Appendix C:
Ancient DNA Analysis of Whale Bones from Huu7ii

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In this study, we applied ancient DNA analysis for species identification to 101 bone samples excavated from Huu7ii, an ancient Nuu-chah-nulth village site in Barkley Sound, western Vancouver Island, in 2004 and 2006. The samples were taken at the Pacific ID laboratory at the University of Victoria in January 2008 and analysed at the ancient DNA facility in the Department of Archaeology at Simon Fraser University, Burnaby, British Columbia during 2008. The collected samples had been morphologically examined at the Pacific ID laboratory, although the fragmented state of most examples did not allow an identification beyond cetacean. The ages ranged from 550–330 BP to 1560–1320 BP based on ^{14}C dating (Frederick et al. 2006).

Barkley Sound is situated along a modern, and probably ancient, whale migration route between Alaska and California/Hawaii. Humpback (*Megaptera novaeangliae*), grey (*Eschrichtius robustus*) and killer whales (*Orcinus orca*) frequently come into the sound, while some pods are known to be resident to the region. The sound has been the territory of Nuu-chah-nulth cultural groups for at least 2000 years (McMillan 1998, 1999). The Nuu-chah-nulth were famed as whalers; along with their Makah relatives to the south, they were the only Northwest Coast peoples to set out on active whaling pursuits. The importance of whaling is highlighted in their oral tradition, rituals, and everyday life (e.g., Sapir et al. 2004).

Whaling was a prestigious undertaking and only a chief could conduct a hunt. The ethnographic record for the Nuu-chah-nulth contains numerous references to whaling and the preparations prior to such an event (Drucker 1951; McMillan 1999; Monks et al. 2001; Sapir et al. 2004). The whaler needed to be prepared by spring, so he would be ready when the whales came. This included material and spiritual preparation (Kirk 1986). According to their beliefs, the hunted whale only allowed the whaler to take it if the whaler was worthy. In the hunt, a whaler and his crew paddled out in a large canoe to get close to the whale, handthrust a harpoon into the animal, played out a line attached to the harpoon head that held large floats to buoy and tire the whale, and manoeuvred the dead or dying animal back to the beach near their village. The success of the hunt depended on size and strength of the animal, as well as the capability of the whaler. Humpback whales are relatively slow swimmers and tend to be rather docile, whereas grey whales can be aggressive and more dangerous to hunt (Banfield 1974).

Not every targeted whale was eventually brought to the beach; many were lost at sea. If the hunt was successful, the meat and blubber were distributed by the whaler, according to specific rules related to status and kinship. The saddle, which included the dorsal fin, belonged to the successful whaler and was set up for ritual display. After the initial distribution, any meat and blubber left on the beach was free for anyone to take. Whales that became stranded on a beach generally belonged to the chief whose territory encompassed the beach, although occasional conflicts over drift whales are recorded (Monks et al. 2001; Mulville 2005; McMillan 1999).

Although butchering occurred on the beach, some bones with meat or blubber attached may have been transported into the village, eventually ending up in the midden (Mulville 2005). Additional whale elements were brought onto the site for oil extraction (Monks 2005). Other bones, including skulls, mandibles, and vertebrae, were stacked as trophies or displays of whaling prowess. Many whale bones in the site, however, were taken there as raw material for the manufacture of a wide range of everyday objects. The number of identifiable bones using standard zooarchaeological methods is thus very limited. Finally, it is hard to identify hunted versus drift whales in the archaeological record. Direct evidence for hunting includes embedded portions of mussel shell cutting blades from the harpoon heads, as is reported for other excavated Barkley Sound sites (McMillan

and St. Claire 2005; Monks et al. 2001), or other directly associated whaling gear, which generally only survives in water-saturated sites (Kirk 1986; Mulville 2005). Apart from the basic species identification, this study also investigates whether genetic analysis can be used as a direct line of evidence to detect active whaling in the archaeological record.

Materials and Methods

Samples were selected from skeletal remains that were morphologically identified as cetacean or whale. All samples used in this study came from within the House 1 platform in the village portion of the site (none came from the earlier component on the higher terrace behind the village area). Each sample was photographed and a 1.5 g piece was cut using a hacksaw. To prevent cross-sample contamination, a new blade was used to cut each sample. The work surface and tools were bleached after handling each sample. Samples were then transferred to the ancient DNA facility in the Department of Archaeology. Bones were decontaminated using the laboratory protocol developed by Yang et al. (1998, 2003). Due to the rough and porous surface of the bones, the outer layer was not removed using sandpaper prior to decontamination. Instead, the length of time the samples remained in the decontamination solution was increased. Samples were placed in 50 ml tubes and submerged in 10 ml of 100% commercial bleach for 8–10 minutes and then rinsed twice with ultrapure water to remove any bleach residual. Following this, the samples were treated with 1N HCl for one minute and then neutralised using 1N NaOH. Samples were then rinsed once in ultra pure water, and soaked in water for about five minutes. Finally, samples were UV-irradiated for 30 minutes, then flipped over and irradiated for another 30 minutes. After the irradiation, the bones were crushed and stored in 15 ml tubes at –20˚C.

For DNA extraction, the modified silica-spin-column method (Yang et al. 1998) was used. Approximately 0.4 g to 0.6 g of the crushed bone was transferred into a new 15 ml tube and incubated overnight in 4 ml of lysis buffer (0.5M EDTA ph 8.0; 0.5% SDS; 0.5 mg/ml proteinase K) at 50˚C. On the following day, samples were spun and 3.5 ml of the supernatant was concentrated to 80–100 µl using 30k Amicon filters (Millipore). Samples were then purified using Quiaquick spin columns (Quiagen, Hilden, Germany), and 100 µl of elution buffer was used to remove the DNA from the spin column. This step was repeated and both eluted solutions were stored at –20˚C for PCR amplification.

To identify the species of the sample, a PCR was carried out using primers targeting a region of the cytochrome b gene. Cytochrome b is a conservative gene which is commonly used for species identification. A 30 µl PCR reaction was set up containing 1.5x buffer, 2mM $MgCL_2$, 0.2mM dNTP, 1 mg/mL BSA, 0.3µM of each primer, 2.5U AmpliTaq Gold, and 5 µl DNA sample. PCR amplification was carried out in an Eppendorf™ Mastercycler Personal with an initial 10 minute denaturing period at 95˚C, followed by 60 cycles of 95˚C for 30 seconds (denaturation), 54˚C for 45 seconds (annealing), and 70˚C for 45 seconds (extension) for 60 cycles, with a final extension of seven minutes at 70˚C. A 2% agarose gel was used to visualize the outcome of the reaction.

Positive PCR reactions were purified using a Quiaquick purification column (Qiagen, Hilden, Germany) following the manufacturer's manual. Samples were sequenced at Macrogen Ltd in Seoul, South Korea. Results were visually edited and species identification was confirmed using the NCBI database tool BLASTn and phylogenetic analysis of other close-related species. All humpback whale samples were also confirmed using another DNA marker (D-loop), resulting in no discrepancy of species identity.

Results and Discussion

Table 1 shows the results from the DNA species identification using cytochrome b. The retrieval rate of analysable DNA at Huu7ii was 85%, which is a good result for ancient DNA analysis. The 101 analysed samples from Huu7ii returned four whale species: humpback, grey, finback, and right. Humpback whale is the most common species identi-

Table 1: Species ID summary of the analysed Huu7ii samples based on ancient DNA.

Species	N	%*
Humpback Whale *(Megaptera novaeangliae)*	70	83.33
Grey Whale *(Eschrichtius robustus)*	11	13.09
Finback Whale *(Balaeonoptera physalus)*	2	2.38
Right Whale *(Eubalaena japonica)*	1	1.19
No species ID	17	(N.A.)
Total	***101***	***99.99***

* % out of all identified bones

fied in this assemblage. The difference between humpback whale and the next leading species (grey whale) is considerable. A similar result has been found for the analysis of whale remains from Ts'ishaa, on Benson Island in Barkley Sound. The sites of T'ukw'aa and Ch'uumat'a, at the western edge of the sound, also had a very similar pattern of whale species present, although that analysis was based on visible morphology rather than DNA (Monks et al. 2001). The fact that all these sites have similar proportions of whale remains, with humpbacks the dominant species, distantly followed by greys, suggests that this is a general pattern in Barkley Sound. Additionally, the similar species distribution supports the assumption that this analysis is based on an unbiased, random collection of samples.

Table 2 shows the distribution of identified whale species by excavation unit. These include units excavated in both field seasons and that extend across the entire excavated portion of the investigated house platform at Huu7ii. This distribution shows that humpback whale remains, for example, were not concentrated in one area but were found across the House 1 deposits.

There is no direct evidence of hunting activity, such as embedded portions of mussel shell harpoon heads, in the skeletal assemblage, so we cannot simply assume that the whale remains resulted from active hunting. Other explanations must be explored. All the identified species are found in shelf edge and coastal waters in the region. However, grey whales swim closer to the shore and frequent coastal waters more often than humpback whales, making grey whales theoretically more likely to be stranded on the beach. If this were the case, we might anticipate a higher proportion of grey whales in the skeletal assemblage. The alternative explanation, that this assemblage is based on the preferential hunting of humpback whales, is more consistent with the observed dominance of humpback whale elements in the assemblage. Ethnographic accounts for the Barkley Sound region state that both humpback and grey whales were hunted but that humpbacks were present in the sound for much of the year, unlike the migratory greys (Sapir et al. 2004). Grey whales are also faster and more aggressive than humpbacks, making them harder to hunt. The fact that the species distribution is so uneven, with humpback whales predominating, suggests that humpbacks were the preferred target and that this assemblage reflects hunting activity.

This research is an example of how ancient DNA can help when the usual zooarchaeological methods fail due to the fragmented nature of the material, as was the case in this study. Genetic data become more accessible and more meaningful in combination with archaeological and ethnographic knowledge, with multiple lines of evidence coming together to allow for a more complete interpretation.

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Table 2: Species identification by excavation unit.

	Humpback Whale	Grey Whale	Finback Whale	Right Whale	no ID	Samples analyzed
N12-14 E18-20	1					1
N14-16 E16-18	1				1	2
N16-18 E26-28	2	1			1	4
N18-20 E16-18	8					8
N18-20 E30-32	6	2	1			9
N18-20 E34-36	10	2		1	3	16
N10-12 E2-4	14	1			4	19
N12-14 E6-8					1	1
N18-20 E2-4	13	2	1		5	21
N18-20 E6-8	15	3			2	20
Total	***70***	***11***	***2***	***1***	***17***	***101***

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