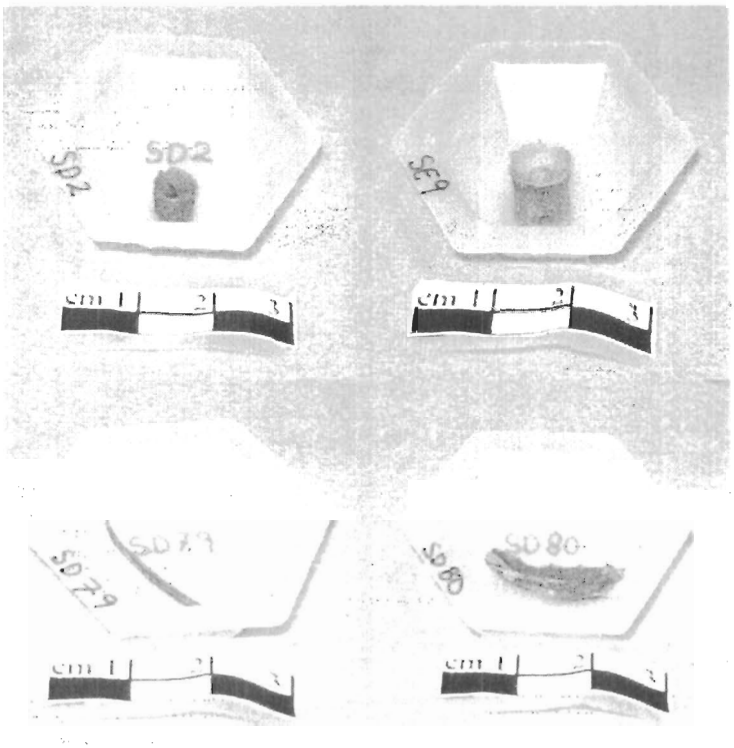

Figure 2 Examples of typical salmon samples used for DNA extraction

Half the bone powder from the vertebral remains, and the entire element for non-vertebral remains was incubated overnight in a lysis buffer (0.5 M EDTA pH 8.0; 0.5% SDS; 0.5 mg/mL proteinase K) in a rotating hybridization oven at 50°C. The samples were place in a centrifuge (LWS-832 Centrifuge, LW Scientific) and spun at 3000 rpm for 30 min until there was a stable interface between bone powder and the solution.

One and a half mL of the supernatant from each sample was transferred into an Amicon Ultra-4 Centrifugal Filter Devices (10 or 30 KD, 4mL, Millipore) for concentration. The Amicon Ultra-4's were then centrifuged until the liquid in the column was reduced to less than 100 µL and purified using a modified silica spin method (Yang, *et al.* 1998) employing QIAquick spin columns (QUIAGEN, Hilden, Germany).

PCR Set-up and Amplification

Three sets of primers were used in this study to amplify two fragments of the control region (D-loop) and a Cytochrome B gene fragment (Cyt B) of mtDNA for *Oncorhynchus* species identifications (Table 3). The primers were designed in a study by Yang *et al.* (2004) using *Oncorhynchus* sequences available in GenBank (www.ncbi.nlm.nih.gov) and published references (Shedlock, *et al.* 1992); these primers can differentiate the five species of Pacific salmon that were under investigation in this study. Initially, PCR reactions were designed to simultaneously amplify both the longer D-loop (Smc7/Smc8) fragment and the Cyt B (Cyt5/Cyt6) fragment. The co-amplification of two separate fragments from a single aliquot of DNA is advantageous as it saves time, conserved lab resources, and serves to preserve DNA samples.

Table 3 Primers for PCR amplification

Primer	Direction	Sequence (5'-3')	Region	Amplicon
Smc7	Forward	AAC CCC TAA ACC AGG AAG TCT CAA	D-loop	249 bp
Smc8	Reverse	CGT CTT AAC AGC TTC AGT GTT ATG CT		
Smc3	Forward	ACT TGG ATA TCA AGT GCA TAA GGT	D-loop	135bp
Smc4	Reverse	CCT GGT TTA GGG GTT TAA CAG G		
Cyt5	Forward	AAA ATC GCT AAT GAC GCA CTA GTC GA	Cyt B	168 bp
Cyt6	Reverse	GCA GAC AGA GGA AAA AGC TGT TGA		

Note: Information based on Yang et al. 2004

PCR amplifications were performed on an Eppendorf™ Mastercycler Personal Thermocycler using a 30 µL reaction volume containing 1.5X Applied Biosystems™ Buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTP, 1.0 mg/mL BSA, 3 µL DNA sample and 2.5 U AmpliTaq Gold (Applied Biosystems). For all samples, the initial PCR reaction took place using a co-amplification of the first D-loop Primer (Smc7/Smc8) and the Cyt B

primer, at a primer ratio of 6:1, and concentrations of 0.6 μM and 0.1 μM of each primer respectively. Due to the length of the amplicon for the initial D-loop primer (249 bp), positive amplification of that control region fragment was not always successful. In cases where PCR inhibition or low template number was suspected, the shorter D-loop primers (Smc3/Smc4) were applied in a simplex PCR using 0.3 μM of each primer to amplify a 135 bp fragment located directly beside, but not overlapping the Smc7/8 region.

The conditions of PCR amplification for all samples were as follows: the initial denaturing took place at 95°C for 12 min, followed by 60 cycles at 95° for 30 sec (denaturation), 56 °C for 30 sec (annealing), 70°C for 40 sec (extension) followed by a final 7 min extension at 72°C. Samples were loaded onto an 2% agarose gel, and fluoresced under a dark reader (Clare Chemical Research Co. USA) using SYBR Green™ (Clare Chemical Research Co.USA). Electrophoresis took place at 100 volts for 30 min.

PCR products were purified using a QIAquick purification method (QIAquick min-elute, QUIAGEN, Hilden, Germany) according to the manufacturer's instructions with some modifications. When possible, purified samples were sequenced for both the Cyt B and longer D-loop fragments using the forward Cyt B primer and using a specially designed reverse primer for the longer D-loop fragment. In cases where there was insufficient amplification of the longer D-loop fragment, the shorter D-loop fragment was sequenced using the Smc3 primer. Samples were sent to the Central Facility of the Institute for Molecular Biology and Biotechnology laboratory at McMaster University for sequencing on a ABI 3100.

In order to decrease the likelihood of contamination from modern salmon sources, modern positive controls were not included in this study. Alternatively, we introduced a

new type of positive controls - ancient salmon sample - into the study to ensure the effectiveness of DNA extractions and PCR amplification. These positive controls (pink, chum and sockeye) were from previously extracted and DNA-identified salmon samples analyzed in a preceding ancient DNA study (Yang, *et al.* 2004).

One non-salmon bone sample was included within our sample for DNA extraction to investigate the possibility of cross-contamination arising due to samples being housed within the same Ziploc bags for extended periods of time. If cross-sample contamination was an issue, then likely the non-salmon bone would yield PCR amplification of salmon DNA. Furthermor, nine samples, (approximately 15% of the tested samples) were repeated from DNA extraction of bone samples through to PCR amplifications and sequencing in order to test the reproducibility of the obtained ancient DNA sequences.

Species Determination

The obtained sequences were compared to GenBank sequences through the BLAST application (http://www.ncbi.nlm.nih.gov/BLAST/) to determine if they would match *Oncorhynchus* sequences, and to ensure that they did not match with any other unexpected species or sequences. Sample sequences were visually edited and base pair ambiguities were examined using ChromasPro software (www.technelysium.com.au). Multiple alignments of the sample sequences and published salmon mtDNA reference sequences were conduced using ClustalW (Thompson, *et al.* 1994), through BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html). A species identification was assigned to a sample if it matched identically or very closely with published reference sequences, and no other evidence, including reproducibility tests or additional sequencing of the same sample indicated a different species. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Kumar, *et al.* 2001).

Contamination Controls

Comprehensive controls were taken during every step of the extraction and amplification procedure to reduce the risk of contamination (Cooper and Poinar 2000; Richards, *et al.* 1995; Stoneking 1995). Protective clothing, including masks, disposable gloves, and Tyvex™ suits with hoods and shoe covers, was worn during bone decontamination, extraction and PCR set-up procedures. Sample preparation, extraction and PCR set-up took place in a positive pressure laboratory, equipped with UV sources for workspace decontamination. Disposable aerosol-resistant plugged pipette tips were used to avoid contamination between samples via communal solutions, buffers and pipettes, and reagents were subjected to UV irradiation in a cross-linker for 20 min prior to use. Additionally, multiple blank extractions and negative controls were run in conjunction with the salmon samples to identify the possibility of systematic contamination.

The DNA extraction lab and the PCR lab are located in separate buildings, with separate ventilation systems, and all equipment, including pipettes, apparatuses, solutions, cameras, etc., is dedicated to either the DNA extraction or PCR laboratories. Steps were taken to prevent the introduction of modern DNA into the ancient DNA processing areas, and no modern salmon DNA has ever been extracted or set up for amplification within the ancient DNA laboratory space. Any positive controls processed along side the ancient samples were recovered from archaeological sites and date to time periods earlier than the samples in this study (approximately 5000BP) (Yang, *et al.* 2004). Since the ancient remains from 5000 BP contain low quantities of DNA, the ancient nature of the positive controls ensures that the possibility of cross-sample contamination is kept at an extremely low level.

Results

Radiographic Analysis

According to Cannon's (1988) method, radio-opaque vertebral bands on salmon vertebrae may represent slowed periods of winter growth and can be used to indicate the age of the spawning salmon, *i.e.* the presence of two dense winter annuli indicates a spawning salmon in its third year (see Figure 3 for examples of salmon of different ages). A total of 158 salmon vertebrae were radiographed and the subsequent age-profiles are present in Table 4. (The radiographic results specific to those samples that underwent ancient DNA analysis are presented in Appendix C).

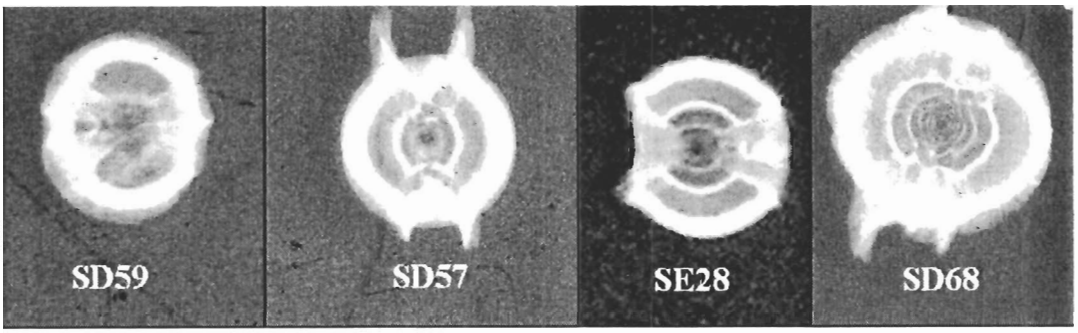

Figure 3 **Examples of radiographed samples. Sample SD59 displays one clear radio-opaque band, SD57 displays two, SE28 displays three and SD 68 displays five radio-opaque bands. Vertebrae are not to scale.**

All of the vertebral samples from specialized structure #9 displayed two obvious radio-opaque rings, and correspond to salmon of three years of age (provided that the rings represent actual annuli). The medium sized residential housepit, #3, demonstrated

approximately 90% three-year-old salmon, and 10% four-year salmon. Vertebrae with only one identifiable band, or salmon of two-years of age, were only identified in the small residential housepit, and composed 12.7% of the sample. The remaining sample consisted of 84.5% three-year-old salmon, and 2.8% six-year-old salmon (two vertebrae displaying five radiographic annuli). Due to the difficulty in distinguishing annuli caused by blurred x-rays or overlapping rings, 20 (approximately 13%) of the vertebrae could not be assigned vertebral counts, and were not included in the date set.

Table 4 **Results of radiographic analysis of vertebrae from both residential and specialized structures**

Structure	2-year-old salmon	3-year-old salmon	4-year-old salmon	5 year-old salmon	6-year-old salmon	Total
#12	9 (12.7%)	60 (84.5%)	0	0	2 (2.8%)	71
#3	0	38 (90.5%)	4 (9.5%)	0	0	42
#9	0	25 (%100)	0	0	0	25

Note: #12 is the small residential housepit, # 3 is the medium residential housepit and #9 is the specialized structure. #107, the second specialized structure did not contain any vertebrae elements. The table only includes those vertebrae that displayed identifiable rings.

PCR Amplification

Strong amplifications of at least one of the co-amplified D-loop or Cyt B fragment were observed for over 90% of the samples. An example of successful PCR amplifications can be seen on Figure 4. Occasionally, co-amplified samples displayed an unexpected third band of approximately 200 bp in length (Figure 5). All samples displaying the three clear bands were later identified through sequencing as sockeye, however, the presence of the additional band did not seem to affect sequence quality or subsequent species determination.

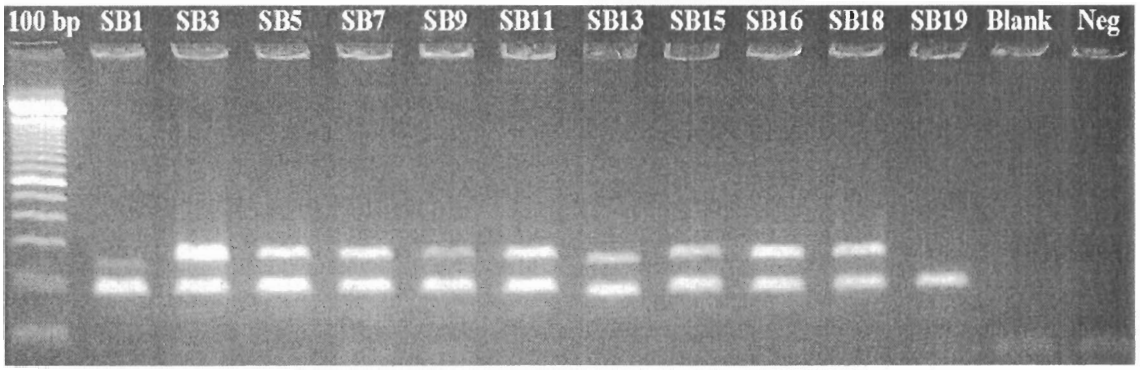

Figure 4 **Electrophoresis gel image of successful PCR co-amplifications of Cyt B (lower band) and D-loop (upper band) fragments. Note: SB# is for individual salmon remains, Blank for blank extraction, Neg for PCR negative control, and 100bp for 100 base pair ladder (InVitrogen, Varslbad, CA).**

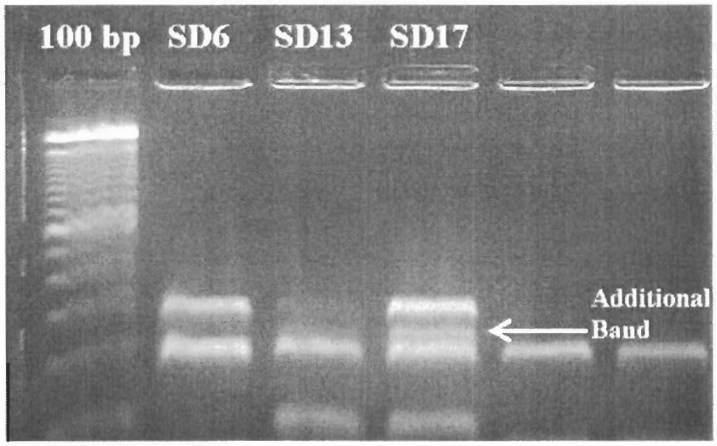

Figure 5 **Electrophoresis gel image produced with PCR displaying the additional third band that characterized some sockeye samples.**

Six of the samples (SE11, SE26, SD2, SD4, SD14 and SD23) did not yield any detectable PCR amplification for either the longer D-loop or Cyt B fragments. DNA degradation and/or PCR inhibition are likely responsible for the failed samples, although damage incurred to ancient DNA templates by x-ray irradiation could not excluded as a factor. All six samples were re-amplified in a simplex reaction using the shorter D-loop primers Smc3/Smc4, only one of the aforementioned 6 samples, SD23, achieved positive

amplification of the shorter D-loop fragment when a lower concentration of DNA (0.1 μM) was applied. No PCR amplification was observed for all blank extracts or the negative controls during the analysis.

Direct Sequencing

All 55 of the 60 samples that produced strong D-loop or Cyt B bands returned generally clear sequencing results. The process of co-amplification may occasionally produce an increase in primer-dimmers (the product of two primers binding together) and they cannot always be completely removed through purification. These primer-dimmers, along with some non-specific PCR products may produce base pair ambiguities and 'messy' areas at the beginning of the sequence electropherograms. Occasionally, some messy beginnings were observed, however, they did not adversely affect the species identification of the samples. Samples that produced ambiguous sequences due to either excessively weak or strong signal strengths were re-sequenced in order to achieve clearer results. Examples of clear and messy electropherograms can be seen in Figures 6 and 7 respectively.

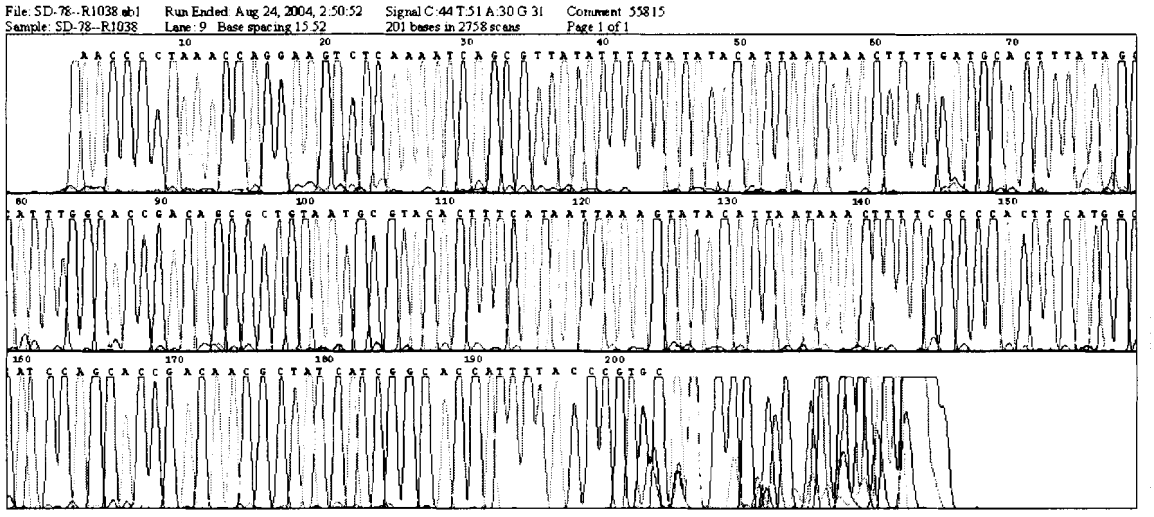

Figure 6 Electrophoresis of a clear sequence, even with signal strength below 100.

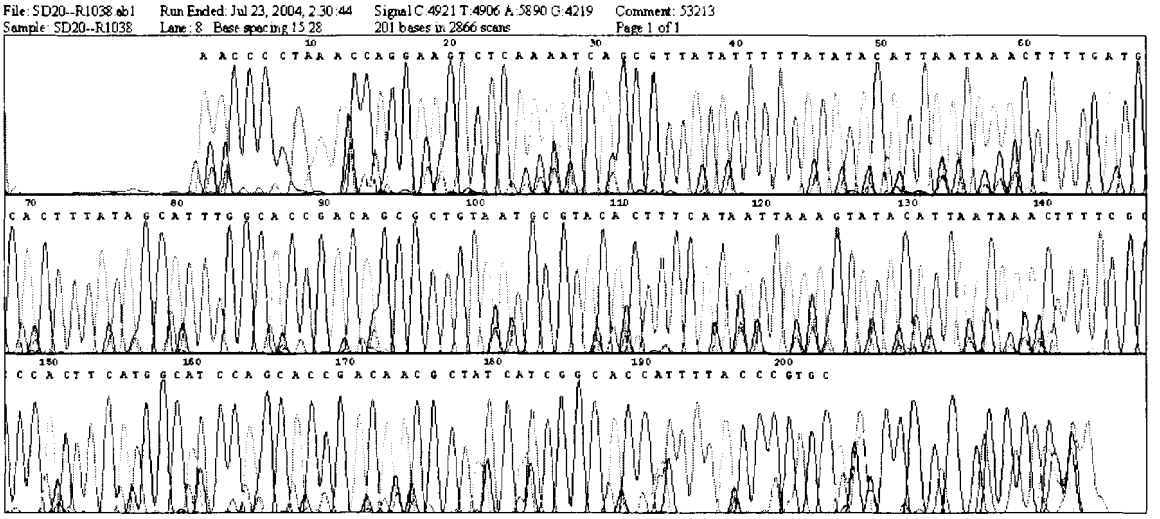

Figure 7 Electrophoresis of a 'messy' sequence (note signal strength is very high).

Species Identification

Species identification was confidently made for all positively amplified samples based on the results of direct sequencing. Sample sequences inputted into BLAST searches always produced a match with a member of the genus *Oncorhynchus*. In total,

three different species of pacific salmon were identified: sockeye (*O. nerka)*; chinook (*O. tshawytscha);* and coho (*O. kisutch).*

Sockeye salmon made up the bulk of the 55 identified samples (81.8%) while only 8 (14.5%) and 2 (3.6%) samples were identified as chinook and coho, respectively. A summary of success rates and species composition by housepit is presented in Table 5.

Table 5 Sample size, success rate and species composition for each structure

Structure	Tested Sample	Successful Sample	Success Rate	Sockeye	Chinook	Coho
#12	22	19	86.4%	17	2	0
#3	17	15	88.2%	12	1	2
#9	11	11	100.0%	10	1	0
#107	10	10	100.0%	6	4	0

Sequence Analysis

As expected for Pacific Northwest salmon populations and for restricted time period from which the samples obtained, low sequence variations were observed for all amplified PCR sequences. Several studies have indicated that salmon have generally low mtDNA variability compared to other fish (Bernatchez and Wilson 1998; Billington and Hebert 1991) which may be the result of the relatively recent post-glacial recolonization of the Pacific Northwest (Park, *et al.* 1993). Even analyses of the hyper-variable region of the D-loop of both modern and ancient salmon indicated low levels of inter-species variability (Nielsen, *et al.* 1994; Park, *et al.* 1993; Yang, *et al.* 2004), although the mtDNA NADH3 gene was found to have a slightly elevated level of genetic diversity within salmonids (Churikov, *et al.* 2001).

Only one haplotype of each species was identified within each of the species for the three amplified fragments, with the exception of the Cyt B amplifications of chinook remains and the SMC3 amplification of SD23. Alignments of the individual haplotypes with the modern reference sequences for the two D-loop fragments and the Cyt B fragment can be found on Figure 9, 10 and11, respectively. Detailed multiple alignments of all successfully amplified samples for all three fragments can be found in Appendix D, Figure D1, D2 and D3.

The chinook Cyt B fragment yielded two haplotypes distinguished by only a single base pair difference; samples SD 66 and SD68 both displayed a G at position 132, instead of the A that was identified in all other chinook Cyt B sequences. Analysis of the region indicated that positions 130-132 code for the amino acid glutamine; the mutation of A to G at position 132 would not affect the amino acid code, as glutamine is coded by both CAA and CAG. Generally mutations are more common in the non-coding D-loop region than they are in the region that codes for the Cytochrome B gene. However, since amino acid production at position 130-132 is not affected, the mutation is too unusual. Since the mutation was identified only in samples SD66 and SD 68 which are assumed to represent the same salmon individual, it is difficult to ascertain if the haplotype is individual to that salmon or if it is present in a greater portion of the population.

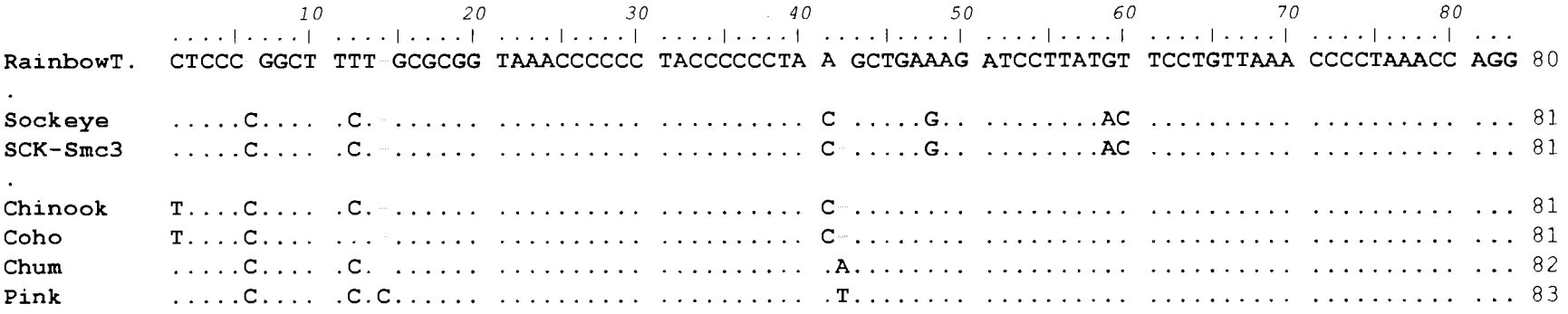

Figure 8 **Part of the amplified D-loop sequence by primers Smc3 and Smc4. All reference sequences were from Shedlock et al. (1992) except rainbow trout (NC_001717). The dots indicate identical base pair to the rainbow trout on the top and the dashes represent insertion/deletion when comparing to the rainbow trout sequence. SCK- refer to sockeye sequences from ancient remains. Heteroplasmy may be present at position 37, peaks of equal magnitude for both T and C are present in one of the samples.**

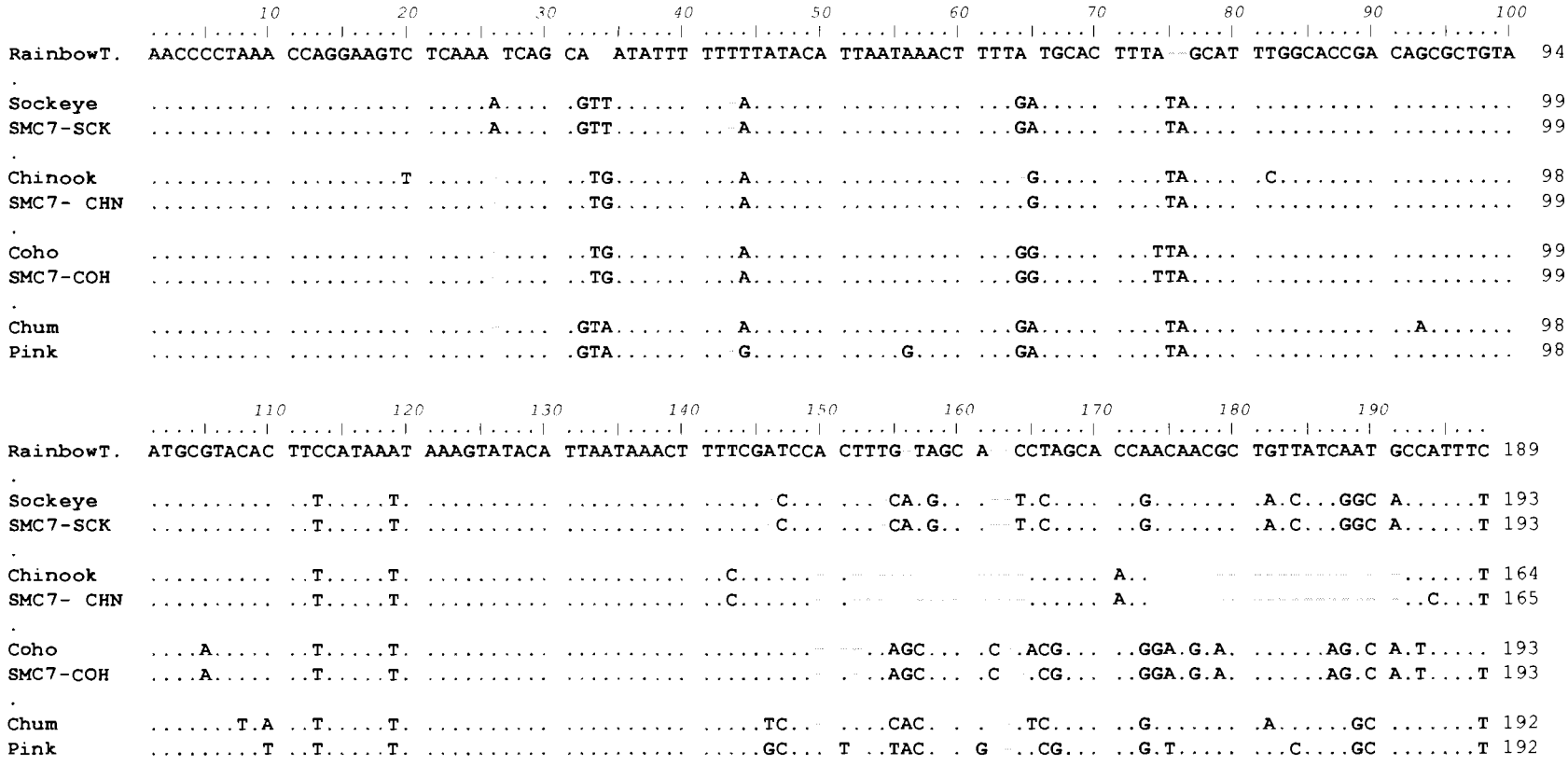

Figure 9 **Part of the amplified D-loop sequence by primers Smc7 and Smc8. All reference sequences were from Shedlock et al. (1992) except rainbow trout (NC_001717). SCK-, CHN- and COH- refer to sockeye, chinook and coho sequences from ancient remains, respectively.**

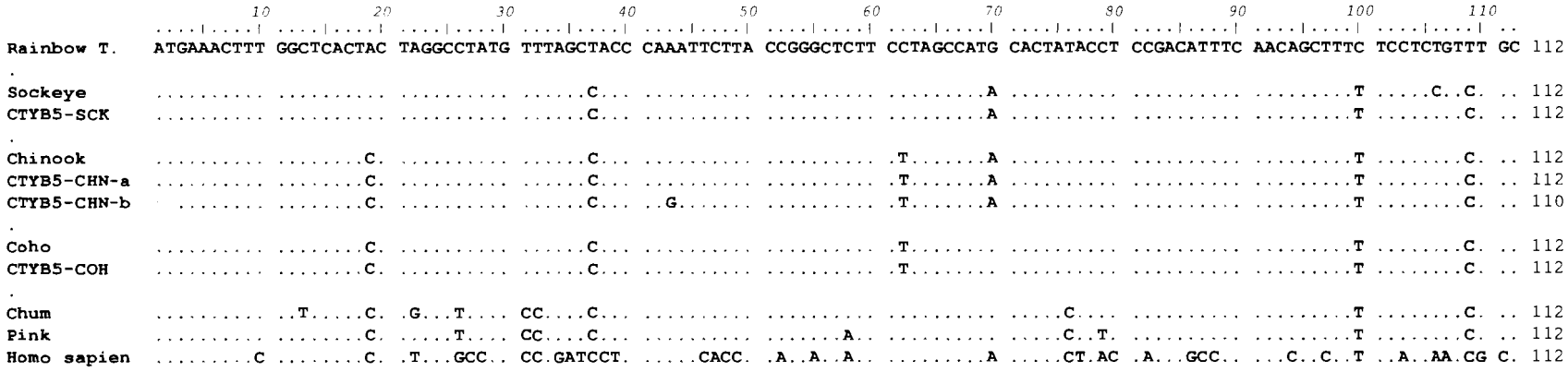

Figure 10 **Part of the amplified Cyt B sequence by CytB5 and CytB6. Reference sequences were retrieved from GenBank: rainbow trout (NC_001717), chum (AJ314561), coho (AJ314563), sockeye (AJ314568), pink (AJ314562), and chinook (AJ314566). SCK-, CHN- and COH- refer to sockeye, chinook and coho sequences from ancient remains, respectively.**

Potential heteroplasmy was identified in the shorter D-loop amplification of sample SD23. Appendix D, Figure D4 displays the multiple peaks present in sample SD23 at position 67, and absent in the two other samples. The multiple peaks could be representative of heteroplasmy, a situation in which a single individual, or single cell contains two different types of mitochondria. It is also possible that the additional peak could be caused by a sequencing or primer error, and additional amplifications and/or sequences would clarify whether the multiple peaks were caused by heteroplasmy or not. However, since the mutation did not affect species identification these procedures were not undertaken.

Although few individual differences were found between the samples, several differences between the amplified fragments and the modern reference sequences obtained from Genbank were evident. For example, although the amplified coho, and the majority of the chinook Cyt B sequences did not differ from modern reference Cyt B sequences, the reference sockeye sequence displayed a transition to a C from a T at position 106. The nucleotide-pair substitution of a pyrimidine to another pyrimidine (ex. T to C) is more likely than a transition from pyrimidine to a purine (ex. C to A) and is therefore not unexpected.

Within the longer amplified D-loop fragment, both chinook and coho sequences differed from the reference sequences. The chinook sequences displayed two transitions at positions 20 and 82 respectively, as well as an insertion at position 64 and a transition at position 193, indicating greater diversity with the reference sequence. However, species identification was not affected by these mutations as the deletion of 30 base pairs that characterizes the chinook salmon D-loop was evident within the ancient chinook sample sequences (this deletion was visible on the electrophoresis gel as a shorter second

band as compared with the other species, see Figure 4, sample SB1). The coho sequence displayed only two differences from the reference sequence, an insertion at position 151 and a deletion a position 164.

The slight difference between the amplified samples and the reference sequences are likely a reflection of regional and temporal variation. In spite of the differences, species identities were confidently assigned to three species, and phylogentic analysis demonstrated that the individual haplotypes in the study grouped most closely with their respective reference sequences (see Figure 12).

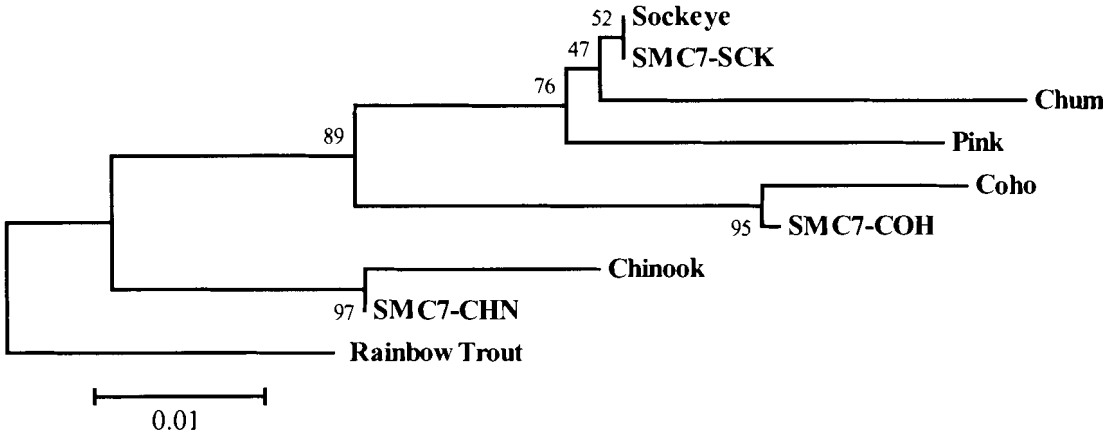

Figure 11 **The phylogenic relationship between the ancient samples and their modern *Oncorhynchus* counterparts. The phylogeny was produced using the Neighbor-Joining method and bootstrapping with 1000 replications. Tree is rooted using Rainbow Trout as an outgroup.**

Species Distribution by Structure

The distribution of species by housepit is depicted in Figure 12. Table 5 and Figure 6 both demonstrate that sockeye made up the majority of the remains in each of the housepits. The highest percentage of chinook remains (40%) was identified in #107, one of the specialized structures. In the other specialized structure, #9 only a single vertebrae was identified as chinook, accounting for 10% of the sample. Of the two

residential structures, the medium housepit, #3 contained sockeye, chinook and coho remains, while the small housepit, #12, contained only sockeye and chinook.

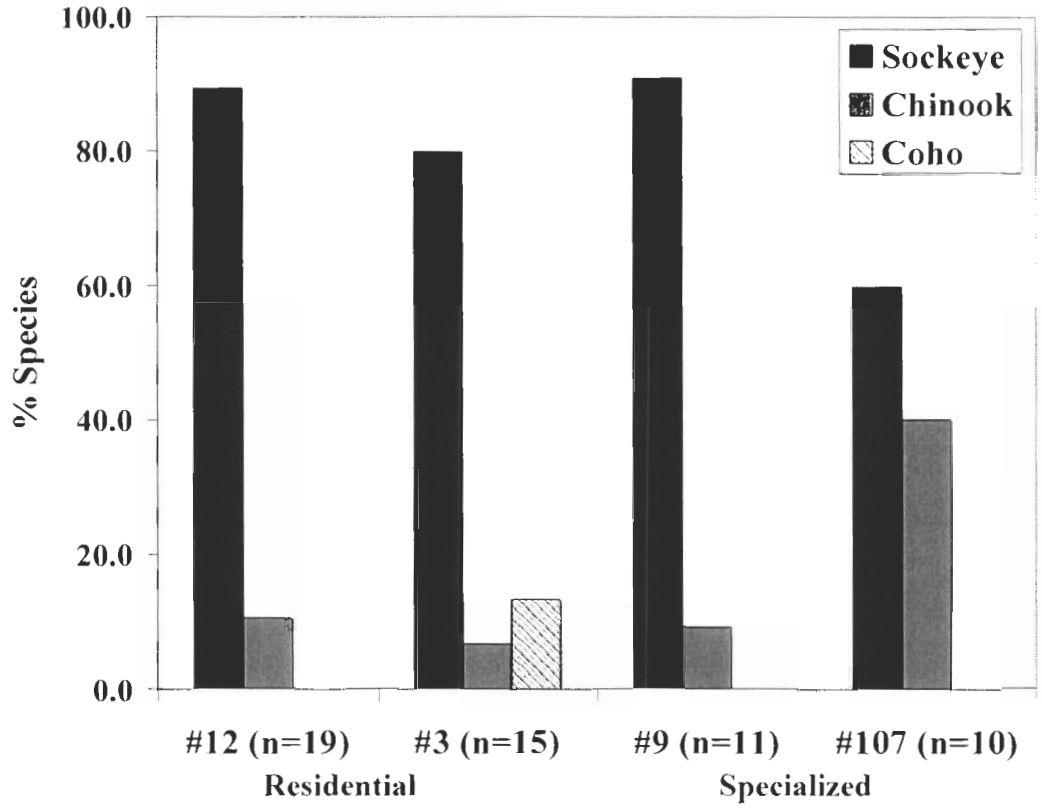

Figure 12 **Bar chart displaying the percentage of each species identified within each of the structures ('n' is the total number of identified remains).**

Species Distribution by Age Class

Table 6 presents a summary of the species identified in each age class. Although the sample size for two-year-old and four-year-old salmon are quite low, all salmon below six-years of age were identified as sockeye, while the two six-year-old vertebrae were identified as chinook. Unfortunately, the age of the only other chinook vertebrae in the sample could not be identified, although it was similar in size to the large six-year-old vertebrae. Additionally, since no vertebrae were identified as coho, an age profile for this

species could not be identified. Appendix C lists the combined results of DNA species identification and radiographic ring counts of each of the successfully identified samples.

Table 6 Species composition by radiographically identified age-group

Species	Two-year-olds	Three-year-olds	Four-year-olds	Six-Year-olds	Unidentifiable
Sockeye	2	30	1	0	3
Chinook	0	0	0	2	1

Discussion

Authenticity of Ancient DNA Results

Ancient DNA results must be proven authentic before they can be used to address research questions, and several publications have outlined the protocols required to ensure authenticity (Cooper and Poinar 2000; Paabo, *et al.* 1989; Poinar 2003). These protocols include the separation of the pre-PCR and post-PCR work spaces, and the use of ancient DNA dedicated equipment including clothing, equipment and reagents. The workspace should be equipped with UV light and should be cleaned using a 5% sodium hypocholorite solution after every use (Handt, *et al.* 1994). Other authentication protocols include the analysis of both positive and negative controls along side the ancient DNA samples and multiple extractions from the same bone sample should be carried out in order to reproduce the original results (Stoneking 1995). Furthermore, ancient DNA sequences should be scrutinized to ensure that they "make phylogenetic sense"; ancient or extinct species should demonstrate an affiliation with modern related species (Handt, *et al.* 1994). There should be an inverse relationship between amplification efficiency and amplification length, as fragments longer than approximately 500 bp are not expected to amplify effectively in ancient samples (Paabo, *et al.* 1989). Additional authentication can be obtained through cloning the amplified products to test the ratio of authentic to exogenous DNA. Finally, DNA analysis of other faunal remains recovered in association with the samples could be tested to test if other faunal remains contain amplifiable ancient DNA (Cooper and Poinar 2000).

Contamination Controls

The degradation and damage to ancient DNA means that even minuscule amounts of modern DNA can exceed the targeted DNA during PCR (Richards, *et al.* 1995); therefore, comprehensive contamination controls were taken during every step of the extraction and amplification procedure. Human contamination was unlikely an issue as primers were designed to avoid binding with human DNA. However, since most of the samples had been stored in contact with other salmon bones, and had possibly been exposed to modern reference samples during morphological comparison, cross-contamination with both ancient and modern remains was potentially an issue.

Samples were prepared within a dedicated Ancient DNA lab in which no modern DNA is processed. Protective clothing was worn during bone decontamination, extraction and PCR set-up procedures. This protective clothing included masks, sterile disposable gloves, and Tyvex™ suits with hoods and shoe cover. The disposable gloves were changed repeatedly. Protective clothing worn in PCR lab included sterile disposable gloves and coveralls. All equipment, including pipettes, apparatuses, solutions, cameras, etc. are dedicated to either the Pre-PCR or PCR laboratories, and clothing and shoes worn or in the PCR lab remain outside the vicinity of the extraction lab.

Sample preparation, extraction and PCR set-up took place in a positive pressure laboratory, equipped with UV sources for decontamination. Disposable aerosol-resistant plugged pipette tips were used to avoid contamination between samples via communal solutions and buffers. The Pre-PCR lab and Post-PCR lab are located in separate buildings, with separate ventilation systems, and same-day travel between the PCR lab and the Pre-PCR lab is generally prohibited. Steps were taken to prevent the introduction of modern DNA into the ancient DNA processing areas.

In addition, materials that reduce the amount of steps required for DNA extraction were employed preferentially. For example, DNA was concentrated using the Amicon Ultra-4. The Amicon Ultra-4 was selected over other concentrators, such as the Centricon™ 30 column because it requires substantially less steps for concentration, thus reducing the likelihood of contamination and human error.

The contamination controls undertaken in this study were successful at eliminating any systematic contamination as no PCR amplification was observed in non-salmon bone samples, blank extracts and PCR negative controls. Multiple salmon species were identified in the remains, possible heteroplasmy was identified in SD23, and two haplotypes of the chinook CytB sequences were identified, indicating that several different salmon individuals were present in the samples. Several differences were observed between modern reference sequences and ancient samples, another indication that modern salmon was likely not a source of contamination.

Decontamination Measure

Rigorous measures were undertaken in an attempt to negate the effects of any possible contaminants present in the sample. The scrupulous bone decontamination measures included both chemical and physical methods. Although, the chemical decontamination methods may seem overly severe, successful extraction and amplification had been conducted using this approach (Yang, *et al.* 2004). In fact, batches of samples that were not immersed in HCl and NaOH displayed the lowest success rates for PCR amplification. This suggests that potential deaminization caused by the emersion of samples in HCl is not overly harmful, and moreover, that rinsing the samples in HCl and NaOH may be effective at removing PCR inhibitors from the samples. The presence of PCR inhibitors may be inferred by the brownish colour of a

sample (O'Rourke, *et al.* 2000), which was noted in some of the failed samples (see Sample SE11 - Appendix D, Figure D5,). Diluting the PCR reaction solution with ddH2O may also increase the yield of amplifiable DNA. The dilution reduces the concentration of the PCR inhibitors and may allow for the enzyme to amplify the target DNA more effectively (O'Rourke, *et al.* 2000). Failed samples were re-run using only 0.1 μM of DNA from the first elution after purification, or using 3 μM from the second elution. The only sample to successfully amplify did so using a 0.1 μM of DNA, suggesting that PCR inhibition was the cause of at least one failed samples.

Species Identification

All the results from this study indicate the species determined for the samples were correct. All obtained sequences matched exactly or very closely with published reference *Oncorhynchus* sequences, and when analyzed phylogenetically, samples always grouped most closely with their reference counterparts. Two fragments of *Oncorhynchus* mitochondrial DNA from all samples were sequenced, except for SD32 which experience DNA degradation subsequent to PCR amplification. All samples displayed a match between the species identified using the Cyt B fragment and either of the D-loop sequences (see Table A1). Additionally, temporally isolated repeat extractions of more than 15% of the samples returned identical species identification as the original experiments and positive controls run along side the original samples always matched previously obtained species identity.

Phylogenetic Analysis

Analysis of the ancient sample haplotypes demonstrated that the results did make phylogenetic sense (Figure 12). The tree produced from these samples is in general agreement with previously conducted phylogenetic analysis of nuclear and mitochondrial

DNA from Pacific salmon species (Domanico, *et al.* 1997). Within the genus, chinook and coho are closely related, forming one sub-group, while pink, chum and sockeye form another subgroup (Domanico, *et al.* 1997; Healey 1991:313). As expected, the sister taxa of sockeye, pink and chum form one subgroup (Figure 12). Unexpectedly, the sister taxa of coho and chinook did not form a second subgroup. This discrepancy is likely caused by the fact that the short 200 bp sequence of mtDNA being analyzed contained a significant deletion within the chinook sequence, producing more variation between the chinook and coho sequences than would be observed through the comparison of the entire genome of the species. A phylogenetic tree produced using the conserved CytB region (results not shown) grouped chinook and coho within the same clade. Therefore, the resulting atypical phylogenetic tree is not indicative of errors in amplification or species identification, but rather a result of the limited genetic information being compared.

DNA Recovery Success Rate

Although a success rate of over 90% is rather high in ancient DNA projects, there is little doubt that the DNA obtained was authentic. The methods and primers used in this study have already demonstrated success in distinguishing species of salmon in previous studies (Yang, *et al.* 2004). Also the relatively recent nature of the remains should be expected to yield viable DNA, as much older salmon bone samples (up to 7,000 BP) from the Pacific Northwest have produced positive PCR amplifications. Additionally, an inverse relationship between amplification success and fragment length was observed, as samples that readily amplified the shorter D-loop fragment (135 bp) and the Cyt B fragment (168 bp) were not always successful at amplifying the longer D-loop fragment of 249 bp (See samples SB19, SD22, SD23 – Appendix C).

It is apparent that the DNA in the salmon bones at Keatley Creek is particularly well preserved, as DNA was successfully extracted and amplified from fragments as small as a single salmon rib. The success of ancient DNA extraction and amplification is perhaps attributable to the processing of the salmon remains prior to deposition. The filleting and drying of the salmon for winter storage could have slowed the bacterial degradation of the DNA, preserving it more effectively.

Although x-ray irradiation may induce several types of damage to DNA, such as single or double strand-breaks, base modification, DNA-protein cross-links, and oxidized DNA bases (Douki, *et al.* 1999), most x-ray damage occurs as a result of hydroxyl radicals which are produced by the irradiation of water (Cadet, *et al.* 1999) . Because the archaeological bone in this study was desicated, and the DNA was protected by the bone matrix, damage incurred by x-ray was probably quite minimal.

Radiographic Analysis

Due to the lack of reliable morphological criteria for salmon species identification, DNA species identities obtained from this research could only be compared with the results from radiographic analysis of salmon vertebrae. Based on our radiographic analyses of the studied vertebrae, we found that 100% of the samples from the specialized structure (#9), 98% of the samples from the small residential housepits (#12), and 90% of the samples from the medium residential housepit (#3) displayed two obvious radio-opaque rings. If these radio-opaque rings represent true annuli, the vertebrae would correspond to salmon three-years of age (Cannon 1988). The remaining 10% of samples from the medium residential housepit displayed three radio-opaque bands, corresponding to salmon of four years of age.

These results displayed some inconsistencies with previous research conducted by Berry (2000) who applied the same radiographic method to analyze the salmon remains from five residential pithouses at the Keatley Creek, including housepits #12 and #3 analyzed in this study. Berry's radiographic study determined that 100% of the salmon vertebrae x-rayed from housepit #12 and 90% of the salmon vertebrae from #3 were identified two-year-old salmon. Since pink salmon has a fixed spawning age of two years while others have much broad range of spawning ages, Berry interpreted the one-ring salmon to be pink, rather than other species. In the medium housepit, the remaining sample consisted of salmon with three- and four-year growth rings and unidentifiable to species based on the radiographic method alone.

The differences observed between the radiographic age profiles developed in this study and that of Berry's previous study are likely the result of inter-observer subjectivity as well as the ambiguities inherent in the radiographic method. During the radiographic analysis undertaken in this study, it was noted that there was often great difficulty in assessing whether the second radio-opaque band present in the center of the vertebrae represented a true annulus or not. After examining a subset of Berry's x-ray results, it seems likely that he did not include the central radio-opaque band in annuli counts, which would result in age determinations that were consistently one year younger than the results achieved in this study. If the salmon age profiles determined in Berry's study are increased by one year, the results from the two radiographic studies become very similar.

Our DNA analyses clearly indicated that the majority of the remains (containing two-, three-, and four-year-old salmon) were identified as sockeye; the sample did not contain a single pink salmon. The vigorous contamination controls conducted in this study as well as the results observed assure the authentic nature of ancient DNA in this

study. Any technical errors in the species identification method can be excluded since pink salmon remains from another archaeological site were processed alongside the studied samples as positive controls, and another on-going salmon project completed in the same DNA lab has yielded 30 pink salmon identifications.

Moreover, the identification of vertebrae with three-year growth rings as sockeye is not in conflict with sockeye spawning ecology in the region. Currently, sockeye salmon usually spawn between four or five years of age, but may spawn as young as two years of age. It has also been noted that the Fraser River streams contain a greater proportion of young spawning sockeye than other areas of their habitat (Burgner 1991:11). The two vertebrae displaying five rings were both identified as chinook salmon, which corresponds to the known chinook spawning age range of three to eight years (Fraser River Action Plan 1995a; Healey 1991:375-378). This observation has demonstrated the potential utility of radiographic analysis for the identification of chinook vertebrae in the region, however the large age range of spawning sockeye in the area of Keatley Creek demonstrate its intrinsic limitation in terms of other species identification.

The discrepancy between the results of Berry's radiographic species identification study and ancient DNA analysis are most likely the consequence of the inherent shortcomings of the radiographic species identification method, *i.e.*, indirectly inferring species identification based on apparent spawning ages alone and the subjective element involved in clearly recognizing annuli. Ancient DNA analysis, on the other hand, directly targets species-specific DNA sequences, rendering an unambiguous species identity.

Salmon Subsistence of Early Lillooet People

Based on the salmon species identified in the remains at Keatley Creek, the subsistence and fishing practices of the Lillooet can now be more accurately reconstructed. It is not surprising that sockeye, chinook and coho were identified in the salmon remains at Keatley Creek. Late runs of sockeye and chinook, and early runs of coho are available today from August to October, the months in which the Lillooet were accumulating food stores for the winter (Alexander 1992). Late runs of sockeye and chinook are also leaner than their spring counterparts, and would have been less difficult and time-consuming to preserve.

The most striking discovery of this study is the lack of pink salmon in all of the four studied housepits and its implications for diet reconstruction at Keatley Creek and other archaeological sites in the areas. It is likely that the lack of pink salmon in the studied salmon remains reflects the absence of pink salmon in the Lillooet region around 1,200 B.P. Like chum, pink salmon currently tend to only spawn approximately 250 km above the mouth of the Fraser River; few pink and chum spawn in the Lillooet region (Heard 1991:137). There is some evidence to suggest that pink salmon may have been spawning further upstream prior to the Hell's Gate landslide in 1913 (Fraser River Action Plan 1995b). However, historic fishery records from the turn of the century suggest that pink salmon rarely spawned as far upstream the Fraser Valley as Bridge River, one of the presumed fishing locations of the Lillooet (Romanoff 1985). Additionally, Romanoff's (1992:228) ethnographic study of current Lillooet fishing practices noted that pink salmon may not have been available until fish ladders were installed downstream at the 6 Mile fishery near Lillooet – a major migration impediment location. Despite the uncertainty concerning pink spawning zones, if pink were present in the Lillooet fishing

areas prehistorically it is likely that they were not as populous as chinook and sockeye due to their small size and weaker swimming abilities (Kew 1992).

The lack of pink salmon remains at the site is unlikely due to any known cultural discrimination against pink salmon. Although pink were not considered a preferred species ethnographically (Kennedy and Bouchard 1978; Teit 1906), they are less oily than other species and thus require less labour to dry and preserve. Although sockeye and chinook have a richer taste and a higher fat content they are more difficult to preserve and their flesh may become rancid if not dried sufficiently (Romanoff 1992). Additionally, due to their smaller size, pink salmon tend to run along the shallow edges of the water, and are reputed to be among the easiest salmon to catch (Berry 2000). In the lower reaches of Fraser River, the timing of pink spawning runs often coincides either with late sockeye and chinook runs, or early coho runs; it would be unlikely that pink salmon would be discriminated against in the Lillooet region if they were spawning contemporaneously in large numbers with the other species.

It is important to note that the relative abundance of each salmon species at the site mirrors its overall availability in terms of contemporary salmon spawning stocks. Although salmon stocks will vary from year to year, around Bridge River, sockeye salmon usually outnumber both chinook and coho by hundreds of thousands or even millions (Kew 1992; Salo 1991). Coho make up a very small portion of the Fraser River spawning stocks in the Lillooet region and may number only within a few thousand (Kew 1992). Sockeye salmon are also easier to catch than chinook, as they are generally smaller (especially if spawning at two- or three-years) and would be swimming closer to the river's edge.

Salmon Storage

Some differences in species composition can be observed based on the skeletal element analyzed. For example, the only two coho samples identified in the sample were both from head fragments, and five of the eight chinook samples were obtained from non-vertebral elements. A study by Butler and Chatters (1994) at Keatley Creek suggested that the density of skeletal elements in the salmon bone assemblage at Keatley Creek played a minimal role in survivorship; the correlation between the density of elements and their survivorship at the site was insignificant. Within the large residential housepit that was examined, there was a high survivorship of the rather dense vertebrae, as well as the fragile basipterygium and coracoid, while only a few of the dense jaw bones were recovered. Butler and Chatters postulated that since the site is further away from the fishing areas, heads were probably removed in order to reduce the weight of the catch returned to the site.

There are traditionally two different preservation techniques when drying salmon for storage. In one situation, the entire fish is dried as a single fillet and includes the backbone, while in the second situation, the fish is separated into two fillets and the backbone is removed prior to drying (Kennedy and Bouchard 1992:291-298; Romanoff 1992:235; Teit 1900:234). The first technique was generally applied to the leaner later runs of salmon in the fall, while the second technique was applied to oilier species If the second technique was applied differentially to chinook or coho, then a dearth of vertebrae from those species may occur. Another preservation technique involves the drying of salmon heads (Teit 1900:235), or the smoking of the fatty heads of chinook for storage (Kennedy and Bouchard 1992:293-294). If chinook were selected preferentially for fish head preservation then we might see a greater number of chinook head fragments preserved at the site compared to other species.

It could be suggested that species such as coho and chinook were being stored differently than sockeye, therefore displaying differences in the proportion of vertebral versus non vertebral elements identified per species. Although a Chi-Squared test of distribution of salmon species by vertebral and non vertebral elements was not significant at the 0.05 level ($p>0.10$), the small sample size may account for the lack of statistical significance.

Environmental Variables Affecting Salmon Access

The presence of salmon species and the abundance of individual species can be informative in reconstructing early salmon fisheries and cultural activities, as well as in the identification of key environmental variables affecting resource accessibility. The absence of pink and chum, without known cultural discrimination for these species, suggests that overall salmon availability was a key factor in Lillooet resource use. If current and historic fishery records are any indication of past species profusion, then the abundance of salmon within each of the species spawning runs would also be a factor in salmon accessibility, since relative quantity of each salmon species at the site reflect its current overall spawning stock abundance. Finally, the presence of chinook, sockeye and coho at the site indicate that the timing of the spawning run was likely not a key factor in salmon acquisition; these species spawn over a long period of months, from July to November, demonstrating that the Lillooet were not confining salmon fishing and storage to only a few months of the year. However, it is likely that most sockeye and chinook were caught and dried in August, when the fish were leaner and the weather was hot enough to dry the fish (Kennedy and Bouchard 1992:272-3).

The identification of the baseline environmental variables affecting species exploitation dramatically alters not only the picture of general salmon fishing practice but

also the pattern of preferred species distribution in native communities and the degree to which economic stratification would have affected access to species. With the environmental variables in mind, the distribution of species between housepits can be assessed in order to identify the cultural factors that would have affected species distribution.

Species Distribution

As mentioned previously, the most ubiquitous species in the remains is sockeye salmon. Percentages of sockeye from each of the tested structures are graphed in Figure 5, and it is evident that in all cases sockeye salmon makes up the greatest percentage of the remains. However, there do seem to be some differences apparent in the distribution of the chinook and coho between the four structures. Structure #107, apparently a ritual structure, contains the greatest percentage of chinook salmon, although this pattern is not repeated in its associated structure, #9. However, recovery of other material remains suggests that different activities seem to be represented in each of these structures.

As originally hypothesized, the medium-sized residential housepit (#3) did contain a greater diversity of salmon species as compared to the small residential housepit (#12), as well as a lower percentage of the most 'common' species, sockeye. Although housepit #12 did contain two chinook salmon remains, and therefore displayed a higher overall percentage of chinook than #3, it should be noted that the vertebrae in question were judgmentally sampled due to their unusually large size in comparison to the rest of the assemblage and it is likely that both vertebrae belong to the same salmon individual. Due to the inclusion of both large vertebrae, the overall percentage of chinook in the remains of the small housepit is probably overemphasized. Provided that the two judgmentally sampled chinook vertebrae are not included, Chi-squared tests of

species distribution across all four housepits indicates that the results are significant at the p=0.025 level, demonstrating a difference in the species accessed by the structures. However, with such a small sample set, the inclusion or removal of a few samples can easily affect the results of statistical analysis, so although initial differences are apparent in the distribution of salmon species, they are not dramatic.

Economic Stratification and Species Restrictions

The small sample size of the ancient DNA study makes it difficult to assess if access to different species *per* se was a characteristic present among the Lillooet. However, the differences observed between the housepits may indicate that cultural factors were affecting the distribution of salmon resources. With pink salmon no longer occupying a central position in terms of salmon resources available to the Lillooet, concepts of cultural preference and the effects of economic stratification on resource distribution must be re-evaluated.

It is unlikely that coho salmon was a common staple of the Lillooet since it occurs today in low frequencies and it was identified prehistorically in only a singly housepit, with only two coho remains identified in that structure. However, coho does spawn a few months after the major runs of sockeye and chinook and may have been a more important resource when insufficient quantities sockeye or chinook were caught in July and August.

The two remaining species, sockeye and chinook, were both initially considered preferred species due to their higher oil content compared to other species. However, of these two species, chinook are usually fatter because sockeye do not eat on their migration upstream and typically lose some of their fat (Romanoff 1992:259). Although chinook and sockeye spawn at varying times throughout the spring and summer, they run concurrently in July and August in the Lillooet region, reputedly the months in which the

greatest concentrations of large chinook arrive, as well as the months in which most salmon were dried for winter storage (Kennedy and Bouchard 1992:272). The most noticeable difference between the two species is their size, with chinook typically being a much larger fish. Kennedy and Bouchard (1992: 272) report that although "large spring [chinook] salmon run together with the sockeye, the springs are found at greater depths"; a platform may have been necessary to catch the large chinook. A Lillooet fisher explains,

> "These platforms that the old Indians used to fish ... They always go deep. They build platforms out. They put the net down maybe six of (*sic*) seven feet from the surface ... It doesn't matter how much sockeye is on the surface. The sockeye go on the surface. Deep down when they get one they'll get a spring salmon" (Romanoff 1992:232).

Due to their smaller size, younger spawning age, and greater abundance, sockeye salmon in comparison with chinook would have been easier to catch and preserve. Additionally, ethnographic evidence suggests that individuals owning fishing platforms had primary access to chinook, while sockeye were available from public fishing areas (Romanoff 1992). With only chinook and sockeye present at the site in significant numbers, it is likely that economic stratification would have precluded general access to chinook while sockeye would have occupied the place as the common staple, (assuming that sockeye intra-species differences in terms of spawning periods and other characteristics was not a factor in accessibility).

Due to the large sample size of Berry's (2000) radiographic study, its results may still indicate the presence of selective access of salmon resources at the site, even if the salmon ages were consistently identified as one year too young. The radiographic study demonstrated that the size of the salmon (which is associated with its spawning age) was correlated with the size of the pithouse, with poorer households accessing primarily small

young salmon, while large pithouses had access to greater numbers of older and therefore larger salmon. The radiographic results of the present study (Table 4), also indicated that only the small residential pithouse (#12) displayed salmon of two years of age, while all other structures displayed salmon of at least three-years of age. This differential age distribution of salmon between structures requires some explanation.

It can be suggested that the two- and three-year-old spawning sockeye accessed by the smaller residential housepits are probably smaller, weaker swimmers, and have less body fat than four-year spawning sockeye. Fishery studies have noted that immature spawning salmon, or 'jacks' are around two-thirds of the size of many four-year-old spawners (McMurrich 1912:14), possibly indicating that the younger spawners may have been similar to pink salmon in some important respects– *i.e.*, easier to catch near shorelines and perhaps easier to process for storage . Therefore, the distribution of different sized salmon may reflect differential access to prime fishing locations where larger salmon could be procured, reinforcing the pattern visible in the distribution of chinook remains at the site.

Another interesting, through entirely speculative possibility, may be that the size differences visible between the housepits, and the rather young spawning ages of sockeye observed in housepit #12 may be related to the differential exploitation of kokanee, the nonanadromous form of sockeye (also known as *O. nerka kennerlyi*). Although the two forms of sockeye are quite similar morphologically, kokanee normally mature earlier and at a smaller size than the anadromous form (Gustafson, *et al.* 1997). Whereas sockeye typically mature around four years of age, with a weight of approximately 5 kg, their landlocked counterparts develop within 2-4 years entirely within lake systems, and typically only weight around 1 kg at maturity (Burgner 1991:59; Ricker 1940). The

difference in their spawning age and size is likely a reflection of the economic costs of anadromous migration (Wood and Foote 1996). Intriguingly, kokanee are present in the Keatley Creek area, living and spawning in the Seton and Anderson lakes on the western side of the Fraser River (Mathewes 1978:97). In fact, both anadromous sockeye and landlocked kokanee spawn in the creeks around the Seton and Anderson lakes around September and October (Mathewes 1978:97). Moreover, Kennedy and Bouchard (1978; 1992:274) report that in early winter, dead kokanee 'known as 'floaters' were collected by the Lillooet from the shores of Seton Lake, and were boiled or barbequed as a staple food. Although the sources for these fish were more than 25 km from Keatley Creek, it is possible that poorer families procured the bulk of their staples from these less desireable locations.

Although they can spawn sympatrically, there seems to be some restriction in the gene flow between the two populations (Foote, *et al.* 1989); however, genetic studies have indicated that distinguishing kokanee from sockeye based on DNA alone may be somewhat challenging (Foote, *et al.* 1989; Gustafson, *et al.* 1997; Taylor, *et al.* 1996; Wood and Foote 1996). Although some kokanee and sockeye populations are genetically distinct, there is some evidence to suggest that sympatric populations may be more similar to each other than they are to their respective morphs in other watersheds (Foote, *et al.* 1989; Taylor, *et al.* 1996). This may be the result of kokanee populations having evolved from anadromous sockeye populations on a variety of independent occasions (Foote, *et al.* 1989; Taylor, *et al.* 1996; Wood and Foote 1996).

If the populations of kokanee and sockeye in the Keatley Creek area are genetically distinct, then Ancient DNA may hold the key to determining if the Lillooet were exploiting both the anadromous spawning sockeye and their landlocked

counterparts. Unfortunately, due to the lack of kokanee mitochondrial DNA reference sequences available in genetic databases, as well as the rather limited amount of genetic data analyzed in this study, the prospect of differential kokanee consumption must for now remain only an appealing supposition.

Conclusions

Ancient DNA analysis was successfully applied to salmon remains recovered from four structures located at the archaeological site of Keatley Creek for the purposes of species identification. The results of 60 salmon remains from two specialized and two residential structures have demonstrated that: 1) DNA is well preserved in the 1,200+ year-old salmon remains from the site; 2) there were at least three species of salmon stored and consumed at Keatley creek, including sockeye, chinook and coho; 3) sockeye was the most abundant species while no pink salmon were identified in the remains; 4) the radiographic method of salmon species identification based on vertebral annuli and spawning ages could not accurately differentiate three of the four species of salmon thought to be present at Keatley Creek; 5) as predicted, the results indicated that larger residential housepits displayed greater species diversity than small residential housepits, and differential access to restricted species (chinook) may characterize some specialized structures; 6) size may be a determining factor in the distribution of salmon resources through access to prime fishing locations; however, 7) the dramatic link between economic stratification and the distribution of preferred salmon species *per se* suggested in previous studies was not observed in this ancient DNA study.

Although this study noted some disadvantages in the radiographic method of species identification, the dismissal of this method is by no means advocated. While ancient DNA analysis may provide accurate species identification, the destructive, time-

consuming and costly nature of the technique precludes the investigation of large sample sizes. Conversely, in radiographic studies, hundreds of vertebrae may be analyzed quickly and relatively inexpensively. An obvious solution would be to combine the two methods, taking advantage of the assets of each technique. For example, once an age profile has been developed for a large proportion of the vertebrae at the site, ancient DNA analysis could be applied to select vertebrae from each age class, in order to examine if there is a correlation between the age of the salmon and its species. Combining the methods surmounts many of their individual shortcomings, providing more accurate species identification data for a sizeable number of remains.

In this study, accurate species identification through ancient DNA analysis has allowed for a clearer illustration of Lillooet subsistence practices and its relationship with the cultural development of the early peoples in the region. DNA analysis has clearly excluded pink salmon as the staple salmon species, and has provided new information concerning prehistoric species presence and absence in portions of the Fraser River. The results of this study are contrary to previous views that pink salmon had once been the dominant species in the Fraser River around Lillooet; it now appears that this was not the case. Rather, the historically documented dominance of sockeye seems to have persisted at least for the last 1,000-2,000 years. Additionally, if the radiographic method is accurately identifying spawning ages of two- and three-years in sockeye, the behaviour of spawning sockeye in the Middle Fraser may have changed drastically over the past thousand years. This species abundance data will be important in correctly interpreting other archaeological subsistence evidence in the area, and in the reconstruction of early regional ecological and environmental conditions.

Due to the small sample size undertaken in this study, clear differences between structures and the salmon species contained within were difficult to generate. This study suggests that the relationship between economic stratification and species distribution at Keatley Creek is visible but clearly not as dramatic as previously assumed, although differential access to differently sized salmon may still characterize some structures.

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Appendices

Appendix A: Pacific Salmon Life Histories

The information presented below concerning salmon life histories has been complied from Department of Fisheries and Oceans literature (Fraser River Action Plan 1995a; 1995b; 1996), International Pacific Salmon Fisheries Commission Bulletins (Henry 1961; Killick 1955; Killick and Clemens 1963; Thompson 1945) and sources within the book *Pacific Salmon Life Histories* (Healey 1991; Heard 1991; Salo 1991; Sandercock 1991)

Sockeye Salmon (*O. nerka*)

Sockeye typically spawn from June to September, laying their eggs in outlet rivers, inlet streams or lakeshores. Fry emerge from their redds in late winter or spring, and although they may migrate to the ocean within a few months, most sockeye fry populations rear in lakes downstream from their spawning area for one to two year. Smolts migrate to the ocean during the months of May and June and typically spend 1.5-3.5 years at sea, where they increase rapidly in size. Sockeye normally mature and spawn at four years of age, although their spawning age-range is from two to eight years of age. Due to the average four-year spawning age, the Fraser River sockeye stocks usually vary in a four-year cycle, with one 'big run' followed by three 'poor runs'. Immature male spawning sockeye are referred to as 'grilses' or 'Jacks' while immature females are referred to as 'Jills'; in the Fraser River these two- and three-year-olds can make up a significant portion of the spawning population (Burgner 1991:11; Killick and Clemens 1963).

Adult sockeye are usually silvery in appearance, with a greenish-blue back faintly speckled with black. When spawning, they undergo a striking colour transformation, developing bright red sides, pale green heads and dark jaws, with males developing a

hooked jaw. The average fork length and weight of spawning sockeye is 65 cm and 3.0 kg respectively, although older fish have an average weight of 5.5 kg. On the other hand, two-year-old grilses may be significantly smaller at two-thirds of the size of four-year-old sockeye, with average lengths of about 46 cm (McMurrich 1912:14).

Chinook or Spring Salmon (*O. tschawytscha*)

Chinook salmon usually spawn between June and September; they are also referred to as spring salmon, because some populations return to their native streams to spawn as early as April and May. Once chinook fry emerge, between late March to early May, the three types of chinook salmon, 'immediate', 'ocean-type' and 'stream-type', display differences in fry behaviour. 'Immediate' fry migrants will swim to salt-water directly after emergence; 'Ocean-type' fry will migrate to the ocean within 60-150 days of emergence, migrating around early fall; while 'stream-type' chinook will spend one to two years in the river mainstem or in natal freshwater streams before migrating to the ocean as smolts. Chinook usually spend 1.5-4.5 years at sea and their age at maturity ranges from two to eight years. In Canada, the Fraser River is largest producer of chinook, with most spawners between the ages of three to five years old. Those chinook that spawn in rivers farther north tend to return at older ages, with five, six and seven year-old spawners being most abundant.

Adult chinook salmon have silvery sides, white bellies and dark blue-green backs and heads. When spawning, adult males will develop enlarged teeth and hooked snouts while the colour of both sexes darken, a reddish hue characterizes their fins and bellies. Chinook salmon grow very rapidly during their last year in salt water and they are the largest of the Pacific salmon species, with an average length and weight of 90 cm and 7-9kg, respectively. However, chinook can vary widely in size, sometimes reaching up to

45 kg and their overall size seems to be a result of food acquisition and feeding behaviour rather than strictly a consequence of spawning age (McMurrich 1912).

Coho Salmon *(O. kisutch)*

Coho, also known as silver salmon, occupy a wide range of freshwater spawning environments, from large watersheds to small tributaries. Fry usually remain in freshwater streams, small tributaries or channels for one to two years after emergence, and smolt migration to the sea frequently takes place in spring. They spend the least amount of time at sea, usually 0.5-1.5 years, and consistently mature and spawn around 2-4 years of age. Coho return to their native streams to spawn quite late in the year, between the months of October to December. Adult coho are bluish-black with silvery side, and black spots on their back and caudal fins. When spawning, both species display reddish to maroon sides, with dark backs and heads, while the males will develop a prominent hooked snout. They are one of the smaller species, with average lengths and weights of 55cm and 4.5kg, respectively.

Chum or Dog Salmon (*O. keta)*

Within British Columbia, chum salmon usually spawn in late September into January (although they can spawn as early as July in the Northern reaches of their habitat). In the Fraser River, most chum stocks spawn in the lower Fraser River below Hope, B.C. Their proximity to tidal waters allows chum fry to migrate seawards within hours or days of emergence, (usually during mid-March and April,) although in larger rivers they may spend up to a month making their way to the ocean. Juveniles predominantly spend mid- to late summer in estuaries and coastal waters before migrating well out to sea. Chum usually spend 2.5-4.5 years at sea and spawn around two to seven years of age, although within B.C. they predominantly spawn around three to

five years of age (Fraser River Action Plan 1996). Chum salmon are normally metallic greenish-blue with white tips on the pelvic and anal fins. When spawning their appearance alters drastically; they display reddish sides, dark bars, and grey or black blotches. The large teeth and hooked snout of spawning males earned the species their nickname 'dog salmon'. Average lengths and weights of chum are range between 65-75cm and 3.5-5kg, respectively, though they can grow as large as 15kg.

Pink or Humpback Salmon *(O. gorbuscha)*

Pink salmon are the smallest of the Pacific Salmon species, with average lengths and weights of 45cm and 1.5-2.5 kg, respectively. Their small size is due to their fixed spawning age of two-years, which also resulting in distinct breeding groups and mating exclusivity of even- and odd-year broods. Inshore migration in preparation for spawning takes place from July to September, with peak spawning runs occurring in October. Like chum, pink fry return to the sea within a few hours or days of emergence in April. Juveniles habitually rear in estuaries close to shore until September, when they move off into the ocean. Although they are small in size, pink migrate extensively and distantly in the ocean, and grow rapidly until their maturity. Pink salmon typically have metallic-blue backs and silvery bellies with oval black spots on their back and tails. When spawning, the species turn a pale grey or green with yellowish sides. Pinks are also known as "humpbacks" or "humpies" due to the humps males develop on their back during spawning, in addition to hooked jaws.

The Fraser River has largest pink salmon runs in Canada, and the bulk of the stocks spawn within 250 km from the mouth of the Fraser. Other B.C. spawning rivers include the Thompson River, Seton Creek, Bridge River, Harrison River and Chilliwak River stocks, although some minor stocks spawn as far north as Quesnel River. Because

they are small, pink salmon are particularly susceptible to migration impediments, like rock slides, poor water quality, drought, parasites, and predators. For examples, the Hell's gate Rock slide in 1913 blocked the migration of many millions of pink salmon from reaching the upper reaches of the river (Fraser River Action Plan 1995b; Heard 1991:137-138).

Appendix B: Summary of Sample Proveniences, Element Type and Species Identity.

ID	Structure	Square	SubSquare	Strata	Level	Strata Type	Element
SA2	107	E	16	3	1	Floor	Rib
SA4	107	D	11	-	-	Floor	Vertebral Spine
SA5	107	D	11	3	-	Floor	Non-vertebral
SA6	107	D	10	3	-	Floor	Non-vertebral
SA7	107	D	10	3	-	Floor	Non-vertebral
SA8	107	E	3	3	1	Floor	Non-vertebral
SA9	107	E	11	3	4	Floor	Non-vertebral
SA10	107	E	6	3	4	Floor	Non-vertebral
S11	107	G	14	3	1	Floor	Non-vertebral
SA12	107	G	14	3	1	Floor	Non-vertebral
SB1	9	I	16	VIII	1	Floor	Vertebra
SB3	9	H	13	VIII	1	Floor	Vertebra
SB5	9	F	2	VIII	2	Floor	Vertebra
SB7	9	D	11	VIII	2	Floor	Vertebra
SB9	9	F	2	VIII	2	Floor	Vertebra
SB11	9	C	3	VIII	1	Floor	Vertebra
SB13	9	B	3	VIII	2	Floor	Vertebra
SB15	9	H	12	VIII	2	Floor	Vertebra
SB16	9	D	15	VIII	1	Floor	Vertebra
SB18	9	F	6	VIII	2	Floor	Vertebra
SB19	9	G	8	VIII	1	Floor	Vertebra
SE1	3	F	3	VI	F#1	Pit Fill	Vertebra
SE9	3	F	3	VI	F#1	Pit Fill	Vertebra
SE11	3	F	12	III	1	Floor	Vertebra
SE15	3	M	2	III	1	Floor	Vertebra
SE21	3	AA	6	III	-	Floor	Vertebra
SE23	3	EE	14	III		Floor	Vertebra
SE25	3	EE	14	III	-	Floor	Vertebra
SE26	3	U	7	III	1	Floor	Vertebra
SE35	3	AA	1	III	-	Floor	Vertebra
SE40	3	U	5	III	1	Floor	Vertebra
SE45	3	M	16	III	1	Floor	Vertebra
SE47	3	M	16	III	1	Floor	Vertebra
SE48	3	A	5	III	1	Floor	Non-vertebral
SE49	3	M	11	III	1	Floor	Non-vertebral
SE50	3	M	10	III	1	Floor	Non-vertebral
SE51	3	N	9	III	1	Floor	Non-vertebral
SE52	3	E	16	III	1	Floor	Non-vertebral

ID	Structure	Square	SubSquare	Strata	Level	Strata Type	Element
SD2	12	J	4	III	-	Floor	Vertebra
SD4	12	F	16	III	-	Floor	Vertebra
SD6	12	J	16	III		Floor	Vertebra
SD9	12	J	13	III	1	Floor	Vertebra
SD13	12	J	4	#6	-	Pit Fill	Vertebra
SD14	12	J	4	#6	-	Pit Fill	Vertebra
SD17	12	D	11	#3	2	Pit Fill	Vertebra
SD20	12	D	11	#3	2	Pit Fill	Vertebra
SD22	12	D	-	#3	2	Pit Fill	Vertebra
SD23	12	D	-	#3	2	Pit Fill	Vertebra
SD24	12	D	-	#3	2	Pit Fill	Vertebra
SD25	12	D	-	#3	2	Pit Fill	Vertebra
SD32	12	D	-	#3	2	Pit Fill	Vertebra
SD57	12	D	-	#3	Bag D	Pit Fill	Vertebra
SD66	12	D	-	#3	Bag B	Pit Fill	Vertebra
SD68	12	D	-	#3	Bag B	Pit Fill	Vertebra
SD70	12	D	-	#3	Bag B	Pit Fill	Vertebra
SD76	12	J	5	III	1	Floor	Non-vertebral
SD77	12	J	15	III	-	Floor	Non-vertebral
SD78	12	J	15	III	-	Floor	Non-vertebral
SD79	12	J	13	III	1	Floor	Non-vertebral
SD80	12	J	4	#6	-	Pit Fill	Non-vertebral

Note: N/A indicates Not Applicable, - indicates unknown.

Appendix C: Summary of Sample Element Type, Radiographic Results and Species Identity.

ID	Structure	Elements	Radio-graphic Rings	Sequencing Results	
				CytB	D-loop
SA2	107	Rib	N/A	Chinook	Chinook
SA4	107	Vertebral Spine	N/A	Sockeye	Sockeye
SA5	107	Non-vertebral	N/A	Sockeye	Sockeye
SA6	107	Non-vertebral	N/A	Sockeye	Sockeye
SA7	107	Non-vertebral	N/A	Sockeye	Sockeye
SA8	107	Non-vertebral	N/A	Sockeye	Sockeye
SA9	107	Non-vertebral	N/A	Chinook	Chinook
SA10	107	Non-vertebral	N/A	Chinook	Chinook
S11	107	Non-vertebral	N/A	Sockeye	Sockeye
SA12	107	Non-vertebral	N/A	Chinook	Chinook
SB1	9	Vertebra	-	Chinook	Chinook
SB3	9	Vertebra	2	Sockeye	Sockeye
SB5	9	Vertebra	-	Sockeye	Sockeye
SB7	9	Vertebra	2	Sockeye	Sockeye
SB9	9	Vertebra	-	Sockeye	Sockeye
SB11	9	Vertebra	2	Sockeye	Sockeye
SB13	9	Vertebra	2	Sockeye	Sockeye
SB15	9	Vertebra	2	Sockeye	Sockeye
SB16	9	Vertebra	2	Sockeye	Sockeye
SB18	9	Vertebra	2	Sockeye	Sockeye
SB19	9	Vertebra	2	Sockeye	Sockeye*
SE1	3	Vertebra	2	Sockeye	Sockeye
SE9	3	Vertebra	3	Sockeye	Sockeye
SE11	3	Vertebra	2	NO PCR	
SE15	3	Vertebra	2	Sockeye	Sockeye
SE21	3	Vertebra	2	Sockeye	Sockeye
SE23	3	Vertebra	2	Sockeye	Sockeye
SE25	3	Vertebra	2	Sockeye	Sockeye
SE26	3	Vertebra	3	NO PCR	
SE35	3	Vertebra	2	Sockeye	Sockeye
SE40	3	Vertebra	2	Sockeye	Sockeye
SE45	3	Vertebra	-	Sockeye	Sockeye
SE47	3	Vertebra	2	Sockeye	Sockeye
SE48	3	Non-vertebral	N/A	Chinook	Chinook
SE49	3	Non-vertebral	N/A	Coho	Coho
SE50	3	Non-vertebral	N/A	Sockeye	Sockeye
SE51	3	Non-vertebral	N/A	Sockeye	Sockeye
SE52	3	Non-vertebral	N/A	Coho	Coho

ID	Structure	Elements	Radio-graphic Rings	Sequencing Results	
				CytB	D-loop
SD2	12	Vertebra	2	No PCR	
SD4	12	Vertebra	2	No PCR	
SD6	12	Vertebra	2	Sockeye	Sockeye
SD9	12	Vertebra	1	Sockeye	Sockeye
SD13	12	Vertebra	2	Sockeye	Sockeye
SD14	12	Vertebra	2	No PCR	
SD17	12	Vertebra	2	Sockeye	Sockeye
SD20	12	Vertebra	2	Sockeye	Sockeye
SD22	12	Vertebra	2	Sockeye	Sockeye*
SD23	12	Vertebra	1	Sockeye	Sockeye*
SD24	12	Vertebra	2	Sockeye	Sockeye
SD25	12	Vertebra	2	Sockeye	Sockeye
SD32	12	Vertebra	2	-	Sockeye
SD57	12	Vertebra	2	Sockeye	Sockeye
SD66	12	Vertebra	5	Chinook	Chinook
SD68	12	Vertebra	5	Chinook	Chinook
SD70	12	Vertebra	2	Sockeye	Sockeye
SD76	12	Non-vertebral	N/A	Sockeye	Sockeye
SD77	12	Non-vertebral	N/A	Sockeye	Sockeye
SD78	12	Non-vertebral	N/A	Sockeye	Sockeye
SD79	12	Non-vertebral	N/A	Sockeye	Sockeye
SD80	12	Non-vertebral	N/A	Sockeye	Sockeye

** Samples were amplified using SMC3/SMC4 primers, N/A indicates Not Applicable, - indicates unknown.

Appendix D: Colour Figures and Photos

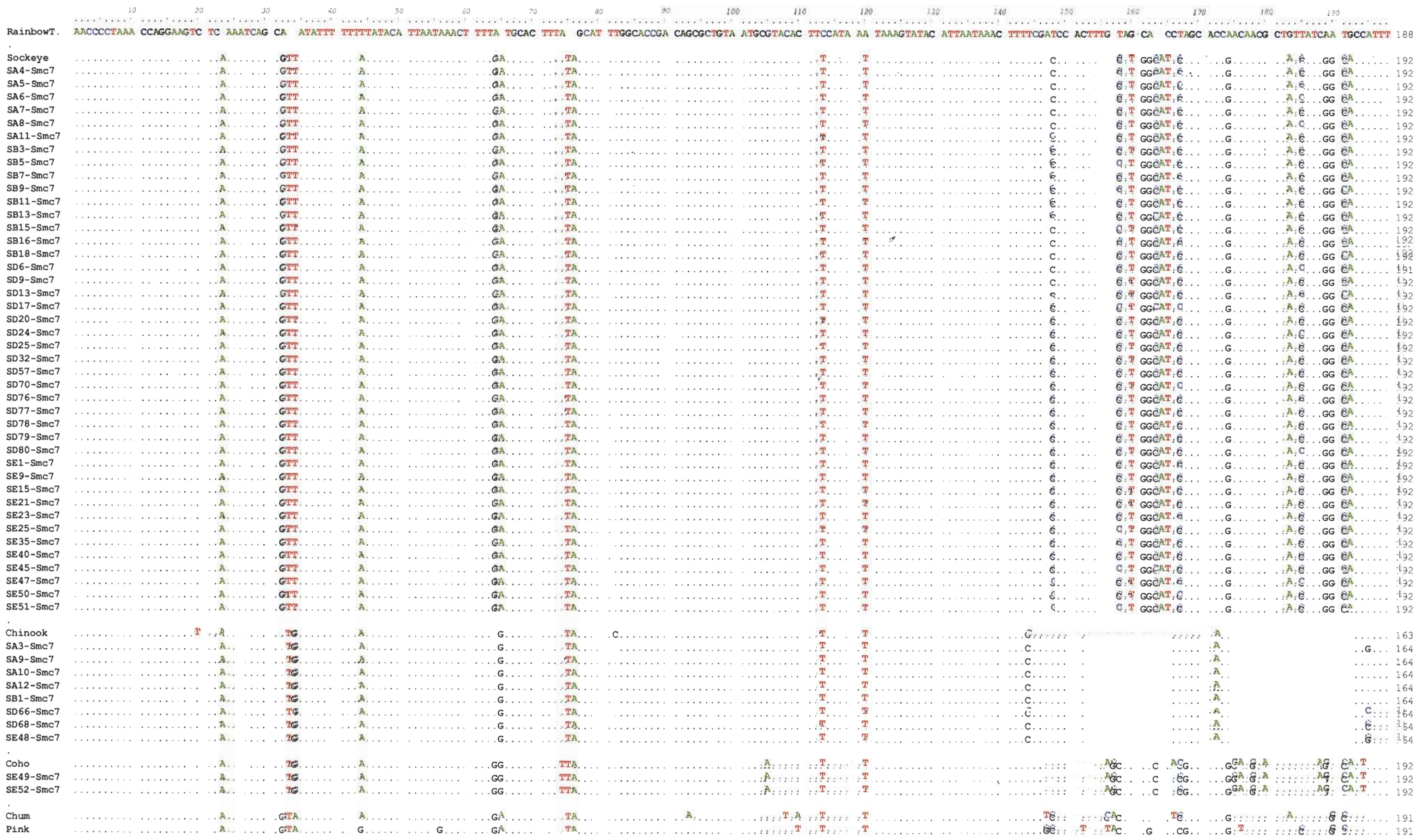

Figure D1 **Part of the amplified D-loop sequence by primers Smc7 and Smc8. All reference sequences were from Shedlock et al. 1992 except rainbow trout (NC_001717). Sample names based on Table A1 and primer Smc7/Smc8.**

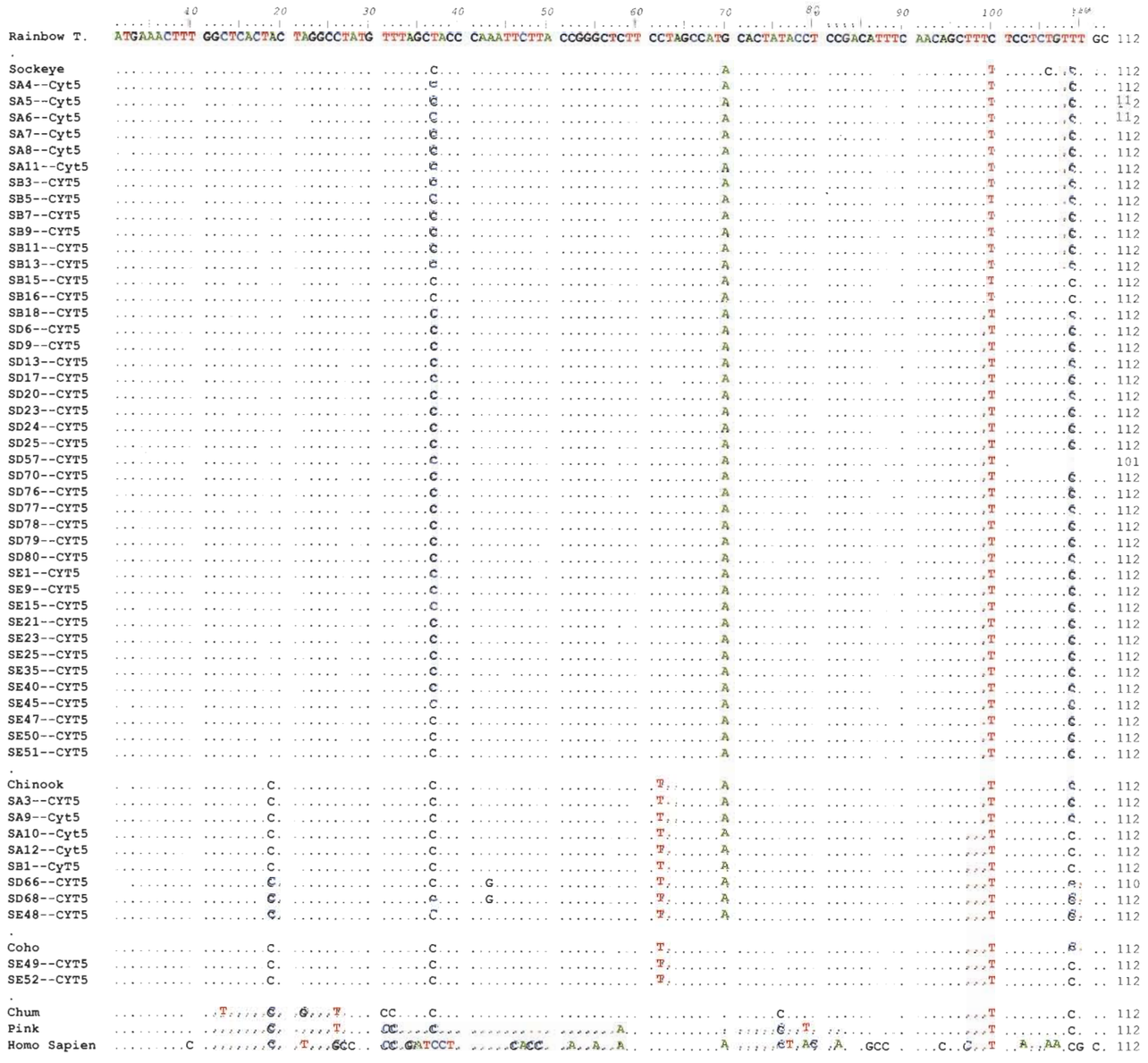

Figure D2 **Part of the amplified Cyt B sequence by CytB5 and CytB6. Reference sequences were retrieved from GenBank: rainbow trout (NC_001717), chum (AJ314561), coho (AJ314563), sockeye (AJ314568), pink (AJ314562), and chinook (AJ314566). Samples names based on Table A1 and on primer CytB5/CytB6.**

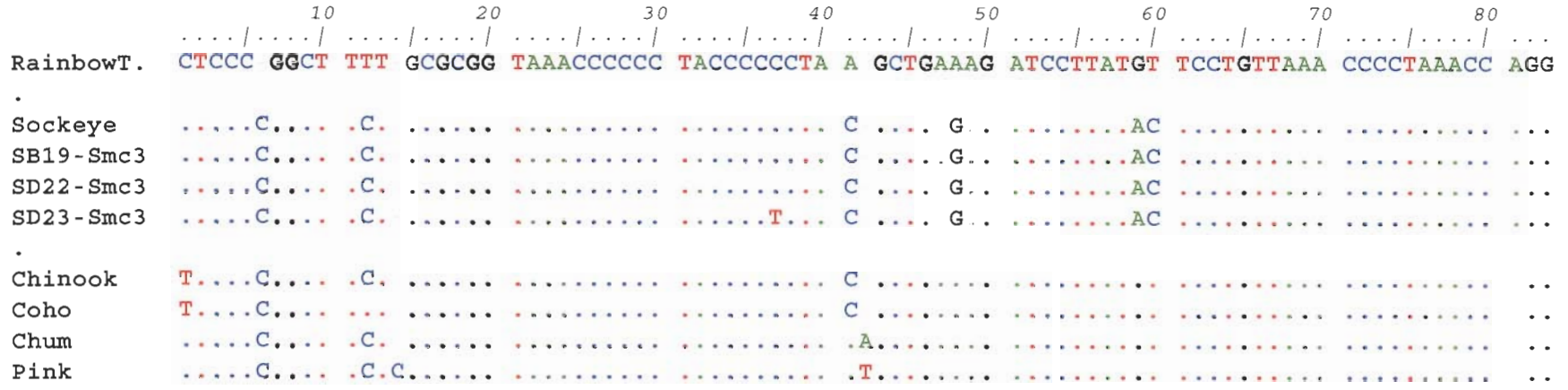

Figure D3 **The amplified D-loop sequence by primers Smc3 and Smc4. All reference sequences were from Shedlock et al. 1992 except rainbow trout (NC_001717). The dots indicate identical base pair to the rainbow trout on the top and the dashes represent insertion/deletion when comparing to the rainbow trout sequence. Heteroplasmy may be present at position 37, peaks of equal magnitude for both T and C are present in one of the samples.**

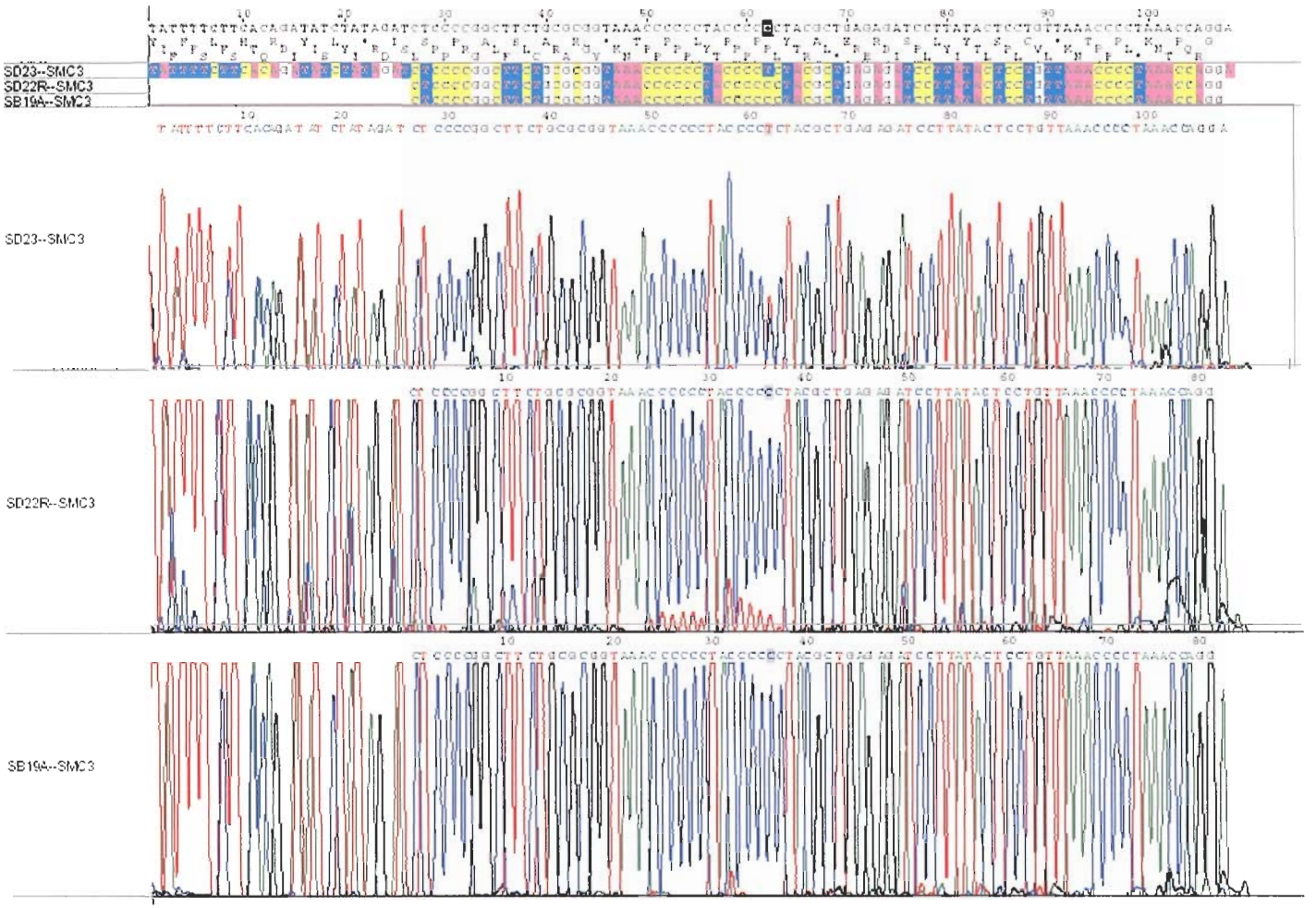

Figure D4 **Electropherogram of a potion of the amplified D-loop sequence by primer Smc3 and Smc4. Heteroplasmy may be present in SD23-SMC3 as peaks of equal magnitude for both T and C and present at position 62.**

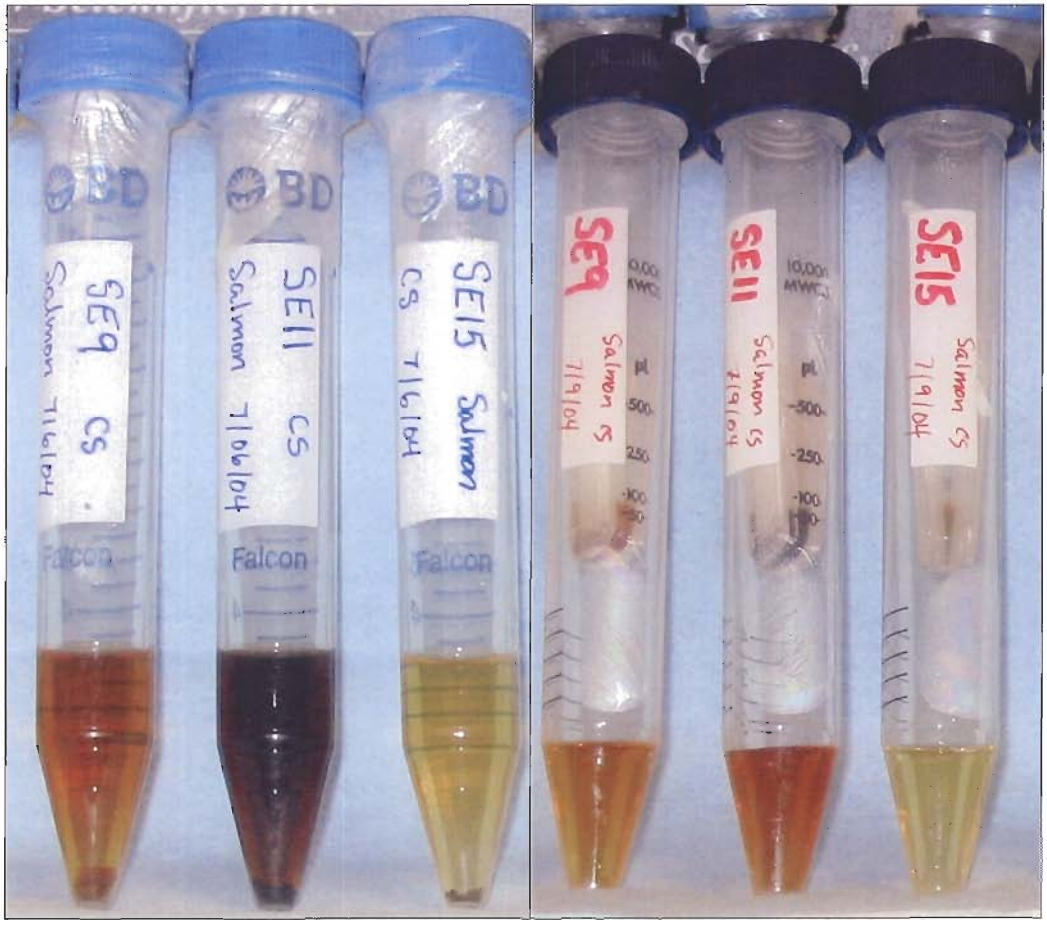

Figure A5 **Photo displaying dark browning colour of Sample SE11, a sample which failed to amplify successfully for any of the fragments. The brown colour may indicate of the presence of PCR inhibitors.**