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Journal of Archaeological Science 32 (2005) 1378–1389

Journal of Archaeological SCIENCE

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Ancient DNA investigation of prehistoric salmon resource utilization at Keatley Creek, British Columbia, Canada

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Received 13 January 2005; received in revised form 14 March 2005

Abstract

This study applied ancient DNA techniques to achieve accurate species identifications for the archaeological salmon remains recovered from the 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. 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 differences among the structures could still be observed.

Keywords: Ancient DNA; Species identification; Keatley Creek; Salmon bones

1. Introduction

Differential access to resources and unequal distribution of wealth are critical factors in the development of complex societies [13,45]. 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 [12,14,17]. 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 [38,43]. 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 (*Oncorchynchus 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

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doi:10.1016/j.jas.2005.03.016

their rich taste [3,43]. 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 [14]. 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 [3,9,17]. 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 [4,5,29,30,34,47]. Additionally, DNA techniques for species identification of archaeological Pacific salmon remains from the Northwest Coast of North America have already been developed [7,46].

This study employed DNA techniques to obtain accurate species identification of ancient salmon remains to test the hypothesis surrounding differential access to salmon resources at Keatley Creek, an archaeological site located in the Interior Plateau of southwest British Columbia. This site was selected for study as it 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 [17,19,27]. 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 indicated that there were dramatic differences in terms of the distribution of preferred salmon species among residential structures of varying sizes [3].

This study analyzed 60 salmon remains that were recovered from two specialized structures and two residential structures of differing sizes, and examined their diversity in terms of species abundance and composition to investigate the effects of economic stratification on the distribution of preferred salmon species at Keatley Creek.

2. Background

2.1. Archaeological context

The Keatley Creek site was a winter pithouse village located near the modern town of Lillooet, BC (Fig. 1) [20]. It is one of the largest prehistoric village sites in western Canada, and at its peak it may have had a population of over 1000 individuals [19]. It was occupied from 3500–1100 BP although the majority of the remains from this study were recovered from the Kamloops Horizon occupation dating to approximately 1200 BP. The housepits at Keatley Creek vary in size, from over 20 m to approximately 5 m in diameter, and house size is thought to be related to wealth and political power, with smaller houses generally being poorer than larger ones [17]. Storage facilities, floral, faunal, and lithic remains all vary between housepits of different sizes [17]. Although the sample size of fully excavated housepits is small, in almost all cases, the larger housepits show the greatest diversity and density of material and subsistence remains, indicating a "difference in the economic foundation of the large versus the smaller housepits" [15]. As well as residential housepits of varying sizes, the site also contains what appear to be specialized structures on the periphery of the site that may have been used for ritual purposes. These structures often contained a greater diversity of material remains than clearly residential small structures, as well as increased prestige goods [18].

2.2. Ethnographic accounts and species preference

In general, of all five salmon species (smaller pink, coho and larger sockeye, chum, and chinook) differ from each other in many ways, and their attributes affect both their desirability and their availability. Ethnographic accounts from the Lillooet region suggest that oily salmon, such as chinook and sockeye, were preferred as their greater caloric content was valuable for keeping warm in the winter months [15]. However, the high oil content of chinook and sockeye, especially in the early runs, makes them much more difficult to dry, and they require a greater labour investment for preservation. Ethnographic records suggest that corporate-groups in the Lillooet region with access to greater labour forces would have preferential access to these species [15].

Additionally, the preferred species of chinook and sockeye typically spawn during their third to fifth year of life and are usually larger than pink salmon which spawn at two years of age. Smaller salmon tend to run along the sides of the river, while larger salmon individuals, especially chinook, tend to swim within the deeper central river portions. Pink salmon and some sockeye would have been accessible from public fishing areas [36], whereas the most productive fishing locations would have been areas where the river was restricted, or where rocks extended into the waterway; platforms were sometimes built in these latter areas in order to access greater quantities of large salmon, such as chinook, and ethnographically, these platforms and fishing rocks were owned by families [36,37]. Ethnographically, preferential access to prime harvesting locations and the availability of labour generated inequalities within the Lillooet group in terms of access to the preferred species such as sockeye and chinook [15].

An earlier study conducted by Berry [3] attempted to assess the distribution of salmon species in select residential housepits at Keatley Creek using radiographic analysis of salmon vertebrae to estimate the spawning

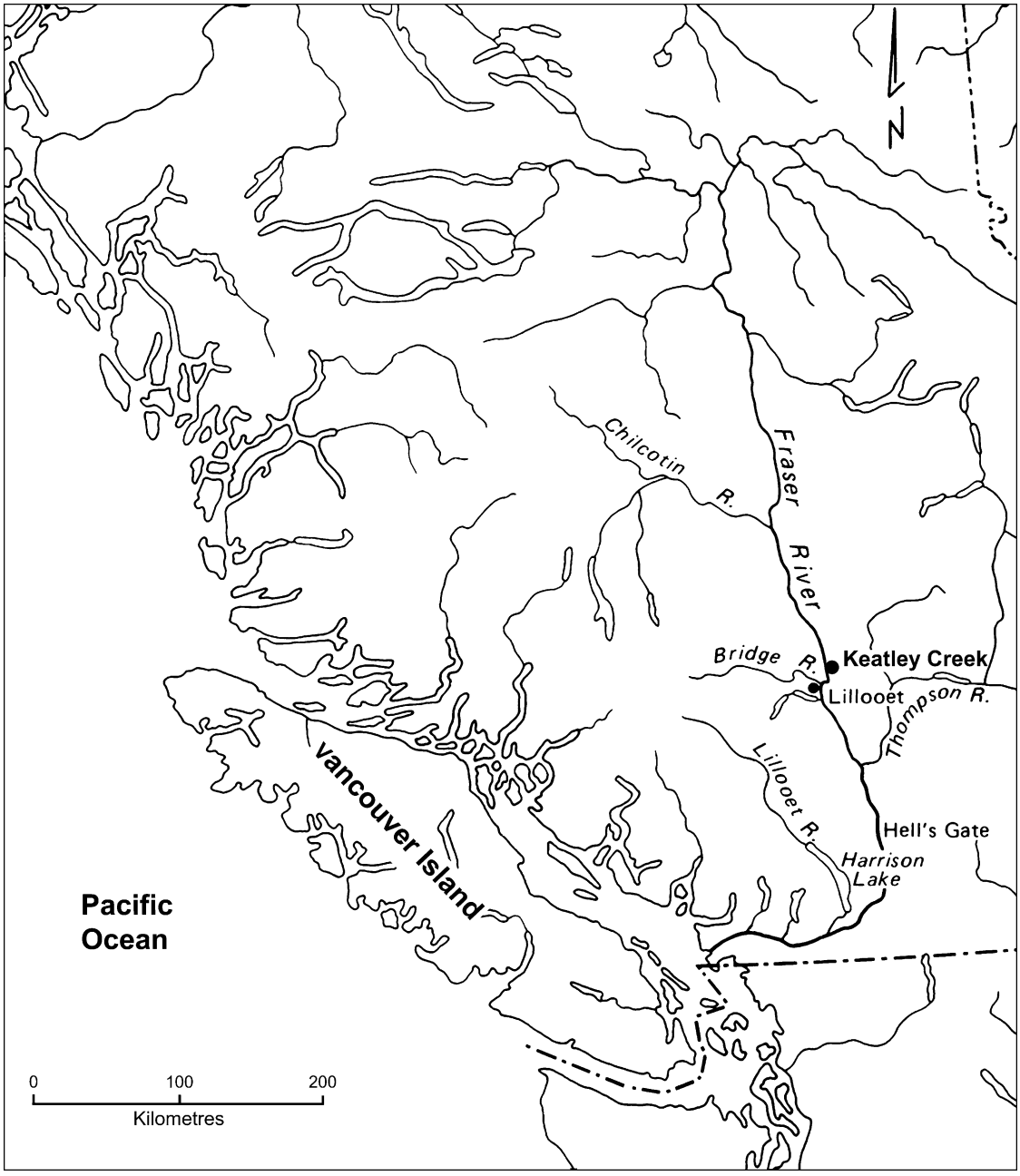

Fig. 1. Location of the Keatly Creek site (after Hayden and Ryder [20]).

ages of the remains. 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 [8,10]. The narrow radio-opaque bands visible on the X-ray are thought to correspond to the dense bone laid down during slowed winter growth and while the wider radiolucent bands are thought to correspond to periods of increased summer growth [8]. Age determination, based on annual growth rings, can be correlated with known spawning ages of salmon as a first step in species identification. However, salmon species with overlapping spawning ages (i.e., chinook, sockeye, coho and chum) cannot be distinguished from each other based on age determination alone. This method, however, is thought to be useful at distinguishing pink salmon from all other species, as it is the only species that spawns at two years of age.

Berry's [3] 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 pithouse 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. 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. 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 were not able to identify which additional species were differentially accessed by members of the larger households.

Prior to the advent of ancient DNA analysis, the radiographic method was one of the few means by which salmon resource distribution could be analyzed. However, the obvious drawbacks to the methods are that species identification must be inferred based on current spawning ages and only vertebral elements may be included in analysis. Ancient DNA analysis was undertaken, therefore, to investigate in a more accurate fashion which salmon species were being utilized by residents of Keatley Creek and to test if the application of ancient DNA analysis could identify the differential access of salmon resources between residential and specialized structures.

3. Materials and methods

3.1. Salmon remains

A total of 60 salmon remains recovered from four structures at Keatley Creek were selected for ancient DNA analysis; the characteristics and contents of the structures are summarized in Table 1. 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 [17,26]. Housepit #3 contains 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 [17]. Structures #107 and #9 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 [18]. Structure #9 contains an unusually large number of prestige goods and faunal remains, as well as articulated salmon vertebrae, fins, tails, and head elements. Structure #107 is located next to #9, and conversely contains very few cultural or faunal remains, although it does contain an unusual storage pit [17]. Ethnographies suggest that the site would have been used primarily in the winter months [2,43], and therefore 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.

When available, the salmon remains analyzed in this study were recovered from the living floor context. However if few floor samples were available, as in housepit #3 and #12, samples were also chosen from storage pits located within the respective housepits and dating to the same occupation as the living floor. Dates for the living floor context and associated storage pits are based on relative dating techniques associated with projectile point styles and artefact types as well extensive radiocarbon dating at the site completed by Simon Fraser University radiocarbon lab and Beta Analytic [16]. All of the living floor and storage pit samples used in the study were inferred to have been deposited between 1500 and 1100 BP (Late Plateau to Early Kamloops Horizon) – a period of relative cultural stability. The number of salmonid samples analyzed from each housepit is listed in Table 2.

Table 1
Characteristics of the four studied structures

Structure	Type	Size in diameter	Faunal remains	Artifacts and features
#12	Residential	Small (6 m)	Predominantly fish and unidentified mammal remains	Ephemeral hearth
#3	Residential	Medium (12 m)	Thousands of salmon remains in storage pits, floor remains contain dog, artiodactyla, and unidentified mammal	Freshwater shells, prestige lithic items, one main hearth and ephemeral hearths
#9	Specialized	Small (7.9 m)	Thousands of salmon remain, 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 (8 m)	Few fish remains, no vertebrae	Rock lined hearth, some unusual lithics remains (fan-tail bifaces), two large storage pits

Note: the information in this table is from published data [13,14,20].

Table 2
The 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

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. All of the samples were selected randomly from the salmon remains of each unit, except two vertebrae from housepit #12, which were judgementally sampled due to their unusually large size. Salmon vertebrae were selected preferentially over other elements due to the fact that radiographic analysis for age identification could be completed in addition to DNA analysis. Structure #107 did not contain any vertebral remains and therefore only head, fin and rib elements were available for DNA analysis. One non-salmon sample from the remains was included in this study as a control to monitor for possible contamination.

3.2. Radiographic analysis

In a previous study, radiographic analysis was conducted on selected salmon vertebrae from the Keatley Creek site to estimate the spawning ages of the remains [3]. 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 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 80 kVp at 15 ma at a distance of approximately 60 mm for 1.5 s. Spawning age (age at death) of the salmon was identified by examining the radiographic annuli on the vertebrae using the method set out by Cannon [8]. 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.

3.3. Ancient DNA analysis

One half of larger vertebra, whole small vertebra or a fragment of head, rib or fin element was processed for DNA extraction using the method developed by Yang et al. [46]. Sample decontamination protocols included a 50–100% commercial bleach rinse, followed by 1 N HCl and 1 N NaOH rinses; samples were subsequently subjected to UV irradiation for 30 min on each side. Three sets of primers (Smc3/Smc4, Smc7/Smc8 and CytB5/CytB6) [46] were used in this study to amplify two separate fragments of the control region (D-loop) and a Cytochrome *b* gene fragment (Cyt B) of mitochondrial DNA (mtDNA) for *Oncorhynchus* species identifications. 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 co-amplified the first D-loop primer set (Smc7/Smc8) and the Cyt B (CytB5/CytB6) primer set, at a primer ratio of 6:1, with primer concentrations of 0.6 μM and 0.1 μM, respectively. One advantage of co-amplification is that it serves to preserve DNA samples, as two separate fragments are amplified from a single aliquot of DNA.

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 in 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 s (denaturation), 56 °C for 30 s (annealing), 70 °C for 40 s (extension) followed by a final 7 min extension at 72 °C. Purified PCR products were sent to the Central Facility of the Institute for Molecular Biology and Biotechnology Laboratory at McMaster University for sequencing on an 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 DNA samples – 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 [46].

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. Approximately 15% of the tested samples were repeated from DNA extraction of bone samples (nine salmon remains) through to PCR amplifications and sequencing in order

to test the reproducibility of the obtained ancient DNA sequences.

3.4. Species determination

The obtained ancient DNA sequences were BLAST compared through GenBank to determine if they would match *Oncorhynchus* sequences, and to ensure that they did not match with any other unexpected species or sequences. Multiple alignments of the sample sequences and published salmon mtDNA reference sequences were conducted using ClustalW [42]. A species identity was assigned to a sample if it matched identically or was within only a few base pair difference from published reference sequences, and no other evidence, including reproducibility tests or additional sequencing of the same sample indicated a different species.

3.5. Contamination controls

Comprehensive controls were taken during every step of the extraction and amplification procedure to reduce the risk of contamination [11,35,41]. The same contamination controls listed in Yang et al. [46] were undertaken in this study including: (1) the use of protective clothing including masks, disposable gloves, and Tyvex™ suits; (2) the use of disposable aerosol-resistant plugged pipette tips; (3) the use of a positive pressure laboratory equipped with UV sources for workspace and material/reagent decontamination; (4) the inclusion of multiple blank extractions and negative controls for contamination detections and (5) the use of positive controls recovered from an archaeological site dating to time periods earlier than the samples in this study (approximately 5000 BP). Additionally, steps were taken to prevent the introduction of modern DNA into the ancient DNA processing areas; no modern salmon DNA has ever been extracted or set up for amplification within the ancient DNA laboratory space.

4. Results

4.1. PCR amplifications

Strong amplifications of at least one of the co-amplified D-loop or Cyt B fragment were observed for over 90% of the samples (Fig. 2 and Table 2). Five of the samples did not yield any detectable PCR amplification using any of the three primer sets. 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 be excluded as a factor. No PCR amplification was observed for all blank extracts or the negative controls during the analysis.

4.2. Species identification

Species identification was confidently made for all positively amplified samples based on the results of direct sequencing. Sample sequences entered into BLAST searches always produced a match with a member of the genus *Oncorhynchus*. Multiple alignments of the longer D-loop fragments and the Cyt B fragments can be found in Figs. 3 and 4, respectively. Three different species of Pacific salmon were identified: sockeye (*O. nerka*), chinook (*O. tshawytscha*) and coho (*O. kisutch*).

As expected for Pacific Northwest salmon populations [46] and for restricted time period from which the samples obtained, low sequence variations were observed for all amplified PCR sequences. Studies of both ancient and modern salmon have suggested that these low levels of genetic diversity may reflect the relatively recent post-glacial recolonization of these species [32,40,44,46]. Only one haplotype was identified within each of the species for the three amplified fragments, with the exception of the Cyt B amplifications of chinook remains which yielded two haplotypes

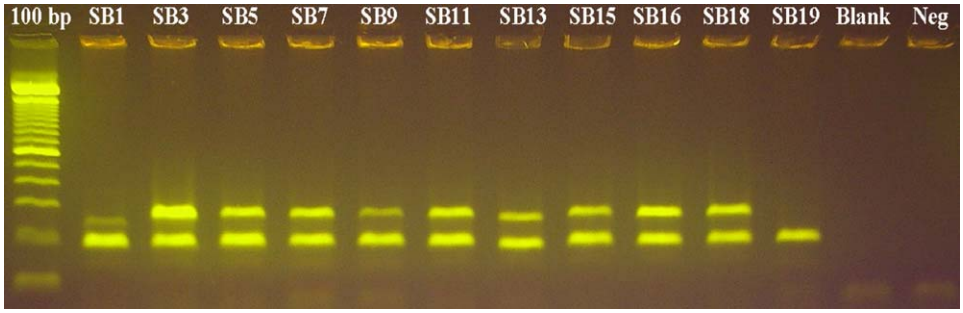

Fig. 2. Electrophoresis gel (2% agarose) image produced with PCR products fluoresced under a dark reader (Clare Chemical Research Co., USA) using SYBR Green). SB# is for individual salmon remains, Blank for blank extraction, Neg for PCR negative control, and 100 bp for 100 base pair ladder (Invitrogen). A deletion of 30 base pair characterizing the chinook salmon D-loop was visible on the electrophoresis gel as a shorter second band as compared with the other species (sample SB1). Unbalanced co-amplification occurred in SB1 and SB19, likely due in SBI end a relatively lower quantity of longer fragments in the sample.

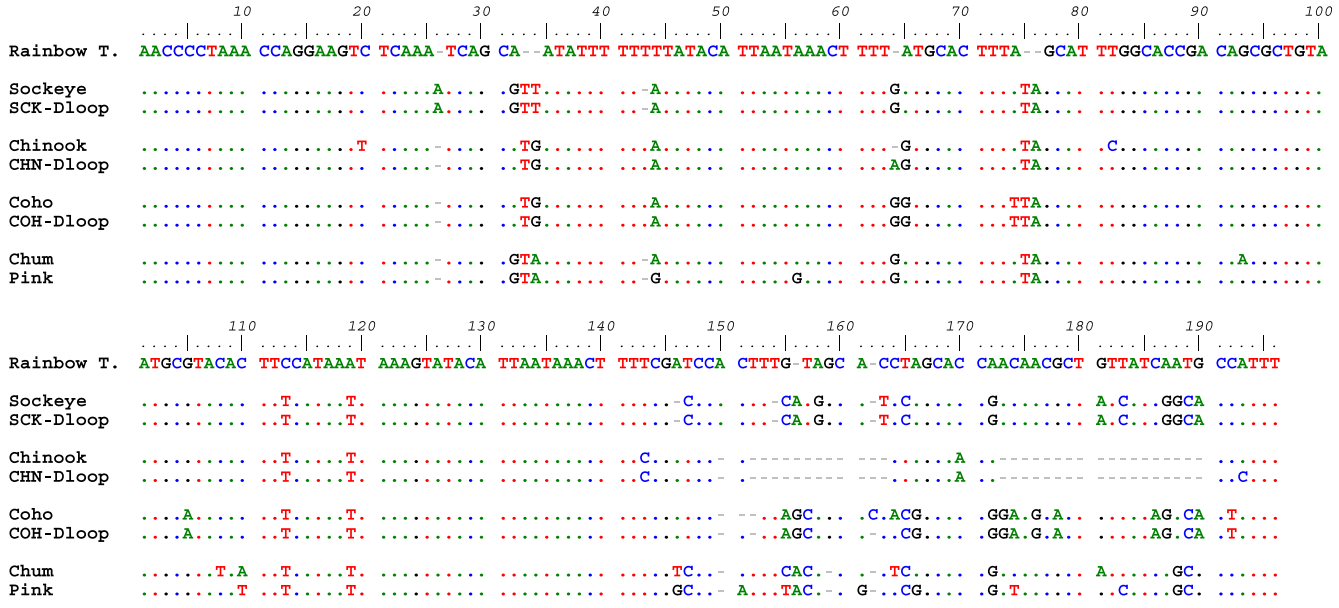

Fig. 3. Part of the amplified D-loop sequence by primers Smc7 and Smc8. All reference sequences were from Shedlock et al. [26] except rainbow trout (NC_001717). The dots indicate identical base pair to those of the rainbow trout on the top while the dashes represent insertion/deletion. SCK-Dloop, CHN-Dloop and COH-Dloop refer to sockeye, chinook and coho sequences from ancient remains, respectively.

(distinguished by only a single base pair difference – see Figs. 3 and 4). However, differences between the amplified D-loop fragments and the modern reference sequences obtained from Shedlock et al. [39] are evident with three and two base pair differences for chinook and coho, respectively, likely a reflection of regional and temporal variations.

In spite of the differences, species identities were confidently assigned to three species since all amplified D-loop and Cyt B fragments from each sample yielded the same identity. 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. The composition of the samples by species can be found in Table 2, and the distribution of species by housepit is depicted in Fig. 5.

5. Discussion

5.1. Authenticity of ancient DNA results

The ancient DNA results must be proved authentic before they can be used to address research questions [11,23,31,33]. Authenticity of ancient DNA samples and confidence in the species identity assigned can be generally demonstrated through the precaution measures implemented and results obtained in this study: (1) a dedicated ancient DNA facility was used; (2) a vigorous decontamination protocol including both physical and chemical decontamination of the samples was employed prior to DNA extraction; (3) no PCR amplification was observed in the non-salmon bone sample, blank extracts and PCR negative controls; (4) multiple haplotypes and multiple salmon species were

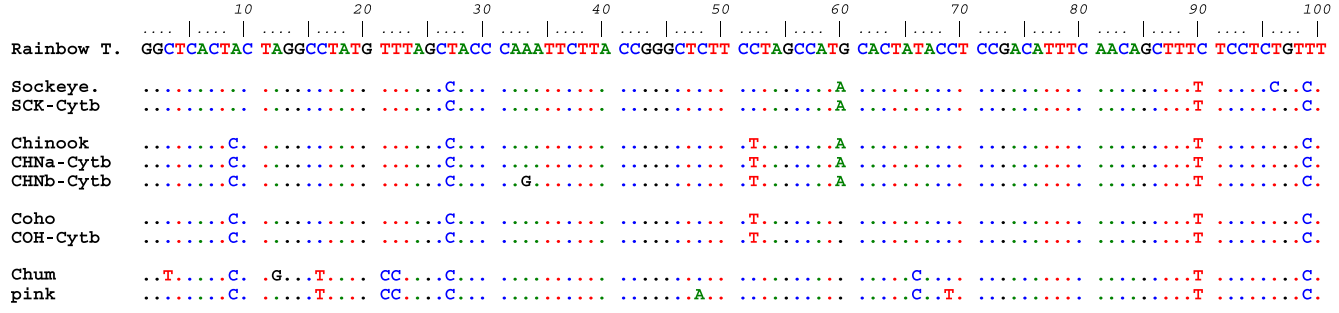

Fig. 4. Part of the amplified Cyt B sequence by primers CytB5 and CytB6. Reference sequences were retrieved from GenBank: rainbow trout (NC_001717), chum (AJ314561), coho (AJ314563), sockeye (AJ314568), pink (AJ314562), and chinook (AJ314566). The dots indicate identical base pair to those of the rainbow trout on the top while the dashes represent insertions/deletions. SCK-Cytb and COH-Cytb refer to sockeye and coho sequences from ancient remains, respectively while CHNa-Cytb and CHNb-Cytb are for the two chinook haplotypes from the remains.

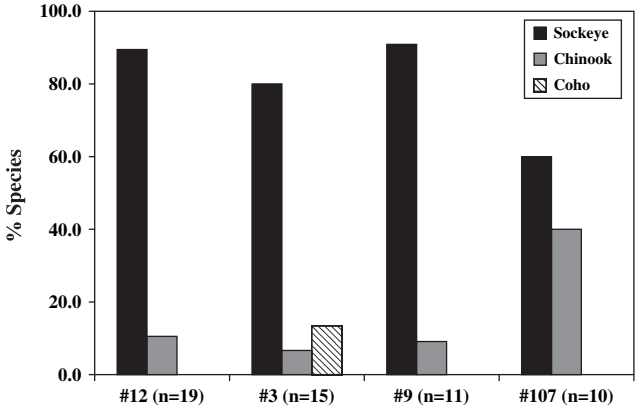

Fig. 5. Bar chart displaying the percentage of each species identified within each of the structures (n is the total number of the identified remains). Note: the results of the chi-squared test of randomly selected samples indicated that the distribution of species among structures was significant at the $p < 0.025$ level, once the two judgmentally sampled chinook vertebrae from HP#12 were removed from the sample set (and then $n = 17$).

obtained from the remains; (5) all obtained sequences matched exactly or very closely with published reference *Oncorhynchus* sequences; (6) all samples demonstrate a match between the species identity obtained with a D-loop sequences and with that of the Cyt B sequence; (7) temporally isolated repeat extractions of more than 10% of the samples returned identical species identification as the original experiments; (8) the recent antiquity of samples (1200 BP) should be expected to yield authentic DNA since much older salmon bone samples (up to 7000 BP) from the Pacific Northwest have produced positive PCR amplifications [46] and (9) the methods and primers used in this study already demonstrated success in distinguishing species of salmon in previous studies [46].

5.2. Species identification

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. According to Cannon's [8] 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. Based on our radiographic analyses of the studied vertebrae, we found that 100% of the samples from the specialized structure (#9), 85% of the samples from the small residential housepit (#12), and 90% of the samples from the medium residential housepit (#3) displayed two obvious radio-opaque rings, corresponding to salmon of three years of age. The remaining 10% of samples from the medium residential housepit displayed three radio-opaque bands, corresponding to salmon of four years of age. Two-year-old salmon were only identified within the small residential housepit (#12) and made up approximately 13% of the sample.

These results displayed some inconsistencies with previous research conducted by Berry [3] who applied the same radiographic method to analyze the salmon remains from five residential pithouses at the Keatley Creek. 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 two-year-old salmon, rather than three-year-old salmon as identified in this study. Since pink salmon has a fixed spawning age of two years while others have much broad range of spawning ages, Berry interpreted the two-year-old salmon to be pink, rather than other species [3]. 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 [3].

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, we 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 ring 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 for salmon above two years of age. If the salmon age profiles determined in Berry's study are increased by one year, the results from the two radiographic studies become far more 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 two- and 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 [6]. 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 [21]. This observation has demonstrated the potential utility of radiographic analysis for the identification of chinook vertebrae in the region; however, the overlapping spawning ages of salmon 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 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 rings. Additionally, the possibility exists that rings visible radiographically may not always correspond directly to years of life, creating additional ambiguity in the identification of spawning ages. Ancient DNA analysis, on the other hand, directly targets species-specific DNA sequences, rendering an unambiguous species identity.

5.3. Salmon subsistence of early Lillooet people

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 [1]. 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 area. 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 1500 BP. Like chum, the majority of pink salmon stocks currently tend to only spawn approximately 250 km up the Fraser River, within the Lower Fraser mainstem and tributaries [22]. There is some evidence to suggest that greater amounts of pink salmon may have been spawning further upstream prior to the Hell's Gate landslide in 1913 [24]. 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 [36]. Additionally, Romanoff's 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 [38]. 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 [25].

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 [43], 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 [38]. 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 [3]. In the lower reach 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.

The abundance of sockeye and the absence of pink from the remains illustrate a different picture of early salmon fishing activities in the region than previously imagined. Without the less-desirable species of pink salmon ubiquitously present at the site, the qualities affecting species preference must be reconsidered for the available three 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 [6,25]. Coho make up a very small portion of the Fraser River spawning stocks in the Lillooet region and may number only around a few thousand [25]. 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.

5.4. Economic stratification and species preference

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 modeling natural changes in salmon runs over time. The identification of three species (with the absence of pink salmon) could dramatically alter 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 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. As mentioned previously, the most ubiquitous species in the remains is sockeye salmon. Percentages of sockeye from each housepit are graphed in Fig. 5, and it is evident that in all cases sockeye salmon makes up the greatest percentage of the remains. Due to their smaller size, younger spawning age, and increased 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 [15], while sockeye were available from public fishing areas [38]. With only chinook and sockeye present at the site in significant numbers, it is likely that economic stratification would have precluded access to chinook while sockeye would have taken 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).

Overall, there seem to be some differences apparent in the distribution of the preferred species, chinook, 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 stood out visibly from the rest of the assemblage and were therefore judgementally selected for analysis due to their unusually large size 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 judgementally 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.

Due to the large sample size of Berry's [3] radiographic study, its results may still indicate the presence of some selective use of access in terms of salmon distribution at the site, even if the salmon ages were consistently identified as one year younger. 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 this study 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. Although DNA demonstrated that pink salmon were not present within the sample, differential age distribution of salmon between structures requires some explanation, and we cannot exclude the possibility that salmon size was a determining factor in access and distribution. 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. Studies [28] have suggested that immature spawning salmon, or 'jacks' are around two-thirds of the size of many four-year old spawners. This indicates that the younger spawners may have been similar to pink salmon – i.e., easier to catch near shorelines and less nutritionally desirable. Therefore, the distribution of differently sized salmon may reflect differential access to salmon resources.

Although there is ample evidence of economic stratification at Keatley Creek from analysis of other archaeological aspects, this study was not able to demonstrate the dramatic link between stratification and the distribution of salmon species per se at the site indicated by previous studies.

6. 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 1200+ 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 were the most abundant species while contrary to previous studies, 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 and (5) the dramatic link between economic stratification and

the distribution of preferred salmon suggested in previous studies was not observed in this ancient DNA study. However, the preliminary results demonstrated that differential access to preferred species (chinook) may characterize some specialized structures, and that larger residential housepits displayed greater species diversity than the small residential housepits.

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 1000–2000 years. Additionally, if the radiographic method is accurately identifying a 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 links between the salmon species contained within structures of different sizes were difficult to generate. This study suggests that the relationship between economic stratification and salmon species distribution at Keatley Creek is visible but clearly not as dramatic as previously assumed.

Acknowledgements

Many thanks to Dr. Aubrey Cannon for first bringing our attention to the possibility of ancient DNA analysis on ancient salmon remains from the site, and subsequent discussions and assistance regarding the radiographic method. Our thanks go out to Andrew Barton, Shannon Wood and Teresa Trost for assistance during the sampling and radiographing the salmon remains, to Karl Hillis and Kathy Watt for technical assistance in the SFU ancient DNA laboratory, and to Dr. Dana Lepofsky for discussion and assistance during the design of the project. We also thank three anonymous reviewers and Dr. Richard Klein for many valuable suggestions for improvement of this paper. This study was supported in part by Yang's grants from the Social Science and Humanities Research Council of Canada and SSHRC/SFU Small Grant.

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