ANCIENT DNA ANALYSIS OF NORTHEAST PACIFIC HUMPBACK WHALE (*MEGAPTERA NOVAEANGLIAE*)

by

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Abstract

The main goal of this ancient DNA-based study was to analyze archaeological whale skeletal remains from the west coast of Vancouver Island, British Columbia to investigate population genetic diversities of humpback whales pre-dating industrial whaling. This study also examined whale hunting practices of early indigenous people by revealing potential species selections. Nuu-chah-nulth people are believed to have hunted whales for millennia and numerous whale bones have been recovered from archaeological middens from the region.

Whale skeletal remains (N=264) from two archaeological sites (Ts'ishaa and Huu7ii) were analyzed using ancient DNA techniques, with 84% of the samples yielding amplifiable DNA. Nearly 79% of the samples were identified as humpback whale based on cytochrome b and D-loop regions of mtDNA. The analysis was carried out in a dedicated ancient DNA facility, including strict contamination controls and multiple repeats of both PCR and sequencing. No systematic contamination was detected over the course of this study, further supporting the authenticity of the ancient DNA data obtained.

The mtDNA haplotypes of 105 of the humpback whales was determined using a 344bp D-loop sequence assembled from multiple overlapping DNA fragments. The genetic diversity of ancient humpback whales (π=0.0147 and h=0.804) falls within the range of modern Pacific humpback whales. Since some of the major genetic signatures can still be observed in today's populations, results indicate a strong resilience despite industrial whaling during the 19th century.

The majority of whale remains in this study were identified as humpback whale and to a lesser degree as grey whale (13%), supporting the notion that the ancestors of the Nuu-chah-nulth people probably practised whaling almost 5000 years ago. Humpback whale could be more easily targeted using traditional techniques based on the whale's speed and proximity to the shore. Other species such as finback and right whale (among others) only appear in archaeological records younger than 2000BP, which may indicate an improvement of hunting techniques over time.

Keywords: ancient DNA; whaling; *Megaptera novaeangliae*; humpback whale; phylogeography; Nuu-chah-nulth

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Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Whales have been a valuable source of meat, oil and other materials for thousands of years and can be found in the oral traditions of cultures around the world. While hunting large land mammals such as bison or mammoth would have been challenging for humans in the past, hunting large sea mammals in small boats on the open ocean would have been even more difficult. Nevertheless, the use of whale evolved from utilizing stranded whales to sophisticated whaling techniques, which included elaborate mythology surrounding the animal and the act of whaling. While traditional whaling has been observed globally in coastal regions, it remains uncertain when traditional whaling first developed.

On the other hand, industrial whaling is well recorded, starting with Basque whaling in the 16th century. Industrial whaling in the 19th and early 20th centuries greatly reduced the size of whale populations worldwide, driving some populations to local extinction, including the Atlantic grey whale. Since the mid-twentieth century, the recovery of whale populations has been variable, from almost no changes in some populations such as the right whale, to an increase beyond expectations as is the case with North Pacific humpback whales. Research over the years has attempted to establish a solid baseline of humpback whale population size, biogeography and genetic diversity, in an effort to aid future management decisions surrounding indigenous whaling, as well as commercial claims on whale stocks (Calambokidis et al. 2001, Calambokidis et al. 2004,

Jackson et al. 2007, Baker and Clapham 2004, Witteveen et al. 2004).

This study investigated the potential of using ancient DNA analysis to provide additional information on Northeast Pacific humpback whale populations prior to industrial whaling. The study analysed 264 archaeological whale bone samples from two sites in Barkley Sound, which is located on the West Coast of Vancouver Island, British Columbia: Ts'ishaa, located in the central part of Barkley Sound on Benson Island (part of the Broken Group Islands) and Huu7ii, located on the north end of Diana Island (part of the Deer Group Islands). Both sites were important Nuu-chah-nulth villages and both demonstrated archaeological evidence for active whaling. The overall goal of this study was to use previously excavated whale bones from these two archaeological sites to investigate archaeological as well as biogeographic questions. The specific objectives of this research were:

1) To investigate the antiquity of whaling in Barkley Sound and the impact the development of whaling had on species diversity in the archaeological record;

2) To establish genetic diversity of Northeast Pacific humpback whale populations prior to the industrial whaling industry of the twentieth century and to describe the changes in genetic diversity of this population through time; and

3) To highlight the possible contributions of molecular archaeology to biogeography and conservation biology.

The results of this analysis indicated that people hunted humpback whales

for at least 5000 years, with species other than humpback and grey whale being present only in layers more recent than 2000 BP. Based on mitochondrial DNA (mtDNA) analysis, the genetic diversity of humpback whales in Barkley Sound did not change substantially over the last 5000 years. The study revealed several contributions of molecular archaeology to various disciplines. For example, the results of this research shed light on the antiquity of whaling in Barkley Sound and will help reveal modes of marine subsistence development. This study also demonstrated that phylogeographic data of pre-industrial whaling populations can aid in the investigation of the long term changes of humpback whale population size and genetic diversity in order to evaluate current population status.

While modern studies have investigated potential changes over time based on current population variation, this study uses data collected from various points in the past, and thus provides a baseline of population distribution and genetic diversity. This study not only investigates the antiquity of whaling and the genetic diversity of past whale populations in Barkley Sound, but also investigates how archaeology, zooarchaeology, ancient DNA analysis, and ethnographic data contribute to modern conservation biology. The use of molecular techniques, especially DNA, has revolutionized both biogeography and archaeology in the past 20 years (Riddle et al. 2008, Hadly et al. 2004). Ancient DNA has the unique ability to provide data comparable to modern DNA.

Humpback whales are a cosmopolite species, even today. In the North Pacific, three subpopulations are further subdivided into multiple feeding grounds in colder waters, only to mix during migration and at the breeding grounds located

in warmer regions. These complex migration and breeding patterns only came to light with extensive studies of over 6000 individuals (Calambokidis et al. 2008, Baker et al. 1998b, Baker et al. 2004). Barkley Sound is part of one of the feeding grounds, encompassing southern British Columbia and Washington, USA. An almost year-round presence of humpback whales in the area could have fostered the development or settlement of whaling cultures in Barkley Sound.

Archaeological and ethnographic data from the Nuu-chah-nulth territory have revealed a long history and extensive tradition in connection with whales and the hunt of whales on the Northwest Coast. Zooarchaeological data suggest that humpback whale was the common species hunted in the area for at least 2000 years (Monks 2001). However, in most cases whale bones are not identifiable to the family or species level, and cannot be used to track species distribution or to investigate changes in hunting patterns through time.

The general background information for this dissertation regarding ancient DNA, archaeology and humpback whale biology is discussed in Chapter 2 while more specific background on Nuu-chah-nulth culture and whale biology can be found in Chapters 5 and 6, respectively. Chapters 3 and 4 describe the methodology of this study, with Chapter 4 focusing on the authenticity of the produced sequences. For this study, ancient DNA analysis was conducted to provide species identification, as well as the mitochondrial haplotype of a subset of the humpback whale samples. Chapter 5 discusses the results of the species identification and its relevance to archaeology. Chapter 6 is focused on the phylogeography of the humpback whale population present in the dataset,

discussing the results of the genetic diversity and comparing ancient DNA data to previously published research results. The general discussion in Chapter 7 includes a discussion on the ability of archaeology to aid biogeographic research, framing the separate archaeology (Chapter 5) and biogeography (Chapter 6) results within a broader context and outlining the potential for future research.

Chapter 2: General Background

Ancient DNA Analysis in Archaeology

Molecular Archaeology

Molecular archaeology implements ancient DNA and protein analysis to study archaeological samples. The spectrum of materials analysed range from faunal remains and cooking residues, to floral remains and artifacts (excavated or museum specimens) made from organic material. The following chapter will discuss ancient DNA and specifically molecular archaeology research.

Ancient DNA research started 30 years ago and the initial 10 years were filled with discoveries about contamination, DNA damage and realistic DNA survival expectations. Prior to the development of the polymerase chain reaction (PCR) technology (Mullis et al. 1986), ancient DNA research on museum samples during the late 1980s used molecular cloning techniques to achieve the DNA quantities necessary for analyses. The first published studies analysed the phylogenetic position of the quagga, an extinct zebra subspecies (Higuchi et al. 1984) and an Egyptian mummy (Pääbo 1985). With the adoption of PCR technology, ancient DNA analysis became more viable to a wider field of researchers in palaeontology, archaeology and anthropology. Initially, research focused on phylogenetic analyses of extinct species and early studies of human

evolution such as the first Neanderthal DNA research published in 1997 (Krings et al. 1997). However, research quickly evolved beyond such questions to almost all levels of anthropology and archaeology. Anthropologists in Europe have been using ancient DNA since the 1990s to analyse burial populations (Handt et al. 1996) and famous interments, such as the Romanovs, a Russian Tsar family (Gill et al. 1994). Over the past 10 years however, with technological developments and access to larger datasets, the focus has shifted towards understanding species phylogeography, which involves comparisons between populations and population genetics of extinct and extant species, including *Homo sapiens*. One of the largest studies to date (Shapiro et al. 2004) analysed over 400 samples for phylogenetic and phylogeographic details of North American bison. With the advent of emulsion PCR and next generation sequencing (currently 454 pyrosequencing by Roche, Illumina/Solexa, and SOLiD by ABI), researchers are moving towards analysing entire genomes of specimens, such as the cave bear, mammoth and *Homo neanderthalensis* (Ronaghi 2001, Noonan et al. 2006, Hebsgaard et al. 2007, Wall and Kim 2007). Future advances, which aim at larger datasets at lower overall costs, will enable researchers to document extinct populations and environments more comprehensively, allowing for more inclusive comparison of modern and ancient datasets, and extending the time frame for statistical analyses by millennia (Knapp and Hofreiter 2010).

Technical Challenges

Ancient DNA techniques are essentially the same as modern DNA

techniques, but age and preservation of samples introduces certain constraints to the practical work. With the increase in research on ancient DNA, knowledge of these constraints and problems has improved, leading to the development of guidelines on proper research in ancient DNA laboratories. The application of ancient DNA techniques does not hinge on a sample's specific age, but rather the environmental conditions under which it has been stored.

The major objectives are to detect or prevent contamination, and to detect sequence damage, such as deamination, that could lead to incorrect results in the final analysis (Hansen et al. 2001, Lindahl 1993). This detection is accomplished by studying multiple sequences and, if necessary, bacterial cloning. However, cloning has become less accepted as necessary (Kemp and Smith 2010). Another way of detection is the use of Uracil-N-Glycosidase (UNG), an enzyme that removes uracil, and one which is used during the early stages of PCR to distinguish between taphonomic C-T changes and non-degraded unique base pair changes (Pääbo et al. 2004, Hofreiter et al. 2001a). Apart from base pair damages, the most visible result of degradation is the reduction in amplicon length; that is, while well preserved samples yield amplicon length of >300bp - with the "normal" range being between 150bp and 200bp per amplification reaction - badly preserved samples yield amplicons in the range between 60bp and 100bp (Pääbo et al. 2004, Gilbert et al. 2005, Hofreiter et al. 2001b, Bollongino et al. 2008b, Willerslev and Cooper 2005).

Ancient DNA work is prone to contamination because of the low concentration and degraded state of the DNA. PCR conditions in ancient DNA

work are designed to amplify short fragments of low quantity DNA. Therefore, even relatively small quantities of modern DNA that would not be a problem in a modern laboratory, can easily outcompete the ancient templates. Thus, laboratories and workflow are designed to prevent possible introductions of non-specimen DNA, and special procedures such as UV irradiation and bleach treatment are used to remove possible contamination. The measurements are even stricter when working with ancient human DNA, including early anatomical modern homo sapiens and Neanderthals. Potential sources include the excavation team, laboratory team, washing the bone at site, laboratory chemicals used and cross contamination during preparation. The guidelines include strict contamination controls, multiple extractions and PCR amplifications, and inter-laboratory controls where needed (see Chapter 4 for details and application in this study). Contamination is one of the limiting factors for anthropological and archaeological studies involving ancient DNA (Mulligan 2006). Ancient DNA research requires planning to include possible contamination prevention measures during excavations and special handling of sample material (Bollongino et al. 2008b, Mulligan 2006).

DNA preservation has been subject to various studies and the factors that determine the survival of biomolecules over time are still under investigation. Some important factors in DNA survival are temperature, water, time, and soil composition, especially pH-level (Bollongino et al. 2008b, Smith et al. 2003, Axelsson et al. 2008, Gilbert et al. 2006). However, the effects of soil composition are not fully understood. In addition, interactions between those components can lead to different chemical reactions, complicating the predictions even further.

Chemical components in the burial environment may also create PCR inhibition, another problem that remains unsolved and poorly understood. Inhibition can either be caused by chemicals in the soil or by chemical changes in the DNA strand. While some of the chemical inhibitors are known, such as humic acid and tannins (King et al. 2009, Sutlovic et al. 2005), many of the activities and cross reactions in soil environments are not understood. In some cases of inhibition, the DNA strand is intact but chemical or biological agents prevent the amplification of the DNA. Removing these agents would lead to amplifiable DNA, but could also lead to a lower concentration of remaining DNA (Gilbert et al. 2003, Willerslev et al. 2004, Kemp et al. 2006).

The age of samples yielding amplifiable DNA generally ranges between 100 and 100 000 years. Researchers have used amino-acid racemisation to predict DNA survival (Hofreiter et al. 2001b). However, amino-acid racemisation and protein survival has not yielded the desired success in predicting DNA survival, and recent studies refute the idea that amino-acid racemisation can reliably predict DNA survival (Collins et al. 2009). Statistical analyses predict a maximum survival, under ideal conditions, of about 100 000 years (Collins et al. 2002). Since most samples are not stored under ideal conditions, thermal age (see also http://thermal-age.eu/) has been introduced as a way to possibly estimate DNA survival rate (Willerslev and Cooper 2005, Smith et al. 2003).

Molecular archaeology and generally ancient DNA research have never been isolated fields, but both were primarily focused on historical and pre-historical questions. Current research increasingly emphasises the importance of

multidisciplinary approaches (Leonard 2008). Also, research design in current ancient DNA studies includes more hypotheses testing and is thus moving away from sample-based data exploration. One important trend in ancient DNA work is to combine various analytical methods with ancient DNA, including stable isotopes, direct dating, modern comparison, as well as material culture archaeology and ethnographic data. Using multiple lines of evidence improves the interpretation of archaeological remains.

Depending on the hypothesis to be tested, different genetic loci are analysed during a study. Because of the level of degradation, mtDNA is generally preferred over nuclear DNA. The amount of mtDNA is significantly higher than nuclear DNA: Each cell contains one copy of nuclear genome and up to 1000 copies of mitochondrial genome. Additionally, mtDNA is non-recombinant which makes it possible to trace maternal lineages through time. Whether mitochondrial genes or the non-coding control region ("D-loop") are studied depends on the question asked. Species identification is often done using the more conserved cytochrome b gene or COI while intra-population studies utilize the generally faster mutating D-loop. In addition, targeted fragments should be less than 200bp if possible, while in good conditions, targeting 300bp or more is possible. Overlapping fragments assure the sequence authenticity and provide additional repeats of the amplified fragment.

Addressing Archaeological Questions

Ancient DNA research has been applied to a wide variety of archaeological questions. Burial populations can be analysed to determine kinship (Gerstenberger et al. 1999, Hummel and Herrmann 1996) and ancient DNA analysis is frequently the only way to detect discrepancies between the burial and historical documents. On an individual level, remains are being analysed to trace living descendants and possible cultural affiliations. Examples of these studies include the DNA analysis of Kwaday Dan Ts'inchi (Monsalve et al. 2002), Ötzi the Tyrolean ice man (Rollo et al. 2006), and the remains of individuals from Nevada (Kaestle and Smith 2001).

The first Neanderthal DNA was sequenced in 1997 using the specimen discovered near Düsseldorf, Germany. Based on a 380bp mtDNA fragment of the HV1 region on the D-loop, the Neanderthal was excluded from the direct ancestry of modern *Homo sapiens* (Krings et al. 1997). More individuals were subsequently analysed and the results corroborated the result that the Neanderthal D-loop haplotype cannot be found in modern humans. In 2006, two research groups, one in Berkley, USA (Noonan et al. 2006) and one in Leipzig, Germany (Green et al. 2006) used pyro-sequencing (454 technology) and emulsion PCR to sequence the entire Neanderthal genome. The results of the two studies differed substantially due to contamination and the German research group used the results sequenced in Berkley for further analysis (Wall and Kim 2007, Krause et al. 2007). Another widely discussed study was published in 2003 analysing two 24 000 BP Cro-Magnon individuals (Caramelli et al. 2003). The results of this study suggested that the Cro-Magnon *Homo sapiens* fell well within the range of modern *Homo sapiens*

mtDNA variation. Despite strictly following the protocols for authenticating ancient DNA, discussions remained as to whether this result was indeed reliable (Pääbo et al. 2004). In a follow up study, the Caramelli research group published a sequence of a Cro-Magnon individual taking all possible contaminant sequences in the analysis under consideration (Caramelli et al. 2008). To detect possible contamination, Caramelli's team determined the HV1 haplotype of the Paglicci 23 Cro-Magnon specimen by typing two bone fragments as well as typing all individuals involved in the excavation and subsequent analysis. The 152 clonal sequences of the Paglicci 23 specimens were compared to the reference samples collected from the excavation and lab members. The researchers concluded that the Cro-Magnon sequence, which carries the Cambridge reference sequence haplotype, is different from all potentially contaminating personnel and thus should be considered authentic (Caramelli et al. 2008).

Plant and animal domestication is an archaeological topic that has been pursued for a long time (Zeder 2006). The origin of domesticated cattle in Europe, for example, has been a prominent issue in ancient DNA research over the past 15 years. Even as modern DNA research has illustrated the geographic patterns of haplotypes, migration and admixture over time play a crucial part in the misinterpretation of these modern patterns (Bollongino et al. 2005, Loftus et al. 1999). Ancient DNA research has ultimately led to more realistic interpretations regarding the origin and domestication events of European cattle (Bollongino et al. 2005, Beja-Pereira et al. 2006, MacHugh et al. 1999). Other domestication events that have been analysed using ancient DNA are that of the horse (Vila et al. 2001,

Jansen et al. 2002), that of the dog (Verginelli et al. 2005, Vila et al. 1999) and that of the pig (Larson et al. 2005, Larson et al. 2007), among others.

Food staples and food procurement studies can aid in understanding the culture and settlement patterns in a region. Cannon and Yang (2006) showed settlement seasonality based on different salmon species in Keatley Creek, British Columbia. Haak et al. (2005) analysed the lactose gene in central European Neolithic populations to trace the onset of dairy use, a substantial change in diet that is still poorly understood. Originally, humans lost the ability to digest milk after childhood; today many Europeans retain the ability to digest milk protein as adults. This change probably happened during the Neolithic when cattle domestication made cow milk readily available (Haak et al. 2005, Burger et al. 2007).

Through plant DNA analysis, the change from wild teosinte to domestic maize was tracked in South and Central America (Freitas et al. 2003, Jaenicke-Despres et al. 2003). These archaeological or paleontological plant DNA analyses can be used for environmental reconstruction, seasonality studies, and the detection of possible resource depressions (Willerslev and Cooper 2005, Gugerli et al. 2005). In terms of human populations, plant DNA can help to understand past lifeways and cultural adaptations (Zeder 2006, Jaenicke-Despres et al. 2003, Kaestle 2002, Kaestle and Horsburgh 2002). When including coprolites as an ancient DNA source, questions can also include the diet of extinct species or ancient populations (Iniguez et al. 2003, Loreille et al. 2001).

Plant and animal DNA can be used by archaeologists to reconstruct past environmental conditions and resources, as well as changes over time. This

information is not only relevant to archaeologists, as it can also be important for modern biologists. Archaeologists have produced environmental and resource reconstructions to establish population distribution and boundaries. Lee Lyman, for example, used zooarchaeological methods in several instances to distinguish indigenous taxa from exotic ones (Lyman 2006, Lyman and O'Brien 2005). In Lyman and O'Brien's study (2005), several subspecies considered indigenous today were revealed to be historic migrants from different areas. The ability to analyse these patterns enables biologists to evaluate current distributions and the optimal steps that need to be taken in conservation areas. This study could have been amended by using DNA to investigate these questions which would have allowed for a more detailed analysis, helping to re-evaluate and assist in the conservation of threatened species.

The option to use archaeological material to extend the window of accessible data for aDNA studies has been discussed for years but rarely used. Modern conservation biology relies strongly on genetic data to establish phylogeography, population movements and boundaries, and biological distances between populations. Such data can also be collected through ancient DNA analyses of archaeological and palaeontological sample sets and compared to modern data. Investigating extinction events can assist in investigating modern populations in unstable environments, which is relevant research for current conservation biologists and future conservation management policies.

Archaeology and Conservation Biology

Conservation biology

In 2008, almost 25% of known mammal species were listed as threatened with extinction on the International Union for Conservation of Nature (IUCN) red list for endangered species (Vié et al. 2008).

Conservation biology has expanded over the past 15 years as the impact of human action on the environment has become more visible. A large body of theoretical work supports today's conservation biology research and practice (Bowen 1999, Amos and Balmford 2001, Desalle and Amato 2004), the goal of which is sustainable equilibrium between a species' habitat and the ecosystem, as well as between species and human requirements. The term 'conservation biology' was coined in 1978 by Michael Soulé at a conference at the University of California. Here, Soulé distinguished 'conservation biology' from other fields, such as 'wildlife management'. Conservation biology, according to Soulé, should be focused on the general biodiversity of the ecosystem and its long term development; furthermore, biodiversity should be preserved for its intrinsic value (Soule 1985).

Conservation initiatives are often forced to make decisions based on incomplete information and under time constraints (Halpern et al. 2007, Frankham 2005). With more accumulated data, the theoretical framework and case studies are improving this situation, making more informed decision possible. Part of the problem is identifying the correct management unit; that is, management decisions regarding population transplantation or hybridisation often lack the knowledge of

previous population boundaries and might lack the foresight to predict the outcome and effectiveness of a plan (Wiens and Graham 2005, Lyman and Cannon 2004). By investigating past population boundaries, possible hybridization zones and gene flow, the ideal population for re-introduction can be chosen (Lyman 2006, Lyman and Cannon 2004). Analysing these population boundaries can also predict the survival chance, based on the environmental conditions of the past population (Brito 2005, Li et al. 2005).

Today, genetic analyses are an important component of conservation biology and are used to investigate current and past population conditions through species identification, population identification and by establishing population genetic diversity and demographic histories (Desalle and Amato 2004, Avise 2004). A population's genetic diversity is a critical factor in a species' survival; a high genetic diversity enhances a population's ability to adapt to environmental changes, and improves its survival chances (Hedrick 2001). Identifying breeding units and establishing the genetic diversity is vital in developing appropriate conservation protocols. However, since fauna and flora are subject to continuous environmental changes, it is difficult to establish the natural condition of a species or ecosystem prior to significant human alteration (Lyman 2006). Additionally, other limitations such as small sample sizes and the need for immediate response hamper the accuracy of conservation biology work.

Research over the past decades has provided evidence that factors for species' survival are multidimensional including genetic, demographic and environmental components. While species diversity enhances the function of an

ecosystem, genetic diversity is relevant for a species as well as for the resilience of the ecosystem, especially when species diversity is low (Reusch et al. 2005, Hughes and Stachowicz 2004). In some cases, species collapses at a a substantially faster rate than previously expected. Research suggests that collapsing ecosystems were already damaged prior to modern human impact (Jackson et al. 2001, Jackson 2001, Newsome et al. 2007).While this problem has been observed in land ecosystems, the lack of detailed knowledge of the various factors appears to be exaggerated in marine ecosystems. Marine ecosystems are still poorly understood in most cases, making it difficult to establish effective marine conservation areas. In addition, the invisibility of most of the marine wildlife, and especially the depletion thereof, obstructs policy making in favour of human resource use. While the disappearance of land animals is more noticeable, destruction of marine resources only becomes visible if this resource is exploited by humans. As Sloan and Jackson point out (Jackson et al. 2001; Jackson 2001), protected areas are often a fraction of the theoretically needed size because of human use requirements and lack of sufficient data.

To mitigate the lack of long term data, estimates of historic population size and distribution are currently based on historic and ethnographic documents (such as written documents of early settlers or explorers, and logbooks from fishing vessels). The use of zooarchaeological resources can dramatically improve the amount of information by estimating population size, as well as a species' habitat extent and habitation ecology directly from the past populations themselves (Lyman 2006, Lyman and Cannon 2004, Lyman 1996, Rastogi et al. 2004).

Ancient DNA analyses, Zooarchaeology and Phylogeography

Environmental research in archaeology has helped identify the environmental conditions in which past populations lived and tries to detect the changes in those environments over time. These environmental changes can have implications on human cultural society, as well as variations in technology. Archaeologists have investigated prey choice, hunting pattern and faunal use in general, shedding light on important subsistence patterns of past populations. Results from these studies can be used as additional evidence to detect cultural changes or the relationship between people and their environment.

Using ancient DNA analysis, research can reveal population demographics and phylogeography prior to human impact. Newsome et al. (2007) used stable isotopes and ancient DNA to analyse the past populations of northern fur seal in relation to modern seal distribution. According to Newsome's analysis, regional extinction of northern fur seal in California predated European contact but did not affect the fur seal populations to the north. According to this, Newsome claims, the previous collapse can be attributed to hunting rather than climate change.

Ancient DNA research can also distinguish between the effects of human impact and environmental changes on animal and plant populations (Hadly et al. 2004). Importantly, if research can reveal long term change, sensible management decisions can be made with more accurate predictions of future outcomes. Without DNA (that is, using only morphological analyses), the analysis of museum, archaeological or palaeontological material may be handicapped by environmental plasticity, especially in small sample sets (Hadly et al. 2004).

As Leonard (2008) noted, Pleistocene faunal studies can be used to analyse the adaptation and extinction of mega fauna, whose pattern might help to predict the survival abilities of extant species during the current or future climate change (Hofreiter et al. 2004, Leonard et al. 2008). Hadly et al. (1998) used ancient DNA to detect different responses to climate change in gopher and vole populations from Lamar Cave in Yellowstone Park, USA. By subdividing their sample set in time, the authors' data demonstrated the changes in genetic diversity and population sizes that had occurred in these two species over almost 3000 years (Hadly et al. 1998).

While some early researchers have presented romantic notions of past people living in harmony with their environment (Raymond 2007), research has since provided evidence of resource overexploitation (Jackson et al. 2001; Jackson 2001), both land and marine, leading to changes in prey size, resource depression and finally changes in prey choice (Raymond 2007, Broughton 2002). In addition to overexploitation, species introductions have led to artificial biodiversity increases, disturbing the balance of the invaded ecosystem. For example, invasive species and the subsequent environmental changes have been investigated in island populations in Hawaii, Australia and Polynesia (Prebble and Wilmshurst 2009, Kennett 2006). Being able to detect pre-historic changes resulting either from natural occurrence or due to human intervention can be relevant background information when dealing with rapid ecological destruction today, and can help predict future response to ecosystem deteriorations.

In an effort to reconstruct phylogeographic patterns of past populations, several resources have been used by archaeologists including pollen and faunal

analysis. While pollen analysis mostly detects species presence/absence as well as the use of plants, faunal analysis can detect species presence/absence, species abundance (based on the minimum number of individuals, MNI or the number of identified specimens, NISP), and individual identification markers such as age, sex and size. Middens in archaeological settlements contain only faunal material deposited by local people. Culture has an important influence on material deposition because the unintentional sampling process by humans may distort the picture gained from these analyses, incorrectly implying the absence of a species in an environment. On the other hand, some species would not be visible in the fossil record without human action because of their life history, such as marine organisms like fish or whales**.** Additional lines of evidence from palaeontological research can help to distinguish between natural changes and cultural inference where available.

A study published by Lee Lyman 15 years ago looked at the application of zooarchaeology to conservation biology and wildlife management questions (Lyman 1996). Having the ability to identify invasive species, changes in ecological communities and species distribution, zooarchaeological assemblages can be important windows into past ecosystems. Only a few studies in conservation biology have made use of the long term data available in zooarchaeology. Lee Lyman's zooarchaeological study on pygmy rabbits in eastern Washington revealed the original species distribution to be more extensive than the known range today (Lyman 2004). In addition, his analysis concluded that the diminished range of the pygmy rabbit probably correlated with the reduction of big sagebrush

distribution, used by the pygmy rabbit for food and shelter. The relatively close distance between the northern and southern habitat in conjunction with archaeological records of migration between the rabbit groups indicated that both populations might be closely enough related to substitute the diminished northern population in Washington State (Lyman 1996).

Virginia Butler's (Butler 2000, Butler and Delacorte 2004) research on fish species in Owens Lake demonstrates that the inclusion of r/K life history strategies can be important factors when investigating ecosystem changes and species survival over time. For this study, fish remains from seven archaeological sites in the Owens Valley were compared between sites as well as over a temporal scale. Results suggest that small fish (speckled dace and pupfish) were rare, either naturally or because they were not targeted by fishers during that time, and that the size of fish present in those sites became smaller over time. Importantly, Butler notes that the absence of a species does not necessarily imply the absence in the ecological community, but can be related to cultural practice (Butler and Delacorte 2004). Changes in size of prey can be due to environmental factors, or a sign of overharvesting of larger fish (Butler and Delacorte 2004, Pauly 1995). Her analysis of different species' survival patterns over a 7000 year period can help fisheries authorities explain modern patterns and make appropriate choices for future policies.

As mentioned above, analysing extinction events helps in the investigation of modern populations in unstable environments, and provides relevant results for current and future environmental management policies. Using ancient DNA,

genetic properties of extinct populations can be analysed and applied to modern populations (Shapiro et al. 2004). The modern focus on climate change provides a venue for ancient DNA research on population genetic diversity and extinction to make this research relevant to modern questions. The basic information has been provided previously for various species, including extinct megafauna species, as well as Pleistocene fauna (Bunce et al. 2005, Cooper et al. 2001, Cooper et al. 1996). However, in many cases, the connections are not being made between data and the possible implications for modern conservation biology (DeMaster et al. 2006).

The idea that historical data in conservation biology has a very limited time depth has extensive implications if the goal is to return an ecosystem to its natural state. As Jackson and others points out (Jackson et al. 2001; Jackson 2001), this historically recorded state is often not even the natural state of the ecosystem. In most cases, human activity has already altered this natural environment. Recent data indicate that previous overfishing or overhunting could have weakened the ecosystem to a state that would have allowed for a faster collapse (Jackson et al. 2001; Jackson 2001, Rick and Erlandson 2009). Since fauna and flora are subject to continuous environmental changes, it is difficult to establish the natural condition of a species or ecosystem prior to significant human alteration unless long term records are available (Lyman 2006). A long term ancient DNA study, including archaeological and if available palaeontological data has the ability to reveal the true baseline for a species population size, density and genetic diversity. If carried out as an interdisciplinary project, including both archaeological data and modern

conservation biological data, the project would have the ability to determine not only this urgently needed baseline but also the changes of this population over time, independent of climactic changes (Butler 2003). Accurate baseline information would help policy makers in long term planning, especially when facing severely depleted ecosystems and current climate changes.

Finally, Jackson and others point out that assumptions about 'natural' ecosystems depend on people's needs as well as expectations based on previous knowledge (Jackson 2001, Jackson and Johnson 2001). Archaeological data might adjust these expectations by revealing the original baseline, thus demonstrating "what could be" and aiding in economic and ecological decision-making.

Archaeological and Ethnographic Record

The whale bones analysed in this study are associated with the Nuu-chah-nulth on the West Coast of Vancouver Island, British Columbia, and reflect the life-ways of past people. In order to put the samples into an appropriate archaeological context and to correctly interpret the ancient DNA data, the following section discusses the archaeology and ethnography of the dataset. The geographic focus of this study was the traditional homeland of the Nuu-chah-nulth, who have a long history and extensive tradition in connection with whales and the hunting of whales.

Archaeological research has demonstrated continuous settlement and the use of whales for more than 4000 years (Arima et al. 1991, Inglis and Haggarty

1986, McMillan 2009), allowing this research to investigate past whaling practices prior to industrial whaling, European contact, and the use of modern technology. The following section deals with Nuu-chah-nulth archaeology and ethnography at Barkley Sound.

Nuu-chah-nulth History at Barkley Sound

The Nuu-chah-nulth people are part of the Northwest Coast culture area which reaches geographically from today's southeastern Alaska to southern Oregon (Suttles and Jonaitis 1990). The Northwest Coast is culturally and linguistically distinctive with 16 languages from five different language families, a variety not seen anywhere else in Canada. Numerous groups live in the Northwest Coast area; Nuu-chah-nulth, Kwakwaka'wakw and Coast Salish share Vancouver Island (McMillan and Yellowhorn 2004). Nuu-chah-nulth territory reaches from Cape Cook in the north, all the way along the West Coast of Vancouver Island to just south of Barkley Sound, bordering in the north on Kwakwaka'wakw territory, in the south on Ditidaht territory and in the southeast on Coast Salish territory (McMillan 1999, Arima et al. 1991, Sumpter et al. 2002). The name Nuu-chah-nulth was only adopted by the tribal council in 1978. "Nootka", a term introduced by Captain Cook was previously used for people all along the west coast of Vancouver Island (McMillan 1999).

Archaeological research in Nuu-chah-nulth territory has traced human settlement back to at least 5000 BP (McMillan and St.Claire 2005). Almost all of

the oldest sites in Nuu-chah-nulth territory are situated on the outer coast (McMillan and St.Claire 2005). Due to the sea level changes over the past 10 000 years, sites older than 5000 BP may be either below water or further inland than today's shore line (McMillan 1999, 1998). Research in other areas of the West Coast has suggested human occupation reaching back to 8000 BP (McMillan 1999, Hobler 1990, Carlson 1996). The sites excavated so far, however, in conjunction with ethnographic data, yield an impressive amount of data.

Based on archaeological material excavated at Yuquot in Nootka Sound and Hesquiat just south of Nootka Sound, Mitchell (1971,1990) coined the term "West Coast culture type" to describe the common traits visible in the material culture of the area. While Dewhirst originally believed this culture type to be continuous and relatively static, later research has revealed variations and additions to the original set of characteristics as well as variations over time and space (McMillan 1999).

The West Coast Culture type is typified by a relative lack of stone tools, especially in later periods. Archaeological excavations at sites in Barkley Sound have found cultural deposits similar to sites in the Strait of Georgia prior to 2000 BP (specifically Locarno Beach stage) and West Coast culture deposits after 2000 BP (McMillan 1998). The most commonly preserved tools in Barkley Sound are made from bone and shell and are associated with fishing gear, indicating a specialisation in marine resources. Implements of wood and bark, were not preserved in archaeological record except at wet-sites such as Ozette and Nitinat Lake Site (McMillan 1998, 1999). Ozette was a major Makah village on the Olympic Peninsula, in Washington State. The village was buried under a mudslide

around 300 BP and yielded remarkable amounts of fauna, flora and material culture. Organic material such as basketry and hunting gear were particularly well preserved. As McMillan (1998) points out, the lack of preserved wooden and fibre artifacts in many archaeological sites may mask a major part of Nuu-chah-nulth material culture variation through time. Excavations at Ozette suggest that basketry might be a better indicator for local variation and group affiliation (McMillan 1999, Croes 1977).

Archaeological and ethnographic records indicate that people in Barkley Sound changed settlement patterns from year round occupation to seasonal settlements (McMillan 1999, McMillan 2009). It is unclear when the seasonal pattern of movement between winter and summer villages developed. Sites such as Ts'ishaa and Ch'uumat'a, for example, exhibit the oldest excavated deposits in Nuu-chah-nulth territory, and analysis of the archaeological remains suggest a year-round settlement (McMillan and St.Claire 2005). The pattern of bi-annual movement seems only possible after the amalgamation of groups that expanded in territory (Arima et al. 1991, McMillan 2009). Just prior to or around the first contact with European explorers, war led to amalgamations, and to the ethnographically-known territories (St. Claire 1991), substantially changing the political picture in Barkley Sound. Several of the local groups living in Barkley Sound, were either incorporated or destroyed by the winning party, with some groups fusing because small groups of survivors were unable to defend themselves and other groups disappearing completely, leaving almost no traces in the ethnographic record (McMillan 1999, 2009).

The first recorded contact with European traders and seafarers on the Northwest Coast was in 1774 (St. Claire 1991, McMillan 1999). Between 1774 and 1795, a more or less continuous contact took place through the trade of sea otter pelts. After the decline of sea otters in the region, contact ceased for almost 70 years before Euro-Canadians permanently settled in the area. Disease and conflicts between European seafarers and traders, and the local Nuu-chah-nulth groups decimated the native population in Barkley Sound. From an estimated 25 000 in 1774, the population dropped to about thousand in the late 18th century (McMillan 1999, Arima et al. 1991, McMillan 2009).

Society

Northwest Coast cultures have been described as stratified and ranked semi-sedentary hunter-gatherer societies (McMillan 1999, Ames and Maschner 1999, Arima and Dewhirst 1990, Drucker 1951). The lowest class, people without any rights, were slaves who could make up to 25% of the society (Ames and Maschner 1999).

The local group, comprised of the chief and his extended family, was the basic political unit (McMillan 2009, McMillan and St.Claire 2005, Drucker 1951). Local groups were able to trace their heritage back to one common ancestor. Marriage between groups fostered widespread social networks, which allowed individuals to move between groups by switching residence (McMillan 1999). This pattern was also important in trade and for defence. Allied groups also sometimes shared winter villages (Drucker 1951). Despite some cultural and linguistic

differences, Nuu-chah-nulth, Ditidaht and Makah shared essentially a common culture (McMillan 1999).

The household was the main economic unit, containing several related families, and usually between 30 and 40 people (McMillan 1999). Chiefs were the heads and owners of a household but their prestige depended on the productivity of the unit. Ideally, a chief was the eldest son of an eldest son. Chiefs were seen as elders rather than monarchs but their authority and power was real (McMillan 1999). Commoners had no property and no rank and thus could move easily between groups and villages. They often were skilled trade workers, their skills being handed down for generations in the family. Special skills were courted by chiefs, sometimes allowing common individuals to gain prestige (McMillan and St.Claire 2005). Chiefs owned hunting and collecting grounds, and gave access to relatives and followers as needed. Importantly, the first harvest and the first hunted animal of the season belonged to the respective chief (Monks 2005). Similarly, drift whales were property of the local chief; in some cases, drift whales could lead to altercations between chiefs when the location or source, for example as a result of an attempted hunt, was disputed (McMillan and St. Claire 2005).

Chiefs were ranked within each village as well as the region. The higher the rank, the more display was needed during potlatches. A chief from an outer coast whaling village and his descendants generally had highest ranking position in potlatch (Drucker 1951, Kirk 1986). The system of inheritance was based on primogeniture, with chiefly rights and ranks being typically passed to eldest sons; however, the rules regarding the sex and birth rank were mutable. Females tended

to inherit “portable wealth” like names, songs, dance and ceremony, and still followed the patrilocal residence scheme, while male descendants inherited non-portable wealth such as hunting grounds and locations for fish traps (St.Claire 1991, Drucker 1951).

For at least 5000 years, Barkley Sound was inhabited by people adapted to a marine lifestyle. Fishing and collecting of marine resources contributed considerably to the daily life and subsistence. The richness of marine species available at Barkley Sound is evident in the archaeological record (Fredrick 2011, Fredrick and Crockford 2005) One of the resources which permeated Nuu-chah-nulth life on all levels was the humpback whale.

Megaptera novaeangliae

Cetaceans are comprised of the groups odontoceti (or toothed whales) and mysticeti or baleen whales. Humpback whales are rorquals within the group of baleen whales. Baleen whales are filter-feeders, inhaling large amounts of water and pressing the water back out, catching krill and small fish between the baleen. Eleven species of baleen whales in three families are known, including blue whale (*Balaenoptera musculus,* the largest whale), grey whale (*Eschrichtius robustus)* and humpback whale (*Megaptera novaeangliae*) (Clapham et al. 1999).

Megaptera novaeangliae (Borowski 1781) is the only member of the *Megaptera* family. While it belongs to the Balaeonopteriadae (rorquals) family, studies on the genetic relations between humpback whales and other

Balaeonopteriadea come to different results respectively depending on the loci used (Sasaki et al. 2005, Jackson et al. 2009, Arnason et al. 1993). Like other baleen whales, humpbacks exhibit throat grooves, allowing the whales to extend the volume of their mouth during feeding. These grooves reach from the lower jaw to the area around the navel, depending on sex and species. The body of M. *novaeangliae* is shorter and rounder in build than other baleen whales. The fluke of a humpback whale is easy to distinguish by its serrated edge, and the flipper grows to about 1/3 of the entire body length. The body color is usually black with a white, or a variation of black and white lower part. The variation of the fluke color is used for individual identification, especially for photo identifications. Humpback whales exhibit protuberances, with hair follicles around the face which are a unique feature to this species.

While all humpback whales belong to the same species, some variation has been described between populations in terms of body color and length. According to Slijper (1979) North Pacific humpback whales are generally darker and a bit smaller than those in the southern oceans. Due to the overlap in phenotypic variation, however, these two attributes are not sufficient for distinguishing membership in a particular population. Several studies have reported cultural transmission of behaviour, suggesting that further inter-group interactions not directly observable must be happening (Ramp 2010, Canning et al. 2011). Canning et al. (2011) report that certain behavioural traits seem to be based on cultural transmission, in the case of their study feeding and rolling behaviour. Investigating the evolution of whale songs, Garland et al. (2011) report that the songs change

every year and are ocean specific, although not population specific as the variation was observed to travel from one population to the next, in some cases crossing ocean basins.

Humpback whales have between 350-370 black-brown keratinous baleen on each side of their upper jaw with a length of about 60cm (Slijper 1979, Winn and Reichley 1985). This enables them to feed on krill, plankton and schools of small fish like mackerel, anchovy and herring. Humpback whales inhale large amounts of water with their food. Feeding occurs at the summer grounds and to a smaller extent during migration. Otherwise, humpback whales are fasting and accessing the energy stored in their fat deposits called blubber. Slijper (1979) notes that whales that were caught in their winter grounds, or on their way to the summer grounds yielded considerably less fat and oil, supporting the notion that whales live off of this energy for most of the year.

Female humpback whales are up to 1.5 m longer than male humpback whales exhibiting body lengths of up to 19 m, and average lengths of around 14-15 m. The body weight can reach up to 53 t (Reeves and Kenney 2003, Perrin et al. 2002). Adult female humpback whales have a birth interval of two to three years. Humpback whales either mate on their way to or at the winter breeding ground. The gestation period is about 11.5 months and calves are usually born at the winter breeding grounds in tropical waters. While most calves are born mid-winter, some births occur during migration, before reaching the breeding grounds (Perrin et al. 2002). Humpback whales return to their birth ground on a regular basis, especially female whales, who return for the birth of their offspring. Newborn calves

stay close to the mother and are nursed for about a year; during the last six months, calves also feed on solid food. A newborn calf is between four and five meters long, and both male and female whales are seen to protect calves when necessary. Sexual maturity is reached at about five years while physical maturity is reached at about 14-30 years (Slijper 1979, Reeves and Kenney 2003, Perrin et al. 2002). The life expectancy of humpback whales is unknown. However, some harpoon heads embedded in modern whales have been dated to at least 80 years, indicating the longevity of these mammals.

The history of whaling

While species and modes of hunting have been analysed from the archaeological record and oral tradition, little is known about how and why people started to hunt large sea mammals. The transfer from using stranded to hunted whales is especially difficult to discern. Various indigenous coastal groups around the world claim to be the first whalers. This section provides a short survey of whaling practices worldwide.

Multiple regions have evidence of whale use but no evidence of active whaling. For example, the faunal material of archaeological sites in Tierra del Fuego included (<1% NISP) whale bones dating as far back as 7000 BP uncal. (Piana 2002). However, archaeologists have concluded that the technology used, including the canoes and harpoon heads found in those sites excludes large cetaceans from being actively hunted (Piana 2002). Sites in Alaska and Greenland

as well as the Okhotsk Sea have been dated to 2000 years and older. Befu and Chard (1964) report whalebones in shell midden and pictures of whales on artifacts and harpoon heads from Okhotsk Sea sites that could have been used for whaling. The culture lasted from "centuries before Christ until the 17th century" (Befu and Chard p.14) when the sites were abandoned. Arctic whalers in Greenland and Alaska have been linked to sites dating as far back as 2000 years (Reeves and Smith 2007, Savelle and McCartney 1999). Furthermore, several Thule sites in Nunavut indicate a whaling culture that is at least 1000 years old, with houses using whale bones as building material, as well as artifacts made of whale bone at sites dating AD1200-AD1600 (McCartney and Savelle 1985). Meanwhile archaeological material from Prince William Sound, on the south coast of Alaska, includes sperm whales as well as unidentified baleen whales dating back to 4400 BP (Yarborough and Yarborough 1998). Finally, in South Korea, Rock Art at Bangu-Dae believed to be 6000 years old includes numerous depictions of whales including whaling activity (Lee and Robineau 2004). A nearby site contains a large amount of whale bones and was occupied between 5000 BP and 1500 BP (Lee and Robineau 2004). However, the difficulties in dating rock art makes these dates somewhat unreliable. In addition, the depiction of whales or possible whaling in cave art is an indirect line of evidence at best. Still, similar cave art has also been found on Vancouver Island, suggesting that it might be possible to infer the cultural importance of whales in these places (Canadian Rock Art Research Associates 1979).

In later centuries, after the 16th century several additional groups took up

whaling after being in contact with foreign whalers from Europe or North America. Groups on the Lesser Antilles, Equatorial Guinea and the Philippines used whaling technology that was likely introduced by whalers in the fifteenth century (Reeves 2002). A similar result has been reported from Tonga (Kandel and Conard 2003, Reeves 2002) In the North Atlantic, archaeological sites containing cetacean elements have been found in Scotland and the Outer Hebrides from the Mesolithic onwards, with an increase during the Bronze Age and Iron Age (Mulville 2002a). Interestingly, a study in an African site revealed only evidence for scavenging whales in a location with frequent drift whale presence, but no evidence for active whaling (Kandel and Conard 2003). In most cases, the onset of whaling cannot be determined accurately, mainly because clear indicators are unknown or disputed (Monks 2005, Savelle and McCartney 1999, Mulville 2002b). While whaling has been a subsistence practice of people worldwide, secure evidence of active whaling is scarce and mostly within the past 2000 years.

The history of modern whaling

Whale hunting is practiced in all regions and all oceans (Reeves and Smith 2007). Industrial whaling moved geographically from the North Atlantic over the South Atlantic and Greenland, to the South Pacific and finally to the North Pacific. Commercial whaling began with the Basques during the eleventh century (McLeod et al. 2008, Jenkins 1971, Baldwin et al. 2010). Whales were not only hunted for personal use and consumption, but also for their tradeable materials such as oil, baleen, skin, bones, and whale meat which was considered fish and was allowed

in Catholic populations for consumption during Lent. Basque whalers mainly hunted bowhead whale with small boats and hand held harpoons in the Bay of Biscay (McLeod et al. 2008). Within 200 years, a decline in whales, the first known record for a decline in local whale populations, led to an expansion of whaling efforts to Greenland in the fourteenth century (Frost 1978, Francis 1990). By 1550, bowhead whaling operations reached Labrador (McLeod et al. 2008, Frost 1978). This time, other nations started to hunt whales, including the Dutch, British and Norwegian fleets. During the eighteenth century, whaling was also conducted by American and Japanese whalers (Reeves and Smith 2007, Tønnessen and Johnsen 1982).

Industrial whaling is usually separated into old style whaling, using sail boats and hand held devices for both shore based and pelagic (open sea) whaling, and modern whaling, using steam powered ships and later, grenade harpoons (Reeves and Smith 2007). The modern harpoon gun was developed by Sven Foyn, a Norwegian whaling captain around 1864 (Tønnessen and Johnsen 1982). The gun could be mounted on deck and equipped with a grenade that would explode on impact (Tønnessen and Johnsen 1982, Webb 1988). Early pelagic whalers stripped the blubber off the whale (a practice referred to as “flensing”) while keeping the whale in the water next to the boat. After 1920, new factory ships could haul the entire whale on board to process the carcass. With the invention of large factory ships that allowed the dismemberment of whales while on the sea, vessels were able to stay in pelagic waters for months at a time (Tønnessen and Johnsen 1982). Where possible, the whale oil was produced immediately by cooking the

blubber on board, as the oil was easier to store and longer lasting than the blubber itself. Whale oil was one of the main products used in Europe and North America, with different qualities depending on the whale species (Tønnessen and Johnsen 1982). At the beginning of the nineteenth century, the whaling industry in America was producing almost 15 million gallons of oil per year, mostly used as fuel in oil lamps (Bardi 2011, Starbuck 1878) but production later declined. Even with the introduction of modern whaling technology towards the end of the nineteenth century, it was impossible to repeat profits gained in the earlier years, because whale populations had been depleted.

While whaling in the Atlantic started in the fifteenth century, the Pacific whaling industry only developed during the eighteenth century. The first whaling vessels to enter the Pacific in 1787 operated off the coast of Chile (Tønnessen and Johnson 1982). From there, operations moved northwards towards California, Japan, Canada and lastly, Alaska. Until the sixteenth century, whaling in Japanese waters was subsistence or small scale commercial whaling. For a long time, Japanese whalers hunted with nets, or bows and arrows, but in most cases relied on drift whales (Hidemura and Fujimoto 1978 as cited in Kalland and Moeran 1992). Smaller cetaceans, such as dolphins and pilot whales, were caught ad hoc with this method or driven into bays and towards the shoreline (Kalland and Moeran 1992). Active whaling using harpoons started in the sixteenth century in this region and net whaling was introduced in 1675 (Kalland and Moeran 1992). For this method, whalers set their nets out on open water, waiting to attack entangled whales with their harpoon.

Whaling grounds in Hawaii and Japan were discovered in 1820 by American and British industrial whalers (Kalland and Moeran 1992). By 1845, almost 300 ships were based in the USA (Tønnessen and Johnsen 1982). A reduction in whaling success by traditional Japanese whalers was noticeable within 10 years (Kalland and Moeran 1992), which led to Japanese whalers turning to American whaling methods. Whaling in Australia started in the eighteenth century, with established whaling stations in New South Wales and Tasmania (Frost 1978). The first deep sea whaling ship for Australia was built in 1805 after an abundance of right and sperm whales were discovered off shore (Frost 1978).

In the North Pacific, modern industrial whaling started late with the discovery of right whales in Alaska in 1848 (Tønnessen and Johnsen 1982, Kalland and Moeran 1992). The first whaling station in the Northeast Pacific was build in San Francisco in 1838, but coastal whaling in California did not start until 1854, catching mainly grey whales. Traditional whaling methods lasted longer in the North Pacific than in any other place because of the remoteness to Europe and the North American east coast, and was only replaced by modern technology around 1900 (Tønnessen and Johnsen 1982).

The first whaling station on Vancouver Island was established in 1905 as part of the Pacific Steam Whaling Company at Sechart in Barkley Sound (Tønnessen and Johnsen 1982). While the first blue whale was caught in August 1905, the station did not commence work until the 1906 season because of technical difficulties. Due to harsh weather during the winter in Barkley Sound, a second station, Page's Lagoon, was opened in the Georgia Strait, close to

Nanaimo, British Columbia. However, this station did not provide sufficient profit and was closed three years later (Tønnessen and Johnsen 1982, Nichols et al. 2002). The two other whaling stations on Vancouver Island were built in Kyuquot and in Coal Harbour, both located on northern Vancouver Island, in 1907 and 1948 respectively. At that time, Kyuquot was considered the "'world's largest whaling station' with a 50% greater capacity than Sechart" (Tønnessen and Johnson 1982, p116). Two more stations were built by the Queen Charlotte Islands Whaling Co. on the Queen Charlotte Islands: Rose Harbour and Nade Harbour. Sechart in Barkley Sound operated between mid-April and late October/early November. Very soon, humpback whales were the main species caught (Nichol et al. 2002). The main product was again whale oil, with some whale meat exported to Japan. The Sechart whaling station caught, with over 75%, the highest amount of humpback whales along the Canadian coast (Nichol et al., 2002). However, in 1917 the whaling station in Sechart closed; most of the other stations closed between 1941 and 1943. Only Coal Harbour operated until the ban on commercial whaling in 1967. In total, whaling stations off Vancouver Island processed over 25 000 whales (Nichol et al. 2002).

An improvement in technology and knowledge about whale distribution did not help the whaling industry to repeat their previous success, even during the increase in demand for whale oil during the First World War (1914-1918). Tønnessen notes that later whaling operations off the shore of Mexico caught predominantly female humpback whales in a late state of their pregnancy, which is considered an "example of the cynical and irrational destruction of whale stocks"

(1982 p. 327). The time between the First and the Second World Wars was again marked by initial success with a sharp and fast decline in catch rates. Apart from the obvious collapse in whale stocks, companies collapsed due to bankruptcy as well, some within two years of their incorporation (Tønnessen and Johnson 1982).

While early attempts to regulate whaling failed, 21 countries signed the Geneva Convention for Whaling in Geneva, Switzerland in 1931. This agreement only regulated the catch of baleen whales and the language was rather unspecific and was later replaced in 1937 by a new regulation which included specifics regarding time and catch quota (Frost 1978). The International Whaling Commission (IWC) was established in 1946 during the inaugural conference in Washington DC, USA. The convention was signed during this meeting by all pelagic whaling nations, with the goal to "provide for the proper conservation of whale stocks and thus make possible the orderly development of the whaling industry" (International Whaling Commission 1946). Since then, the function of the IWC has been to establish and regulate catch quotas, as well as to oversee conservation management of cetaceans and tighten measures of sustainability. In light of an increase in whaling efforts during the 1960s, a moratorium on commercial whaling was put in place in 1984 to allow whale stocks to recover before sustainable whaling could resume. Over the past years, several countries have pushed to end the moratorium at the yearly meetings, arguing that whale stocks have recovered. The yearly meetings of the IWC are today dominated by political rows about the commercial whaling ban, making any proposals next to impossible to pass.

The International Whaling Commission (IWC) meets on a yearly basis, monitoring research progress and continuing the dialogue between nations and whaling opponents. Their memoranda are under fire since it was first implemented. Countries like Iceland, Norway and Japan consider it their right to whale on a regular basis. Japan in particular is featured on a regular basis in the international press because their 'scientific whaling' is seen as a cover for industrial whaling (Gales et al. 2005). Another problem are the catch quotas that are set for five years by a scientific commission of the IWC, and during this process representatives for the whaling industry who are constantly lobbying for an increase in quota. Also, the trade of catch quota can be seen as a problem since this means that different populations are affected. Finally, a major political problem is the definition of 'traditional whaling' by First Nations/Aboriginal groups. Whether a group is granted whaling rights or not depends on how well they can convince the Commission of their long standing tradition. Even if whaling rights are granted, the public often disagrees (Sullivan 2000). Critics of the IWC complain about the ineffectiveness of this organization, as well as the lack of actual power over offenders. For the past five years, Japan and Norway have been trying to convince countries to re-evaluate and subsequently abandon the ban on commercial whaling. Modern conservation biology not only monitors wild populations, but has also reached out to collect samples from meat markets to get a better insight on species and the extent of these hunting efforts (Baker et al. 2000a, Baker et al. 2000b, Baker and Palumbi 1994).

Today, only some traditional whaling groups are allowed to hunt a certain

quota, and only if listed under the aboriginal subsistence whaling scheme set out by the International Whaling Commission. For example, on the Northwest Coast, the Makah on the Olympic Peninsula have the right to hunt and on May 17, 1999 the Makah in Neah Bay successfully took the first grey whale in 70 years with the permission of both the International Whaling Commission and the US Marine Protection (http://www.makah.com/ whalingrecent.html, last accessed October 22 2010).

After thousands of years of hunting, industrial whaling managed to remove an estimated 90% of the world's humpback whale population in less than 200 years. However, recent years have witnessed a remarkable recovery of humpback whale populations. With the increase in data analysed, a complicated network of male and female humpback whale migration in the North Pacific has become apparent. Current biogeography suggests that humpback whale populations have retained their genetic diversity without any major losses; access and time depth of archaeological material in Barkley Sound makes this region an ideal place to investigate these suggestions by examining pre-industrial whaling population genetic diversity.

Chapter 3: Material and Methods

Sample Provenience

The whale bones processed during this study were excavated in Barkley Sound, Vancouver Island during two archaeological projects. The first project included two adjacent sites on Benson Island, Ts'ishaa (DfSi-16) and Himayis (DfSi-17). Benson Island is located in the northwest corner of the Broken Group Islands in central Barkley Sound (Figure 1, location 1). Samples were selected from both the Ts'ishaa main village and Himayis, excavated from 1999 to 2001 (McMillan 2009, McMillan and St. Claire 2005). The age of the samples ranged

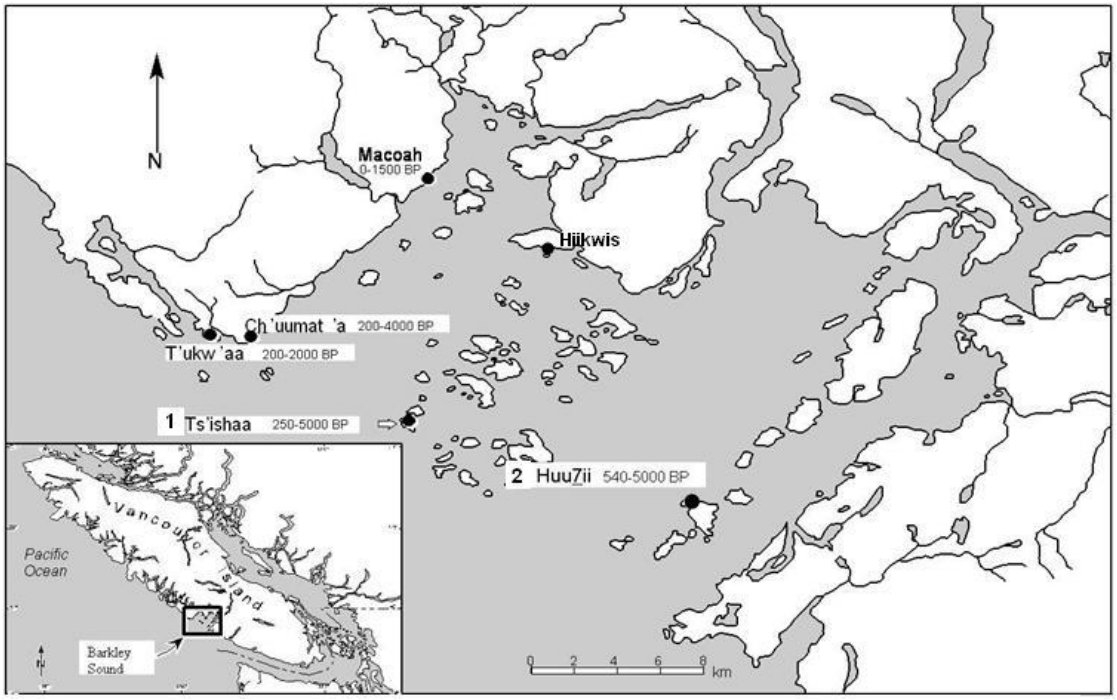


Figure 1: map of Barkley Sound with excavated sites (adapted after McMillan and St Claire 2005) 1: Ts'ishaa (DfSi 16 and 17) 2:Huu7ii (DfSh7)

from 4500 BP to 200 BP.

Huu7ii (Figure 1, location 2, DfSh7) is located in the Deer Group Islands on the eastern side of Barkley Sound. The site at Huu7ii was excavated in 2004 and 2006. Samples were excavated from a large house structure as well as the pre-house floor. Sample ages ranged from 1500 BP to 500 BP. An earlier occupation, located on a raised back terrace dated to 5000BP-3000BP, was not sampled because zooarchaeological analysis had not been done on this part of the excavated material at that time.

Ts'ishaa

Ts'ishaa was the main village of the Tseshaht, a Nuu-chah-nulth political unit. The Tseshaht occupied the Broken Group Islands and the central portion of Barkley Sound. The original homeland of the Tseshaht included Benson Island, and the surrounding islands. Oral traditions indicate that the site of Ts'ishaa was the main village of the Tseshaht people. Ts'ishaa was occupied for around 5000 years (McMillan and St.Claire 2005). Ts'ishaa was a year round occupied village for most of its history, but by early historic times used as a summer camp only when the territory expanded towards the mainland of Vancouver Island.

Ts'ishaa (#1 on figure 1) was comprised of the main village, including a back terrace, and Himayis, with up to six subgroups of the Tseshaht located at adjacent beaches. While the back terrace includes occupation components dating to the mid- Holocene, Himayis was occupied by the lowest ranked group (McMillan and

St. Claire 2005).

According to ethnographic records, there were favourite whaling and sea otter hunting areas in the islets around Benson Island. During the spring, migration routes led grey whales past the Broken Group and Deer Group Islands (McMillan and St.Claire 2005). While few grey whales entered Barkely Sound, humpback whales would enter, especially in fall. According to Sapir's informant (Sapir 1915), humpback whales were frequently sighted at Alberni, Uchucklesaht, Effingham Inlet and the upper sound area from October to March (McMillan and St. Claire 2005). Other areas were known as whaling spots, included Pigot Islet and Turret Island; other areas carried names connecting these locations to whales, such as Dodd Island ("where there are many whales") or Gilbert Island ("whale oil here") (McMillan and St. Claire 2005, Sapir 1915).

European contact in the late eighteenth century led to dramatic changes in the life ways of the Tseshaht. These changes, which had begun prior to European contact, included increased warfare, reduced population, and population amalgamations or absorption of groups, leading to larger territories in the end (McMillan 2009, McMillan and St.Claire 2005, McMillan et al. 1982). At the end of these amalgamations, by about 1815, Tseshaht territory included the entire Broken Group Islands, as well as much of the central Barkley Sound coastline and Alberni inlet. At this time, Ts'ishaa was used only as a summer camp, occupied between May and August. The major winter village shifted to Hiikwis on the Sechart Channel of upper Barkley Sound and then to the top of Alberni Inlet (McMillan 1999, 2009, St. Claire 1991).

Huu7ii

Huu7ii (#2 on figure 1), located on Diana Island in the Deer Group Islands in Barkley Sound and is the ancestral village of the Huu-ay-aht people (McMillan 2009, St. Claire 1991). Huu7ii was initially recorded in 1984 (Mackie and Williamson 2003, McMillan 2009) and was excavated in 2004 and 2006. Huu-ay-aht territory is located in the eastern portion of Barkley Sound, including the Deer Group Islands and the southeastern part of Barkley Sound. Diana Island is part of the original Huu-ay-aht territory, but the current boundaries are much larger due to expansion through amalgamations (McMillan 2009, Inglis and Hagarty 1986, St. Claire 1991, Fredrick 2011). Locations of ten to twelve previous house platforms were easily visible based on their flat surfaces, partially bounded by midden ridges (McMillan 2009, p. 627, Mackie and Williams 2003). The occupation at Huu7ii lasted from approximately 1500 BP to 400 BP (McMillan 2009, Fredrick 2011). The site was abandoned prior to European contact (McMillan 2009, St.Claire 1991). The elevated back terrace dates to 5000-3000 BP (McMillan 2009). Based on these specifications, Huu7ii was occupied roughly at the same time as Ts'ishaa and of a similar size. The excavation took place primarily on the largest house platform, which is presumed to indicate the house with the highest social status (McMillan 2009). Samples came from house floor as well from the "pre-house" deposit below the house floor. No samples came from the back-terrace dating to 5000-3000BP

Samples

Ts'ishaa samples were zooarchaeologically analysed by Gay Fredrick and Susan Crockford prior to this study (Fredrick and Crockford 2005). Huu7ii whale bones were sampled by the author based on prior zooarchaeological analysis recorded in a database. The site location data and zooarchaeological species identification for samples used in this study can be found in Appendix A. While samples were well preserved in terms of genetic material, the morphological preservation was poor. Due to the fragmented conditions, most bones were not identifiable beyond genus level; at Ts'ishaa, for example, only 874 of the 1541 samples identified as sea mammal were identified to at least the order level (McMillan and St.Claire 2005). Out of samples identifiable to at least order, 254 were identified as cetacean (McMillan and St.Claire 2005). Samples classified as "cetacean" or "whale" were selected for the ancient DNA analysis. Ts'ishaa samples were selected to minimize possible resampling of individuals (McKechnie 2010 pers.comm.), although some samples were labelled "possibly same bone".

No MNI (Minimal number of individuals) was established during the zooarchaeological analysis (McKechnie 2010 pers. comm). The fragmentation did not allow for the selection of specific skeletal elements, an otherwise common practice in zooarchaeology to prevent sampling an individual multiple times (Reitz and Wing 1999). The poor physical preservation can be attributed to the butchering process as well as natural taphonomic processes. Only a few bones were of value for artefact manufacture. However, most of the whale skeleton remained at the beach and was discarded (Monks et al. 2001). The majority of bone material found

in middens was probably attached to blubber or meat distributed after the hunt (Monks et al. 2001). Specimens excavated were identified as “whale” and only in few instances was a species identification or re-connection of bone fragments attempted. How many individuals are represented in this sample set may be relevant because re-sampling of individuals would potentially create a bias towards a haplotype, influencing the genetic diversity of the population.

The question of redundancy of the sample set was approached via three different analyses. The initial approach was to establish the sex of each bone [a], followed by a zooarchaeological review of two independent researchers [b]. Finally, the location of the sample was matched with the genetic haplotype and the species identification [c]. Results for [a] and [b] are discussed in Chapter 5; results for [c] are discussed in Chapter 6.

Samples for DNA analysis were labelled with consecutive numbers, starting with WH26. For the statistical analysis, sample labels were changed to PT (Ts’ishaa) and PH (Huu7ii). The total dataset of 264 bone samples was comprised of 163 samples (WH 26-WH 176) from Ts’ishaa and 101 samples (WH 180-WH 279) from Huu7ii. The age of the samples used over the course of the analysis was provided by Alan McMillan and Iain McKechnie based on direct and indirect C^{14} dating and occupation times.

Method

Sample preparation and extraction

Prior to sampling, pictures were taken of each bone. Sampling of the bones was done using a handsaw blade available in hardware stores. To avoid cross sample contamination, blades were used only once and then disposed of, and the work surface was wiped with a 10% bleach solution between samples. The same procedure was applied to the sampling process at Simon Fraser University (SFU) for the Ts'ishaa samples as well as the sampling process at the University of Victoria for the Huu7ii samples. About 1.5g of bone was cut, bagged in new sample plastic bags and stored at room temperature until use. Laboratory ID numbers were assigned in consecutive order starting with the number 26 with the prefix WH denoting whale. Sample numbers 1-25 were used during a previous study (Watt 2003). Samples that had been identified as "possibly one bone" in the Ts'ishaa collection were designated by -1 and -2 (e.g. WH 176-1 and WH176-2). The cut portion of the bone was taken into the decontamination room of the ancient DNA laboratory (Fig 2, Chapter 4). After taking a picture of the bone fragment, the samples were decontaminated. In spite of existing laboratory protocol, the sample surface was not removed using sandpaper. After initial attempts, this step was abandoned due to the fragile nature of the bones. Instead, the time the sample remained in bleach and acid was increased. Bones were rinsed in UV-irradiated HPLC H_2O and incubated in 100% commercial bleach (Javex©) for 8 to10 minutes. The bleach was discarded and the sample rinsed twice in UV-irradiated HPLC H_2O to remove residual bleach. An acid wash was performed by submerging the

sample in 1N HCl for one minute and then neutralising with 1N NaOH. A final rinse in UV-irradiated HPLC H_2O was performed after discarding the NaOH, followed by soaking the bones in UV-irradiated HPLC H_2O for about 5 minutes. After soaking, samples were UV irradiated for 30 minutes, flipped and UV irradiated for another 30 minutes. Bones were then crushed and stored in 15 ml tubes (Sarstedt) at -18°C .

The extraction procedure followed standard laboratory protocol (Yang et al. 1998): 0.5-0.7 g of crushed bone was incubated in 3.5 ml lysis (0.5 M EDTA, pH 8.5, 0.5% SDS, 0.5 mg/ml proteinase K) overnight at 50°C in 15 ml Sarstedt reaction tubes. The tube lids were wrapped in Parafilm™ and further wrapped in paper towel and foil to control for possible leakage. After incubation, samples were spun and 3 ml of the supernatant was transferred onto a 4 ml Amicon 30 k filter. Samples were spun until the supernatant was concentrated to ≤100 µl. The duration of this step varied from 1 hour to 2.5 hours, depending on the sample. After concentrating, 600 µl commercial PE buffer (QiaQuick PCR purification kit or nucleotide removal kit) was added to the Amicon, and the resulting solution transferred into a PCR purification silica based spin column and spun to bind the DNA to the filter column.

The remaining steps followed the manufacturer's protocol. The column was washed in two steps using 500 µl of PB wash buffer each time. The column was transferred to a new collection tube, and 100 µl of elution buffer was added. The column with the elution buffer was incubated at 64°C until the solution started to drip through the filter. The column was then spun at 13 000 rpm/ 15 871 x g and

the solution was transferred into a new 100 µl tube for storage. A second elution was produced similarly and stored in a separate 100 µl tube. Both elutions were stored at -18°C. If the second elution was discoloured, a third elution was produced in an attempt to recover non-inhibited DNA. At least one blank control per lysis master mix was included with each extraction and included in PCR amplifications.

PCR setup and amplification

For the PCR setup, a premix was used containing 1.5x Buffer, 1x MgCl, dNTP, BSA and H_2O. This premix was prepared, alliquoted and frozen in the ancient DNA laboratory. This allowed for a continuous contamination control between researchers and a quality control of the PCR premix. To prepare a master mix, primers (15 pmol/ µl stock solution) and AmpliTaq Gold™ (Applied Biosystems) were added to the premix. The final volume of a PCR was 30 µl with 5 µl of sample DNA, and contained 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl2, 0.2 mM dNTP, 1.0 mg/ml BSA, 0.3 µM of each primer, 5.0 µl of DNA sample and 2.5-3.5 U AmpliTaq Gold™ or AmpliTaq Gold LD™. Negative controls were included with each PCR setup. PCR conditions were 10 minutes at 94°C, followed by 60 cycles of 94°C at 45 seconds, 54/56 or 52°C for 30 seconds, and 72°C at 40 seconds and concluded by a final extension step of 72°C for 10 minutes (see Chapters 5 and 6 for details). After the PCR reaction, samples were stored at -18°C until further handling. The success of the PCR was analysed on a 2% Agarose gel with a 100 bp ladder (Invitrogen) using SYBRgreen (Invitrogen) as

staining agent. Successful samples were purified using QiaQuick PCR purification kit and eluted in 12-15 µl of the PE Buffer (Qiagen, Hilden, Germany). Purified PCR products were stored at -18°C.

Purified samples were sequenced at Macrogen Ltd. in Seoul, Korea, and at McMaster University in Hamilton, Ontario. To improve the reading quality, samples were sequenced using sequencing primers designed approximately 5-10 bp into the original PCR product.

Table 1: Primer used in this study

	name	5'-3'	length
Cyt B	WL-F1	ATGACCAACATCCGAAAAACAC	22
	WL-R182	GTTGTTGTGTCTGGTGTGTAGTGTATT	27
D-loop	WL-F86	AATTCGTGCATGTATGTACTACCACTAA	28
	WL-F167	CACCACGAGCAGTTAAAGCTC	21
	WL-F22	CCACCATCAGCACCCAAAGC	20
	WL-R258	TGCTCGTGGTGTARATAATTGAATG	25
	WL-F360	TATGTATAATCGTGCATTCAATTAT	25
	WL-R569	GCGGGTTGCTGGTTTCAC	18
	WL-F101	TGTACTACCACATAACCAACTGATAGCA	28
	WL-R308	ATCTAATGGAGCGCCCATAGAGATC	25
	WL-F322	AGCATGCCGCGTGAAACCA	19
	WL-R588	CTGAGTCCGTGCAAGCCC	18
Sequencing Primer	WL-Fn86	AATTCGTGCATGTATGTACTACCACATA	28
	WL-F86s	ATAGCACCTTCCATGGGTATGTATA	25
	WL-F322s	TTGGCAGGGATCCCTCTTCT	20
	WL-R588s	AATACCAAATGTATGAAACCTCAG	24
ZFX/Y	WL-ZFXY F1.1	CACCAAGAAGAAGTACCGCTGTAC	24
	WL-ZFXY R1.2	CGTGAGAGAAATGCTTCCCACAT	23
	WL-ZFXY R2.3	ATTCAATAACCCTTGTTCAGCTGTC	25
	WL-ZFXY R2.4	TTGCTGTGGACTGCCAAAAGG	21
	WL-ZFY F2.2	GGTGCATAAGGAAAAAGGAGCT	22
	WL-ZFX F2	AGGCCATTGAATGCGATGAA	20
SRY	WL-SRY F2	GATCAGTAAGCACTTGGGATACGAC	25
	WL-SRY R2	GTATTTGTCTCGGTGCATGGCTC	23

Sequencing Analysis

Sequences were analysed visually using ChromasPro© (Technelysium Pty Ltd). After a manual control of the sequences, the overlapping sequences were assembled in ChromasPro and compared to published modern sequences in GenBank (http://www.ncbi.nlm.nih.gov/nucleotide/). Only reliable sequences were included in the final alignment. Quality control included analysing double-peaks and stutter pattern, comparing absolute height to background noise and finally, comparing sequences to repeats. Unreliable sequences were either re-sequenced, shortened or excluded, depending on the sample.

Finalised sequences were aligned using BioEdit© (http://www.mbio.ncsu.edu/BioEdit/bioedit.htm). Phylogenetic trees were calculated using BEAST (see Chapter 6 for details) with finback whale (*Balaeonoptera physalus,* NC_001321.1/GI:5819095) as outgroup species, based on the analysis of the mitochondrial genome by Sasaki et al. (2005). The program jmodeltest (Posada 2009, Posada 2008) was used to choose the best model for the tree using the Bayesian Information Criterion (BIC). BEAST tree outputs were prepared using FigTree Version 1.2.3 (http://tree.bio.ed.ac.uk/software/figtree) The Bayesian skyline plot was also computed using the BEAST software package, using a population set ("popset") for California as a reference population (Jackson et al.2009). BEAST analyses were carried out on the Bioportal (www.bioportal.uio.no) cluster and the computational biology service unit (http://cbsuapps.tc.cornell.edu, Beast@BioHCP) at Cornell University.

Inhibition

Forty-five samples remained dark during the extraction and purification process. The subsequent elution had a discoloration that varied between pale yellow to black. These samples either failed to amplify during PCR, or only amplified in low frequencies. The inhibitors present appeared to have similar qualities to DNA as they would bind to the silica filter during the purification step and only move past the filter when the elution buffer (EB) was added to the filter.

To test the hypothesis that the discoloration is a sign of inhibition, successfully amplified samples were spiked with discoloured samples. PCR was carried out as described above, but with the addition of potentially inhibited extract. In all cases, even 1 µl of additional DNA led to a complete drop out of PCR product. McGrath (pers. comm., 2010) used the discoloured whale samples to spike artificial DNA in order to test the changes in the efficiency of the artificial control sample. Even a dilution of 10^4 of the inhibited sample inhibited the PCR reaction completely. In an attempt to remove the inhibitor, a repeat silica step was carried out on previously extracted samples as described in Kemp et al. (2010). In this case, repeating the silica purification step did not improve the success rate of the inhibited samples. However, Kemp repeated the step up to 4 times, a variation which was not tested in this study (Kemp pers. comm. 2007). WH43 was used for further tests to determine possible venues to remove the inhibitor because a partial profile was obtained from this sample despite strong inhibition and almost black first elution and discoloured second and third elution. The first test increased the HCL incubation during the decontamination step to 5

minutes; the second test heated the decontaminated bone sample (0.5 g) to 85°C for 90 minutes. The heating step was tested because McGrath (2010) hypothesised that certain inhibitors might appear during the heating process (e.g Maillard products) and dissolve when heated further. This heating could have occurred during oil extraction at the site (Monks 2001). Both samples were extracted using the standard silica-spin extraction method (see Sample preparation and extraction for details above). In addition, WH43 was extracted using a DTAB/CTAB extraction method, the protocol based on Manen et al. (2003), Gustincich et al. (1991) and Allen et al.(2006) and modified for the SFU Forensics Laboratory by Jason Moore. Samples were incubated for 30 minutes at 65°C in DTAB Buffer (5.5% DTAB, 1 M NaCL, 70 mM Tris-HCL, 30 mM EDTA), inverting the sample 3 times. After centrifuging the samples for 10 minutes at 4400 rpm the supernatant was transferred into a new tube and washed with the same amount (in this case 700 µl per sample) of Phenol-Chloroform-Isoamylacohol (25:24:1) twice, moving the top (aqueous) phase between steps into a new tube. After removing the Phenol Chloroform Isoamylalcohol, 1200 µl CTAB Buffer (0.5% CTAB, 40 mM NaCL) was added and the sample was incubated at room temperature for 30 min. To pellet the DNA, the solution was centrifuged for 5 minutes at 4400 rpm. After removing the supernatant, the pellet was washed in 100 µl 1.2M NaCL and 250 µl 95% EtOH, followed by a wash step in 70% EtOH. The solutions were centrifuged and the liquid was removed between steps. After removing the 70% EtOH, the sample was dried at room temperature for 1 hour or until dried completely. The DNA pellet was re-

suspended in 100 µl TE Buffer (10 mM Tris-HCL, 1 mM EDTA). The WH43 extract was still slightly discoloured, but most of the discolouration was removed during the Phenol-Chloroform step.

Subsequent PCR amplifications comparing the three extraction variations yielded results for the sample incubated in HCL for 5min and the sample incubated at 85°C for 1.5h but no improvement for the sample extracted using DTAB/CTAB and Phenol-Chloroform. While this was only an initial test, results point to simple changes that could be incorporated if this problem arises in the future to gain access to otherwise inhibited DNA. It should be noted that WH26 worked well and did not exhibit any inhibition in the first extraction, but was discoloured in the second extraction. Repeated extraction of WH43 did not change the inhibition of the sample. More research is needed in this area to pinpoint the source of the inhibition as well as the optimal way of removal.

Chapter 4: Contamination Control and Ancient DNA Authenticity

Contamination and DNA damage are two of the limiting factors for anthropological and archaeological studies involving ancient DNA (Mulligan 2006). To provide a measure of authenticity to ancient DNA research, control points have been discussed over the past years (Pääbo et al. 2004, Hofreiter et al. 2001b, Willerslev and Cooper 2005, Gilbert et al. 2003, Cooper and Poinar 2000). These points are considered guidelines rather than fixed rules, taking the variability of ancient DNA research into account. Depending on sample age, history and origin, authors agree that guidelines may be adjusted to be more or less stringent as seen fit.

Guidelines for quality control and reliability of ancient DNA research have been expanded and improved, and include strict contamination controls and measures of authenticity. These guidelines (Gilbert et al. 2005, Willerslev and Cooper 2005, Cooper and Poinar 2000) currently contain nine criteria to establish authenticity of ancient DNA research as outlined below:

(1) An *isolated work area* with dedicated preparation and PCR set-up room, separated from all post-PCR activities. This also includes dedicated lab equipment to prevent carry-over of PCR products or modern DNA into the pre-PCR area.

(2) *Negative controls during extraction and amplification* as a screening

process for contaminated set-ups, both for cross contamination between samples as well as contaminated chemicals.

(3) *Appropriate molecular behaviour* which includes amplification of shorter DNA fragments and a lower available template compared to modern, non-degraded samples.

(4) *Reproduction of samples* should be part of the protocol to control for deamination and amplification errors. The reproduction schedule should include re-extraction and re-amplification of samples to ensure that the result is indeed reliable.

(5) The *cloning* of PCR products is used frequently to ensure the reliability of the sequence and control for damage and contamination (i.e. both should be random and absent in the majority of clones).

(6) An *independent replication* in a separate laboratory helps to control for ubiquitous contamination within a lab as well as problems with different extraction and amplification protocols.

(7) The observation of the *biochemical preservation* of samples should generally point to a better preservation of younger samples.

(8) A *quantification* of samples, today usually done with real time PCR, is used to verify differences between ancient DNA samples and modern controls.

(9) Finally, *associated remains* can be used to establish preservation and presence of DNA at a site. When working on human DNA in particular, associated remains of animals, which are less prone to contamination, can be an

important line of evidence.

These key criteria have been published to help the researcher design sound experiments and, at the same time help, readers evaluate a published result, but they do not guarantee reliable results. Common sense and multiple lines of evidence are needed to support the result established during the study (Pääbo et al. 2004, Gilbert et al. 2005, Hofreiter et al. 2001b). Gilbert et al. (2005) include questions that need to be asked when evaluating obtained results. Researchers should explicitly include the background of the sample such as age, environmental history and preservation of the sample to evaluate the likelihood of DNA survival, the contamination risk of the sample, including handling history and macromolecular preservation (Gilbert et al. 2005). Similarly, data should be scrutinized against possible contamination, jumping PCR or other PCR errors. Finally, an overall assessment of the data makes sense in order to see if there is further evidence to support the result. These strict measures paired with a critical self assessment help to distinguish authentic results from artificial results based on contamination or DNA damage.

Contamination control

The ancient DNA laboratory at SFU's Department of Archaeology is a facility designated for ancient DNA analysis. Protocols and work-flow are designed to prevent or detect possible contamination and are outlined below.

The ancient DNA facility at SFU is made up of two general areas: the

ancient DNA area and the post-PCR/modern DNA area. The ancient DNA laboratory (Figure 2) is divided into three separate rooms for sample decontamination, extraction and PCR set up respectively. A positive air-pressure system is included to minimize the transfer of aerosol and dust contaminants between rooms, and from outside the laboratory. Material and samples can only be transported from the ancient DNA laboratory into the post-PCR laboratory; they cannot be returned to the ancient DNA laboratory ("One-Way-Rule"). Lab material and equipment introduced into the ancient DNA laboratory are, where possible, subjected to bleach treatment prior to use. Sample tubes are additionally UV irradiated prior to use.

When working in the ancient DNA laboratory, researchers must change from street-clothes into scrubs designated for the laboratory. These scrubs are only worn inside the lab and on the way between the change room and the ancient DNA laboratory. This dedicated clothing includes a set of shoes, which get changed again at the entrance into the laboratory. The same procedure applies to the post- PCR laboratory. The post-PCR laboratory consists of one room for all post-PCR work, and was located in a different building (Robert Brown Building) than the ancient DNA laboratory. The post-PCR laboratory has been relocated to Saywell Hall (still separate from the ancient DNA laboratory) in the spring of 2008. The new facility, equipped with separate ventilation, can only be accessed via a separate entrance. The same strict conditions apply to the new facility, including the ban from entering the hallway of the archaeology department after the use of the post-PCR laboratory. Scrubs used in the post-

PCR laboratory do not leave the area.

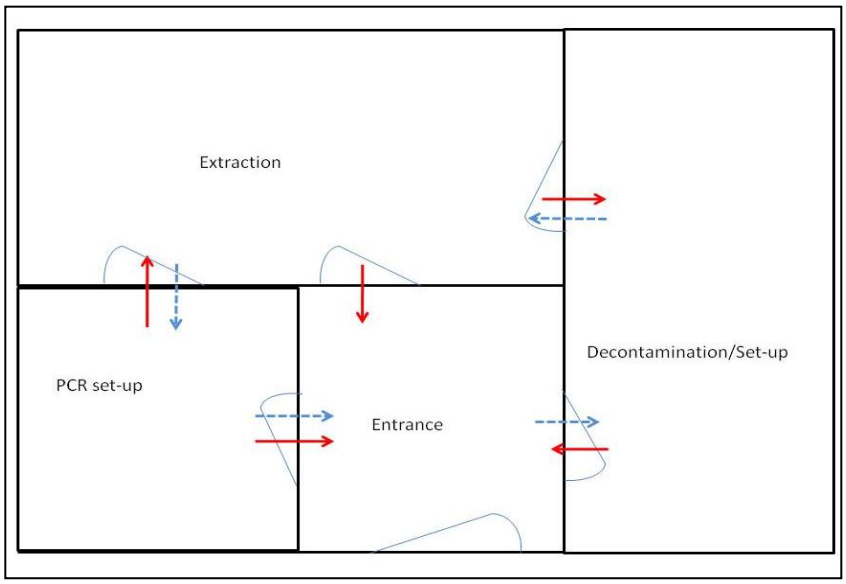


Figure 2: Ancient DNA laboratory at SFU. Red arrows (solid) indicate positive pressure airflow. Blue arrows (dashed) path of samples. Material leaving the PCR set-up room will not be re-entered into the Decontamination room.

Protocols implemented at SFU

The strict separation of pre- and post-PCR work was observed during this study. Pictures were taken in a separate archaeology laboratory area at SFU prior to entering the ancient DNA laboratory. No modern whale samples were extracted in the ancient DNA laboratory at any time. Individual bone samples were stored in new plastic bags and transferred into the ancient DNA area for decontamination (see Chapter 3, for details). Following PCR setup, the PCR reactions were transferred to the post-PCR area. All further steps were carried out in the designated post-PCR area. No material could be returned from the

post-PCR area to the ancient DNA laboratory. The researcher had to shower and change clothes before re-entering the ancient DNA laboratory. Plastic bags containing material to be sequenced were placed in an additional clean plastic bag upon leaving the post-PCR laboratory. The transfer did not pass the ancient DNA laboratory and bags were not opened before shipping.

Internal contamination controls

Internal controls included blank extracts and negative controls as well as extraction and amplification of different species within the same batches. The contamination control set-up in an extraction was called “blank control”, containing all chemicals but no sample. The contamination control set-ups in PCR setups were called “negative control”, containing the complete master mix without sample DNA. The blank control was handled last during all extraction steps to maximize the potential of detecting cross contamination or carry-over. PCR amplification negative controls were handled in the same manner. If PCR set-ups contained variations of chemicals between samples, for example addition of BSA, separate negative controls were included. Some PCR set-ups included a variety of species, which were also used as a contamination control between samples using whale primer. No whale or human DNA was amplified in the 155 negative controls (146 PCR setups for a total of 2099 PCRs) or in the 59 blank extraction control amplifications.

Reproducibility

Because of the potential for contamination, or damage/degradation of

sample DNA, repeating extraction and PCR amplifications is necessary to confirm species and sequence haplotypes. Sequences were confirmed through repeated PCR amplification, repeated sequencing and overlapping primer systems. Both species and D-loop haplotype were reproduced during this study. Sequences were reproduced by repeated PCR and direct sequencing as well as with the overlapping primer system in the case of D-loop sequences. The species of all humpback whale samples identified using the cytochrome b fragment was confirmed using the D-loop primers. Grey whale samples, and a portion of the other species samples collected at Ts'ishaa were also reproduced using this approach. The D-loop sequence was amplified using four overlapping primer-sets. Twenty samples were re-extracted and partially re-amplified (Appendix B). No discrepancy was detected between the cytochrome b and D-loop species identification or within D-loop haplotypes.

Biochemical preservation

Older samples are expected to amplify less and exhibit more deamination (Pääbo et al. 2004, Hofreiter et al. 2001a, Gilbert and Willerslev 2007, Banerjee and Brown 2004). In this study, whale bone samples ranged from 5000 BP to approximately 150 BP. The overall preservation of the samples was excellent, but the rate of samples that yielded no amplifiable DNA increased from 9% in the 2000-0 BP group to 34% in the 5000-3000 BP group. Moreover, the presence of amplifiable nuclear DNA, in addition to the high success rate for mtDNA amplification, supports the observation that the samples at Ts'ishaa and Huu7ii were well preserved.

Sequence alignments from the same individual sample were visually examined for base pair ambiguities and sequence quality.

Overall, the archaeological whale bone samples displayed very good preservation of amplifiable DNA, based on overall success rate and reproducibility. At the same time, the proportion of samples that failed to amplify was comparable to other studies. It was therefore not found necessary to quantify the amount of DNA present.

Phylogenetic sense

Both the species identification and the D-loop sequence were analysed for phylogenetic sense during the study. The species identifications were congruent with ethnographic and biological records of the area; no non-cetacean species were detected and no species detected were outside of their modern geographic range.

Haplotypes identified either match current whale haplotypes or were closely related to known haplotypes. Base pair changes of new unique haplotypes were undisputed based on repeat sequencing and repeat extraction. Samples that were not sufficiently reproduced were not included in the final analysis (Chapter 6).

Cloning

Cloning has been used to identify deamination and contaminations. In this case, all sequences could be confirmed through repeat amplification and sequencing, including deamination and PCR errors. No mixed sequences or

repeat deamination cases were detected. Additionally, cloning is not considered a necessary step in ancient DNA research anymore (Kemp and Smith 2010). While previous research included bacterial cloning as a relevant part of the authentication process, this method has become less common in recent years; indeed, in some cases, labs have completely abandoned bacterial cloning, especially with the advent of next generation sequencing (Kemp and Smith 2010, Bollongino pers. comm. 2010). In a letter to the editor, Kemp and Smith (2010), point out that no consensus has ever been achieved as to how many clones are necessary to validate a sequence. In one of the most extreme cases, Krause et al. sequenced 104 clones to find the Neanderthal sequence they were looking for in two sequences (Krause et al. 2007). On the other hand, most research has sufficient results with 5-10 clones per amplicon (Bollongino et al. 2005, Ludwig et al. 2008, Orlando et al. 2003). Similarly, Malmström and colleagues found in a dog/human contamination study that the consensus would not always lead to the correct result (Malmstrom et al. 2005). Finally, some researchers suggest that a simple repeat direct sequencing offers the same information as the more time-intensive cloning (Orlando et al. 2003). Because of the unambiguous results achieved and the lack of any indication that cloning would provide additional information, sequences were not cloned for this study.

Authenticity of samples

Whale samples yield a lower risk of contamination than other species that are represented in the cultural or environmental context of a laboratory, because

whales are not commonly used for food, clothing or the production of laboratory chemicals (Leonard et al. 2007). The risk of contamination is higher when working with food or commensal species such as pigs, cattle, dogs or cats. Nevertheless, all analysis and protocols were considered non-discretionary to ensure the authenticity of the obtained sequence and to protect the integrity of the laboratory. As described above, samples were analysed in a dedicated facility which included physical and organizational separated pre- and post-PCR work areas. Analyses included extraction controls and negative controls. No contamination was detected over the course of this study. Cytochrome b and D-loop sequences were used as control for species identification in 180 out of 222 samples. While contamination can be difficult to detect (Kemp and Smith 2010, Pääbo 2004), negative controls, implemented protocols and consistency of the acquired sequences are lines of evidence supporting the authenticity of the dataset.

Eighty-four percent of the samples yielded amplifiable DNA and 60 well-preserved samples yielded amplifiable nuclear DNA. While these results demonstrate the excellent survival of the DNA in this dataset, a comparison of samples shows the higher success rate of younger samples over older samples. Compared to 7.6% in samples dating to 2000-0 BP, samples found in layers 5000-3000 BP had a failure rate of 33% at Ts'ishaa. No difference in success rate was found at Huu7ii (84% success; 16% failure) where all samples came from layers dating to 1500 BP and above. The higher success rate of younger samples supports the authenticity of the sequences analysed here,

notwithstanding some of the oldest samples (WH69) yielding excellent DNA.

Samples were not analysed using long fragments (i.e. >200 bp) except for some well preserved samples; instead the overlap of primer systems was used as a control. However, nuclear DNA was present, allowing for successful amplification of sex identifying SRY and Zinc Finger fragments. None of the results indicate that the success rate is unusual or unexpected, given the relatively young age of the samples and the temperate to cool environment. While not yet quantified, the presence of salt water does not seem to have had an adverse effect to the survival of DNA.

The complete haplotype sequences were created with partially overlapping primer systems. Samples were included in the final analysis if they were sufficiently reproduced and yielded sequences from all primer sets. Some additional samples were also included even if one of the primer systems was not reproduced; that is, if it displayed a sufficient sequence quality and a haplotype that matched previously detected haplotypes, it was included. The presence of previously observed haplotypes and expected species provides strong support that the sequences, including the new haplotypes, can be accepted as authentic.

In conclusion, established contamination controls including blank and negative controls which helped to establish the absence of contamination during the analyses, repeat sequencing and the rep-extraction of 10% of the samples supports the authenticity of D-loop sequences and species identifications used in this study.

Chapter 5: Species Identification at Barkley Sound and the Implications for Whaling Practices

Abstract

Excavations have uncovered vast numbers of whale bones from archaeological sites in Barkley Sound, Vancouver Island. These sites were once the villages of the Nuu-chah-nulth people, well known for their prolific whaling capabilities. While whaling gear and harpoon heads embedded in whale bone represent some rare direct evidence for active whaling, the vast quantity of whale bones found in these middens provide indirect evidence in strong support of long term active hunting. This chapter reports a new line of evidence that supports active whale hunting in Barkley Sound for the past 5000 years.

A total of 264 whale bone samples from two previously excavated sites, Ts'ishaa and Huu7ii, were analysed using ancient DNA techniques. Out of 222 successful samples, 79% were identified as humpback whale (*Megaptera novaeangliae*). Grey whale (making up 13% of the samples) and other species were also identified in the remains. The dominance of humpback whales over the entire 5000 year assemblage, as well as the increase in species diversity in later time periods, supports active whale hunting for at least 5000 years, probably longer. These results are a strong line of evidence for the antiquity of whaling in

Barkley Sound and support existing archaeological and zooarchaeological evidence.

Introduction

While industrial whaling is well documented in historical records, the antiquity of traditional whaling by aboriginal whalers was not well understood until recently. Reports from different locations around the world, including Great Britain (Mulville 2002a) and South Korea (Lee and Robineau 2004), have discussed evidence of whaling in each corresponding region. However, direct evidence for whale hunting is either hard to date or absent due to the degradation of the hunting gear. Only embedded harpoons or the presence of whaling gear can strictly be counted as direct evidence. Indirect evidence, such as a large amount of whale bones present at a site and oral traditions can be used as a line of evidence in favour of active whaling. Since the use of whale bones does not prove active whaling because these could also have been obtained through scavenging beached whales, the practice needs to be considered tentatively. While topics such as land mammal hunting and domestication are well documented and analysed in the archaeological record, the hunting of sea mammals and especially the hunting of whales remains less well known. While whaling is an important part of Nuu-chah-nulth history, supported by archaeological and ethnographic data, little data is available to the antiquity of whaling. The whale species that were hunted are only known for the latest

period, based on ethnographic data. Archaeological identification of whale hunting practices is difficult in general because, in most cases, only little direct evidence is available (see Chapter 2 “Whaling”). The reliance on whales as a resource and the importance of these animals to indigenous cultures has led to questions about the origin and antiquity of whaling on the Northwest Coast. The aim of this study was to shed light on the whaling practices at Barkley Sound and to investigate the potential commencement of active whaling in the region, using evidence generated from ancient DNA analysis.

This research is the first comprehensive ancient DNA study of whale remains found in shell middens from the sites of Ts’ishaa and Huu7ii in Barkley Sound. This section will survey Nuu-chah-nulth culture and the migration of humpback whales in the region to create a context for the data presented within this study.

This research was designed:

- To investigate the species used at Ts'ishaa and Huu7ii;
- To investigate changes of species diversity over time; and
- To investigate the antiquity of whaling by Nuu-chah-nulth in Barkley Sound based on species composition in the dataset.

The hypothesis of this research was that the onset of active whaling should be visible as a change in species composition, even if zooarchaeological and archaeological results are ambiguous. Species diversity should differ through time depending on the mode of procurement of the specimens. A midden

collection of scavenged stranded whales should have higher species diversity than a targeted hunted whale population.

The following background highlights the relevant ethnographic and archaeological records in terms of whaling, as well as the natural occurrence of whales in Barkley Sound, through migration and stranding.

The samples in this study were excavated from archaeological sites previously occupied by Nuu-chah-nulth people. Ethnographic and archaeological evidence provides evidence for marine resources, especially whales, being a vital part of their culture and subsistence. As a resource, whales were both advantageous, due to their size and recurring migratory activities, and inconvenient as hunting whale was a dangerous task that required great skills on the part of the hunter as well as his companions. Previous research has not been able to trace direct evidence of whaling beyond 1200 BP, while zooarchaeological research is sparse in terms of species use. Vancouver Island's prime location along major cetacean migration routes made it an ideal place for whaling cultures.

Background

Ethnographic and Archaeological whaling evidence

Oral traditions and archaeological research have revealed a rich history of Nuu-chah-nulth whaling practices. More than 1000 years ago, people on the Northwest Coast of Vancouver Island were hunting whales from canoes with

hand held harpoons (McMillan 1999). Judging from the number of bones uncovered at archaeological sites, whales were an important source of food, oil and raw materials (McMillan 1999, Balasse 2002).

Based on the ethnographic and archaeological records, marine resources were the foundation of Nuu-chah-nulth economy and subsistence (McMillan 1999). On a broad scale, the Northwest coast is rich in resources, though local differences in terrain and general climatic fluctuations lead to variations in resource availability (Ames and Maschner 1999, McMillan 1999). Such climatic fluctuations in Barkley Sound made terrestrial resource availability less dependable than in the northern sounds (Arima and Dewhirst 1990). Fish were an important part of the diet, including bluefin tuna, rockfish, salmon, halibut, lingcod, pacific cod, greenling, herring and red snapper (McMillan 1999). Sea lion, harbour seal and porpoise were hunted, as were several birds, including water fowl (McMillan 1999). While certain resources dominated the economy, indigenous groups used their environmental resources more broadly. Other marine resources exploited throughout the year included clams, mussels and other shellfish, chitons, sea urchins, sea cucumber, crab, and octopus (McMillan 1999, Ames and Maschner 1999, Drucker 1951). During the winter, land mammals were hunted opportunistically (McMillan 1999, Drucker 1950). Plant resources such as salmonberries, cow parsnips, camas bulbs, tiger lily bulbs and clover rhizomes were collected depending on the season. In fall, salmon, halibut, clam and some plants were dried for winter reserves, as was whale to a lesser degree, supplemented by fresh fish caught whenever the winter weather

permitted.

Whales were one of the main marine mammals hunted (Fredrick and Crockford 2005). Whales such as grey whale and humpback whale migrate semi-annually, which made them a generally predictable food source for the Nuu-chah-nulth. While the main hunting season was spring and summer, humpback whales were also present during the winter and in inlets such as Alberni Inlet, Ukluleshat Inlet and Effingham Inlet between November and February (Sapir n.d. notebook XVIII: 11-: in Monks et al.2001). In addition, beached whales ("drift whales") were used when available (Arima and Dewhirst 1990, Monks et al. 2001).

Whales did not only provide meat but also provided raw materials for tools and supplies. An adult whale, weighting about 25-40 t, could provide meat and blubber for the village as well as thousands of litres of oil which was a valuable trading good. Huelsbeck (1988) speculates that the amount of blubber and oil available from whales at Ozette must have led to a resource surplus. Whale oil and sinew were important trade commodities exchanged with other groups for resources locally unavailable in a group's territory, such as salmon, and cedar, which was used for canoes and house planks (Drucker 1950).

In addition to the blubber, oil and meat, the bones were also important resources used as raw material for tools and architectural applications (Mulville 2002b). Not all bones were brought into the village; most of the whale skeleton was left on the beach after being butchered (Monks et al. 2001, Monks 2003). A few bones were taken for raw material while the rest were fragmented during butchering and oil extraction (Monks 2003). The butchering of whales on the

beach instead of in the village is one of the reasons why the number of whale bones found at sites do not accurately reflect the number of whales exploited by these groups (Monks 2001).

Ethnographically, grey whales, and to a lesser degree humpback whales, were the species associated with Nuu-chah-nulth and Makah whaling (Kool 1982, Monks et al. 2001, Drucker 1950). Grey whales arrived in Barkley Sound earlier in spring than humpback whales, which usually reached Barkley Sound in May or June (with some humpback whale remaining in the region almost year round, feeding on small fish in the inlet). Accounts by early explorers and ethnographers describe the impressive numbers of whales present in spring and early summer, during the migration and feeding season, which made manoeuvring small boats and canoes challenging (McMillan 1999, Monks 2001, Sapir 1919). Being part of the feeding ground, Barkley Sound may have been an ideal location for early whalers.

Ethnographic and historical whaling records relate the different behaviours of humpback and grey whales during the hunt. Humpback whales were described as a slower and less aggressive species while grey whales were more aggressive and defensive when attacked (Davis et al. 1997). Other whale species were less available for various reasons. For example, right whales (*Eubalaena japonica*) may not have been frequently available as Vancouver Island is just outside of their current range (Gregr and Coyle 2009). However, right whales are mentioned as one of the hunted species in later ethnographic descriptions (Drucker 1950, Kool 1982). Sperm whales (*Physeter*

macrocephalus) and blue whales (*Balaeonoptera musculus*) generally do not migrate close to shore; this shore distance, along with migration speed and body size, made them more difficult to hunt using traditional shore-based whaling techniques. Kool (1982) has suggested that humpback whales were the most frequently hunted species in Barkley Sound, based on their unique migratory behaviour. Additionally, humpback whales surface more frequently than grey whales, making them more visible to whalers. This theory has been supported by data from Toquaht, an archaeological site in western Barkley Sound where 78% of the identifiable whale bones (N=83) were classified as humpback whale (McMillan 1999, Monks et al. 2001).

For the people in this region whaling was a ritual activity. At the site of Ozette, in Washington State, a large effigy of a whale's dorsal fin, the "whaler's saddle", was found with sea otter tooth inlays (McMillan 1999). This archaeological find implies that some pre-contact whaling rituals were very similar to those known ethnographically (McMillan 1999, Balasse 2002). Several whaling shrines are known in Nuu-chah-nulth territory (McMillan 1999). Ritual preparations were carried out prior to the first whale hunt of the season, and included the whaler's wife and family. Ethnographic research indicates that whalers were prepared to leave for the hunt throughout the entire whaling season in case of a whale sighting (Kirk 1986, Drucker 1950).

Whale lookouts were stationed on off-shore islands and at defensive sites, as these would have had far-ranging views to detect the whales (St. Claire pers. comm 2007). For Ts'ishaa, the open ocean side of Benson Island was also a

spot to observe whales. Whalers would leave after midnight or early morning from outer islands with eight men in a 12 m long dugout cedar canoe (Drucker 1966). Whaling was directed by the chief, who was also the initial harpooner. His canoe was often accompanied by a second boat for assistance, with both crews comprised of male relatives and sometimes slaves (Drucker 1966). The main boat was described by Drucker to contain eight men and specific places for each item. The harpooner would be in the bow, followed by three paddlers on each side and the steersman on the aft (Drucker 1966). The harpoon was placed to the right of the harpooner. Three of the floats were placed on the inside, next to the right paddlers who also had the function to secure a smooth deployment of the lanyard that was attached to the harpoon (Drucker 1966). The toggling harpoon was a three-piece construction: The toggling harpoon head with mussel-shell blade was mounted on a heavy shaft of yew, 3-4.5 m long (Monks et al. 2001, Losey and Yang 2007). A valve made of two bone or antler pieces was attached to the mussel shell blade (Monks et al.2001). The head was attached to several meters of line which in turn was tied to a seal-skin float. This toggling harpoon head would turn and detach from the shaft when driven into the whale (Monks et al. 2001, Sproat 1987). Whales were approached in the morning at daybreak (Sproat 1987). Whalers paddled alongside the targeted whale, which was struck by the harpooner as soon as it submerged with the fluke towards the canoe. The streersman would give notice of the right moment, followed by the harpooners' strike (Drucker 1966). As soon as a whale was harpooned, the men would paddle backwards to avoid being struck by the whale. The floats attached

to the harpoon line served to keep the whale afloat and as marker to track the whale in the water as it swam away (Sproat 1987). A second harpoon was used after the initial struggle to add a second line and more floats to aid in securing the whale (Drucker 1966). When the whale could no longer pull the floats underwater, the hunters would move in. The canoe was paddled close to the whale again, and the whalers used a barbless spear ("killing lance") to kill the whale. One crew member was chosen to dive under the whale and tie the snout closed with rope to prevent the whale from filling with water and sinking. The whale was then towed back to the village and butchered on the beach (Drucker 1951, McMillan 2009).

The whalers hoped the whale would swim towards shore and not out into open water. However, they sometimes had to tow their catch over many kilometres back to the beach. Towing the whale to the beach was a group effort, especially over long distances. The endeavour therefore needed to be well organised and efficient. Fanfare and communal feasting welcomed the killed whale to the village, along with songs and promises to the whale that it would be honoured (Kirk 1985, Drucker 1951).

At most sites in Barkley Sound, whale bones were found in virtually every layer (McMillan 1999), indicating that whales had been exploited, at least opportunistically, for as long as 4000-5000 years. As McMillan (1999) points out, reliable whaling techniques would have supported a larger human population density along the outer coast. In Barkley Sound, this may have been as early as 2000 BP, when the human population increased rapidly (McMillan et al. 2004,

Balasse 2002, St.Claire 1991). In 1785, an explorer named Walker noted that he saw 11 whale skeletons on a beach that had been killed no more than a year prior to his visit in Hesquiaht or Ahousaht territory (Monks et al. 2001). On a different occasion, Banfield observed that the Makah and Ditidaht “kill a lot of whales” (Arima et al. 1991). Accounts of 10 whales killed at the same time (McMillan 1999) and whalers attempting to fill the channel between Benson Island and Clark Island with whale bones as a monument of their achievements (Golla 2000) have been reported as well. On the other hand, John Jewitt, who was a captive in Nootka Sound in 1803, noted in his diary that out of eight hunted whales, only one was killed over a period of 53 days in total (Jewitt 2000). While several accounts indicate an overall relatively low kill rate during whale hunting (Golla 2000), some of the whales injured but not captured during the hunt eventually ended up as drift whales. In these cases, the group that benefited from the drift whale may not have been associated with the initial hunters. The beaches where drift whales might strand were carefully guarded as property of the individual chief. Based on the size of the animal, any species of whale, whether taken or as drift, would have provided a large amount of food for the group.

Ethnographic research recorded seven different species of whale known to the Nuu-chah-nulth (Monks et al. 2001). Humpback *(Megaptera novaeangliae)* and grey whale (*Eschrichtius robustus*) were the most prominent species. Both species migrated in spring and fall past Vancouver Island. Grey whales migrated during the spring closer to the shore and swam slower, making this species

better accessible than during their migration in fall further off-shore (Monks 2001). Right whales, a species preferred for their slow swimming speed and high oil content in other cultures was not commonly mentioned. Finback whale, in some publications translated from the same word as right whale, was not mentioned explicitly. It is possible that this species was lumped with other species for various reasons. In addition to being the second largest whale following blue whale, it sank when it died, as did humpback whale. Baird's beaked whales, the largest of the beaked whales, were known to people on the Northwest Coast but considered inedible (Monks et al. 2001). Blue and sperm whales were considered too large to hunt but probably would have been used when beached. Finally, Orca whales (*Orcinus orca*) were considered good tasting, but generally were dangerous and too fast and thus hard to hunt, making them suitable only for hunters who had to prove their ability (Monks et al. 2001).

Notably, information that can be retrieved from the ethnographic accounts is limited as most of this research occurred after industrial whaling had depleted most whale stocks in the Northeast Pacific. In addition, species naming was not always consistent. Finally, recording happened at a time when whaling was a part of oral tradition and active whaling was no longer taking place (McMillan 2009). Nevertheless, these documents give an important insight into some of the species known in the region.

Several theories have been brought forward to shed light on the origin and age of active whaling. Some researchers speculate that active whale hunting originated early on in the settlement of the Northwest Coast. Other theories state

that whaling developed later as a response to new ecological settings, and as an adaptation to an open ocean environment. According to Drucker, whaling may have emerged to provide a reliable subsistence resource in the face of food shortages (Drucker 1950). Another hypothesis is that whaling developed before people came to the Northwest Coast (Lantis 1940). In that case, people would have brought their whaling techniques along when moving into the new territories such as Vancouver Island.

There is some debate as to the initial motivation behind whaling practices, whether for economic or prestige purposes (McMillan 1999, McMillan and St.Claire 2005, Arima and Dewhirst 1990). Dewhirst has noted that whaling could have been economically less important than currently assumed. On the other hand, Huelsbeck (1988) concludes, on the basis of finds at Ozette in Washington State, that whales could have accounted for up to 80% of the meat and oil present or represented by faunal elements. As Yesner points out in the case of Thule whaling, fewer bones per individual are found on an archaeological site when compared to smaller animals (Yesner 1988), making a correlation between zooarchaeological material and subsistence importance more difficult.

While convincing arguments regarding the whale's economic importance have been proposed (Huelsbeck 1988, Golla 2000), the reason for active whaling as opposed to the use of beached whales remains speculative. Early indications of whale material usage were observed in Yuquot in Nootka Sound, Vancouver Island, where whale bones were found throughout the deposit. In addition, barnacles were found in layers dated to 2000 years ago that are associated most

often, but not exclusively, with humpback whales. However, archaeologists were not able to confidently identify the whale species present at the site, or to determine whether these whales were actively hunted. Similar results were found in Hesquiaht sites, located just south of Nootka Sound and in Toquaht Sites (McMillan 1999). Currently, the oldest known potential evidence for whaling is a humpback scapula with marks that could have been caused by a harpoon head from Ch'uumat'a, in a layer dated to approximately 3000-2500 BP (Monks et al.2001). Dewhirst (1978) has speculated the full development of whale hunting technology at 1200 BP and younger, based on the appearance of toggling harpoon valves. The fact that most hunting gear does not survive in the archaeological record, makes definitive answers difficult (Monks et al. 2001). However, the sheer abundance at sites in Barkley Sound can be considered a strong line of evidence that Nuu-chah-nulth people in this region did not rely on drift whales, but were engaged in whaling activities (McMillan and St. Claire 2005, Monks et al. 2001). The development of whaling this early is certainly supported by the fact that Barkley Sound is located along the migration routes for humpback and grey whales.

Humpback Whale Migration

The migration of sea mammals, specifically humpback whales, is an adaptation to the environmental fluctuations of their habitat (Rasmussen et al. 2007, Clapham 2001, Corkeron and Connor 1999). The seasonal pattern of

ocean productivity, which is generally very predictable, allows for a steady migration pattern over thousands of kilometres. Baleen whales are known as the mammal with the longest annual migration route (Rasmussen et al. 2007, Lagerquist et al. 2008, Straley et al. 2009). Except for one group of humpback whales in the Arabian Sea (American Cetacean Society 2004, Mikhalev 1997), all humpback whale populations migrate between cooler areas during the summer months and tropical areas during the winter. North Atlantic humpback whales migrate between Greenland and Iceland to feeding grounds in the West Indies. Southern Atlantic whales migrate between Antarctic whaling grounds and Brazil, Australia, New Zealand and possibly the west coast of Africa (Baker and Medrano-Gonzalez 2002, Reeves et al. 2004). North Pacific humpback whales are separated into three stocks: eastern, central, and western Pacific (Calambokidis et al. 2004, Witteveen et al. 2004, Baker et al. 1998b). Since the seasons are reversed between the Northern and Southern hemispheres and thus the migration between warmer and colder regions is reversed, genetic exchange between the populations is limited (Baker and Clapham 2004, Roman and Palumbi 2003).

Despite earlier assumptions of an even ratio of females and males in winter and summer, more males migrate to the breeding grounds than females in order to mate (Brown et al. 1995). Migration patterns vary not only by sex, but also by age and reproductive class. The energy requirements of the individual during feeding and breeding times (Perrin et al. 2002) are considered the link between migration and resource patchiness (i.e. the mix of high and low

availability of resources) (Clapham 2001, Corkeron and Connor 1999, Pomilla and Rosenbaum 2005). For example, pregnant females migrate to the feeding ground first, followed by immature whales of both sexes and adult males. The migration to the breeding ground happens in reverse order, starting with adult males. In addition to these different migration times, there is little pressure to migrate for immature individuals and non-breeding females. Some individuals do not migrate in a season or travel only part of the way (Rick and Erlandson 2009, Alter et al. 2009, Witteveen et al. 2009). Wiley et al. (1995) point out that it might be more adaptive for juvenile animals to stay in latitudes closer to the feeding ground, rather than migrating all the way to the resource depleted breeding ground. Thus, whales can be found in and around the feeding ground year round, potentially creating the illusion of local pods.

Whale Stranding

Alternatively to active hunting, people have frequently been using drift whales (also called stranded whales) (Monks et al. 2001). While the majority of stranded marine animals belong to the class Cetacea (Perrin et al. 2002, Geraci and Lounsbury 2005), baleen whales such as humpback whales are not commonly beached onshore. Even so, humpback whale strandings are known, and have increased in frequency since the 1980s. Wiley et al. (1995) link most of these beaching cases to incidents associated with commercial fisheries or cargo ships. The increased frequency may also be due to an overall increase in population size or changes in the migration pattern (Perrin et al. 2002, Geraci

and Lounsbury 2005). Geraci and Loundsbury (2005, p107) report “the stranding frequency of humpback whales on the Northwest Pacific Coast (including Alaska) to an average of more than 1/year but less than 1/month”, which is less than the grey or finback whale for example. The highest frequency of humpback whale stranding is reported from the Canadian Maritime region, which suggests an average of more than 1 per week (Geraci and Lounsbury 2005).

The reasons for whale strandings are diverse and not well understood (Wiley et al. 1995, Mustika et al. 2009). Collisions with ships, entanglements, and lesions from sonar use are some of the anthropogenic reasons for stranding. On the other hand, weakness due to natural illness can cause whales to strand as well. Lastly, some species such as killer whales strand themselves on purpose in an attempt to catch prey. In these cases, the whale generally has the ability to return to the ocean (Perrin 2009).

Modern observation of whale strandings and the species composition of stranded whales is relevant to the current study because one of the questions, especially in the absence of direct evidence such as embedded harpoon heads, is whether a whale bone found in the archaeological record is likely a hunted or more likely a stranded whale.

Material and Methods

The samples in this study were excavated from two archaeological sites previously occupied by Nuu-chah-nulth people. Ancient DNA analysis was

applied to 264 whale bones from these two sites in Barkley Sound; 163 bones from Ts'ishaa and 101 samples from Huu7ii. Samples from Ts'ishaa were dated to 5000 BP to 0 BP (Appendix 1). Samples from Huu7ii were from the house floor excavation and dated to 1500 BP to 200 BP. Ts'ishaa samples were selected with the goal of sampling as many individuals and as few duplicates as possible (Ian McKechnie personal comment, 2010). Similarly, Huu7ii samples were selected based on zooarchaeological identification as "cetacean", sampling one bone per sample bag to minimize re-sampling individuals.

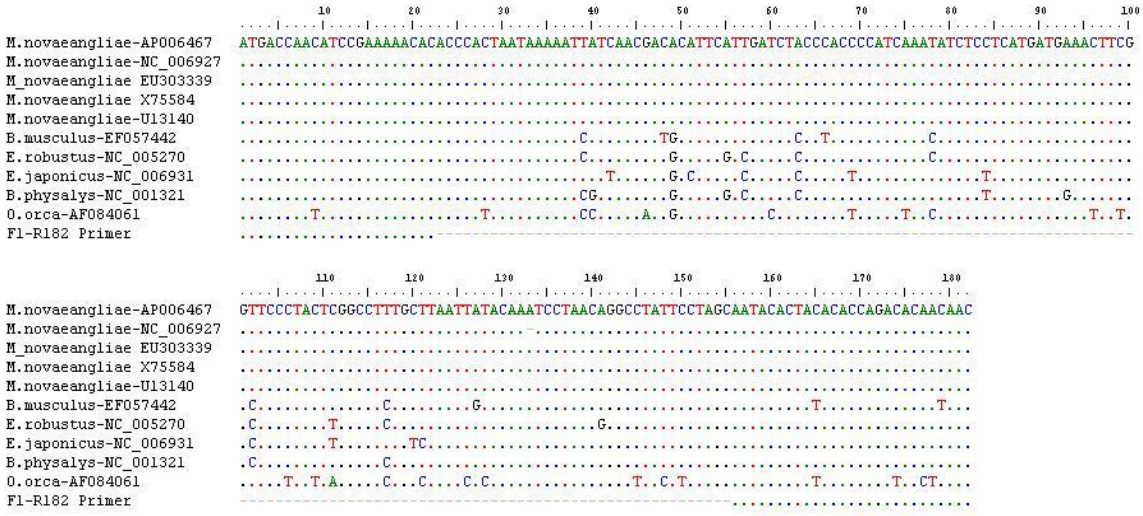
Ancient DNA analysis was carried out as described in chapter three. To identify the species of a sample, PCRs were performed using primers targeting 133bp of the cytochrome b (cytB) gene on the whale mtDNA (Table 2, Figure 3). Cytochrome b is a conservative gene which is commonly used for species identification. PCR was carried out in 30 µl reactions per sample, as described in Chapter three. The PCR was run under the following conditions: 94°C/ 5min followed by 60 cycles of 45s at 94°C, 30s at 54°C and 40 seconds at 64°C. The final extension was at 72°C for 10 minutes. Samples were stored at -18°C until further processing. Presence of PCR amplicons was established on a 2% agarose gel with SyBrGreen stain. Positive PCR reactions were purified using a Qiaquick purification column (Qiagen, Hilden, Germany) following the manufacturer's manual. Samples were sequenced at McMaster University's sequencing facility and at Macrogen Ltd. in Seoul, South Korea using either the forward or reverse Primer (Table 2).

Results were visually edited and species identification was confirmed

using the NCBI database tool BLAST using the blastn algorithm, along with phylogenetic analysis of other closely related species in Bioedit (Fig 3). All humpback whale samples were also confirmed with the D-loop sequence (see Chapter 5).

Sex identification was attempted using SRY and ZFX/Y co-amplification to estimate a minimum number of individuals. The sex determining region Y (SRY) is located on the Y-chromosome. The SRY gene encodes the male sex determining SRY protein (with testis as the determining factor). The Zinc Finger gene (ZFX/Y) used in this study is located on both the X and the Y chromosome. Primers for sex identification were designed to amplify different lengths depending on the ZFX or ZFY binding position, using one general and two specific primers (X and Y) per reaction. The goal was to create an electropherogram-based sex identification based on the presence or absence of the SRY band with an additional ZFX/Y length polymorphism as positive control. SRY was only sequenced to confirm that the amplified sequence was indeed whale SRY.

Figure 3: Cytochrome B alignment of species identified in Barkley Sound.



GenBank Sequences (see accession numbers next to species) for this alignment.

Table 2: Cytochrome b Primer used for species Identification.

Name	5'-3' sequence	Length	Frag. Length	Location*
WLF1	ATGACCAACATCCGAAAAACAC	22bp	182/133bp	14206-14225
WLR182	GTTGTTGTGTCTGGTGTGTAGTGTATT	27bp		14361-14387

* Location based on humpback whale sequence X75584 (Sasaki 2002)

Results

Out of 264 whale bone samples processed in total, 222 yielded amplifiable DNA. This constitutes an overall success rate of 83.3% for all samples. The analysis established a species identification for 138 (84.6%) out of 163 samples of the Ts'ishaa samples and 84 (83%) out of 101 of the Huu7ii samples. Well preserved samples yielded an mtDNA fragment length of 300 bp (covered by F101/R569), and the amplification of nuclear DNA for sex identification

demonstrated excellent preservation of DNA. Samples that did not yield amplifiable DNA were classified as “no species ID” in the tables. Species diversity was calculated excluding these failed samples.

Forty-five (17%) out of 264 samples remained discoloured after the purification and final elution. The discoloration varied from a light tint to black, and could not be removed by repeated purification steps as described in Kemp et al. (2006). PCRs of discoloured samples were generally at least partially inhibited. However, in some cases a second clear elution was obtained and yielded amplifiable DNA. Discoloured samples WH43 and WH175 and WH185 were used to spike artificial DNA samples that had previously amplified and run in a real-time PCR setup to determine the extent of the inhibition. The whale samples completely inhibited the amplification of the artificial DNA, even when diluted 1:10 (McGrath, pers comm 2010) (see Chapter 3 for more details).

The species distributions at the two sites can be found in Table 3. Out of the 222 samples identified, 78.8% were identified as *Megaptera novaeangliae*. At Ts’ishaa, 76% (105/138) of the identified samples were humpback whale, whereas 83.3% (70/84) of identified samples at Huu7ii were humpback whale. The second most common species was grey whale, accounting for 13% of all identified samples.

Table 3: Species distribution at Ts'ishaa and Huu7ii.

	Ts'ishaa		Huu7ii	
Species	**N**	**%***	**N**	**%***
humpback whale (*Megaptera novaeangliae*)	105	76	70	83.3
grey whale (*Eschrichtius robustus*)	18	13	11	13.1
finback whale (*Balaeonoptera physalus*)	9	6.5	2	2.4
blue whale (*Balaenoptera musculus*)	2	1.4	0	
right whale (*Eubalaena japonica*)	3	2.2	1	1.2
Orca ("killer whale", *Orcinus orca)*	1	0.7	0	
Identified samples	138	100%	84	100%
No species identification	25		17	
Total samples analysed	163		101	

* percentage is calculated out of identified samples only.

The species distribution at Ts'ishaa and Huu7ii both show a similar majority of humpback followed by grey whales. The Ts'ishaa set also had two blue whales and one killer whale. The samples identified from Huu7ii included humpback and grey whales as well as two finback whales and one right whale. No blue whales or killer whales were found in this dataset. The chi square test result ($X_{(2)}$= 3.78, p=0.151) between sites indicates that there is no significant difference between Huu7ii and Ts'ishaa in terms of species distribution. The categories used in the chi-square were "humpback whale", "grey whale" and "other species", lumping finback, right whale, orca and blue whale into one category.

Table 4: Species identification by time depth for Ts'ishaa and Huu7ii.

Time (yBP)	humpback	grey	others	Chi square[1] within site	Chi square[1] between site
Ts'ishaa					
0-2000	86 (79%)	8(7%)	15 (14%) Fin(9)blue(2) right(3) orca (1)	χ^2= 14.14, p=0.0008	
3000-5000	19(65.5%)	10(34.5%)	0		χ^2= 3.78, p=0.151
Huu7ii					
500-1000 (house)	47(96%)	2(4%)	0	χ^2= 9.76, p=0.007	
1000-1500 (pre-house)	23(66%)	9(26%)	3(8.5%) Fin(2) right(1)		

1 For the chi-square, "other species" were considered as one category. "within site" compares the two time period. "Between sites" compares sites and lumps time. Yates correction was used because the expected frequency of some categories was less than 5.

The species distribution in the Ts'ishaa dataset can be roughly grouped by date into 2000 BP to 100 BP and 5000 BP to 3000 BP. The gap between 3000 BP and 2000 BP is based on a gap in the excavation record. The part of the site that would have been occupied between 3000-2000 BP has not been excavated (McMillan pers. comm. 2010). Humpback and grey whales were present over the entire span of 5000 years, while other species (blue, finback, orca and right whale) were only present in the last 2000 years.

Huu7ii samples can be separated into house (900-0 BP) and pre-house (~1500-1000 BP) deposits, with the house deposit further divided into early and late levels. The species diversity at Huu7ii includes only humpback and grey whales in the house deposit, while humpback, grey, right and finback whales were found in the pre-house deposit.

Sex identification to individualise specimens

Sex identification was attempted as a means of estimating the minimum number of unique individuals. To circumvent allelic dropout, the presence of the Y chromosome was tested together with the presence of the Zinc Finger gene (ZF), which is present on both the Y and the X chromosome. Allelic drop out is prevalent in ancient DNA and can be avoided by designing short primer amplicons and repeat amplification (Butler et al. 2003, Burger et al. 2007). While a humpback whale reference sequence (GI:134287128, Nishida et al. 2003) was available for SRY, no reference sequence and no modern whale DNA was available to establish an lab-internal reference sequence for humpback whale ZFX/Y. A grey whale sequence was used to design ZFX (GI: 13991118 and GI: 13991116) and ZFY (GI: 13991120) primer. The primer system for SRY and ZFX genes were tested separately before analysing samples in a multiplex set-up. The goal of this approach was a simple agarose gel-based screening of the multiplex.

As seen in Figure 4, the results were not consistent, even within a well preserved sample. In addition, it was determined that the planned screening of the presence or absence of the Y chromosome would not yield sufficient information to estimate a minimum number of individuals as opposed to loci with more than two alleles.

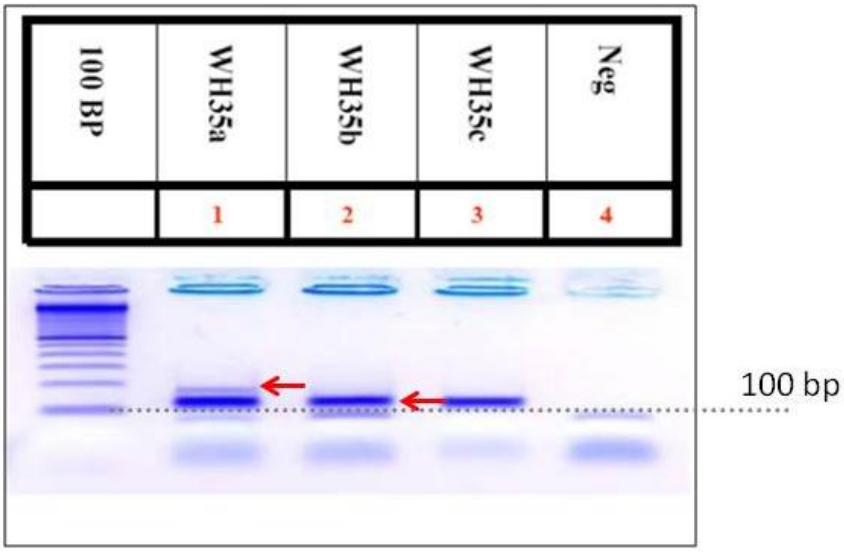


Figure 4 Sex Identification using ZFX and SRY primer system. Note that all lanes are amplifications of the same sample (WH35). WH35a+b indicate SRY/ZFX co-amplification, WH35c SRY only amplification. WH35a successfully co-amplified at both loci (upper band=ZFX, lower band SRY), WH35b is missing the ZFX band. "Neg"control exhibits primer-dimer.

In an effort to narrow down the possible number of individuals sampled, two researchers (B.T. Fothergill (BTF), C.F. Speller (CFS)) evaluated the database (Appendix A) independently to estimate how many individuals could be determined. The summarized results can be found in Table 5. Fothergill notes that numbers provided could be overestimated because no detailed element identification was provided. Identifying an MNI would be in this case "at best guesswork" (Fothergill pers. comm. 2010). When adding the species into the database, the number of possibly re-sampled specimens increases, even though the DNA analysis is missing 42 samples not identified to a species level. When counted by unit and layer, the total increased to 94 samples with species identification. By adding haplotypes to the table, the total minimum number increased to 132 (50%).

Table 5: Number of individuals based on location by researcher.

	CFS	BTF	Incl. DNA species ID (UA)	Inc species ID and haplotype (UA)*
DfSh 7 (N=101)	20	27	33	51
DfSi 16 (N=152)	31	58	56	75
DfSi 17 (N=11)	2	4	5	6
Total (of 264 samples)	53	89	94	132

* haplotypes determined for part of the sample set only: DfSh7=44, DfSi16=63, DfSi17=3. Remaining samples: species identification only.

Discussion

The objective of this study was to identify the species used at Ts'ishaa and Huu7ii, to investigate the changes of species diversity over time, and to investigate the antiquity of whaling by Nuu-chah-nulth in Barkley Sound based on species composition in the dataset. Furthermore, this research attempted to reveal when the people in this region began active whaling, and which whale species were hunted in Barkley Sound. To answer these questions, a total of 264 whale bone samples were processed from two locations in Barkley Sound on the west coast of Vancouver Island: 163 samples from Ts'ishaa and 101 samples from Huu7ii. Eighty-three percent (83.3%; 222 out of 264) of the samples yielded amplifiable DNA and 78.8% of the successful samples were identified as humpback whale. In addition, grey whale, right whale and finback were identified from both sites, and blue whale (n=2) and orca (n=1) were identified from Ts'ishaa only. The orca sample was identified despite a primer mismatch (Figure 3). Humpback whales were the most common species and were consistently

found over the entire period of almost 5000 years. Grey whales were the second most common species present in all time periods. All other species were restricted to the last 2000 years and found in lower frequencies. At Huu7ii, species other than humpback and grey whale were only found in the pre-house levels (older than 900 years). The similar frequency between Huu7ii, Ts'ishaa and published zooarchaeological results from the Toquat sites in Barkley Sound (McMillan 1999, McMillan and St.Claire 2005, Monks et al. 2001) indicate that the sample set was, in both cases, large enough to identify the major species composition.

The results of this study indicate that the Nuu-chah-nulth at Ts'ishaa and Huu7ii made use of the feeding ground and hunted predominately humpback whales. While the intial analysis indicates a change in species diversity after 200BP, a χ^2 comparison between Humpback and Grey whale as well as between either of those and the "other species" category reveals that only if grey whale is included, a significant difference over time is observed. The same is true for Huu7ii comparison between the house floor and the pre-house level. This suggests that grey whale changed significantly over time but neither humpback nor "other species". The taxonomic unevenness of large baleen whales is an important line of evidence suggesting that the people in Barkley Sound actively took whales as early as 5000 BP. If no whaling had taken place, higher taxonomic evenness and fewer whale bones in general would have been found in both sites in earlier times. If whaling had started after 5000 BP, a decrease in species diversity or change taxonomic evenness should have been found.

This is the first study of indigenous whaling practices using ancient DNA techniques. By identifying species present in shell middens, archaeologists can gain insight into the lives of past peoples, including cultural and religious beliefs. Previous interpretations of whaling practices relied solely on ethnographic and zooarchaeological research. Ethnographic records can be highly informative and were collected diligently, however the time depth is limited (St. Claire 1991) and the socio-political context of the time the data is recorded needs to be considered (McMillan 2009). Furthermore, the possibility that species may have been named differently or misidentified by early researchers and explorers needs to be taken into account when incorporating such material. One such case is the confusion between right and finback whale (Monks 2001). Monks et al. (2003) previously discussed the MNI using the NISP and MNE, but came to a number of individuals as N=1 for similar reasons as mentioned above. The comparison of haplotype, species identification and location from a subset of 105 samples (see Chapter 6) indicates that very few samples are indeed likely from the same individual. A zooarchaeological analysis of the whale bone assemblage at Toquaht, a site located in the northern part of Barkley Sound (Figure 1), yielded similar results in terms of humpback whale prevalence in the sample set (McMillan 1999). However, this study was only possible on a relatively small number of bones (NISP=89, Monks et al. 2001) complete enough for accurate identification. Zooarchaeological analyses performed on samples from Ozette, WA, indicated that the species distribution at the Makah site was almost equal between humpback and grey whale (Huelsbeck 1988), possibly due to the geographically

exposed nature of Ozette, being located on a direct line along the humpback and migration route. The exposed geography potentially constrained the time frame during which hunting could take place to calm month while whales in Barkley Sound could be hunted during calmer winter month as well due to a slightly more sheltered geography. Alternatively, the difference between Ozette and Barkley Sound is due to choices made by whalers based on cultural or political incidences.

These studies have made important contributions to the interpretation of indigenous whaling practices. However, the DNA collected in this study present a more indepth analysis of the larger archaeological assemblage. In addition, the DNA analysis here provides archaeologists with an unambiguous species identification of the highly fragmented material. At Ts'ishaa for example, only 57% of the sea mammal bones were visually identified to a species level (Fredrick and Crockford 2005). If only complete or somewhat complete bones are used for identification, a species-specific use pattern, for example ritualistic treatment of orca, could skew the overall interpretation.

The consistency of humpback whales as the prevalent species over the entire 5000 year period analysed supports the hypothesis that Nuu-chah-nulth active whaling is at least 5000 years old. If people had relied exclusively on stranded whales, the taxonomic evenness should have been higher, with no single species standing out as humpback whale does in this case. In addition, the amount of whales should have been considerably less in general. This point has been previously argued by archaeologists as a line of evidence supporting the

antiquity of whaling in Barkley Sound (McMillan and St.Claire 2005, McMillan 1998, Monks et al. 2001). With grey whale being the second most important species, the two species identified ethnographically as the preferred species hunted in this region, are indeed the most common in this sample set. The assemblages seen in Ts'ishaa and Huu7ii however suggest a targeted hunt: a consistent majority of humpback whale has been established through the entire 5000 year dataset, along with a consistent amount of cetacean bone over the entire time (Fredrick and Crockford 2005). While the people at Ozette hunted humpback and grey whales equally (Monks et al. 2001, Huelsbeck 1988), the fact that sites in Barkley Sound provided mostly humpback whale remains supports that humpback whales were the traditional choice in Barkley Sound. Given that the distance from Barkley Sound and Ozette to the deep ocean (depth of >100m) is approximately equal, indicating that both groups would have taken to boats as opposed to hunting from the shore, the difference in species selection can potentially be attributed to human behaviour. Alternatively, as discussed above, the somewhat different access to more sheltered waters in a Sound instead of an open coastline may expanded the time when whales could be hunted and thus guided the selection towards predominantly hunting humpback whales.

Humpback whales were in many ways the "right whale to hunt" in Barkley Sound; for example, humpback whales swim closer to the shore and even into the Sound in search of herring. Ethnographic literature even refers to humpback whales entering the Sound and its inlets on a regular basis (Drucker 1951, Monks et al. 2001). This would have impacted past whalers' choices as they would have

been equipped with cedar canoes, limiting their range on the open ocean. Humpback whales are one of only a few species that come close to the shoreline, while most other species in the Northeast Pacific remain in open water (Gregr et al. 2000). Hunting whales that remained greater distances away from the shore would have necessitated better seafaring and hauling technology. In contrast to grey whales, humpback whales spend more time close to the water surface, making them more easily detectable to the outlook and hunter (Slijper 1979). Therefore, humpback whales would have been the ideal species, especially in the early years of active whaling. If Barkley Sound was part of a feeding ground, the historical and ethnographic accounts of large numbers of whales can be explained by this fact, instead of the previously proposed resident population which would be uncommon for humpback whales. Feeding grounds are frequented by whales year round, depending on the individual's reproductive status (Valsecchi et al. 2010, Craig et al. 2003).

The ratio of humpback and grey whales in the archaeological record could be based on behavioural differences exhibited by these species when approached. Humpback whales are more docile than the aggressive grey whale. While hunting humpback whales still entailed great risks for the whaler, a preference for humpback over grey whales seems reasonable. However, research at Ozette (e.g Huelsbeck 1988), shows an almost even distribution of humpback and grey whales in the zooarchaeological record, indicating that grey whale was within the hunting range in terms of distance from shore, behaviour and technological advances. The statistically significant decline of grey whale in

Barkley Sound over time may have had a variety of reason. Political changes could have change the access to grey whales, A natural change in grey whale distribution in the region could have lead to less access. Finally, a change in culture could have simply lead to decline in grey whales being targeted. However, at this time, none of these options can be justified with evidence.

Other species were generally considered to be unsuitable to hunt, being either too fast or too large and in most cases, migrating further offshore than humpback whales (Monks et al. 2001, McMillan and St. Claire 2005). Finback, right and blue whales were generally considered as beached whales only (Monks et al. 2001). While no “other species” were found in deposits prior to 2000 years, the Chi square indicates that the absence of these species prior to 2000 years is not significant.

Successfully hunting a blue whale would have been extraordinarily difficult, based simply on sheer size and speed, suggesting that blue whale specimens may indeed have been from beached animals. The presence of finback and right whales can more likely be attributed to active whaling or whaling attempts. A change in species actively approached would make sense if the technology of the harpoon or the canoe improves. The earliest modern form of toggling harpoon is found in the archaeological record at Yuquot and Hesquiat Harbour around 1200 BP (McMillan 1999, Dewhirst 1978) but intermediate forms are to be expected. However, whether these species were hunted or scavenged is impossible to determine. Additional samples around 5000 BP and older may yield species. Because the change of species diversity is not significant between

older and younger samples additional samples older than 3000 and especially older than 5000 are needed to clarify if there are additional species present in these older layers or if the current taxonomic diversity remains the same.

A potential change in species diversity and taxonomic evenness could be a sign of political or cultural changes as well. This change could be either through trade between groups, or through expanding territories, giving the chief extended access to beaches (St. Claire 1991, McMillan 2009). The original territory of Ts'ishaa has only limited exposed areas and few beaches (Sumpter 2005). However, the expansion of the territories of the Tseshaht is not visible in the archaeological record of Ts'ishaa and according to ethnographic accounts only happened after contact with Europeans (McMillan and St.Claire 2005), postdating the change in species diversity by 1700 years. On the other hand, archaeological and ethnographic evidence indicates a possible cultural change prior to 2000 years (McMillan 2009, McMillan 1998). Earlier people related to Locarno Beach culture in the Strait of Georgia were replaced (or absorbed) by people from the north carrying the West Coast culture type. Incoming people could have brought a different species preference or a higher acceptance for beached whales. McMillan and St. Claire place the possible change in culture to around 2500 BP (McMillan and St. Claire 2005, p.105), right between earlier and later samples in this dataset. One change may be an increase in population size at Barkley Sound, forcing whalers to go after more and larger whales for subsistence purposes.

While finback, right, orca and blue whales were only found in later

deposits, these species were found at Huu7ii in the earlier beach (pre-house) deposits (>1000 BP) but not in the house deposit (1000-500 BP). The significance of this will only be resolved after further analysis of other sites in the region. It is possible that finback and right whales were treated differently from humpback or grey whales, and if so, this cultural difference could perhaps explain their presence or absence in the archaeological record. While this scenario of cultural differences is the most likely explanation based on the current data, alternative arguments explaining the observed species distribution based on the natural occurrence of the hunted species should be discussed, including information on migration patterns, habitat distribution, and species behaviour.

To explain the difference in species distribution between earlier (5000-3000 BP) and later (2000-0 BP) time periods based on natural causes, a change in migration patterns or an overall change in species distributions in the North Pacific could be the cause. Migration patterns of several species have been linked to water temperature, with changes of as little as 1°C possibly altering the timing of migrating species (Slijper 1979). Climate change on the Northwest Coast has been recorded at around 4000 BP. Before 4000 BP, the climate was warmer and dryer than today, as recorded in marine deposits (Chang and Patterson 2005, Dallimore et al. 2005). After 4000 BP, the climate became wetter and colder, accompanied by El Niño events, causing fauna and flora to change (Benson et al. 1997, Clague et al. 1982). On a related note, one of the factors that could have played a role in the change of species distribution might have been the end of the neoglacial sea ice as described by Crockford and

Frederick (2007). The retreat of the sea ice in Alaska, for example, might have caused an expansion of whale migration further north. If climate change was the reason for the altered species distribution, the observed change should have occurred earlier than 3000 BP. To further clarify if changes similar to those observed herein occurred during that time, sampling should be concentrating on the period just before 5000-4000 BP and after the ~3500-2000 BP climate change event.

Apart from natural changes in whale distribution, human behaviour is the alternative option to explain the different species distributions, as well as the changing species frequencies over time. Three scenarios could link human behaviour to the change in species distribution observed between 3000 BP and 2000 BP. Firstly, the distribution change could indicate the onset of active whale hunting, with no discrimination between species. This would imply that the earlier distribution of humpback and grey whales remains is based on beached whales only. The absence of right and finback whales in earlier periods could be due to the limited sample size (n=29) from this period. This however, would be contrary to modern evidence that baleen whales are less likely than odontocete whales to beach (Mustika et al. 2009, Borsa 2006). For example, a study in Indonesia found only 3 cases of baleen whales out of 35 strandings (8.5%) over a period of 20 years (Mustika et al. 2009). As well, Borsa (2006) reports 423 stranded animals in 72 single animal events and 5 mass strandings (32-199 individuals) over a time of 128 years with only 5 reported baleen whales over the entire time period. Dolphins and large toothed whales, especially sperm whales, made up

the rest of the beached species. Monks argues that an increase in beached whales is to be expected with an increase of whaling activity as more whales would potentially be struck but lost (Monks 2003).

The second scenario would be a shift to more effective whaling techniques. Active whaling probably progressed through several phases. The change from 62% humpback to 78% humpback whales during this time period may be a sign that Nuu-chah-nulth whaling had developed into the highly skilled, technologically advanced enterprise for which the Nuu-chah-nulth would be famous. This scenario may explain the relative increase in humpback and an increase in finback and right whale but not necessarily the change in grey whale species since these should increase instead of decrease.

Thirdly, the species distribution could be due to a change from full time occupation to seasonal summer occupation of sites in Barkley Sound. In this case, the hunted species would be dependent upon the migration cycle of the different species or temporal residency. All species identified in this study migrate during roughly the same time period with at least humpback whales being present almost year-round. As described above, ethnographic research indicates that most whaling took place in late spring and summer during later time periods, at times when humpback whales would have been found in nearby waters more frequently. This ethnographically described practice is supported by reduced accessibility to the ocean during the fall and winter seasons, as storms would have jeopardized any whaling endeavours.

Samples collected at Ts'ishaa are distributed over 5000 years with a gap

between 2000 and 3000 years as this part of the site was not excavated. The results show a shift in species distribution through time which is statistically significant for grey whale but not statistically significant for other whale species. Initially, species other than humpback and grey whales were assumed to be stranded rather than hunted whales. However, alternative explanations are possible. Generally, a change in species distributions can be an indication of changing natural or anthropogenic influences. Natural factors influencing population shifts include climate change and natural population expansion or decline. Changes on an anthropogenic level include modifications to belief systems, political alterations, and warring. In addition, technology transforms and develops over time, as does the knowledge base for understanding and manipulating the environment.

The species identification at Ts'ishaa should be considered an initial result worth further investigation notwithstanding the small sample size for the earlier time period (5000-3000 BP). To explain the change in species distribution, ethnographic and archaeological evidence play a major role. Possible anthropogenic factors could be either the beginning of whaling, or a new development in whaling capability.

While the question of possible species shifts for sustainability reasons cannot be answered conclusively, inferences can be drawn from the different lines of evidence regarding human behaviour towards the environment. First, the spiritual world that surrounded the daily life of the Nuu-chah-nulth guided their behaviour towards animals and other natural resources (McMillan 1999, Drucker

1951, Kirk 1986, Monks et al. 2001). This helped to balance the immediate need for a resource and the need for sustaining those resources for future generations. A second point in the case of whaling, are the pre-requisites for a hunt: animals were not randomly hunted, but could only be hunted by specific people, usually of the highest ranks, and required rigorous spiritual preparation (Drucker 1951, Monks et al. 2001). Such behaviour and regulations can be viewed as environmental, although it might not have been a conscious environmentalism. Since intent cannot be tracked through the archaeological, this would at best be a philosophical discussion. Another factor limiting the impact of human behaviour on the environment in this case was the need for a highly advanced technology required for a successful hunt. The technology used for whaling, though improving over time, was probably not efficient enough at this time to cause serious harm to whale populations. In his diary, for example, John Jewitt reports several unsuccessful whaling attempts by Maquinna, the chief he was held by (Jewitt 2000). The size of whale would also limit human impact. Hunting a whale was more difficult and dangerous than hunting a deer or salmon. Not only would travelling by water be a hindrance, but moreover, the size of the animal and the hugely uneven distribution of flexibility and power between the hunter and the prey, would limit the number of whales caught. Genetic diversity discussed in Chapter 6 does not indicate any loss but a stable diversity and population size.

To date, the oldest potential evidence for whaling in Barkley Sound has been dated to over 2000 BP, from Ch'uumat'a (Monks et al.2001, McMillan 2009). Unfortunately, archaeological evidence of whaling is extremely rare due to

limited preservation of the materials used. Active whaling evidence includes harpoon heads embedded in whale bones and actual whaling gear, most of which only preserves in wet sites. Whaling gear, especially the harpoon valves, were also kept for their spiritual and social relevance, so the cultural taphonomy skewed the archaeological record further (see also Monks et al. 2001). While the presence of whale bones is not evidence of active whaling in a strict sense (Monks 2005, Mulville 2002b), the large amount of whale bone found in Ts'ishaa and Huu7ii can be considered a strong line of evidence (McMillan 2005; 2009, Monks 2001; 2003). Archaeological evidence of whale use over the entire time, and ancient DNA evidence showing a continuous preference for humpback whales over 5000 years, could be considered sufficient evidence to conclude whaling took place at least 5000 BP, and probably longer.

The increase of species diversity in the time after 2000 BP is not related to the introduction of active whaling. Three possible explanations have been discussed here, including natural cause, technological advances and political changes. It is possible that the change included both technological advancements, such as toggling harpoon heads like those found at Yuquot and Hesquiat Harbour (McMillan 1999, Dewhirst 1978), as well as increased knowledge of successful hunting techniques and whale behaviour, and a different perception towards beached whales. In later times, with an increase in human population size and political upheaval, it may have been more important to hunt faster and larger prey species in order to further increase the prowess of the hunting chief to solidify his position during war times; this would ultimately lead to

more daring hunting excursions.

It is unlikely that the species distribution changes discussed here were the result of the initial invention or introduction of whaling, as whale remains were found at Ts'ishaa throughout the entire 5000 year period. As mentioned earlier, modern records show that baleen whales are not commonly beached, despite their frequent close proximity to the shore. The species distribution at Huu7ii is probably different because the two excavated areas included the house floor, as well as the pre-house level. The species differences could therefore be a reflection of differences in the use of an area, occupation patterns, or political changes. Similarly, an overall increase in sample size dating to 5000-3000 BP might change the species distribution.

Further studies using samples dating to the time prior to 5000 BP might illuminate the consistency of species choice and may find the onset of whaling, which should be the change from random species to a selective humpback whale hunt. While the change from hunting to domestication has been documented in land mammals, the change from scavenging to hunting large sea mammals has not been shown in the archaeological record. Both share the intentional modification of species used by a group; in land mammals, the keeping of animals with later control of breeding, and in sea mammals, from picking "whatever is available" to developing technologies that enable humans to choose the hunted species and hunting it. This can be due to either the change in sea level or due to the possibility that people moving to Barkley Sound already brought the technology with them.

Conclusions

Prior archaeological analyses of assemblages excavated on Nuu-chah-nulth territory, as well as recorded oral traditions, have demonstrated the importance and extensive use of whales over the past 4000-5000 years. However, no direct evidence supports active whaling until about 2500 BP (McMillan 1999, Monks et al. 2001). To date, the oldest evidence of active whaling in Barkley Sound dated to 2500 BP and a harpoon head embedded in a whale bone dated to 500 BP (McMillan and St. Claire 2005, McMillan 2009). Due to the highly fragmented nature of the assemblages, zooarchaeological identification to the species level has been practically impossible in many sites. In this study, ancient DNA techniques were used to analyse a dataset of 264 samples from two sites dating to between 5000 BP and ~100 BP. The data herein provide evidence suggesting that whales were being hunted in the region at least 5000 years ago.

The consistently high numbers of humpback whales and, to a lesser degree grey whale, can be used as an indicator of the time depth of whaling. Other species appear after 2000 years in the archaeological record at Ts'ishaa and Huu7ii which may be due to the higher amount of samples taken from these layers. At least a portion of these "other" species are likely drift whales, especially the blue whale samples. If all of these samples are exclusively from stranded whales, they should also appear in the record prior to 3000 BP. Incorporation of data from other sites in the region would be useful, including a longer time period

for Huu7ii samples to clarify the long term pattern, similar to that found for Ts'ishaa.

This study is the first of its kind, using molecular archaeology as a tool to detect ancient whaling practices and species diversity. While ancient DNA cannot provide definitive answers, it is a strong line of evidence that, in combination with biological, archaeological and ethnographic data, supports the hypothesis that active whaling was practiced by the Nuu-chah-nulth by 5000 years BP. Further research along the Northwest Coast, as well as in other whaling regions, could shed light on the early development of whaling nations worldwide.

Chapter 6: Population Genetic Diversity of Northeast Pacific Humpback Whale (*Megaptera novaeangliae*) from an Ancient DNA Perspective

Abstract

Archaeological excavations in Barkley Sound, Vancouver Island unearthed whale bones covering a time period from 5000 BP to 200 BP. Using ancient DNA techniques, archaeology can trace genetic changes of populations over time creating a baseline for genetic diversity. To investigate pre-industrial whaling genetic diversity of *Megaptera novaeangliae* (humpback whale), 344 bp of the D-loop sequence of 105 humpback whales samples from two sites in Barkley Sound were analysed using ancient DNA techniques. Results were compared within the dataset as well as with published extant data from North Pacific feeding grounds.

Results indicated that the genetic diversity and haplotype diversity of humpback whales in the Northeast Pacific did not change during industrial whaling in the nineteenth and twentieth century. Population size estimates (Ne) based on this dataset range between 1500 and 2500. However, previous research has suggested that population size estimates based on D-loop diversity alone only provide partial results, so further investigation is needed to obtain the reliable pre-industrial whaling population size. The study herein found success rate, as well as the presence of amplifiable nuclear DNA suggests that the

material is sufficiently preserved for future DNA analyses including nuclear DNA marker such as STR or SNPs.

While the lack of change supports the reliability of modern DNA investigation concerning the historic population status in this case, haplotypes not recovered from modern humpback whale samples highlight the ability of ancient DNA analysis of archaeological material to support modern biogeography investigations and conservation biology.

Introduction

For millennia, whales were hunted in all areas of the world as a source for meat, oil and raw material for subsistence and trade purposes. During the industrial whaling phase in the nineteenth and twentieth century, up to 90% of the population of humpback whales was destroyed. While today, humpback whales seem to have recovered, and perhaps even reached their pre-nineteenth century population size, other whale species, for example the eastern Pacific grey whale or the North Atlantic right whale, are still threatened by extinction.

Recently, efforts have been made to estimate the historic population size and genetic variability of humpback and other whale species. Based on whaling logbooks and recent mtDNA analyses, Roman and Palumbi (2003) estimate the historic population size of humpback, finback and minke whales to be up to twenty times higher than the current estimates of the International Whaling Commission (IWC). The implications of these results would be a long term

whaling ban until a sufficient population size is reached. This study was challenged in a report to the IWC in 2005 (Clapham et al. 2006). Despite detailed historic records and modern studies, the baseline of humpback whale population genetic diversity and population size remains under debate. The increase of Northeast Pacific humpback whale population estimates from 1500 in 1966 to 20 000 in 2006 (Calambokidis et al 2008), with increases of 4-7% over the last years suggests a swift recovery of population. Because the ban on whaling hinges on sustainable population sizes, it is vital for humpback whales that biologists are able to accurately predict original population size and genetic diversity.

Using ancient DNA analysis can fill potential gaps in the modern record such as pinpointing lost haplotypes or potentially lost populations. In addition, historic documents of industrial whaling can be avoided because they are potentially flawed by underreporting, not reporting *struck-but-lost* (potentially fatally harpooned animals that were not captured), misrepresenting and miscounting.

The objectives of this study were to investigate the past genetic diversity of humpback whales in the Northeast Pacific, to analyse the changes over time by comparing ancient DNA results with modern published data, and to investigate the population size of humpback whales based on ancient DNA and modern comparative material.

Background

Humpback whale populations

Humpback whales in the Northeastern Pacific were hunted in the late nineteenth and early twentieth century. After industrial whaling effectively ended in the 1960s, and especially after the official moratorium on whaling in 1983, research on the biology and later genetic diversity of whales, and in particular humpback whales increased rapidly. However, because of their large habitat and long migration, population structure studies on humpback whales are challenging. Information about the social structure improves the understanding of the demographics, especially in cases of migrating animals, and thus may help to explain genetic and stable isotope patterns observed.

Genetic analysis has shown populations to be structured based on feeding and breeding grounds, but the social interaction is largely unknown (Clapham et al 1996, Valsecci et al. 2002, Ersts et al 2003). The fact that populations are genetically segregated was initially surprising because of the lack of barriers within an ocean (Baker et al. 1998). Humpback whales return to their natal breeding and feeding ground. The analysis by Alter et al. of the Baja California grey whale population reveals that the fidelity to the breeding ground varies, with some animals returning every year while others only return every couple of years (Alter 2009, see also Craig et al. 1997). Witteveen (2009) reports high site fidelity at feeding aggregations, based on genetic and trophic levels observed (see details below). Reports of humpback whales re-sighting in other feeding grounds are not uncommon though (Stevick 2004, Olavarria 2007, Friday 2008). These

results indicate that while feeding populations are somewhat coherent groups, they are not closed, especially to neighbouring groups.

Within a feeding population, humpback whales were observed to loosely interact with most individuals, but recent studies have uncovered different levels of interaction. Ramp et al. (2010) found associations between non-lactating females and males, as well as between non-lactating females over the course of their study. Female-female bonds lasted longer, reaching up to 6 years. The connections were maintained during the summer and disassociated during fall migration only to re-assemble in the following spring when returning to the feeding ground. Most associations of humpback whale pairs last between 2 weeks and a summer, with several years being an exception (Ramp et al. 2010). Females were more social than males, especially during the feeding season. The reason for this sex specific behaviour is unclear but has been investigated in other species. Typical theories include survival of offspring and predator pressure in general. However, in the case of humpback whales, lactating female are less frequently found in female-female groups for a longer period of time and instead group with their offspring. DNA analysis of pairs could help to reveal if the observed pairings are in fact kin-based relations.

While affiliations seem to be somewhat sex specific, there is no sexual segregration in terms of habitat use observable, but a class based foraging differentiation, with juveniles foraging on the outer areas of a feeding area.

The group behaviour of humpback whales is different between feeding and breeding grounds, along with a different sex ratio between male and female

in the different regions, varying between 1.5:1 to 2.5:1 in breeding areas depending on the study and probably the time. In breeding grounds, as well as on migration, population structure is male-biased with juvenile animals being underrepresented. According to Brown et al. (1995), population patterns during migration resemble more closely breeding than feeding populations. Previous research has already determined a class specific migration, differentiating juveniles from non-lactating females, males and lactating females. In addition, not all animals migrate every year, depending on their energetic needs (Craig and Herman 1997, Pomilla and Rosenbaum 2005, Felix and Botero-Acosta 2011).

Witteveen's comparison of the Alaskan sub-regions of southeast Alaska, Prince William Sound and Kodiak Islands reveals not only potential subpopulations for these areas, based on re-sighting, DNA and stable isotope analysis, but also differences between inshore and offshore animals, at least at Prince William Sound visible on a trophic level. The differentiation of inshore versus offshore was a research based differentiation, not based on oceanographic or biological facts (Witteveen et al. 2009). Further research may reveal other feeding grounds with similar differentiations between inshore and offshore individuals. Witteveen's study also suggests that the current classification of a central pacific population may need re-classification as more humpback whale data is available for a more differentiated landscape analysis (Witteveen et al 2009).

Research on modern humpback whale populations reveals complex migration networks between the feeding and breeding grounds of humpback

whale populations in the Pacific Ocean. Importantly, researchers are trying to determine not only the current genetic diversity, but are also establishing a baseline for whale genetic diversity and population size prior to industrial whaling. These data would provide more accurate estimates for carrying capacities and hunting quotas. Roman and Palumbi (2003) assessed current genetic diversity as well as historical whaling records to estimate pre-industrial whaling humpback population size. According to Roman and Palumbi, the population size in the North Atlantic probably ranged between 156 000 and 401 000 individuals. The current estimate of humpback whales by the IWC for the Northwest Atlantic is 11 600, and "at least 10 000" for the North Pacific (http://www.iwcoffice.org/ conservation/estimate.htm. retrieved Dec 10th 2009). Calambokidis (2008) et al. estimate the North Pacific population to be at least 20 000 animals.

The seasonal pattern of ocean productivity, which is in general very predictable, allows for a steady migration pattern over thousands of kilometres. North Atlantic humpback whales migrate between Greenland and Iceland and to breeding grounds in the West Indies. Southern Atlantic whales migrate between Antarctic whaling grounds and Brazil, Australia, New Zealand and possibly the west coast of Africa (Baker and Medrano-Gonzalez 2002, Reeves et al. 2004). Five populations are recognized in the Southern Oceans based on their feeding area (I-V) (Rasmussen et al. 2007, Baker et al. 1998a, Baker et al. 1993). North Pacific humpback whales are separated into three subpopulations: the western stock migrates between Japanese waters and the Aleutian Islands, the central stock migrates between Hawaii and the feeding ground in Prince William Sound

and western Alaska (Calambokidis et al. 2001, Witteveen et al. 2004), and the eastern North Pacific stock migrates between Mexican breeding grounds and California/Oregon, Washington/southern British Columbia and southeast Alaska/northern British Columbia during the summer feeding time (Witteveen et al. 2009, Baker et al. 1993). A stable isotope study by Witteveen et al. suggests that some of the whales feeding in British Columbia belong to the California/ Oregon/ Washington population (Witteveen et al. 2009). While these stocks are somewhat defined, some whales seem to move between these defined feeding and breeding grounds, for example migrating between Japanese waters and Oregon (Darling and Cerchio 1993).

Since the seasons are reversed between the Northern and Southern hemispheres, genetic exchange between the populations is limited (Baker and Clapham 2004, Roman and Palumbi 2003). The average swimming speed of migrating humpback whales has been measured at 4.2km/h and can vary between 4 and 9.5 km/h (Lagerquist et al. 2008), but can reach a speed of up to 12km/h for a short period of time. Calves usually stay close to the mother and those groups are slower during migration than solitary females and male humpback whales (Lagerquist et al. 2008, Stevick et al. 2003).

Industrial Whaling

Long before whaling was a lucrative business, subsistence whaling was carried out worldwide in coastal communities. Nuu-chah-nulth hunted whales using dugout canoes made of cedar trees and harpoons, outfitted with seal bladder floaters for at least 5000 years (Chapter 5). Industrial whaling is thought to have begun in the 1600s with the Basques, who hunted bowhead whale (*Balaeonoptera mysticetus*) and right whale (*E. glacialis*) (McLeod et al. 2008, Higdon 2008, Logan and Tuck 1990); it quickly spread across the world. Within 200 years, a large scale whaling industry developed and covered the Atlantic and Pacific Ocean from Greenland to Antarctica (Tønnessen and Johnsen 1982).

During the nineteenth century, whaling technology advanced rapidly and floating factory ships and exploding harpoons were developed, supporting a worldwide whaling industry. Whaling companies harvested regional populations until it became unprofitable, and then expanded their harvest to new species and/or new whaling grounds. Species were hunted systematically; right whales and bowhead whales were the first species that were targeted, followed by other species such as sperm whales and grey whales. *M. novaeangliae* (humpback whales) was one of the last species hunted because of its relatively small size and poor oil quality in comparison to right whales or sperm whales. However, the collapse of the right whale, bowhead and grey whale populations led to an intensified hunt of humpback whales.

Around Barkley Sound, whaling stations opened in 1903 and 1905 and operated until 1965. In some cases, whaling stations operated less than 10 years

before the whale stocks collapsed, causing companies to go out of business. The harvesting of humpback whales became unviable before the Second World War but was continued after the war in an effort to keep the industry alive. The International Whaling Commission (IWC) began protecting humpback whales by ratifying a ban on whaling in 1963. A general moratorium on whaling was decided in 1985 and was supposed to last at least 10 years. Industrial whaling practices reduced the worldwide humpback whale population by an estimated 90% (Baker 2003). For the past 30 years, research has been assessing the population structure and genetic diversity to determine the current state of these stocks. With an increase in data collected, including fluke sightings and genetic evidence, feeding and breeding ground relationships have been determined. Using ancient DNA techniques, phylogeographic data can provide a new time depth and potentially improve the current interpretation.

Material and Methods

Material

For this study, 105 humpback whale samples from Huu7ii and Ts'ishaa, two sites archaeologically excavated, were analysed using ancient DNA techniques. Sixty-one samples from Ts'ishaa and 44 samples from Huu7ii, dating between 5000-0 BP were included in the final analysis (Table 6). The material was excavated between 1999 and 2001 in Ts'ishaa (DfSi16 and DfSi17), and

between 2004 and 2006 at Huu7ii (DfSh7) by teams lead by Alan McMillan and Denis St.Claire. The age of the samples is based on site stratigraphy, including 14C dates from Ts'ishaa and Huu7ii. The zooarchaeological analysis was carried out at the University of Victoria in Victoria, BC. Samples were taken from shell midden and house floors. The 105 samples were part of a larger collection processed for this study (see Chapter 5 for details). Samples that could not be sufficiently reproduced were excluded from this study.

Both archaeological sites had large amounts of whale bones in shell midden and the house floor. For at least 5000 years, people were utilizing whales in Barkley Sound, primarily humpback whales. Ethnographic records reveal a high density of whales during spring and summer in this area and whale sightings year round with whales swimming into the Sound and upstream (McMillan 2009, StClaire 1991, Monks et al. 2001, Monks 2003).

Table 6: humpback whale samples used for mtDNA genetic diversity analysis.

WH_ _	Site	Unit	Level	Layer	Quad	DBD	Element	Age[1]	H [2]
26	DfSi 16	S58-60/W64-66	8	E		0.40-0.50	1 fragment	3600	CD3
31	DfSi 16	N10-12/W102-104	feature 57	C		Jul-84	"flat peice of crania- may be part of WB # 00-14 but does not seem to fit in" G. Frederick	250-1000	E1
37	DfSi 17	S5-7/W11-13	14			4.10-4.20	likely a whale but….	250-500	A1
39	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	fragment of "whale bone # 5"	3500-4500	E1
42	DfSi 16	S4-6/W98-100	2	A		3.00-3.10	thin slice of a scpula? associated with "fauna feature" from this level	250-500	CD3
44	DfSi 16	S10-12/W25-27	4	A		1.80-1.90	small cancellous fragment	250-500	A1
46	DfSi 16	S14-16/W25-27	37	G		4.70-4.80	solid rib fragment	1500-2000	CD3
50	DfSi 16	S16-18/W25-27	14	B		2.28-2.38	1 fragment	1000-1500	A1
55	DfSi 16	S60-62/W63-64	6	C		0.05 above-0.05 below	1 fragment	3500-4500	CD3
57	DfSi 16	S8-10/W98-100	7	A		2.60-2.70	cancellous fragment	250-500	A1
58	DfSi 16	S12-14/W25-27	28			4.10-4.20	small rib fragment	1000-1500	E3
59	DfSi 16	S14-16/W25-27	20	C		3.00-3.10	rib frag	500-1000	E1
60	DfSi 16	S10-12/W25-27	12			2.60-2.70	small rib frag	250-1000?	A1
64	DfSi 16	S58-60/W64-66	10	E		0.40-0.50	1 fragment	4500-5000	CD4
65	DfSi 16	S14-16/W25-27	36	G		4.60-4.70	large whale?	1500-2000	CD3
66	DfSi 17	N43-44/W53-55	2	A		4.85-4.95	1 small fragment	250-500	E1
68	DfSi 16	N10-12/W102-104?	?	?		?	WB 00-4 from feature 56 or 57? piece #2	500	E1

WH_ _	Site	Unit	Level	Layer	Quad	DBD	Element	Age[1]	H [2]
69	DfSi 16	S56-57/W50-52	17+			shovel test	dense	4500-5000	E1
70	DfSi 16	S14-16/W25-27	21	C		3.00-3.10	2 mandible frag	1000-1500	CD7
71	DfSi 16	S8-10/W98-100	10	A		2.90-3.00	unfused phalange	4000-5000	E2
72	DfSi 16	S12-14/W25-27	19			Mar.18	ribfrag from precise depth	500-1000	A1
73	DfSi 16	N0-2/W98-100	7	A		5.70-5.80	cancellous frag	250-500	E1
74	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	"whale bone # 5"	3500-4500	E1
76	DfSi 16	S56-58/W66-68	3	B		0.20-0.30	uncertain element	3500-4500	CD4
77	DfSi 16	N2-4/W102-104	5	C		6.00-6.10	2 fragments	250-500	A1
78	DfSi 16	N10-12/W102-104?	see feature record-2000				WB 00-3 from feature 56 or 57	500	E1
79	DfSi 16	S12-14/W25-27	13	A		2.60-2.70	rib fragment	500-1000	E1
81	DfSi 16	N4-6/W102-104	8	C		6.35-6.45	1 fragment	500-1000	E2
82	DfSi 16	S54-56/W77-79	7	A		0.10-0.00 above	ephyisis	3500-4500	CD3
85	DfSi 16	N10-12/W102-104	see feature record	see feature record		see feature record	WB 00-7 from a feature (either 56 or 57)	500	CD3
86	DfSi 16	S12-14/W25-27	18	B		3.70-3.80	large rib frag	500-1500	E1
88	DfSi 16	N2-4/W102-104	23	D		7.80-7.90	I lage vert frag	500-1000	CD3
89	DfSi 16	S14-16/W25-27	17	B			2 rib frags	1000-1500	CD5
92	DfSi 16	N2-4/W102-104	31	D		8.60-8.70	1 rib frag	1000-1500	E1
96	DfSi 16	S12-14/W25-27	33	F		4.60-4.70	2 fragments of ephiysis	1500-2000	CD6
97	DfSi 16	S14-16/W25-27	5	A		1.50-1.60	vetebral epiphysis	250-500	A1
101	DfSi 16	S12-14/W25-27	14			2.70-2.80		500-1000	CD5
102	DfSi 16	N10-12/W102-104				Jul-84	cranial frag from the large feature	500-1000	E1

WH_ _	Site	Unit	Level	Layer	Quad	DBD	Element	Age[1]	H [2]
106	DfSi 16	S62-64/W62-64	3	B		0.50-0.40	vert disc	3500-4500	CD3
107	DfSi 16	N4-6/W102-104	16	D		7.15-7.25		500-1000	CD3
112	DfSi 16	N4-6/W102-104	13	C		6.85-6.95	rib ?	500-1000	E1
113	DfSi 16	N4-6/W102-104	3	A		5.85-5.95	small frag	250-250	A1
125	DfSi16	N2-4/W102-104	20	D		7.50-7.60	unid level	500-1500	CD7
126	DfSi 16	S6-8/W98-100	9	A		2.85-2.95	vert disc	250-1000	CD3
127	DfSi 16	S10-12/W98-100	5	A		2.10-2.20		250-500	A1
129	DfSi 16	S10-12/W98-100	12	A		2.80-2.90	rib frag	250-1000	CD3
131	DfSi 16	S2-4/W98-100	2	A		3.5-3.6	small frag	0-500	A1
132	DfSi 16	S12-14/W25-27	14			2.7-2.8		500-1000	CD5
136	DfSi 16	S14-16/W25-27	3	A		1.25-1.35	large fragment	250-500	E1
138	DfSi 16	N0-2/W98-100	9	A		5.90-6.0	phalanx complete	250-500	A1
141	DfSi 16	S10-12/W25-27	15	A		3.10-3.20		500-1000	E1
142	DfSi 16	S12-14/W98-100	3			1.60-1.70	2 frags from same level bag	250-500	A1
143	DfSi 16	N4-6/W102-104	2	B		5.75-5.85	rib frag	250-500	E1
145	DfSi 16	S8-10/W98-100	1	A		1.85-2.10	vert disc	250-500	A1
146	DfSi 16	S12-14/W98-100	8	A		2.20-2.30	large vert disc frag	250-500	A1
150	DfSi 16	N4-6/W102-104	4	B		5.95-6.05	unid level	250-500	A1
151	DfSi 16	S12-14/W98-100	10	A		2.40-2.50	2 frags	250-500	A1
156	DfSi 16	S8-10/W98-100	1	A		1.85-2.10	small frag	250-500	A1
163	DfSi 16	S10-12/W98-100	5	A		2.10-2.20	cranial?	250-500	E4
165	DfSi 16	S14-16/W98-100	12	A		2.15-2.25		500-1000	E1
169	DfSi 17	S5-7/W11-13	6			3.30-3.40	whale? Large fragment in paperbag, verebrae frag?	250-500	E1

WH_ _	Site	Unit	Level	Layer	Quad	DBD	Element	Age[1]	H [2]
185	Dfsh7	N18-20/E34-36	16	D	NE	003.80-003.85	Vertebra (undet)	650-850	E4
186	Dfsh7	N18-20/E34-36	18	D	NE	003.90-003.95	Unidentified (fragments)	650-850	E4
193	Dfsh7	N18-20/E34-36	32	F	NE	004.60-004.65	Unidentified (fragments)	1200	CD3
196	Dfsh7	N18-20/E2-4		F		5.2	Vertebrae (complete)	1350	E2
198	Dfsh7	N10-12/E2-4	14	D	SW(NW?)	3.48-3.53	Vertebra (undet)	450-650	CD3
199	Dfsh7	N18-20/E2-4	12	C	NW	4.05	Rib	650-850	E3
203	Dfsh7	N14-16/E16-18	10	B1	SE	003.90-003.95	whale rib	650-850	E1
208	Dfsh7	N16-18/E26-28	19	C	SW	004.20-004.25	Caudal vertebra (undet)	900	E2
209	Dfsh7	N16-18/E26-28	17	C	SE	004.10-004.15	Phalanx (undet)	900	CD1
210	Dfsh7	N18-20/E2-4	21	D	SW	4.55-4.60	Unidentified (fragments)	1100	A1
212	Dfsh7	N18-20/E2-4	14	C	SW	4.20-4.25	Vertebra (undet)	650-850	E1
213	Dfsh7	N18-20/E6-8	28	E	SW	4.90-4.95	Unidentified (fragments)	1300	CD2
214	Dfsh7	N18-20/E6-8	31	E	NE	5.05-5.10	Vertebra (undet)	1300	E1
217	Dfsh7	N18-20/E6-8	13	C	SE	4.15-4.20	Unidentified (fragments)	650-850	A1
219	Dfsh7	N18-20/E6-8	31	E	NW	5.05-5.10	Unidentified (fragments)	1300	CD3
220	Dfsh7	N18-20/E6-8	18	E	SE	4.40-4.45	Vertebra (undet)	1100	E4
221	Dfsh7	N18-20/E6-8	18	E	NE	4.40-4.45	Vertebra (undet)	1100	A1
222	Dfsh7	N18-20/E6-8	18	E	SW	4.40-4.45	Vertebra (undet)	1100	A1
223	Dfsh7	N18-20/E6-8	28	E	SE	4.90-4.95	Unidentified (fragments)	1300	E1
225	Dfsh7	N18-20/E6-8	29	E	NE	4.95-5.00	Unidentified (fragments)	1300	E2
227	Dfsh7	N18-20/E2-4	18	C	NW	4.40-4.45	Unidentified (fragments)	650-850	A1
229	Dfsh7	N18-20/E2-4	21	D	NW	4.55-4.60	Unidentified (unknown element)	1100	CD3
230	Dfsh7	N18-20/E2-4	32	F	NW	5.10-5.15	Unidentified (fragments)	1300	E1

WH_ _	Site	Unit	Level	Layer	Quad	DBD	Element	Age[1]	H [2]
233	Dfsh7	N18-20/E6-8	35	G	SW	5.25-5.30	Vertebra (undet)	1350	A1
241	Dfsh7	N18-20/E6-8	20	E	SW	4.50-4.55	Vertebra (undet)	1100	E3
246	Dfsh7	N10-12/E2-4	16	E	SW	3.58-3.63	Unidentified (fragments)	450-650	CD3
247	Dfsh7	N10-12/E2-4	20	G	NW	3.78-3.83	Vertebra (undet)	650-850	CD3
249	Dfsh7	N10-12/E2-4	20	F	NE	3.78-3.83	Vertebra (undet)	650-850	A2
250	Dfsh7	N10-12/E2-4	21	G	NW	3.83-3.88	Unidentified (fragments)	650-850	E1
251	Dfsh7	N10-12/E2-4	21	G	SE	3.83-3.88	Unidentified (fragments)	650-850	E2
252	Dfsh7	N10-12/E2-4	20	F	NW	3.78-3.83	Vertebra (undet)	650-850	CD3
253	Dfsh7	N10-12/E2-4	20	G	SW	3.78-3.83	Rib	650-850	A1
254	Dfsh7	N10-12/E2-4	16	E	NW	3.58-3.63	Unidentified (fragments)	450-650	CD3
256	Dfsh7	N10-12/E2-4	10	C	SE	3.28-3.33	Vertebra (undet)	450-650	E1
257	Dfsh7	N10-12/E2-4	14	E	SW	3.48-3.59		450-650	E1
261	Dfsh7	N10-12/E2-4	12	C	SW	3.38-3.43	Unidentified (fragments)	450-650	CD3
265	Dfsh7	N18-20/E16-18	13-16	B	SW	4.0-4.20	whale bone near feat.42	650-850	A1
268	Dfsh7	N18-20/E16-18	13	B	SW	4.0-4.1		650-850	E1
271	Dfsh7	N18-20/E30-32	16-19	D/E/F	NE/NW	4.05-4.35	whale rib, three pieces almost complete	650-850	E4
275	Dfsh7	N18-20/E30-32	15-19	D/E	NW	4.2-4.25	epiphysis	650-850	CD3
276	Dfsh7	N18-20/E30-32	16-19	D/E	NW/NE	4.05-4.2	vertebrae	650-850	A1
277	Dfsh7	N18-20/E30-32	19	E	NW	4.2-4.25		650-850	A1
278	Dfsh7	N12-14/E18-20	Nov-13	C	SE	3.85-3.95		650-850	CD3
279	Dfsh7	N18-20/E30-32	16-17	D/E	SW	4.05-4.25		650-850	E1

[1]=age estimate based on calibrated radiocarbon dates at Ts'ishaa/Huu7ii, age(stat)[2] =average used for statistical analysis. Estimated dates for statistics for Huu7ii provided by A.McMillan. Site: DfSi16/17 Ts'ishaa, DfSh7=Huu7ii; [2]**H**=haplotype determined in this study

Methods

Only samples identified as *M. novaeangliae* (humpback whale) during the DNA species identification analysis (see Chapter 5) were incorporated in this analysis. PCR analysis was carried out as described in Chapter three. D-loop sequences were amplified using four overlapping primer systems (see Table 7) designed to target a 400bp region (Figure 5). This variable region was used in previous studies (Baker 1994, Baker and Medrano-Gonzalez 2002, Witteveen 2004) for population genetic purposes. The D-loop is frequently used for population genetics of different species in ancient DNA studies because it is usually the most variable mtDNA sequence available, and is suitable for studies on levels below the species level.

Without primer system F22/R258, the first 100 bp of the D-loop are not covered, therefore omitting the mutation distinguishing haplotypes A+ and A- (position 28 of the humpback whale D-loop based on Genbank reference sequence NC_006927). The D-loop primer system was later optimized to overlap with previously published sequences for whale haplotypes. Additionally, primers WL-F86 and WL-F167 were developed to replace WL-F101 and WL-F320, which were located on polymorphic regions. Primers were designed using NetPrimer© (PremierBiosoft International, Palo Alto, USA). Because the analysed part is missing these variable sites, the nomenclature of the final haplotypes includes the haplogroup as used widely in humpback whale genetics, followed by a simple numbering to distinguish different haplotypes.

Table 7: D-loop Primers used

locus	Name	5'-3' sequence	length
D-loop	WL-F86	AATTCGTGCATGTATGTACTACCACTAA	28
	WL-F167	CACCACGAGCAGTTAAAGCTC	21
	WL-F22	CCACCATCAGCACCCAAAGC	20
	WL-R258	TGCTCGTGGTGTARATAATTGAATG	25
	WL-F360	TATGTATAATCGTGCATTCAATTAT	25
	WL-R569	GCGGGTTGCTGGTTTCAC	18
	WL-F101	TGTACTACCACATAACCAACTGATAGCA	28
	WL-R308	ATCTAATGGAGCGCCCATAGAGATC	25
	WL-F322	AGCATGCCGCGTGAAACCA	19
	WL-R588	CTGAGTCCGTGCAAGCCC	18
Sequencing Primer	WL-F22s	CTCAGCACCCAAAGCTGAAATTCTA	25
	WL-R258s	GAATGCACGATTATACATCTCTAGG	25
	WL-F86s	ATAGCACCTTCCATGGGTATGTATA	25
	WL-F101s	AACTGATAGCACCTTCCATGAG	22
	WL-R308s	AGCGCCCATAGAGATCATTTGAC	23
	WL-F322s	GTGAAACCAGCAACCCGCTT	20
	WL-R588s	ATACCAAATGTATGAAACCTCAG	23

PCR was carried out in 30 µl reactions per sample as described in Chapter three. The PCR was run under the following conditions: 94°C/ 5 minutes followed by 60 cycles of 45 seconds at 94°C, 30 seconds at 54°C and 40 seconds at 64°C. The final extension was at 72°C for 10 minutes. PCR amplifications that failed initially were re-amplified using an annealing temperature of 52°C.

The presence of PCR amplicons was established on a 2% agarose gel with SyBrGreen stain. Positive PCR reactions were purified using a Qiaquick purification column (Qiagen, Hilden, Germany) following the manufacturer's manual. Samples were sequenced at McMaster University's sequencing facility and Macrogen Ltd in Seoul, South Korea, using either the forward or reverse primer (Table 2). To optimize the sequencing result, nested sequencing primers

were designed located approximately 10 bp to the 3' end of the original PCR primer.

All sequences recovered from each individual sample were aligned in ChromasPro© and the final sequence was exported to Bioedit. Variable sites were exported to an Excel spreadsheet to confirm the haplotype designation. Haplotypes were assigned by comparing the ancient DNA sequences to data published in the NCBI Genbank and by using published description of haplotype and haplogroup features (Medrano-Gonzales et al. 1998). Specific haplotype designations for haplotype F were provided by Baker and Steel (Splash, 2010, CS Baker, D.Steel pers.comm) Because different research groups have used variations of beginning and end points of the D-loop sequence, the final overlap of modern and ancient DNA was 344 bp (Figure 5), truncating the F322/R588 sequence.

Statistical Analysis

Statistic analyses, included bayesian coalescence analysis and a unique sequence tree were carried out using the BEAST software package V1.6 and V1.7 (Drummond and Rambaut 2007, Drummond et al.2012) while population statistics was calculated using Arlequin V1.5 (Excoffier et al. 2007). The appropriate substitution models for tree and skyline plot were chosen using jmodeltest (Posada 2008).

The BEAST (Bayesian Evolutionary Analysis Sampling Trees) program package implements Bayesian MCMC algorithm (Drummond et al. 2002, Drummond and Rambaut 2007) for phylogenetic and demographic analysis. In BEAST priors are optimized to infer posterior probabilities of the model parameters using Bayesian statistics. The MCMC (Markov Chain Monte Carlo) algorithm is “a stochastic algorithm that produces sample-based estimates of a target distribution [*in this case posterior distribution*] of choice” (Drummond and Rambaut, 2007; Drummond et al.2002)

The outcome of the chain is then used as a sample parameter. A long chain creates a well mixed distribution, meaning the estimate is more accurate. Bayesian re-analyses the probability of an outcome based on certain prior and posterior information under inclusion of phylogenetic models (Salemi et al. 2009). The Bayesian MCMC algorithms eventually can weight trees based on their posterior probability. Since the age of the sample can be included in the analyses, BEAST is ideal for the analysis of ancient DNA datasets.

The Bayesian skyline plot was produced to detect changes in population size over time. For this step, all 105 ancient DNA sequences were used and combined with a partial population set from GenBank (PopSet: 289473606), published by Jackson et al. (2009). The final size of the dataset used for the skyline plot included 124 samples in total spanning 344 bp.

In subsequent analysis, the dataset was adapted to test the robustness of the skyline plot result. Instead of the entire 105 aDNA sequences, 46 were used based on the MNI analysis (Table 12: changes in genetic diversity based on minimum and maximum estimated individuals). In addition, datasets were run with 70 and 35 sequences, removing the first 500 years because this time frame appeared to have been sampled unevenly based on the genetic diversity indices (Table 12). Because no modern dataset was available and the Jackson dataset may not be optimal, runs were carried out with (1) samples from Washington and Oregon only(n=19) (2) samples from Washington, Oregon and Mexico (n=100) and (3) the entire popset from Jackson et al (n=100, same as (2)) and a popset from Alaska (Jackson et al.2009). None of the tested alternatives provided a more reliable skyline plot than the initially one.

The clock was not fixed to any published mutation rates because the data contained ancient DNA data. Since ancient mutation rates may vary from modern clock rates, the estimated clock might be the more accurate because the substitution rate increases when ancient DNA data is added to a dataset (Kuhn, pers. comm. 2009, Ho et al. 2005, Navascues et al. 2009). Previous studies used substitution rates between 1.5 $x10^{-8}$ bp^{-1} $year^{-1}$ to 2.0x $10^{-8}bp^{-1}$ $year^{-1}$. (Roman

and Palumbi 2003), with the latest published substitution rate for the whale D-loop being 3.9% per million years((Jackson et al. 2009).

The Bayesian skyline plot was calculated using HKY+G as a substitution model (Hasegawa et al. 1985, Yang 1993). The tree prior settings for the initial analysis was the constant population size with 15 groups and a piecewise constant skyline model. Operator weights were changed as follows for the skyline plot: up/down substitution rate, subtree and narrow exchange: number of taxa/2, wide exchange and wilson balding number of taxa/10 (Kuhn, pers. comm. 2008). All other operator weights were left at a default rate or adapted based on recommendations from previous runs. For the statistical analysis in BEAST, dates before present (in full years) were attributed to the samples. The dates were based on radiocarbon dates of the layers, or the relative dates of the layers provided with the samples. Dates for Huu7ii were provided by Alan McMillan (pers. com. 2009). Each analysis was run with 95 million MCMC steps to ensure the chains converged to a stationary state. The 95 million iterations were sampled every 9500 steps with an initial burn-in of 10%. The final analysis was run five times to allow the MCMC to start from different points (called “seeds”).

The results of the BEAST runs were imported in Tracer 1.5 (Rambaut and Drummond 2005). Tracer was used to create the Bayesian skyline plot output graph. A Bayesian skyline plot was created in Tracer to estimate the effective population size of the ancient dataset. The Tracer output of the population size over time was exported into Excel (Microsoft Office 2007). The MCC (maximum clade credibility) tree of the skyline plot was created in Tree Annotator (part of the

BEAST package) with a 10% burn-in and visualized using FigTree. The tree in FigTree was rooted at midpoint.

A tree with unique haplotypes was calculated using BEAST with linear population size change and an HKY+G model. For this step, the dataset for the Bayesian skyline plot was truncated to unique sequences only. The final dataset for the tree file was N=17 of both ancient and modern samples, and finback (NC_001321; GI: 5819095) as outgroup. Results were evaluated in Trace V1.5 as described for the skyline plot, and the tree was created in FigTree.v 1.3 (http://tree.bio.ed.ac.uk/). BEAST analyses were carried out on the Bioportal (www.bioportal.uio.no) cluster and the computational biology service unit (http://cbsuapps.tc.cornell.edu, Beast@BioHCP) at Cornell University.

Genetic diversity indices, specifically haplotype and nucleotide diversity, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) were calculated using Arlequin 1.5 using the frequencies of the haplotypes of the dataset used for the BEAST skyline analysis. These indices were compared to modern published data. In addition, possible changes of genetic diversity were tracked over time, following Borges (2007) study on bowhead whales (Table 12).

Results

In total, 105 samples were completed for the D-loop fragment and included in the analysis. 70 additional samples identified as humpback whale in the archaeological dataset originally provided were not included because not all

samples had a complete and reproduced sequence. In addition, samples were included despite a full profile if the authenticity of the haplotypes was in question. However, results suggest that the 105 samples provide an accurate cross-section of the archaeological whale population. The D-loop fragment used for this analysis covers most of the consensus sequence but is missing the first 100bp due to an alignment error early on. Because of this, haplotypes A cannot be distinguished from A-, but this does not invalidate the analysis. To create the highest possible overlap with published data, the alignment (Table 9) was truncated to cover a length of 344 bp from position 120 to position 464 on the humpback whale D-loop (Sasaki et al. 2005). A total of 13 haplotypes were found in the ancient DNA dataset among 105 samples (Figure 5). Except for three unique haplotypes, all haplotypes found here are extant and published in Genbank. All of the new haplotypes are in the CD clade and are different from the next known haplotype by 1 bp. While CD1 was found in PH209 only, CD4 and CD5 are found in 2 and 3 individuals respectively. The differences of the haplotypes were confirmed through repeat sequencing. The three major haplotypes were found in 23, 28 and 29 individuals respectively. All other haplotypes are found in low frequency of 6 individuals or less. Haplotypes present in the analysed dataset belong to the CD and AE clades as published by Baker and others (Baker et al. 1994, 1998, Baker and Medrano Gonzalez 2002). The distribution in A (27.6%), E (40.9%) and CD (31.4%) haplogroups differs from the distribution reported from the California/Oregon/ Washington population as well as the southeast Alaskan population. As discussed below,

Washington/southern BC used to be clustered with California/Oregon but have lately been considered separate feeding aggregations (Witteveen et al. 2009). California's humpback whale population has 43% E and 50% CD with a total of 12 haplotypes (Witteveen et al. 2004, Baker and Medrano-Gonzalez 2002). On the other hand, southeast Alaska has a reported 100% A, and no CD or E samples (Witteveen et al. 2004). None of the samples analysed in this study that are dated to older than 3000 BP display the A haplotype, although the sample size may be too small with only 19 samples.

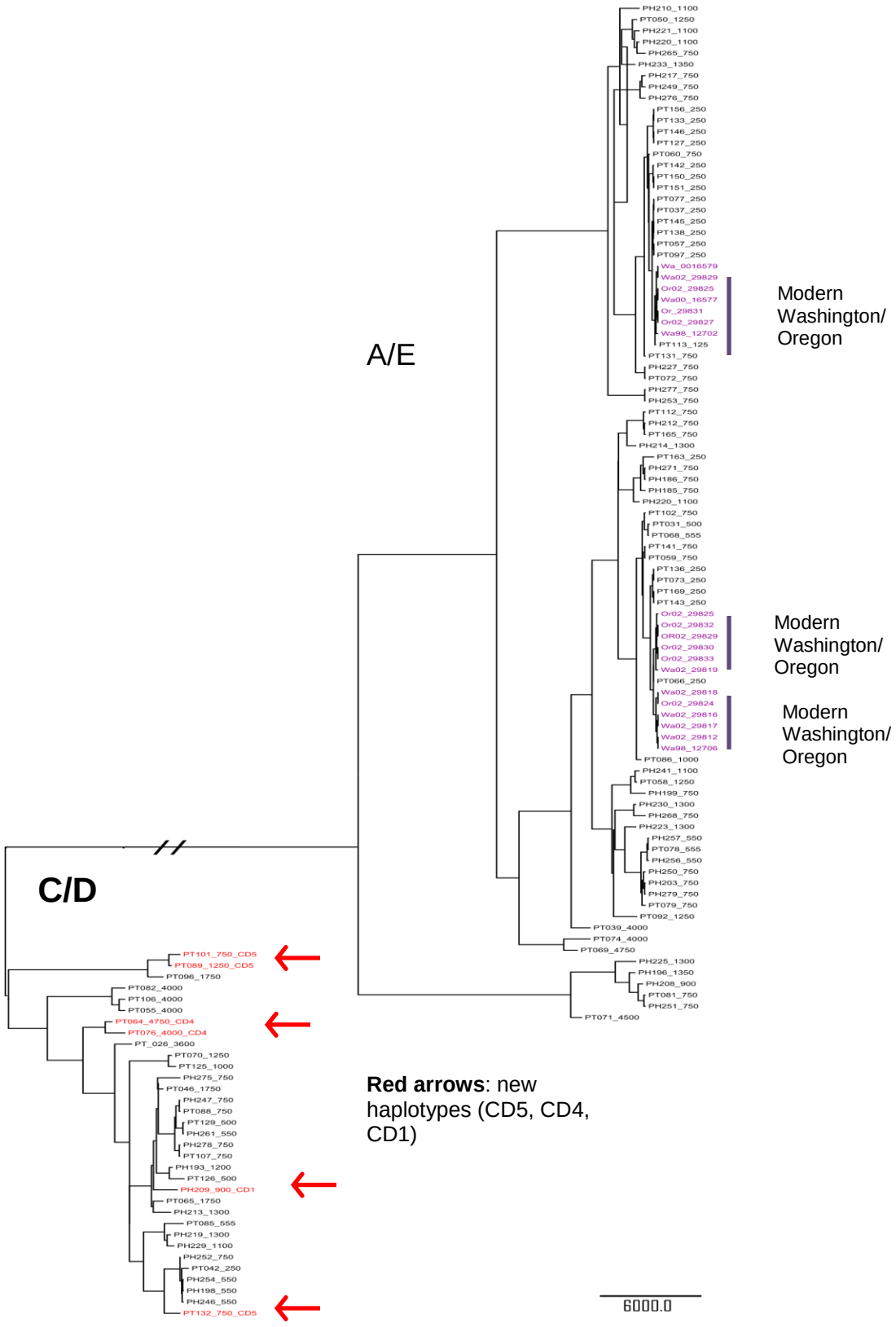


Figure 5: tree of samples used in this study. Ancient haplotypes (red arrows) were not found in Genbank. Modern samples (pink) N=19 from Jackson et al (2009)

The genetic variability within the ancient DNA dataset was assessed using Arlequin and compared to published data in Table 8.

Table 8: nucleotide and haplotype diversity for this study compared to published data. All data in grey is based on published material only.

Source (location sampled)	N	bp	π (S.D.)	h (S.D.)
This study				
Ancient DNA (south British Columbia)	105	344bp	0.0147 +/- 0.008	0.804+/- 0.0196
Modern sample set (Jackson et al.2009)	100	344bp	0.0083+/- 0.0049	0.779+/-0.023
Publish data (Baker and Medrano Gonzalez 2002)				
California	20	297bp	2.4 *±1.32*	0.832*±0.063*
Alaska (central)	8	297bp	0*±0*	0.0*±0.0*

No background: genetic diversity of samples used in this study (ancient DNA and modern GenBank data), calculated using Arlequin. Grey background: previously published values (Baker and Medrano Gonzalez 2003)

The nucleotide diversity of the ancient DNA humpback whales is π=0.0147 +/-0.008, and the haplotype diversity is h=0.804+/-0.0196. The modern population used as comparative material had a nucleotide diversity of π= 0.0083+/-0.0049and a haplotype diversity of h=0.779+/-0.023. To compare these results, Table 8 also includes indices from previous published studies (Baker and Medrano Gonzalez 2002). The results are lower than the published values for the modern California population and above the published dates for modern southeast Alaska population. Because no samples or diversity have been published from the region directly around Vancouver Island, only limited interpretation of these results is possible. Table 8 further includes the number of individuals and the length of the sequences used because both influence the diversity indices.

Table 9: unique haplotypes found in 105 ancient DNA sequences.

absolute location				15612	15620	15632	15648	15725	15732	15733	15734	15749	15750	15751	15752	15753	15755	15759	15802	15803	15866
relative position				5	13	25	41	118	125	126	127	142	143	144	145	146	148	152	195	196	259
NC_006927, Sasaki_et_al.2005				**C**	**G**	**T**	**T**	**G**	**T**	**C**	**C**	**G**	**T**	**C**	**C**	**A**	**C**	**A**	**T**	**C**	**C**
haplotype	aDNA sample	freq	N																		
A1	PH217	0.27	28	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
A2	PH249	0.01	1	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.
E1	PH203	0.27	29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E2	PH208	0.06	6	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	A	.	.
E3	PH199	0.03	3	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
E4	PH271	0.05	5	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
CD1	PH209	0.01	1	T	A	C	C	.	C	.	.	.	.	T	.	.	T	G	.	T	.
CD2	PH213	0.01	1	T	A	C	C	.	C	.	.	.	.	T	.	G	T	G	C	T	.
CD3	PT219	0.23	23	T	A	C	C	.	C	T	.	.	.	T	.	.	T	G	C	T	.
CD4	PT076	0.02	2	T	A	C	C	.	C	.	.	A	.	T	.	.	T	G	C	T	.
CD5	PT089	0.03	3	T	A	C	.	.	C	T	.	.	.	T	.	.	T	G	C	T	.
CD6	PT096	0.01	1	T	A	C	.	.	C	.	.	.	.	T	.	.	T	G	C	T	.
CD7	PT125	0.02	2	-	-	-	C	.	C	.	.	.	.	T	.	.	T	G	C	T	T

Bold: unique haplotypes (CD1,CD4,CD5) not identified in GenBank.
dots in this matrix display an agreement with the top sequence which is the reference sequence published by Sasaki et. al. 2005.

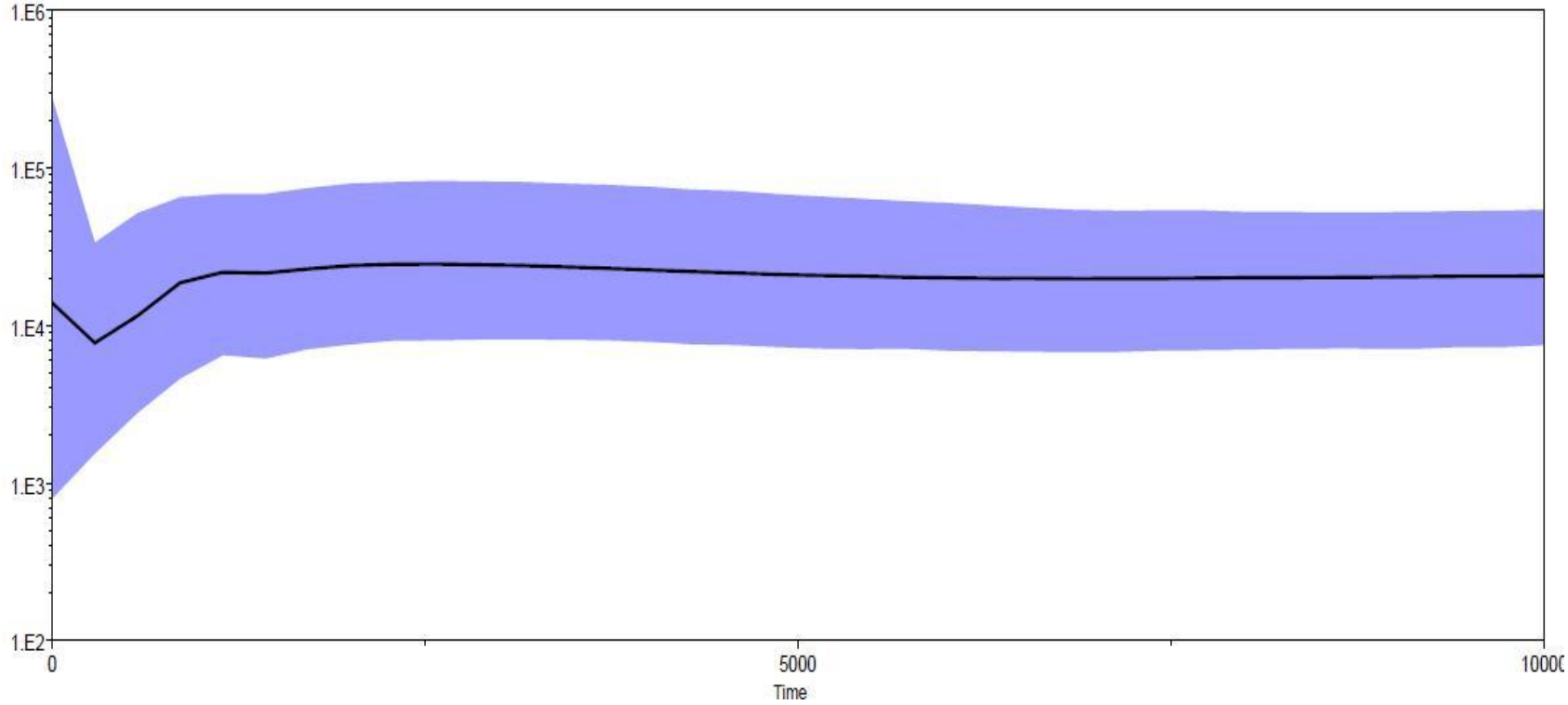


Figure 6: Bayesian skyline plot. X-axis=time (moving backwards in time from left to right); Y-axis =population size. Black line= mean values; blue area= upper and lower 95% confidence interval.

Table 10: changes in population size. All values are raw data (=Ne*tau)

	mean (upper and lower 95%CI)					
Time*	**Run A**	**Run B**	**Run C**	**Run D**	**Run E**	**Mean (all runs)**
0	33679 (224305-411)	31274 (200301-419)	44586 (285391-829)	34832 (237876-403)	39597 (260375-506)	**37275** (241650-514)
500	14550 (37671-4157)	14495 (39128-3584)	15417 (51457-2680)	14521 (37757-4142)	13704 (35710-3806)	**14549** (40345-3674)
1000	28819 (74907-8061)	30191 (83217-8181)	25868 (69191-6526)	28736 (73898-8064)	28507 (76764-8175)	**28150** (75595-7802)
2000	31652 (83688-9169)	29691 (79449-8450)	28974 (80269-7509)	31143 (80912-9101)	30494 (82955-9143)	**30784** (81454-8675)
3000	32495 (92075-9021)	29728 (78321-7980)	29252 (80872-8042)	31919 (89227-8916)	29558 (79906-8625)	**31144** (84058-8517)
4000	29462 (82159-8178)	27247 (74639-7936)	27227 (75195-7761)	28706 (79345-8191)	27117 (75941-7687)	**28395** (77456-7951)
5000	27768 (78950-8136)	25605 (67785-7573)	25067 (67836-7288)	27176 (75927-8069)	25765 (68248-7668)	**26709** (71749-7747)
6000	26676 (73273-8454)	24590 (61003-7851)	23164 (59822-6834)	26000 (67852-8328)	24973 (63729-8071)	**25498** (65136-7908)

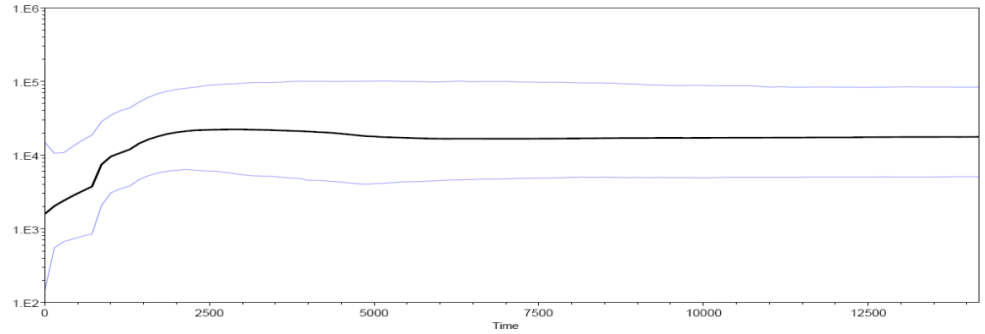
Bayesian skyline plots estimated the substitution rate as above the published rate of 3.9% (between 4.3% and 4.7% depending on the run). All ESS values were around or above 200 for runs with 95 million chains. The substitution rate of 4.3%, being above the modern published rate, has been reported in ancient DNA studies before. Navascues proposed that this elevated rate could be a real change in molecular clock rate in older samples, citing the time dependency of molecular rate theory by Ho et al. (2009), but that it could also be attributed to an oversimplification of population demographic models, for example as in this case assuming that all individuals sampled over 5000 years belong to the same population (Navascues, 2009). The elevated mutation rate could simply

be due to the fragment use in this study, using the highly variable 3' end but not the less variable part of the cetacean control region.

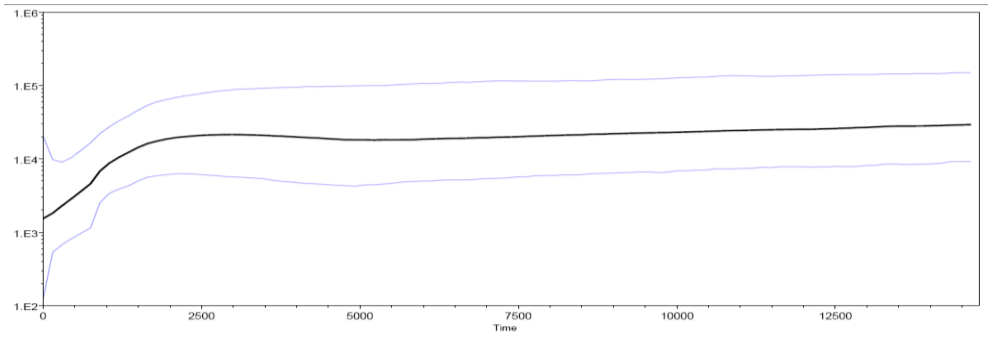
The Bayesian skyline plot results (Figure 6) indicate a stable population size up to about 1000 BP (Tables 10 and 11). After a decline in population size after 1000 BP, the population size increases and exceeds this level in modern times. While this appears to fit the recorded past of humpback whales as the reduction in population seems to coincide with industrial whaling, the reasons for the decrease may be due to sampling strategy. Specifically the change in population size around 1000 BP may be due to a sampling error of the original dataset, as discussed below, in the context of the number of individuals (Table 12). The decrease has to be taken with caution though because changes of the population size range within the 95% confidence interval. The most recent change as well as the increase of the 95% confidence interval may be due to the use of a popset (Jackson et al. 2009) that is not from the same precise geographic area as the ancient DNA samples. Using the ancient DNA dataset alone for a Bayesian skyline plot analyses results in the same long term stable population size as the dataset discussed here.

The Bayesian skyline plot result includes a relatively wide 95% HSP and is almost completely flat. Flatness and large confidence interval are both indicators of a locus with low information content and a poor fitting model. Alternative priors yield the same results (Figure 7), supporting the result that the here used dataset does not have the amount of information needed to correctly infer the past changes in population size.

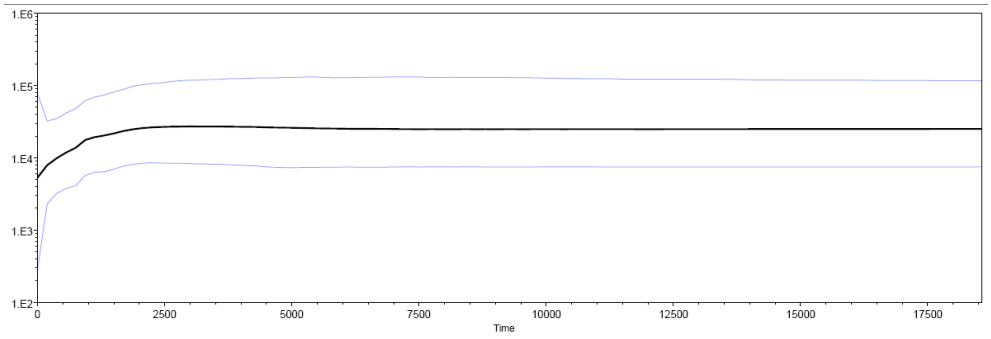
A



B



C



D

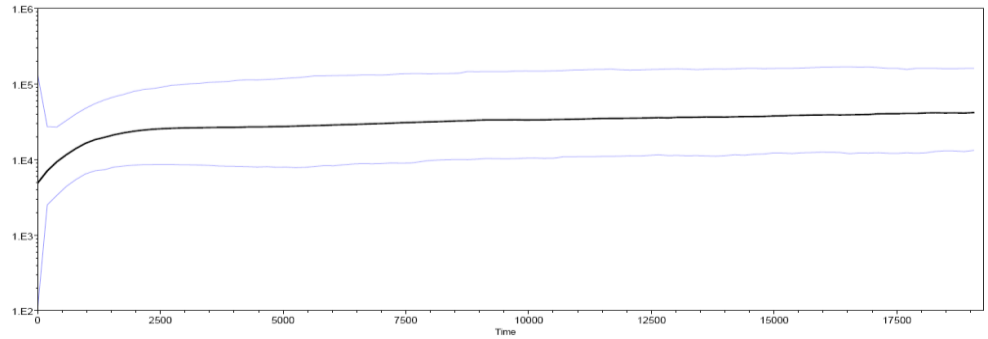


Figure 7: comparison of skyline plots: linear and constant population size prior. 35 ancient DNA samples. A and B: Washington Oregon (n=19), C and D: Washington, Oregon and Mexico (n=100) A and C=constant prior, B and D linear prior

The value on the y-axis of the skyline plot equals the breeding population size (Ne) multiplied by the generation length (tau). To calculate the effective breeding population Ne, this number is divided by the generation length. Because different generation lengths are published, the equation in Table 11 was repeated to cover the span in generation length (Slijper 1979, Perrin et al. 2002, Clapham and Baker 2002), including the lower value of tau=12 used by Roman and Palumbi (2004).

Table 11: effective population size based on Bayesian skyline plot

time	Ne*tau (Table 10)	Ne			
		tau=12	*tau=16*	*tau=18*	*tau=20*
0	37275	3106.3	2329.7	2070.8	1863.8
500	14549	1212.4	909.3	808.3	727.5
1000	28150	2345.8	1759.4	1563.9	1407.5
2000	30784	2565.3	1924.0	1710.2	1539.2
3000	31144	2595.3	1946.5	1730.2	1557.2
4000	28395	2366.3	1774.7	1577.5	1419.8
5000	26709	2225.8	1669.3	1483.8	1335.5
6000	25498	2124.8	1593.6	1416.6	1274.9

Number of Individuals analysed

The approach to control for the re-sampling of individuals was a correlation between location, species/haplotype, and time. Specifically, if two samples were from the same location (unit, layer and level) and were identified as the same species, they were assumed to be the same individual. In particular, several samples in the Ts'ishaa sample set were labelled by the excavating archaeologist as "may be the same individual or bone" (Table 8 and Appendix A).

In some cases, the samples' species identification designated the individuals as different species. In other cases, only one of the two bones yielded amplifiable DNA. However, with the size of a whale and the cultural tradition of sharing a hunted whale, it is more than likely that the same individual would be found in more than one location, and therefore possibly in more than one site.

Using species identification, D-loop sequence polymorphism and zooarchaeological analysis, in correlation with the location in time and space helps to distinguish between broken bone fragments. Using the DNA results presented here, possible additional data were produced. Because no method of counting would have been perfect, the approach was to count samples repeatedly, distinguishing the database in various ways. Sorting the samples in the database "by provided time across all sites" or alternatively "by unit only" would be the worst case scenario, unlikely given the amount of whale bones present. However, differentiating by time and location should produce a more likely result given the amount of whale bones found at the sites, as well as ethnographic data regarding whaling in Barkley Sound (see Chapter 2 and Background of this chapter for details). In addition, the similar species distribution between Huu7ii and Ts'ishaa supports that sampling was random enough to create an accurate result.

The best case scenario was the calculation of individuals per layer (instead of time) per unit. The assumption in this case was that the quantity of whales on site must account for a larger quantity of whales. This would prevent one bone from being sampled multiple times, but would also account for the

overall observation of the large quantity of whale bones at Ts'ishaa and Huu7ii that, as mentioned before cannot be based on few whales (e.g one whale per layer) being hunted (Monks 2001, Monks et al. 2001).

Utilizing the worst case scenario as described above would assume few whales being butchered and shared between sites and a higher fragmentation rate per whale. The worst case scenario does not match descriptions published by early ethnographers; nor does the archaeological record of whale bones support a hypothesis like this. The best case scenario accounts for many whales being caught and a low inter-site distribution between Tseshat and Huuayhat. It is possible that different whale species are represented slightly differently. The amount of whale bone is related to the size of the whale, but also to the procurement method; that is, drift whales should be represented less frequently because these specimens were not necessarily butchered immediately but were decomposing from the inside out, in some cases making only the very outer layer consumable (Monks 2001). In addition, some whales were more suitable for oil extraction than others, which should account for more bone material on site.

Table 12: changes in genetic diversity based on minimum and maximum estimated individuals

		Min	Max	Complete
sequences	**All times**	48	90	105
haplotype diversity		0.88(0.002)	0.813(0.02)	0.804(0.0196)
nucleotide diversity		0.018(0.009)	0.0168(0.009)	0.015(0.008)
Tajimas D		1.25	0.913	1.13
Fst		0.37	1.58	1.87
	5000-3000			
sequences		4	9	9
haplotype diversity		1.0 (0.17)	0.77(0.11)	0.77(0.11)
nucleotide diversity		0.0228 (0.016)	0.017(0.01)	0.017(0.01)
Tajimas D		1.67	1.43	1.43
Fst		**-0.094**	2.77	2.77
	2000-1000			
sequences		18	22	25
haplotype diversity		0.928 (0.034)	0.905(0.035)	0.88(0.035)
nucleotide diversity		0.020(0.011)	0.02(0.011)	0.019(0.01)
Tajimas D		1.25	1.48	1.47
Fst		**-0.58**	0.19	0.53
	1000-200			
sequences		26	59	71
haplotype diversity		0.87(0.03)	0.77(0.028)	0.76(0.029)
nucleotide diversity		0.017(0.009)	0.014(0.007)	0.013(0.007)
Tajimas D		0.87	0.78	0.74
Fst		0.91	2.32	2.55
	1000-500			
sequences		21	41	48
haplotype diversity		0.88(0.039)	0.79(0.034)	0.79(0.03)
nucleotide diversity		0.017(0.009)	0.016(0.009)	0.016(0.008)
Tajimas D		0.75	1.14	1.217
Fst		0.33	2.13	2.53
	500-200			
sequences		5	18	23
haplotype diversity		1.0(0.126)	0.66(0.08)	0.54(0.104)
nucleotide diversity		0.018(0.012)	0.006(0.004)	0.0051(0.0035)
Tajimas D		**-0.628**	**-1.79 (p=0.03)**	**-1.94 (p=0.01)**
Fst		**-1.14**	0.46	0.23

The p-value for Tajimas D and Fst value is only shown if the value is significant. For the haplotype and nucleotide diversity, the standart deviation (SD) is shown in brackets.

The results of the re-sampling (MNI) estimates found in Table 12 can be used to estimate the impact of re-sampling an individual multiple times by comparing the total values to the lowest and highest estimates of individual counts. The minimum number is based on time periods by site, with the time period as provided in the database, but ignoring excavation units. The maximum

estimated number is based on unit and layer, taking provided time periods into account. Except for the instances when the sample size drops to the number of haplotypes, the genetic diversity varies very little between the minimum, maximum and total sample number.

While this table cannot unambiguously establish the number of individuals tested, Table 12 shows that a possible re-sampling did not bias the genetic diversity established using all 105 bone samples. Based on archaeological and ethnographic data, it is unlikely that nearly 50% of the dataset is based on re-sampling.

The SRY system successfully co-amplified ZFX and SRY. Initial, not reproduced tests suggest a ratio of 60:40 in favour of male individuals. This result is based on fragment length identification. While the sequenced amplicons were identifiable as the appropriate loci, this system would have required extensive troubleshooting and no conclusive sequence analysis towards different allels was made. Forty-three out of 62 tested samples (71%) yielded amplifiable nuclear DNA over the course of this study.

Discussion

The aim of this study was to investigate the humpback whale population present in Barkley Sound pre-industrial whaling including the genetic diversity, possible changes over time, and an estimate of the population size based on ancient DNA data analysis. Results indicate that despite large scale whaling

operations in the North Pacific that killed an estimated 28 000 animals over a time of 60 years (Nichols et al. 2002), genetic diversity did not change substantially. Comparisons between early (5000-3000) and late (2000-500) samples indicate little change over a period of 5000 years in terms of population genetic diversity. Population size estimated using ancient DNA data matches previous published data (Rice 1978, Perry et al. 1990, Baker 1993). Based on the current knowledge of the complex migration patterns in the Northeastern Pacific, the here calculated population estimate is likely a partial estimate, and not equal to the complete North Pacific population, in part because this study only used one feeding ground. Adding feeding grounds to the ancient DNA dataset would likely change the final number. In addition, results indicate that the 344bp used in this study do not yield the amount of information needed to infer changes in population size in the past. Additional loci are needed to accurately past population dynamics. Nevertheless, these results are an important insight into past humpback whale population changes over time, and will provide an important line of evidence towards our understanding of pre-industrial whaling population size and diversity, as discussed below.

Genetic diversity

The population genetic diversity in this dataset display similar levels to the genetic diversity found in most North Pacific feeding grounds today (Baker and Medrano-Gonzalez 2002), indicating that the mitochondrial genetic diversity was not lost through industrial whaling. Haplotypes found herein are, with few

exceptions, found in modern Pacific whale samples published in GenBank®. Haplotypes absent from North Pacific datasets today are found in the ancient DNA dataset in low frequency, even if found in several time periods. Haplotypes found in low frequencies are lost in a population more easily through genetic drift, and do not necessarily indicate a substantial loss or change in population size. The three unique sequences found in four individual samples are closely related to the common haplotypes by 1 and 2 base pairs difference (0.3% and 0.6% difference respectively). Due to the low frequency, these sequences could reasonably be discovered in future studies of modern humpback whales.

The lack of change in diversity was expected for several reasons. Humpback whales have a long generation length (16-20 years), a life expectancy of over 40 years and an overall slow mutation rate. The time elapsed since industrial whaling is less than 45 years (1966 but whaling continued in some areas beyond that date), which suggests that two generations or more of the animals are probably still alive. Consequently, industrial whaling caused either no change in genetic diversity, or a change that will become detectable in the future.

The hypothesis that genetic diversity has not changed despite large scale hunting has been discussed previously by Amos (1996) as well as Baker (1995). According to the research and theories provided, human impact happened too recently to have had a substantial impact on whale populations and the mutation rate of the humpback mtDNA was too slow. Based on the slow mutation rate, and the recent halt of industrial whaling, a low genetic diversity of modern populations consequently would not be an outcome of industrial whaling but it has to be

expected that the genetic variability likely remained at the same level as before. Amos argues that the lack of change in genetic diversity does not imply a healthy population and that protection is still warranted: “Inbreeding could be an important factor capable of slowing or preventing recovery, and it is clearly desirable to identify cases where this is so” (Amos 1996 p.658).

Even over a period of nearly 5000 years, the genetic diversity did not change substantially, which supports a stable population over this period. The change in genetic diversity in the subset between 500-200 BP is potentially due to sampling error, as this is the subset with the most loss of samples (down to 21%) during the MNI testing (Table 12). If this is the case, the drop in population size calculated in the BEAST analysis is an effect of this sampling error instead of industrial whaling.

While the genetic diversity for south British Columbia has not been published yet, both California/ Oregon as well as southeast Alaska/ northern BC have been documented. Haplogroup variation matches the variation found in BC today (Baker pers.comm 2010; Witteveen 2004), supporting the hypothesis that southern BC is part of an independent feeding ground, and that the modern population is part of the original population found in BC prior to industrial whaling. Previous theories included an overlap of adjacent feeding grounds or a post-whaling expansion into empty areas coming from California or Alaska. Indeed, Witteveen’s (2009) research using stable isotopes suggests that whales in British Columbia are difficult to distinguish from the California/Oregon whales.

According to Medrano-Gonzales and Baker, the clinal haplotype frequency

might be due to an expansion of North Pacific humpback whales northwards after the retreat of the last glaciations (Baker and Medrano-Gonzalez 2002, Medrano-Gonzalez et al. 1995). The population analysed in this study falls neatly between California/Oregon and southeast Alaska feeding grounds in terms of haplotype distribution. The Alaskan region is the most extreme in terms of cline with almost exclusively AE haplogroup present (Witteveen et al. 2004). Most likely, this is a post-glaciations founders effect, with a small population migrating into this area after the feeding ground became accessible after 10 000 BP. Climate change may have had a similar effect on humpback whale populations as on seal populations. Based on zooarchaeological data by Crockford and Frederick (2007), this happened to fur seal populations found before and after glaciers retreated around 4000 BP (Crockford and Frederick 2007). During the previous contraction of feeding and breeding area, the opportunities for gene-flow within the southern hemisphere were higher (Medrano-Gonzalez et al. 1995). The absence of haplogroup A in the earliest layers could be a stochastic error due to the low sample size (19 samples), or a sign of a change in population structure in the past. Further analysis of a larger sample set dating to 5000-3000 BP and older would reveal whether haplogroup A can be found this early or whether the haplotype diversity changed prior to 2000 BP. The hypothesis that intensive hunting has reduced the genetic diversity is not supported by the dataset analysed here.

Whether the reduction of the population has lead to a bottleneck has been part of cetacean research from the beginning (Baker 1995, Amos 1996). The

results reported here are in concordance with previous findings, suggesting that there was no major loss in mtDNA genetic diversity in the region due to industrial whaling. While further studies are needed to explore the nuclear DNA genetic diversity of past whale populations, studies thus far have found that the current microsatellite diversity is largely matching mtDNA diversity (Roman and Palumbi 2003, Palsboll et al. 2004, Palumbi and Roman in: Estes 2007). Ancient DNA research should therefore concentrate on potential changes in migration pattern and the independence of feeding grounds.

Despite the lack of changes in mtDNA diversity, Amos notes that a reduction in population size could potentially increase the frequency of previously rare deleterious genes, making a possible future extinction more likely. Additionally, current climate change will likely be in direct competition with the potential whaling industry.

The implications of reducing higher trophic level mammals from the marine community have been for example recorded in the case of sea otters in the Northeast Pacific. Here, the hunt of sea otters, beginning with Aboriginal hunters and increasing with fur traders, led to a depletion of sea kelp due to an increase in sea urchins whose predator, the sea otter, had been removed. With the recovery of sea otters, the kelp forest on the North Pacific Coast recovered as well (Estes 1998, 2007, Springer 2008). Based on this example, the whale population may not be the only species at risk in the marine environment.

Modern whales present in southern British Columbia are either remnants of the archaeological population, or are migrating into an empty feeding ground

after the population previously present was wiped out. If the current population is moving into an empty area, the genetic diversity of the stock in southern British Columbia should match the California/Oregon/Washington feeding aggregation. California/Oregon is more likely than Alaska because previous research has suggested the similarity of humpback whales in this area to the California/Oregon feeding ground (Witteveen 2009), based on stable isotopes. If this is the case, the haplotype diversity should be different from the ancient population. However, if current whales are remnants of a pre-industrial whaling population migrating into Barkley Sound, a continuity of genetic diversity and haplotype diversity should be visible. Today's stocks (Baker and Medrano-Gonzalez 2002) may have had more overlap prior to industrial whaling based on population size, and the presence of an intermediate population in British Columbia is therefore possible.

In all scenarios, a severe bottleneck would potentially have left the species with a low genetic diversity. A similar assumption was made prior to this study of Northeast Pacific humpback whales. However, genetic diversity of ancient and extant population suggests that the historic "bottleneck" was passed without creating a detectable genetic bottleneck.

Two different roads of recovery are visible in humpback and right whale populations. Neither humpback whales nor right whales exhibit a loss of mtDNA genetic diversity, but the right whale populations remain at a low population density. If humpback whales have retained their genetic diversity, the population should theoretically be able to recover.

While this is the first study involving humpback whales and the first study

in the Northeast Pacific, research has been published involving historic whale bones. Through these recent studies, which compare different species, biologists can investigate how or why species recover, and how far this recovery is linked to pre-industrial whaling genetic diversity.

Two other studies looking at historic and pre-historic whale bones in past years have come to similar results in terms of genetic diversity. For example, Borge (2007) investigated the genetic diversity of 99 bowhead whales collected in Spitsbergen, concluding that the ancient bowhead whale population had a similar genetic variability despite a severe decline in population, and suggesting a migration between stocks around Spitsbergen and the Chuckchi-Beauford Stock (Borge 2007).

In an ongoing study, Anderung et al. (presented at ISBA 2010 in Copenhagen and pers. comm. 2010) sampled sperm and blue whale individuals from museum samples at the Natural History Museum in London. These samples were collected during industrial whaling in the eighteenth and nineteenth century. In their study, Anderung et al. found 24 new haplotypes in blue whale samples, but no increase in genetic diversity for sperm whale samples. Estimates for blue whales suggest that the population was reduced from 239 000 animals in the Antarctic to 360 individuals in 1960. Today, the population has recovered to 2280 individuals. On the other hand, sperm whales had an estimated 1.1 million individuals. This population was hunted in two steps, both with open boat and the full industrial use of exploding harpoons. In the end, an estimated 360 000 animals were left in the ocean (Whitehead and Planck 2002).

While only ancient DNA can accurately test the pre-industrial whaling population genetic diversity, the population size did not change from earlier estimates that were based on modern samples. Generally, both ancient and modern estimates are relevant to modern biologists and conservation to identify the current population levels and to estimate appropriate hunting quota in a changing habitat of the twenty-first century.

The finding that genetic diversity was similar over the past 4000 years without any significant loss due to commercial whaling explains the swift recovery and the growth rate of 4-7% annually (Calambokidis et al. 2008).

Population size

Based on conventional information, the North Pacific humpback whale population was reduced to an estimated 1500 individuals by 1960, but today has grown to 18 000 to 20 000 individuals (Calambokidis and Barlow 2004). Initial population size estimates (Rice 1978, Perry et al. 1990, Baker 1993) for North Pacific humpback whales were between 15 000 and 20 000 animals prior to industrial whaling. The analysis of the ancient DNA data suggests genetic diversity has not changed much through industrial whaling**.** Using Bayesian skyline plot analysis, the estimated effective population size prior to industrial whaling in Barkley Sound was between 1500 and 2500 individuals. However, the analysis also indicates that the d-loop did not have sufficient coalescence relevant information to construct a reliable skyline plot, suggesting that additional loci are needed to investigate the pre-industrial whaling population size of

humpback whales in the North Pacific.

An annual population increase of 4-7% was reported in 2009, based on data collected during the SPLASH project (Calambokidis et al. 2008). While the overall rate appears to show that humpback whales have recovered well, the complexity of feeding and breeding ground populations has left specific groups at risk. For example, the feeding aggregate of Washington/southern BC (of which Barkley Sound is part of) is an estimated 200-400 individuals while northern BC/southeastern Alaska is an estimated 3000-5000 individuals (Calambokidis 2008, 2009). Similarly, Eastern Pacific humpback whales found off Japan are believed to be at a vulnerable low density.

The possible increase in population has been modeled by Zerbini et al. (2010) and increase rates of 7-8% per year were described as based on “optimistic parameters”. Higher estimates published previously were deemed unlikely to impossible (Zerbini 2010, Clapham 2001, Mizroch 2004, Gunnlaughsson and Sigurjonsson 1990). In cases with very high observed population growth rates, other factors such as the increase in observation or migration between populations might have played an important role (Zerbini 2010). Retaining their population genetic diversity may help the surviving humpback whale population to rebound faster towards pre-industrial whaling levels. If current population sizes exceed the estimated pre-whaling population size without indicators of a halt or reduction in growth rate, it is likely that the previous size has not been reached yet (Palumbi 2002).

While the Bayesian skyline plot indicates a long term stable population

size, further analysis reveals that the d-loop region used in this study lacks the information to accurately infer the past population size of humpback whales. Neither a change in modern or ancient DNA dataset nor a change in tree priors modified the outcome of the skyline plot substantially. Additional analysis were run with 70 ancient DNA samples, removing the 0-500BP time zone that may be a mischaracterization of the population due to sampling error and 35 ancient DNA samples which was the minimum number of individuals without the last 500 years as well (table 12).

If this dataset, which includes temporal data ranging from 500 to almost 5000 years is not informative enough because the first 344bp of the humpback whale d-loop was used, modern conservation efforts relying on the same fragment may misestimate the past population size and carrying capacity, which in the worst case scenario would threatened a vulnerable population by overharvest. Roman and Palumbi (2003) already pointed out the need for additional loci to be included to provide a more accurate population size estimate.

In the case of humpback whales, the d-loop sequence lacks the amount of information necessary to create a reliable population demographic. The flatness of the skyline plot points towards a lack of coalescence historic relevant information, as does the high 95% HSP. The lack of evolutionary information is partially due to the long generation length of 16-20 years and the general slow mutation rate. As Baker and others pointed out before, these may be reasons why a change in population genetic diversity cannot be detected (e.g Baker and

Clapham 2002, Baker et al. 1998).

It has been suggested previously that additional loci, specifically highly informative nuclear loci, need to be added to the analysis to improve the resolution necessary in this case. For example, Hart and Marko (2011), point out that additional loci may provide more information than adding additional individuals. Analysing the present dataset with 105 samples, 70 (without 0-500BP), 46 and 35 respectively, supports this notion as neither of these datasets provided new information, with exception to resolving the artifactual decline in population size around 500-1000BP.

Based on the preliminary results, nuclear DNA is well preserved in the Barkley Sound samples. The herein attempted sex identification using SRY with ZFX/Y as a positive control has been used by most published studies analysing the demographic of cetacean pods.

While this is a preliminary result, the data matches the hypothesised ratio. Humpback whale pods are thought to have a roughly even distribution of male and female animals (Ramp et al. 2010, Brown et al. 1995) in the feeding grounds.

In the larger scope of this study, identifying male and female individuals did not yield the required demographic data. In order to satisfy the need for population demographic data, more individual-specific loci such as STR (following the example by Valsecci et al. 1997) and SNP loci would be more suitable. Using a multiplex of sex-specific and autosomal STRs would have allowed both the sex identity of the individual and also delivered information on

highly variable loci, allowing for a more efficient identification of individual whales. A multiplex of X and Y chromosome-specific loci as well as autosomal loci would help to assess the number of individuals present in the dataset. Ancient DNA studies have successfully used multiplex STR and SNP systems (von Wurmb-Schwark et al. 2009, Kaestle and Horsburgh 2002, Schmidt et al. 2003, Haak et al. 2005). STR loci have been identified in humpback whale samples and could be an appropriate starting point. Often, STR primer systems can be optimised to match the requirement of short amplicons while conserving the locus in question. However, if looking towards coalescence based analysis such as population size changes, alternative fast evolving loci may be more suitable

This study shows that nuclear DNA is retrievable from archaeological humpback whale bones and therefore can play an important part in population demographic analysis. For studies involving archaeology and conservation biology, sex identification should be done using markers such as STR and SNP suitable for population genetic diversity studies and coalescence statistics. By doing so, archaeological data would seamlessly fit into larger datasets of modern whale biology.

However, when adding additional loci, demands are different from current loci used in ancient DNA analyses. As Ho et al (2011) point out, rather than just screening for highly polymorphic loci, highly informative loci with a faster mutation rate are more appropriate. Similar to modern geneticist using statistical programs such as STRUCTURE (http://pritch.bsd.uchicago.edu/structure.html) to estimate the amount of STR loci needed for an accurate population

characterization, potential loci should be tested for mutation rates and general suitability prior to the beginning of such study. While mitochondrial DNA and especially d-loop has been the go-to loci for analysis using ancient DNA for reasons discussed previously, but a case like this one clearly shows the inadequacy of d-loop as a single locus. While this study still provides relevant information on the pre-exploitation genetic diversity and comparison between estimates of past and current population size, in order to obtain an accurate population size estimate, additional loci need to be included in future studies.

Because the current management procedure of the International Whaling Commission (IWC) is based on the identification of discrete feeding and breeding grounds (Jackson et al. 2007, Baker and Clapham 2004, Reeves 2002), the identification of a distinct feeding ground and its population size is relevant for improved management decisions. Specifically, the low population size in southern British Columbia of around 400 individuals would lead to a different catch quota, if any, than the feeding ground in California or southeast Alaska.

The current estimate of 200-400 humpback whales in the southern BC/northern Washington feeding ground is probably still lower than the original population and certainly lower than modeled in this study: an estimated 6000 thousand whales were hunted between 1905 and 1966 in British Columbia, with an additional thousand taken in California (Nichol et al. 2002). Current estimates on pre-exploitation humpback whale population size have been estimated by adding the amount of recorded hunted whales to the current population size estimate. The population size of the ancient population found in Barkley Sound

would have been at least within the same size range as California/Oregon or southeast Alaska today, given these historic data as well as the estimates found using ancient DNA data, even if migration between feeding grounds is taken under consideration.

Analysis to estimate the historic population size and genetic variability of humpback whales has been published both based on historic records and genetic data. Rice (1978) predicted a pre-industrial whaling population size of 15 000 individuals. Based on whaling logbooks and recent mtDNA analyses, Roman and Palumbi (2003) estimated the historic population size of Atlantic humpback, finback and minke whales to be up to twenty times higher than the current estimates of the IWC. The implications of these results are a long term whaling ban until a sufficient population size is reached. This study was challenged in several follow up publications, criticising both the assumptions as well as the techniques used (Clapham et al. 2006, Holt 2004).

Based on the here presented analysis, neither of the mtDNA based population size estimates previously published may be correct. In these cases, comparing estimates based on logbooks and historic documents to DNA based estimates may narrow down a ballpark number. However, a comprehensive understanding of actual historic population size is needed to correctly assess the current status of humpback whale populations. To obtain more precise data, a large-scale effort of several research teams has been collecting skin samples and fluke sightings over the past years. SPLASH now has over 6000 genetic data of worldwide humpback whale individuals (Calambokidis et al. 2008). New

data will likely not be solely based on mitochondrial DNA but include nuclear markers as well.

As noted elsewhere (Baker and Medrano-Gonzalez 2002), the insight into past population size and adaptation to climate change may become relevant amid current climate trends. While environmental changes and possible subsequent changes in carrying capacity are part of the bigger picture, conservation biology investigates optimal management units, which in this case may be breeding aggregates.

Humpback whales migrate between their summer feeding ground in southeast Alaska/northern British Columbia, southern British Columbia/Washington or Oregon/California and their winter breeding and calving ground in California, Mexico and Hawaii (Baker et al. 1998, 2002). However, analyses of nuclear DNA, inherited both maternally and paternally, indicates less geographical group distinctions, suggesting that all three groups are breeding in the same area, and that the geographic mtDNA patterns are the result of female whales returning to their own “birth place” to calve (Baker et al. 1993). With more data available today, the complexity of North Pacific humpback whale populations has become increasingly apparent with several feeding and breeding ground (Baker et al. 2002, Witteveen et al. 2004; Calambokidis et al. 2008) and strong site fidelity towards the feeding ground as opposed to breeding ground. The haplotype diversity supports previously described theory that the maternally directed fidelity following colonization of new feeding grounds has persisted for thousands of years (Baker et al.1990). Since shared haplotypes could be caused

by either gene flow or a recent divergent time (Marko and Hart 2011), modern biology needs to clarify the extend of which modern migration pattern have influence on the genetic differentiation. The complicated migration pattern begs the question in how far the lack of a definite modern comparative population would interfere with the analysis. This is potentially a problem because while general migration patterns are understood, gene-flow and individual migration patterns are still poorly observed.

Beerli (2004) found that missing populations do not necessarily pose a problem, mainly depending on the immigration rate into the population in question: the deviation from the true population size increases when immigration increases. In populations with low or moderate migration rates, Beerli expected more accurate parameter estimates.

Given the humpback whale migration pattern, population size should be estimated using breeding and feeding grounds and compared to investigate how accurate each of these results is. Effective population size estimates in the Bayesian skyline plot only changed slightly when using 19 samples from Washington and Oregon as modern sample set as opposed to 100 samples from Washington/Oregon/Mexico. However, it would be crucial to use a population from Barkley Sound in future analysis to increase the robusticity of the analysis.

Migration has only been estimated between ocean populations so far (Baker and Medrano-Gonzalez 2002, Baker 1990) except for one study on gene flow (Baker 1998, Rizzo and Schulte 2009) for male DNA. A positive effect of sampling feeding grounds, located in more temperate to cold climates, may

mean better preservation of nuclear DNA for additional marker analysis. Adding additional feeding grounds will also provide a more comprehensive estimate for the entire ocean.

The lack of an informative loci (d-loop) as well as the intricate migration pattern may mean that the effective population size estimated from this dataset may not depict the complete population, given the complexity of the population found in the North Pacific today. This restriction is also a problem for previous studies estimating maximal 24 000 animals in the North Pacific (Clapham 2005, Calambokidis 2008, 2009). In order to estimate the population size for the entire North Pacific, nuclear loci and more feeding grounds need to be sampled to establish a more accurate pre-industrial whaling population size. In addition, direct dating of the samples would increase the time estimates of changes in the past.

The initial estimate of pre-industrial whaling humpback whale census was based on whaling records, primarily log books of whaling vessels (Rice, 1978). However, especially after the establishment of the International Whaling Commission and the subsequent ban on whaling, those whaling reports became politically sensitive. For instance, log books from Soviet whaling ships demonstrate examples of massive underreporting, one of the incidences that might have skewed initial estimates (Clapham, 2009, Jackson 2007). In addition, log books only recorded caught whales but not *struck but lost* (animals that were harpooned but not retained by the whaler) or, in some cases counted in “blue whale unit” instead of individual animals. In cases like this, ancient DNA could

provide an additional line of evidence to test the accuracy of historical documents. As Palumbi and Roman pointed out, historical data needs to be seen in context, especially recognizing the limited precision of these estimates (Palumbi and Roman 2007). Using DNA to investigate current and past population size is therefore an important independent line of evidence. This study highlights that ancient DNA has the capability to add an additional line of evidence to studies in modern biology, independent of historical documents that might be missing or recorded inaccurately. The three parts (historical data, modern DNA and ancient DNA) serve as controls against each other to achieve the most precise results. However, this will only be possible when DNA based estimates are not based on the d-loop sequence alone but have been re-estimated using additional nuclear loci. This is true for both aDNA and modern DNA. Finally, gene-flow between populations needs to be quantified to chose accurate analysis units and accurate models for statistical analysis. Future analysis will need to include additional loci as well as alternative statistical analysis, to test the robusticity of the analysis.

While this is not the first research done on ancient whales, it is one of the most extensive; being able to compare humpback whales to other studies unveils that pre- and post-whaling genetic diversity and population size can help to evaluate the trajectory of a species' growth and genetic diversity.

Conclusions

For this study 105 humpback whale samples from two archaeological sites were analysed using ancient DNA techniques. The goal of this study was to investigate the genetic diversity of humpback whales in Barkley Sound, southern British Columbia, to compare these results to modern data, and to investigate the population size of humpback whales based on ancient and extant data. To obtain comparable results, the 344bp of the humpback whale D-loop sequence were analysed. The genetic diversity found in the ancient DNA dataset fits the modern population genetic diversity of North Pacific humpback whale feeding aggregations. The haplogroup variation found differs from published data of adjacent feeding grounds, but may match the unpublished haplogroup frequency of southern British Columbia/ Washington feeding area. Results indicate only a minimal, if any change in genetic diversity between ancient and extant data. Bayesian skyline plot analysis revealed an effective population size between 2500 and 1500 prior to 1000 BP. However, the Bayesian skyline plot analysis failed to infer changes in past population size because the d-loop does not provide necessary information for a coalescence based analysis, making the estimated population size a preliminary result until additional loci have been added. Results of this study confirm modern estimates of humpback whale mtDNA genetic diversity and support the presence of a separate feeding ground at Barkley Sound. Based on the data found here, North Pacific humpback whales have retained most of their genetic diversity despite a loss of 80-90% of their original population size.

Using ancient DNA analysis of archaeological samples to investigate pre-impact genetic diversity has the advantage to reach into time periods not covered by written records or ethnographic data, revealing long term environmental records. Thus, while ancient DNA does not provide a significant change in our knowledge of humpback whale population size immediately prior to industrial whaling, it can contribute to the long term data of humpback whale phylogeography.

Chapter 7: Final Discussion

Archaeology and Biogeography

"Simply stating in an essay that zooarchaeological data are relevant to a conservation problem was an insufficient warrant for its inclusion here. Similarly, simply documenting that the structure of a prehistoric ecosystem was different from a modern one was insufficient." (Lyman and Cannon, 2004, p4)

Lyman and Cannon use this as inclusion criteria for case studies in their 2004 book "Zooarchaeology and Conservation Biology". In order to make a case study relevant to conservation biology, it needs to address current or upcoming problems from a zooarchaeological perspective.

This study investigated the antiquity of whaling and the biogeography of humpback whales in Barkley Sound, aiming to provide data for whale biogeography and possibly conservation biology. In theory, molecular archaeology can contribute to modern biogeography by providing data on past populations and environmental conditions in a similar way as zooarchaeology can contribute to wildlife management. While ancient DNA has been contributing to biogeography, this study tested how molecular archaeology can provide a unique, archaeological perspective.

After carrying out the presented research, the hypothesis that ancient DNA improves the ability of archaeology to contribute to conservation genetics cannot be refuted. However, it should be modified; that is, the impact of human

behaviour on a population, and therefore the impact of archaeological interpretations on datasets, depends on the questions asked and on the genus/species analysed. As discussed in more detail below, while the analysis of ancient humpback whales reinforces results from modern studies, investigations of other species may change the way conservation biology approaches a population. Differences may be due to shorter generation length, faster evolution or having undergone a genetic bottleneck. Molecular archaeological studies on marine mammals and fish from archaeological contexts for biogeography questions will undoubtedly benefit from archaeological lines of evidence pertaining to human behaviour, culture and settlement pattern, as this will help distinguish human behaviour from natural changes.

Despite these modifications, it is unquestionable that ancient DNA has had an enormous influence in all applicable fields and that this research also highlights the possibilities to expand the way data can be interpreted. Previous research has highlighted benefits from the ancient DNA field (Burger et al. 2004, Bunce et al. 2009, Yang et al. 2008, Yang et al. 2005), but often was carried out in isolated circumstances within the field. Molecular archaeology has successfully investigated life ways of ancient people and their environment (Haak et al. 2005, Kaestle and Horsburgh 2002, Losey and Yang 2007, Speller et al. 2005). Ancient DNA has become a regular part of biogeography and is increasingly relevant to our knowledge of a species' history (Di Bernardo et al. 2004, Mantooth and Riddle 2011). Ancient DNA research has been applied to phylogenetic and phylogeographic analysis for conservation biological research before, in some

cases using archaeological material. In previous ancient DNA studies, archaeological samples were often analysed without including cultural or ethnographic data relating to site occupation, continuity or hunting practices, leading to potentially incomplete results in relation to the biogeographic history of a species (Ludwig et al. 2008, Ludwig et al. 2009).

Zooarchaeology has been analysing ancient life ways, human-animal relationships and environmental changes for decades. Including ancient DNA improves not only the specificity of taxonomic identifications, especially in fragmented datasets, but can also detect changes in the species' genetic variability, and distinguish between populations (Bollongino et al. 2005, Hadly et al. 1998, 2008a, Hofreiter et al. 2004). In these cases, ancient DNA improves zooarchaeological research to contribute data pertaining to changes in pre-historic population distribution. These results are not only relevant to biologists, but also to archaeologists while studying, for example, prey choice and subsistence of ancient people. On the other hand, zooarchaeology has paved the way for this study, providing almost exclusively baleen whales to be analysed in this study. A sample set unanalysed by a zooarchaoelogist would have contained a large amount of other cetacean, especially dolphins, which would have cost researchers invaluable time and resources, and invariably would have led to a smaller sample size relevant to the scope of the study.

In her 2008 paper, Leonard outlined how ancient DNA analysis can benefit wildlife conservation by providing prehistoric population and species identifications, gene flow, as well as information on geographic distributions.

While in most cases palaeontological material is the preferred material, the use of archaeological material is sometimes the sole avenue if fauna are not found otherwise, such as fish and sea mammals, or can increase the sample size because of hunting activities (Leonard 2008). This is the case with humpback whales, which otherwise would be invisible in the palaeontological record.

As an alternative to archaeological samples, ancient samples could be obtained from historic museum samples where possible. This has recently been done for the North Atlantic using sperm whale and blue whale samples (Anderung, ISBA presentation Copenhagen 2010). Samples used were obtained from eighteenth century industrial whaling ships. The advantages of museum samples can be a more secure and detailed dating of the individual bone as well as a better estimation of the number of individuals (provided that museum provenience data are accurate). The disadvantage of museum samples is that in many cases the time depth of available samples is only sufficient to detect the most recent population diversity, but cannot detect long term variability. Archived samples may help to detect diversity lost through industrial whaling, but the possibility of detecting lost diversity due to natural causes remains low. Finally, using museum samples from whaling ships obviously does not allow further investigation of the human behaviour of pre-industrial whaling hunters as done in this study. Taken together, remains from archaeological sites are not inferior to non-archaeological sampling.

Archaeology has the unique expertise to provide insight into past human behaviour leading to the formation of a zooarchaeological assemblage, as well

as taphonomic processes which affect those assemblages through time. Ethnographic data may also elucidate human practices that influenced the assemblage and the knowledge that was transferred through generations about these species. Molecular archaeologists have the experience required to analyse archaeological assemblages, including experience relating to questions of DNA degradation and contamination concerns. On the other hand, conservation biology brings the experience and theory of modern population genetics, population migrations, and other animal behavioural issues into the equation. This data can help in interpreting the assemblage from an environmental point of view. In the case of whales, it is unlikely that large numbers of whale samples can be obtained from palaeontological deposits. Additionally, anomalies in an ancient genetic dataset can be compared to the archaeological record to identify possible changes in human behaviour or, alternatively, environmental changes. Ethnographic records may also provide important data relating to animal behaviour, for example the reports from Barkley Sound of whales during the summer and the presence of whales in a particular area year round. This ethnographic information can help direct possible study areas for molecular environmental archaeology.

In the case of whales, using archaeological data is probably the only opportunity to investigate long term migration patterns and feeding ground identity because the presence of ancient whale bones is tied to human behaviour. In the case of humpback whales, archaeological and ethnographic data does not provide unique or essential information relating to biogeography or

conservation biology, beyond the ancient DNA results. However, ancient DNA provides pre-industrial whaling genetic diversity of humpback whales in Barkley Sound which is not just valuable to establish a baseline of pre-whaling mtDNA genetic diversity, but also includes a population size estimate.

Apart from whales, there are many potential avenues when archaeological data becomes relevant, such as when studying the absence or presence of archaeological species to determine which species to reintroduce as the most "natural". For example, the introduction of the Coho Salmon in the upper Columbia River was assumed to be a re-introduction of a lost population. This measurement was deemed necessary after four dams altered the spawning habitat of salmon in the river in the 1970s and increased the mortality (Kareiva et al. 2011, Gustafson et al. 2006). Similarly, the reintroduction of sturgeon into the Baltic Sea required not only the knowledge of species appropriate for the current conditions but also the historic species present in the Baltic Sea (Kirschbaum et al. 2009, Gessner, 2006). Moreover, ancient DNA analysis of shell midden remains can determine the presence of species (for example sturgeon or salmon) while using contextual archaeological data can help to evaluate the settlement pattern. A seasonal settlement pattern might coincide with the absence of certain species, leading to a false negative result over the presence of a species.

In terms of conservation biology, ancient whale population diversity, and the changes in population diversity and size help modern biologists to refine theories about a species' future survival and adaptability. These types of long

term genetic analyses are especially important when studying a species with a slow mutation rate such as whales. Sampling from both archaeological sources and historic museum specimens can reveal a wealth of information, albeit focused on different time periods. If available, museum collections can deliver more samples from a narrow time span, safely identified as different individuals. Often, additional information has been collected such as the species and sex. Archaeological samples, on the other hand, may be collected from a much longer time frame and provide insight into antiquity and change of human behaviour.

While archaeology has the expertise to investigate human behaviour based on an excavated site, this human behaviour is not necessarily relevant to the research question. In terms of hunted fauna, relevant human behaviour includes the seasonality of hunting activities, prey choice, including the choice of phenotypic characteristics, and finally the treatment of the carcass after the hunt, which may include species- specific ritual or secular treatment. Because research involving species identification relies on visible markers also chosen by people, hunting may skew the environmental analysis. On the other hand, DNA analysis relies on mostly invisible markers which cannot be chosen by the hunter. The only exception here could be seasonality, if for example two populations inhabit a region at different times, as is the case with transient killer whales and resident killer whales (Pilot et al. 2010). A different treatment of species depending on the cultural perception could lead to incorrect ethnographic identification of species targeted during these ancient whaling expeditions. Differential treatment of bones depending on hunted vs. beached or preferential

hunted vs. alternatives, leading to differences in bone presence and completeness depending on the species. By using ancient DNA the possibility of biased sampling was removed.

People in Barkley Sound have been hunting whales for more than 5000 years. Over the past 2000 years, species other than humpback and grey whales were caught more frequently, possibly due to an improvement in hunting technology. While there are plenty of historic records, both written documents and logbooks of industrial whaling ships, records of native whalers is more scattered despite its equal relevance. By unveiling native whaling “records” (through ethnographic or oral traditions), questions can be answered concerning both human behaviour and whale biology. Highlighting the antiquity of whaling in non-western civilisations can aid the survival of these traditions and emphasize the prolific ability of these people. In addition, the change of culture and the development of hunting techniques can be inferred from archaeological and ancient DNA data, especially when early hunting tools are lost to taphonomic processes. Ancient DNA can counteract the uncertainties to which historical records are generally subject. Using ancient DNA adds a solid line of evidence in terms of species diversity at a site, independent of the bone fragmentation, as well as the state of a population via population genetic diversity and population size estimates.

The analysis of pre-industrial humpback whaling at Ts’ishaa and Huu7ii suggests a stable population in terms of population size and genetic diversity over more than 4000 years in Barkley Sound. The presence of a feeding

aggregation would have supported the development and maintenance of a whaling culture. Based on the ancient DNA data from this study, it is unlikely that the population at Barkley Sound was a non-migrating pod as previously assumed by archaeologists. Studies on the non-migratory whale subpopulation in the Arabian Sea indicate that local populations are distinct from other pods both genetically and morphologically (Baldwin et al. 2010, Mikhalev 1997). This does not seem to be the case in Barkley Sound. While most whales migrate bi-annually, moving between feeding and breeding ground, analyses have indicated that not all individuals migrate, and some only migrate part of the way (Stevick 2002, Valsecci 2010). Zooarchaeological data account for an ongoing presence of humpback whales over 5000 years at least while ancient DNA results indicate a continuum in population genetic diversity, suggesting that Barkley Sound was a feeding ground then as it is now. If Barkley Sound had not been frequented by the same population, ancient DNA genetic diversity would reveal a different signature from reported modern populations.

The fact that humpback whale genetic diversity is on a similar level today as it was prior to industrial whaling helps to define some long term goals for whale conservation. Having a benchmark of genetic diversity allows us to evaluate population growth found today or lack thereof. Humpback whales in the North Pacific have had a remarkable population growth rate over the past 30 years, while other species, including right whales, have not. Having long term data of conditions in Barkley Sound will help determine the optimal course of action towards sustainable use of humpback whales. Based on the analysis of

mitochondrial haplotypes in this study, humpback whales in this region should be able to recover. However, because of the multifaceted nature of this issue, no definite conclusions can be drawn from one analysis. Analysing nuclear DNA, a different site, as well as ongoing environmental changes will play a vital role before any recommendations can be made. However, based on the results obtained here, the protection of whales may have been timely and successful, and modern genetic data accurately reflect pre-whaling population size. Any management decisions, however, need to be tailored to the local feeding and breeding grounds, given variability of genetic diversity and population size.

If modern data confirms the haplotype diversity in southern British Columbia, the long term stable haplotype diversity could help to make a case to treat the population as a separate feeding aggregate for whaling and catch quota negotiations (Clapham et al. 2008). Further ancient DNA analyses in other feeding grounds can help to answer questions of “what to preserve” such as population boundaries that may have shifted due to a reduction in population size, playing a vital part in answering the question of human impact on humpback whale and subsequently other large cetacean populations. In this case, the apparent absence of changes in population genetic diversity would indicate that while the population was reduced substantially, genetic diversity was preserved, minimizing the long term impact of human interaction. While data from archaeological sites are too recent to detect changes in humpback whale population diversity, it would be possible to detect changes in humpback whale behaviour such as changing migration patterns, which would be genetically

visible in a change in haplotype composition (Baker and Clapham 2004, Baker and Medrano-Gonzalez 2002, Calambokidis et al. 2008, Clapham et al. 2008). In the case presented here, no such change was detected. In addition, to accurately detect migration pattern and changes, multiple feeding grounds would need to be examined (Palumbi 2011). Finally, adding further data would also support the accurate assessment of pre-industrial whaling humpback whale population size for the entire North Pacific.

In case of cetaceans in Barkley Sound, using archaeology and ethnographic data was necessary to interpret the ancient DNA based species identification data correctly but was not necessary to evaluate the biogeography. If compared to Lyman's use of zooarchaeology to conservation biology, ancient DNA improves the zooarchaeological dataset and can do so for other faunal datasets, as well providing conclusive species identifications and genetic diversity markers. Whaling as a high status occupation limited the access to this resource. Moreover, hunting technology developed over 5000 years and eye-witness reports suggest low success rate at times. These ethnographic and archaeological observations support the assumption that traditional whaling techniques probably did not strongly influence the size or composition of whale population in pre-contact time periods. Changes in population genetic diversity and population size through time would have been therefore due to natural causes (in pre-contact times) and more importantly, to the well-documented industrial whaling practices in historic times. While the species composition of the archaeological dataset changed over time, the genetic diversity of humpback

whale did not change substantially, indicating a stable and probably resilient population amidst changes in climate over 4000 years.

In conclusion, molecular archaeology can provide important information for biogeography and possibly conservation biology, especially in the case of marine life that would not be available if not hunted by human settlers. Riddle et al. (2008) point out that palaeontological sites available for phylogeographic studies are rare. Archaeological sites may have species present over centuries and millennia, documenting not only human subsistence practices but also a repository for phylogeographic data. Only DNA analysis can provide the detailed data, unaffected by short term environmental fluctuations, to observe changes over time, making archaeological material directly comparable to modern DNA studies.

The value of archaeological and ethnographic information in interpreting the ancient DNA data depends not only on the question asked but also on the type of fauna analysed. The strength of zooarchaeology to identify genera, species and abundance has been integrated in ancient DNA analysis and can be vital to the analysis, along with archaeological site information, especially in biogeography studies.

Sampling

For this study, 264 cetacean identified samples were analysed using ancient DNA techniques. Results suggest that archaeological sample sets are

viable for population genetic diversity studies such as this one. Neither hunting nor zooarchaeological analysis/selection has led to a non-random selection. The species identification of both sites resulted in similar species frequencies. The population genetic diversity fits the expected outcome when compared to modern data in the North Pacific and preliminarily seem to match the modern population in this area.

While the hunting of land mammals might skew a zooarchaeological dataset because hunters can choose for phenotypic characteristics such as overall size, fur color/length or antler size, the hunt for humpback whales was unlikely based on phenotypic choice but rather on availability. The results of this dataset indicate that hunters selected certain species of whale, but within that species, genetic diversity correctly mimics the source population.

The zooarchaeological nature of the dataset had additional advantages. The sampling by a zooarchaeologist who is familiar with the site helps to maximise the number of individuals. The zooarchaeologist who chose the samples for Ts'ishaa tried to sample each specimen once based on his expertise (McKechnie pers. comm. 2010). Species identified were consistent with ethnographic description of hunting practices. In this study, all except for one sample were large baleen whales, increasing the amount of humpback whales present in the dataset. Without the support of zooarchaeology, the sample set would have included larger amounts of non-baleen cetaceans. Zooarchaeological selection for this study probably created a bias in the species identification because only large cetaceans were sampled (McKechnie pers. comm. 2010,

McMillan pers. comm. 2007, 2010). A future smaller sample set should be tested to test the true species diversity of cetaceans in general at Ts'ishaa and Huu7ii.

The match of general species distribution between zooarchaeological analysis (Monks et al. 2001) and ancient DNA analysis indicates that in sites with reasonable preservation of whale bones ancient DNA analysis may not be necessary for species identifications. However, in cases like Ts'ishaa and Huu7ii, the high degree of bone fragmentation would not allow for confident taxonomic identifications of the material. Ancient DNA analysis provides further information such as changes in species genetic diversity and can help narrow down the number of individuals if appropriate markers are selected (STR or SNP analysis). This information would be helpful in a study narrowing down the MNI and to correlate changes in culture to changes in species abundance. If only species identification is needed, a smaller sample size would be sufficient. Davis (2010) reports that, in a zooarchaeological setting an increase by two magnitudes (100 samples to 10 000 samples) is needed in order to double the number of species in a dataset. The chi-square test comparing Ts'ishaa and Huu7ii and the comparison of species frequency between the two sites are a further indicator that the sampling here is adequate for a species identification study at these archaeological sites.

Future sampling should take genetic properties of the sampled species into account. Because of the slow mutation rate of the humpback whale D-loop and long generation length of the humpback whale, samples should be selected from fewer time periods to improve discrete sample populations over a longer

period of time instead of trying to cover the entire 5000 years. Using archaeological datasets allows for the selection of dated horizons while still collecting sufficient amounts of data. If sampling was isochronous instead of comprehensive, the collected samples would still be viable for archaeological questions. Research using rodents in the Yellowstone National Park, for example, implementing serial sampling of discrete time periods, successfully distinguished environmental changes and population response over several thousand years (Hadly et al. 2004).

The initial idea was to use the haplotype and sex of an individual, in combination with the archaeological provenience information to estimate the number of individual whales. However, even these three variables could not provide a meaningful MNI. While it was possible to distinguish some individuals based on dates and haplotype, all variables are afflicted with uncertainty. Non-recombinant sequences, such as mtDNA, can be used to trace lineages but are unsuitable for individual identification. In this case, nuclear DNA markers would have been helpful. A multiplex system using sex chromosomal and autosomal loci would have provided an optimal amount of information including sex identification and population diversity markers. Second, indirect dating through layers obscured the actual age of the sample. In addition, the date associated with the layer does not necessarily correlates with the year of death, as people might have used the bone for a while before discarding it. While using excavation units as part of the individualisation process is an artificial classification, it helps to account for an estimate of individual whales present at the site.

Overall, results in this study strongly support that the archaeological dataset was sampled on a random basis, providing reliable species diversity as well as humpback whale genetic diversity even with the above discussed uncertainties.

Future studies

This study investigated the antiquity of whaling in Barkley Sound and the humpback whale population genetic diversity in the area. While results support that people were hunting whales over the period analysed and the population remained stable over the last 5000 years, questions remain that are worth investigating in future studies. Two of the possible studies are outlined below.

1. Investigating the age of whaling

The current results support the interpretation of whaling in Barkley Sound but could not identify the onset of indigenous whaling. Future studies should investigate sites older than 5000 years that exhibit signs of whale use. Results here indicate that samples around 5000 BP are well preserved and ancient DNA has the capability to investigate much older samples. Based on previous research, two scenarios are possible: first, it is possible that future investigations will not find a change in species distribution because whaling traditions and technology was introduced by early settlers to Barkley Sound before 5000 BP.

Thus, the trial phase did not happen on Nuu-chah-nulth territory but prior to the first settlement. Alternatively, researchers may identify the onset of whaling. People probably used stranded whales before they actively hunted whales so the species diversity should decrease at around the time that hunting was initiated. Data should be added to cover the time gap (3000-2000) in this study to investigate if changes in whaling technology as reported from the archaeological record, can be linked to changes in species diversity during this time period (Monks et al. 2001, Huelsbeck 1988, Monks 2003). It is possible, that non-DNA zooarchaeological research may not detect this change because of a high fragmentation, making it impossible to accurately identify the change in species distribution.

Finally, this dataset can be used as a comparison to analyse sites elsewhere, for example Nootka Sound. Because the political situation was different, it is possible that zooarchaeological remains in sites are different, both within the Sound as well as between Nootka and Barkley Sound.

2. Investigating genetic diversity in the North Pacific

This study only investigated Barkley Sound and the population using this area as feeding ground. Further feeding grounds in the North Pacific should be investigated to confirm conclusions drawn in this study. Specifically, feeding grounds that were hunted earlier and longer would be beneficial as well as areas of low extant population density and diversity. By comparing feeding grounds, investigations into the antiquity of current migration patterns can be investigated.

In addition, the population size of the entire North Pacific can be established by adding a broader geographic range. Future studies need to include nuclear DNA to improve the resolution of the genetic data. Preliminary results in this study indicate the preservation of nuclear DNA, even in older samples. Lessons learned from this study will also help to improve the sampling strategy for future studies with similar research questions. If available, sampling should be expanded back in time to allow for possible changes. As seen in this study, zooarchaeological data helps greatly to narrow down sample sets. Nevertheless, sites from other areas would need to be tested prior to future studies to establish the presence of humpback whales in sufficient quantities

Conclusion

In this study, whale samples taken from shell middens were analysed using ancient DNA for species identification and D-loop haplotype. Previous archaeological research indicated the use of large whales over the entire time depth of the sites analysed. Ancient DNA results indicate that whaling took place in Barkley Sound for at least 5000 years and that people drew from a stable population of humpback whales using Barkley Sound as a feeding ground. While the sampling strategy in this study can be considered appropriate, improvements could be made: future whale research should consider obtaining more samples from discrete time periods to cover more time as opposed to sampling all-over. Considering the slow mutation rate of the humpback whale D-loop this approach

would not lead to a loss in information. Future studies should be expanded both in time and geographically, including small scale expansions to neighbouring Sounds. Studies like this would provide useful information to whale management in terms of long term population boundaries or changes in populations, especially when reaching further back in time.

Finally, this research highlights both the possibilities as well as the limitations of archaeological data. While in some cases additional information are needed to correctly identify changes found in a dataset, in some cases archaeological data of human behaviour are not needed to use zooarchaeological sample sets. In those cases, the contribution of archaeology is solely through providing the material as well as zooarchaeological and ancient DNA data.

Chapter 8: Conclusions

The goal of this study was to use previously excavated whale bones from two Nuu-chah-nulth archaeological sites to investigate the genetic diversity and population size of humpback whales prior to industrial whaling and to assess the antiquity of whaling in Barkley Sound. A total of 264 whale bone samples from two sites were analysed using ancient DNA techniques. Of those 264 samples, 84% yielded amplifiable DNA which facilitated species identification. One hundred and five samples identified as humpback whale were analysed in the phylogeographic portion of the study. The DNA in the samples was generally well preserved and no contamination was detected over the course of this study.

Humpback whales have been the predominant species for at least 5000 years at archaeological sites in Barkley Sound. Grey whales were the second most frequent exploited species, though considerably less frequently than humpbacks. The whale species diversity at the two sites increases in later times (post 2000 BP). The lack of species diversity, especially the lack of potential drift whales, and the high frequency of the humpback whales, previously reported as preferred hunted species, supports the conclusion that active whaling was taking place for at least 5000 years in Barkley Sound. The increase in species frequency after 2000 BP may be due to either an improvement in technology or an increase in use of beached whales during a time of increased population size in Barkley Sound. Additional samples from earlier layers (3000-5000BP) need to

confirm whether the change in species diversity is accurate or a stochastic error.

The mitochondrial genetic diversity of the ancient DNA sample set of humpback whales is within the range of modern humpback whale populations in the North Pacific. Preliminary comparisons suggest that the mtDNA haplotype frequency matches that of a modern population in southern British Columbia. The estimated effective population size prior to industrial whaling for this dataset ranges between 1500 and 2500 individuals. The Bayesian skyline plot fails to provide a meaningful result because of a lack of information on the d-loop sequence of humpback whales, combined with long generation length of whales. Further analysis is needed to obtain accurate estimates of populations size changes in the past.

Results confirm that the genetic diversity of humpback whales has not changed substantially due to industrial whaling. While further feeding grounds and more importantly more loci are needed to establish the population size for the entire North Pacific, these results indicate that the pre-industrial whale population in Barkley Sound was likely larger than the current 200-400 individual. This is also supported by ethnographic records, accounting for whales inhibiting the passage of boats during the summer. While other areas in the North Pacific are recovering well, the population southern British Columbia/northern Washington may need considerably more time to recover before whaling can resume. Recent data of population growth and the genetic diversity of humpback whales established here, indicates that whales are recovering.

These results support the use of archaeological DNA in phylogeographic

studies of humpback whales to provide ancient population information without having to resort to potentially inaccurate or fraudulent historical whaling records.

This research highlighted both the possibilities as well as the limitations of archaeological contributions to phylogeographic research. Using ancient DNA analysis, a variety of questions for both archaeology and biogeography potentially can be answered. A collaborative approach is the optimal course of action in a destructive and expensive analysis using non-renewable sources such as archaeological bone samples. However, in some cases such the analysis presented herein, the contribution of archaeology to phylogeographic interpretation is limited for neutral markers such as D-loop haplotypes. Advances in ancient DNA techniques will increase the accessibility to nuclear markers for phylogeographic studies. Zooarchaeological analysis was an important part of this study with respect to narrowing down potential samples but no data about Nuu-chah-nulth culture or subsistence practices was used in the phylogeographic interpretation. Results of this analysis suggest a population with a similar genetic markup to the modern feeding population found in southern British Columbia. These results support the initial assumption that archaeological remains can be prime specimens for phylogeographic investigations.

This research extends the evidence for active whaling in Barkley Sound back to 5000 years from previous under 2000 BP (Nootka Sound 2100 BP, Barkley Sound 800 BP) and is the first genetic analysis in Barkley Sound. Furthermore, this is the first study investigating genetic diversity for pre-industrial humpback whale population. This dataset of 105 humpback whale samples

expands the time depth of humpback whale biogeography by 5000 years. The ability to obtain concrete information on the genetic diversity and biogeography of ancient humpback whales indicates that archaeological samples could be a prime target for future studies on large sea mammals worldwide.

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Appendix A: Samples processed including provenience

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 180	1	Dfsh7	N18-20/E34-36	06	C	S end	003.30-003.35	Unidentified (fragments)	incomplete	450-650	humpback	
WH 181	1	Dfsh7	N18-20/E34-36	07	C		003.35-003.40	Unidentified (fragments)	incomplete	450-650	humpback	
WH 182	1	Dfsh7	N18-20/E34-36	10	C	SW	003.50-003.55	Unidentified (fragments)	incomplete	450-650	xx	
WH 183	1	Dfsh7	N18-20/E34-36	11	C	NW	003.55-003.60	Rib	shaft	450-650	humpback	
WH 184	1	Dfsh7	N18-20/E34-36	12	C	NW	003.60-003.65	Unidentified (fragments)	incomplete	450-650	humpback	
WH 185	1	Dfsh7	N18-20/E34-36	16	D	NE	003.80-003.85	Vertebra (undet)	incomplete	650-850	humpback	E4
WH 186	1	Dfsh7	N18-20/E34-36	18	D	NE	003.90-003.95	Unidentified (fragments)	incomplete	650-850	humpback	E4
WH 187	1	Dfsh7	N18-20/E34-36	18	D	NW	003.90-003.95	Rib	incomplete	650-850	xx	
WH 188	1	Dfsh7	N18-20/E34-36	26	E	SE	004.30-004.35	Vertebrae, undisting.	incomplete	1100	humpback	
WH 189	1	Dfsh7	N18-20/E34-36	28	F	NE	004.40-004.45	Unidentified (fragments)	incomplete	1100	humpback	
WH 190	1	Dfsh7	N18-20/E34-36	28	F	NE	004.40-004.45	Vertebrae, undisting.		1100	humpback	
WH 191	1	Dfsh7	N18-20/E34-36	30	F	NE	004.50-004.55	Unidentified (fragments)	incomplete	1100	grey	
WH 192	1	Dfsh7	N18-20/E34-36	30	F	NE	004.50-004.55		incomplete	1100	grey	
WH 193	1	Dfsh7	N18-20/E34-36	32	F	NE	004.60-004.65	Unidentified (fragments)	incomplete	1200	humpback	CD
WH 194	1	Dfsh7	N18-20/E34-36	36	F	NW	004.80-004.85	Vertebra (undet)	incomplete	1200	RIGHT	
WH 195	1	Dfsh7	N18-20/E34-36	38	F	SE	004.90-004.95	Unidentified (fragments)	incomplete	1300	xx	
WH 196	1	Dfsh7	N18-20/E2-4		F		5.2	Vertebrae (complete)		1350	humpback	E5
WH 197	1	Dfsh7	N18-20/E2-4	40	F	NW	5.50-5.55	metacarpal		1300	humpback	
WH 198	1	Dfsh7	N10-12/E2-4	14	D	SW(NW?)	3.48-3.53	Vertebra (undet)		450-650	humpback	A1
WH 199	1	Dfsh7	N18-20/E2-4	12	C	NW	4.05	Rib		650-850	humpback	E6
WH 200	1	Dfsh7	N18-20/E6-8	31	E	SE	5.05-5.10	Phalanx (undet)		1300	humpback	
WH 201	1	Dfsh7	N12-14/E6-8	20	D	NW/SW	4.32-4.44	long bone highly weathered	1350 CAL BP?	1350	xx	
WH 202	1	Dfsh7	N10-12/E2-4	21	G	SW	3.83-3.88	Vertebra (undet)		650-850	xx	
WH 203	1	Dfsh7	N14-16/E16-18	10	B1	SE	003.90-003.95	whale rib		650-850	humpback	E8
WH 204	1	Dfsh7	N14-16/E16-18	07	B1	NW	003.70-003.75	Vertebra (undet)	incomplete	650-850	xx	
WH 205	1	Dfsh7	N16-18/E26-28	19	C	SW	004.20-004.25	Unidentified (fragments)	incomplete	900	grey	
WH 206	1	Dfsh7	N16-18/E26-28	11	B1	SW	003.85-003.90	Vertebra (undet)	incomplete	650-850	xx	
WH 207	1	Dfsh7	N10-12/E2-4	31-33	H	NW	4.33-4.48	vertebrae		1100	xx	
WH 208	1	Dfsh7	N16-18/E26-28	19	C	SW	004.20-004.25	Caudal vertebra (undet)	effectively complete	900	humpback	E5
WH 209	1	Dfsh7	N16-18/E26-28	17	C	SE	004.10-004.15	Phalanx (undet)	effectively complete	900	humpback	CD
WH 210	1	Dfsh7	N18-20/E2-4	21	D	SW	4.55-4.60	Unidentified (fragments)		1100	humpback	A1
WH 211	1	Dfsh7	N18-20/E2-4	14	C	NW	4.20-4.25	Vertebra (undet)		650-850	humpback	
WH 212	1	Dfsh7	N18-20/E2-4	14	C	SW	4.20-4.25	Vertebra (undet)		650-850	humpback	E8
WH 213	1	Dfsh7	N18-20/E6-8	28	E	SW	4.90-4.95	Unidentified (fragments)		1300	humpback	CD
WH 214	1	Dfsh7	N18-20/E6-8	31	E	NE	5.05-5.10	Vertebra (undet)		1300	humpback	E8
WH 215	1	Dfsh7	N18-20/E6-8	27	E	SE	4.85-4.90	Unidentified (fragments)		1300	grey	
WH 216	1	Dfsh7	N18-20/E6-8	13	C	SE	4.15-4.20	Rib		650-850	humpback	
WH 217	1	Dfsh7	N18-20/E6-8	13	C	SE	4.15-4.20	Unidentified (fragments)		650-850	humpback	A1
WH 218	1	Dfsh7	N18-20/E6-8	13	C	SE	4.15-4.20	Vertebra (undet)		650-850	humpback	

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 219	1	Dfsh7	N18-20/E6-8	31	E	NW	5.05-5.10	Unidentified (fragments)		1300	humpback	CD
WH 220	1	Dfsh7	N18-20/E6-8	18	E	SE	4.40-4.45	Vertebra (undet)		1100	humpback	E4
WH 221	1	Dfsh7	N18-20/E6-8	18	E	NE	4.40-4.45	Vertebra (undet)		1100	humpback	A1
WH 222	1	Dfsh7	N18-20/E6-8	18	E	SW	4.40-4.45	Vertebra (undet)		1100	humpback	A1
WH 223	1	Dfsh7	N18-20/E6-8	28	E	SE	4.90-4.95	Unidentified (fragments)		1300	humpback	E8
WH 224	1	Dfsh7	N18-20/E6-8	29	E	NE	4.95-5.00	Vertebra (undet)		1300	grey	
WH 225	1	Dfsh7	N18-20/E6-8	29	E	NE	4.95-5.00	Unidentified (fragments)		1300	humpback	E5
WH 226	1	Dfsh7	N18-20/E6-8	15	C	NE	4.25-4.30	Vertebra (undet)		900	xx	
WH 227	1	Dfsh7	N18-20/E2-4	18	C	NW	4.40-4.45	Unidentified (fragments)		650-850	humpback	CD
WH 228	1	Dfsh7	N18-20/E2-4	18	C	SW	4.40-4.45	Vertebra (undet)		1300	grey	
WH 229	1	Dfsh7	N18-20/E2-4	21	D	NW	4.55-4.60	Unidentified (unknown element)		1100	humpback	CD
WH 230	1	Dfsh7	N18-20/E2-4	32	F	NW	5.10-5.15	Unidentified (fragments)		1300	humpback	E8
WH 231	1	Dfsh7	N18-20/E2-4	38	F	NW	5.40-5.45	Skull (fragment)		1300	humpback	
WH 232	1	Dfsh7	N18-20/E2-4	28	D	SW	4.90-4.95	Rib		1300	grey	
WH 233	1	Dfsh7	N18-20/E6-8	35	G	SW	5.25-5.30	Vertebra (undet)		1350	humpback	CD
WH 234	1	Dfsh7	N18-20/E6-8	17	E	SE	4.35-4.40	Unidentified (fragments)		900	humpback	
WH 235	1	Dfsh7	N18-20/E6-8	36	G	NE	5.25-5.30	Unidentified (fragments)		1300	grey	
WH 236	1	Dfsh7	N18-20/E6-8	35	G	NW	5.25-5.30	Unidentified (fragments)		1300	xx	
WH 237	1	Dfsh7	N18-20/E2-4	16	C	SW	4.30-4.35	Skull		650-850	humpback	
WH 238	1	Dfsh7	N18-20/E2-4	24	D	SW	4.70-4.75	Unidentified (fragments)		1200	humpback	
WH 239	1	Dfsh7	N18-20/E2-4	24	D	NE	4.70-4.75	Phalanx, prox/medial		1200	xx	
WH 240	1	Dfsh7	N18-20/E2-4	18	C1	NE	4.40-4.45	Vertebrae, undisting.		1300	humpback	
WH 241	1	Dfsh7	N18-20/E6-8	20	E	SW	4.50-4.55	Vertebra (undet)		1100	humpback	E6
WH 242	1	Dfsh7	N18-20/E2-4	21	C	SE	4.55-4.60	Skull		1100	xx	
WH 243	1	Dfsh7	N18-20/E2-4	21	D	NE	4.55-4.60	Unidentified (fragments)		1100	xx	
WH 244	1	Dfsh7	N18-20/E2-4	14	C	NE	4.24-4.30	Rib		650-850	xx	
WH 245	1	Dfsh7	N18-20/E2-4	40	F	NW	5.50-5.55	?longer bone??		1300	finback	
WH 246	1	Dfsh7	N10-12/E2-4	16	E	SW	3.58-3.63	Unidentified (fragments)		450-650	humpback	CD
WH 247	1	Dfsh7	N10-12/E2-4	20	G	NW	3.78-3.83	Vertebra (undet)		650-850	humpback	CD
WH 248	1	Dfsh7	N18-20/E2-4	14	C	NE	4.24-4.30	Rib		650-850	xx	
WH 249	1	Dfsh7	N10-12/E2-4	20	F	NE	3.78-3.83	Vertebra (undet)		650-850	humpback	A2
WH 250	1	Dfsh7	N10-12/E2-4	21	G	NW	3.83-3.88	Unidentified (fragments)		650-850	humpback	E8
WH 251	1	Dfsh7	N10-12/E2-4	21	G	SE	3.83-3.88	Unidentified (fragments)		650-850	humpback	CD
WH 252	1	Dfsh7	N10-12/E2-4	20	F	NW	3.78-3.83	Vertebra (undet)		650-850	humpback	CD
WH 253	1	Dfsh7	N10-12/E2-4	20	G	SW	3.78-3.83	Rib		650-850	humpback	A1
WH 254	1	Dfsh7	N10-12/E2-4	16	E	NW	3.58-3.63	Unidentified (fragments)		450-650	humpback	CD
WH 255	1	Dfsh7	N10-12/E2-4	33	G/H	NW	4.43-4.48			1100	grey	
WH 256	1	Dfsh7	N10-12/E2-4	10	C	SE	3.28-3.33	Vertebra (undet)		450-650	humpback	E8
WH 257	1	Dfsh7	N10-12/E2-4	14	E	SW	3.48-3.59			450-650	humpback	E8
WH 258	1	Dfsh7	N10-12/E2-4	21	G	SW	3.83-3.88	Unidentified (fragments)		650-850	humpback	

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 259	1	Dfsh7	N10-12/E2-4	12	D	NE	3.38-3.43	Unidentified (fragments)		450-650	xx	
WH 260	1	Dfsh7	N10-12/E2-4	14	C	NE	3.48-3.53	Rib		450-650	xx	
WH 261	1	Dfsh7	N10-12/E2-4	12	C	SW	3.38-3.43	Unidentified (fragments)		450-650	humpback	CD
WH 262	1	Dfsh7	N10-12/E2-4	27	G	NE	4.13-4.18	Rib		900	humpback	
WH 263	1	Dfsh7	N18-20/E16-18	15	B1	SW	4.1-4.15	long bone epiphysis	complete	650-850	humpback	
WH 264	1	Dfsh7	N18-20/E16-18	13	B	SW	4.0-4.2			650-850	humpback	
WH 265	1	Dfsh7	N18-20/E16-18	13-16	B	SW	4.0-4.20	whale bone near feat.42		650-850	humpback	CD
WH 266	1	Dfsh7	N16-18/E26-28	15	B1	SE	4.1-4.15			900	humpback	
WH 267	1	Dfsh7	N18-20/E16-18	13	B	SW	4.05			650-850	humpback	
WH 268	1	Dfsh7	N18-20/E16-18	13	B	SW	4.0-4.1			650-850	humpback	E8
WH 269	1	Dfsh7	N18-20/E16-18	13	B	SW	4.0-4.1			650-850	humpback	
WH 270	1	Dfsh7	N18-20/E16-18	13	B1	SE	4.0-4.05			650-850	humpback	
WH 271	1	Dfsh7	N18-20/E30-32	16-19	D/E/F	NE/NW	4.05-4.35	whale rib, three pieces almost complete		650-850	humpback	E4
WH 272	1	Dfsh7	N18-20/E30-32	19	D/E	NE	4.2-4,25	skull		1300	Finback	
WH 273	1	Dfsh7	N18-20/E30-32	16-19	D/E	NW/NE	4.05-4.25			1300	grey	
WH 274	1	Dfsh7	N18-20/E30-32	16-19	D/E	NW/NE	4.05-4.25	skull fragment?		650-850	grey	
WH 275	1	Dfsh7	N18-20/E30-32	15-19	D/E	NW	4.2-4.25	epiphysis		650-850	humpback	CD
WH 276	1	Dfsh7	N18-20/E30-32	16-19	D/E	NW/NE	4.05-4.2	vertebrae	complete	650-850	humpback	A1
WH 277	1	Dfsh7	N18-20/E30-32	19	E	NW	4.2-4.25			650-850	humpback	A1
WH 278	1	Dfsh7	N12-14/E18-20	11-13	C	SE	3.85-3.95			650-850	humpback	CD
WH 279	1	Dfsh7	N18-20/E30-32	16-17	D/E	SW	4.05-4.25			650-850	humpback	E8
WH 280	1	Dfsh7	N18-20/E30-32	16-19	D/E	NW/NE	4.05-4.25	cut marks on bone		650-850	humpback	
WH 26	1	DfSi 16	S58-60/W64-66	8	E		0.40-0.50	1 fragment	id'd level	3600	humpback	CD
WH 28	1	DfSi 16	S62-64/W62-64	2	A		0.60-0.50 above	rib frag	killer whale?	3000-3500	humpback	
WH 29	1	DfSi 16	S56-58/W66-68	2	A		0.10-0.20	very dense probably cranial (ear?)		3500-4500	xx	
WH 30	1	DfSi 16	S56-58/W64-66	7	C		0.60-0.70	more chunks from feature# 24		3500-4500	grey whale	
WH 31	1	DfSi 16	N10-12/W102-104	feature 57	C		Jul-84	"flat peice of crania- may be part of WB # 00-14 but does not seem to fit in" G. Frederick	glued fragment	250-1000	humpback	E8
WH 32	1	DfSi 16	S56-58/W64-66	7	A		0.60-.70	large frag from feature 24		3500-4500	humpback	
WH 33	1	DfSi 16	S56-58/W64-66	7	C		0.60-0.70	cranial fragment from feature #24	heavy chunk	3500-4500	grey whale	
WH 34	1	DfSi 16	S14-16/W25-27	37	G		4.70-4.80	small cancellous frag from deep in the id'd unit	from id'd level!	1500-2000	right whale	
WH 35	1	DfSi 16	S12-14/W25-27	27	C		4.00-4.10	large cancellous frag with charcoal	candiate for direct dating?	1000-1500	humpback	
WH 36	1	DfSi 16	S16-18/W25-27	25	C		3.48-3.58	1 fragment		1000-1500	finback	
WH 38	1	DfSi 16	S10-12/W25-27	7			2.10-2.20	unfused phalange		500-1000	grey whale	
WH 39	1	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	fragment of "whale bone # 5"	this is fragment 2 of 2	3500-4500	humpback	E9

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 40	1	DfSi 16	S56-58/W64-66	7	A		0.60-0.70	large fragment of vertebral disc associated with feature #24		3500-4500	xx	
WH 41	1	DfSi 16	S14-16/W25-27	26	D			small frag from unid level		1000-1500	xx	
WH 42	1	DfSi 16	S4-6/W98-100	2	A		3.00-3.10	thin slice of a scpula? associated with “fauna feature” from this level	68cm below surface in same level as lots of bluefin tuna	250-500	humpback	CD
WH 43	1	DfSi 16	S56-58/W66-68	4	E		0.30-0.40	delicate vertebra fragment		3500-4500	humpback	
WH 44	1	DfSi 16	S10-12/W25-27	4	A		1.80-1.90	small cancellous fragment		250-500	humpback	A1
WH 45	1	DfSi 16	S12-14/W25-27	22	B		3.50-3.60	1 fragment		1000-1500	grey whale	
WH 46	1	DfSi 16	S14-16/W25-27	37	G		4.70-4.80	solid rib fragment		1500-2000	humpback	CD
WH 47	1	DfSi 16	S56-58/W64-66	7	A		0.60-0.70	large chunk from associated with feature #24		3500-4500	grey whale	
WH 48	1	DfSi 16	N10-12/W102-104?	see feature record	see feature record		see feature record	WB -00-19	see feature record (56 or 57) around 500 Cal BP	500	humpback	
WH 49	1	DfSi 16	S56-57/W50-52	6	B		0.40-0.50	1 fragment		3000-3500	grey whale	
WH 50	1	DfSi 16	S16-18/W25-27	14	B		2.28-2.38	1 fragment	has charcoal on it…	1000-1500	humpback	A1
WH 51	1	DfSi 16	S10-12/W98-100	4	A		2.00-2.10	fragment with charcoal attached...	(could be a candiadate for dating?)	250-500	humpback	
WH 52	1	DfSi 16	S56-58/W66-68	6	E		0.60-0.70	extensive cancellous frag from level bag	probably associated with feature #24	3500-4500	xx	
WH 53	1	DfSi 16	S8-10/W98-100	12	A		3.10-3.20	whale frag		250-500	humpback	
WH 54	1	DfSi 16	S56-58/W64-66	7	B		0.60-0.70	“cranial frag” from feature #24		3500-4500	grey whale	
WH 55	1	DfSi 16	S60-62/W63-64	6	C		0.05 above-0.05 below	1 fragment		3500-4500	humpback	CD
WH 56	1	DfSi 16	S16-18/W25-27	25	C		3.48-3.58	1 dense rib frag		1000-1500	finback	
WH 57	1	DfSi 16	S8-10/W98-100	7	A		2.60-2.70	cancellous fragment		250-500	humpback	A1
WH 58	1	DfSi 16	S12-14/W25-27	28			4.10-4.20	small rib fragment		1000-1500	humpback	E6
WH 59	1	DfSi 16	S14-16/W25-27	20	C		3.00-3.10	rib frag		500-1000	humpback	E8
WH 60	1	DfSi 16	S10-12/W25-27	12			2.60-2.70	small rib frag		250-1000?	humpback	A1
WH 61	1	DfSi 16	S14-16/W25-27	20	C		3.00-3.10	unid frag	from unid level	500-1000	humpback	
WH 62	1	DfSi 16	N4-6/W102-104	7	C		6.25-6.35	2 frags of lage element		250-500	grey whale	
WH 63	1	DfSi 16	S14-16/W25-27	25	C		3.50-3.60	three fragments from id’d level	unsure whether these are the same element	1000-1500	humpback	
WH 64	1	DfSi 16	S58-60/W64-66	10	E		0.40-0.50	1 fragment	from NW corner of unit	4500-5000	humpback	CD
WH 65	1	DfSi 16	S14-16/W25-27	36	G		4.60-4.70	large whale?	element?	1500-2000	humpback	CD
WH 67	1	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	1 fragment	same text on bag as 171	3500-4500	xx	
WH 68	1	DfSi 16	N10-12/W102-104?	?	?		?	WB 00-4 from feature 56 or 57? piece #2	see feature record, Frederick 2000	500	humpback	E8
WH 69	1	DfSi 16	S56-57/W50-52	17+			shovel test	dense	oldest sample	4500-5000	humpback	E8
WH 70	1	DfSi 16	S14-16/W25-27	21	C		3.00-3.10	2 mandible frag		1000-1500	humpback	CD

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 71	1	DfSi 16	S8-10/W98-100	10	A		2.90-3.00	unfused phalange		4000-5000	humpback	E5
WH 72	1	DfSi 16	S12-14/W25-27	19			Mar.18	ribfrag from precise depth	distal fragment	500-1000	humpback	A3
WH 73	1	DfSi 16	N0-2/W98-100	7	A		5.70-5.80	cancellous frag	2 fragments (1of2)	250-500	humpback	E8
WH 74	1	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	“whale bone # 5”	fragment 1 of 2	3500-4500	humpback	E7
WH 75	1	DfSi 16	S56-58/W66-68	6	E		0.50-0.60	soild chunk		3500-4500	xx	
WH 76	1	DfSi 16	S56-58/W66-68	3	B		0.20-0.30	uncertain element		3500-4500	humpback	CD
WH 77	1	DfSi 16	N2-4/W102-104	5	C		6.00-6.10	2 fragments		250-500	humpback	A1
WH 78	1	DfSi 16	N10-12/W102-104?	see feature record-2000				WB 00-3 from feature 56 or 57	see feature record-2000	500	humpback	E8
WH 79	1	DfSi 16	S12-14/W25-27	13	A		2.60-2.70	rib fragment		500-1000	humpback	E8
WH 80	1	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	1 fragment		3500-4500	grey whale	
WH 81	1	DfSi 16	N4-6/W102-104	8	C		6.35-6.45	1 fragment		500-1000	humpback	E5
WH 82	1	DfSi 16	S54-56/W77-79	7	A		0.10-0.00 above	ephyisis		3500-4500	humpback	CD
WH 84	1	DfSi 16	S54-56/W64-66	5	A		0.70-0.80	2 fragments from level bag	probably the same element	3500-4500	xx	
WH 85	1	DfSi 16	N10-12/W102-104	see feature record	see feature record		see feature record	WB 00-7 from a feature (either 56 or 57)	2 fragments	500	humpback	CD
WH 86	1	DfSi 16	S12-14/W25-27	18	B		3.70-3.80	large rib frag		500-1500	humpback	E8
WH 88	1	DfSi 16	N2-4/W102-104	23	D		7.80-7.90	I lage vert frag	rt scapula?	500-1000	humpback	CD
WH 89	2	DfSi 16	S14-16/W25-27	17	B			2 rib frags		1000-1500	humpback	CD
WH 90	2	DfSi 16	S56-58/W68-70	7	A		0.60-0.70	2 vert frags (in separate bags)	assoc with feature 26	4000-5000	xx/xx	
WH 91	1	DfSi 16	N0-2/W98-100	1	A		5.00-5.20	1 fragment		250-500	xx	
WH 92	1	DfSi 16	N2-4/W102-104	31	D		8.60-8.70	1 rib frag		1000-1500	humpback	E8
WH 93	2	DfSi 16	S56-58/W64-66	7	A		0.60-0.70	2 fragments from level bag		4000-5000	xx/xx	
WH 94	2	DfSi 16	S56-57/W50-52	13	D		1.10-1.20	2 frags from level bag	likely the same element	4000-4500	humpback/xx	
WH 95	1	DfSi 16	S10-12/W25-27	2			1.57-1.67	1 fragment		250-500	grey whale	
WH 96	1	DfSi 16	S12-14/W25-27	33	F		4.60-4.70	2 fragments of ephiysis		1500-2000	humpback	CD
WH 97	1	DfSi 16	S14-16/W25-27	5	A		1.50-1.60	vetebral epiphysis	fragment	250-500	humpback	A1
WH 98	1	DfSi 16	S54-56/W77-79	2	B		0.60-0.50 above datum	1 fragment		3000-3500	grey whale	
WH 99	1	DfSi 16	S56-57/W50-52	15	D		1.30-1.40	vetebral epiphysis		4000-4500	grey whale	
WH 100	2	DfSi 16	S56-58/W68-70	4	A		0.30-0.40	2 frags from level bag	probably the same element	3500-4500	humpback/xx	
WH 101	1	DfSi 16	S12-14/W25-27	14			2.70-2.80			500-1000	humpback	CD
WH 102	1	DfSi 16	N10-12/W102-104				Jul-84	cranial frag from the large feature	Aug-00	500-1000	humpback	E8
WH 103	1	DfSi 16	N0-2/W98-100	7	A		5.70-5.80	cancellous frag	2 fragments (2of2)	250-500	xx	
WH 105	1	DfSi 16	S58-60/W64-66	6	A		0.0-0.10	unid whale frags from id’d level	2001	4000-5000	humpback	
WH 106	1	DfSi 16	S62-64/W62-64	3	B		0.50-0.40	vert disc	from id’d level	3500-4500	humpback	CD
WH 107	1	DfSi 16	N4-6/W102-104	16	D		7.15-7.25		unid level	500-1000	humpback	CD
WH 108	1	DfSi 16	S2-4/W98-100	8	A		4.10-4.20	1 frag	unid level	250-500	humpback	

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 110	1	DfSi 16	N4-6/W102-104	7	C		6.25-6.35	cranial	id'd level	250-1000	finback	
WH 111	1	DfSi 16	N2-4/W102-104	5	A		6.0-6.10	large cranial	id'd level	250-500	grey whale	
WH 112	1	DfSi 16	N4-6/W102-104	13	C		6.85-6.95	rib ?	id'd level	500-1000	humpback	E8
WH 113	1	DfSi 16	N4-6/W102-104	3	A		5.85-5.95	small frag	id'd level	250-250	humpback	A1
WH 114	1	DfSi 16	N2-4/W102-104	7	C		6.10-6.20	large chunk	id'd level	500-1000	BLUE	
WH 116	2	DfSi 16	S58-60/W64-66	10	E		0.40-0.50	cranial frag from NW corner of unit	id'd level	4000-5000	grey whale	
WH 117	1	DfSi 16	S12-14/W25-27	14			2.70-2.80	extracted chunk from large cranial element	may be worth looking at susan's 1999 report	500-1000	humpback	
WH 118	1	DfSi 16	S5-7/W11-13	6			3.30-3.40	vert frag		250-500	humpback	
WH 119	1	DfSi 16	N2-4/W102-104	9	C		6.45-6.55	fragile cancellous		500-1000	humpback	
WH 120	1	DfSi 16	N2-4/W102-104	11	C		6.6-6.7	vertebral disc	id'd level	500-1000	humpback	
WH 121	1	DfSi 16	S6-8/W98-100	10	A		2.95-3.05	scapula frag		500-1000	humpback	
WH 122	1	DfSi 16	N0-2/W98-100	8	A		5.8-5.9	large frag	unid level	250-500	humpback	
WH 123	1	DfSi 16	S12-14/W25-27	21	B		3.40-3.50	cranial		1000-1500	orca	
WH 124	2	DfSi 16	S59/W65.5-66	3	A		0.20-0.10 above	frags of the same vertebra	id'd level	3500-4500	xx/humpback	
WH 125	1	DfSi 16	N2-4/W102-104	20	D		7.50-7.60	unid level		500-1500	humpback	CD
WH 126	1	DfSi 16	S6-8/W98-100	9	A		2.85-2.95	vert disc		250-1000	humpback	CD
WH 127	1	DfSi 16	S10-12/W98-100	5	A		2.10-2.20			250-500	humpback	A1
WH 128	1	DfSi 16	N4-6/W102-104	29	D		8.45-8.55	1 of 2 fragments		1000-1500	humpback	
WH 129	1	DfSi 16	S10-12/W98-100	12	A		2.80-2.90	rib frag		250-1000	humpback	CD
WH 130	1	DfSi 16	S62-64/W62-64	3	A		0.50-0.40 above	rib frag	id'd level	3000-4000	xx	
WH 131	1	DfSi 16	S2-4/W98-100	2	A		3.5-3.6	small frag		250-500	humpback	
WH 132	1	DfSi 16	S12-14/W25-27	14			2.7-2.8			500-1000	humpback	CD
WH 133	1	DfSi 16	S12-14/W98-100	6			1.90-2.00			250-500?	humpback	A1
WH 134	1	DfSi 16	N2-4/W102-104	1	A		5.50-5.70	rib frag	id'd level	250-500	humpback	
WH 135	1	DfSi 16	N4-6/W102-104	29	D		8.45.8.55	frag 2 of 2		1000-1500	xx	
WH 136	1	DfSi 16	S14-16/W25-27	3	A		1.25-1.35	large fragment		250-500	humpback	E8
WH 137	1	DfSi 16	N4-6/W102-104	8	C		6.35-6.45	vert process	unid level	500-1000	humpback	
WH 138	1	DfSi 16	N0-2/W98-100	9	A		5.90-6.0	phalanx complete	unid level	250-500	humpback	A1
WH 139	1	DfSi 16	S10-12/W25-27	11			2.50-2.60			250-1000?	humpback	
WH 140	2	DfSi 16	N4-6/W102-104	28	D		8.35-8.45	2 frags		1000-1500	Finback	
WH 141	1	DfSi 16	S10-12/W25-27	15	A		3.10-3.20			500-1000	humpback	E8
WH 142	2	DfSi 16	S12-14/W98-100	3			1.60-1.70	2 frags from same level bag		250-500	humpback	A1
WH 143	1	DfSi 16	N4-6/W102-104	2	B		5.75-5.85	rib frag		250-500	humpback	E8
WH 144	1	DfSi 16	N2-4/W102-104	9	D		6.40-6.50	cranial or scapula	id'd level	500-1000	humpback	
WH 145	1	DfSi 16	S8-10/W98-100	1	A		1.85-2.10	vert disc		250-500	humpback	A1
WH 146	1	DfSi 16	S12-14/W98-100	8	A		2.20-2.30	large vert disc frag		250-500	humpback	A1
WH 147	1	DfSi 16	S12-14/W25-27	21	B		3.40-3.50	large cranial chunk		500-1000	xx	
WH 148	1	DfSi 16	S12-14/W25-27	24	C		3.70-3.80	small		500-1500	grey whale	

Table 13: samples used including excavation information.

DNA lab		Zooarchaeological and archaeological information provided									DNA lab results	
Sample ID	##	SITE	UNIT	LEVEL	LAYER	Quad	Depth Below Datum	ELEMENT	PORTION	age	DNA species ID	Haplotype
WH 149	1	DfSi 16	S10-12/W25-27	6	A		2.00-2.10	vert disc		250-1000	right whale	
WH 150	1	DfSi 16	N4-6/W102-104	4	B		5.95-6.05	unid level		250-500	humpback	A1
WH 151	2	DfSi 16	S12-14/W98-100	10	A		2.40-2.50	2 frags		250-500	humpback	A1
WH 152	1	DfSi 16	N4-6/W102-104	11	C		6.65-6.75	vert disc		500-1000	humpback	
WH 153	1	DfSi 16	S58-60/W64-66	8	D		0.20-0.30	heavy frag		4000-5000	humpback	
WH 154	1	DfSi 16	S12-14/W25-27	24	C		3.70-3.80			500-1000	humpback	
WH 155	1	DfSi 16	S14-16/W98-100	12	A		2.15-2.25	unid level		500-1000	humpback	
WH 156	1	DfSi 16	S8-10/W98-100	1	A		1.85-2.10	small frag		250-500	humpback	A1
WH 157	1	DfSi 16	N2-4/W102-104	8	C		Jun-30	vert process		500-1000	humpback	
WH 158	1	DfSi 16	S8-10/W98-100	8	A		2.70-2.80			250-500?	humpback	
WH 159	1	DfSi 16	N4-6/W102-104	18	D		7.35-7.45	whale frag		500-1000	humpback	
WH 160	1	DfSi 16	N4-6/W102-104	19	D		7.45-7.55	whale from id'd level "unid whale"		500-1000	grey whale	
WH 161	1	DfSi 16	S6-8/W98-100	14	A		3.35-3.45	very dense element		>1000	Blue whale	
WH 162	1	DfSi 16	S10-12/W25-27	15	A		2.90-3.00	bone from north wall		1000-2000	humpback	
WH 163	1	DfSi 16	S10-12/W98-100	5	A		2.10-2.20	cranial?		250-500	humpback	E4
WH 164	1	DfSi 16	N2-4/W102-104	17	D			unid whale from id'd level	id'd level	500-1000	humpback	
WH 165	1	DfSi 16	S14-16/W98-100	12	A		2.15-2.25			500-1000	humpback	E8
WH 166	2	DfSi 16	N4-6/W102-104	30	E		8.55-8.65	2 whale frags from level bag		1000-1500	xx/finback	
WH 167	1	DfSi 16	S58-60/W64-66	3	A		0.20-0.10 above	2 frags	id'd level	3500-4500	xx	
WH 168	1	DfSi 16	S8-10/W98-100	4	A		2.30-2.40	2 frags of same element	unid level	250-500	humpback	
WH 171	1	DfSi 16	S56-58/W66-68	5	E		0.40-0.50	"bone #1 rib frag"		3500-4500	humpback	
WH 172	1	DfSi 16	S56-58/W64-66	7	B		0.60-0.70	lrag cranial frag from feature # 24		3500-4500	grey whale	
WH 173	1	DfSi 16	S2-4/W98-100	1	A		3.20-3.50	from top of 2000 trench	charcoal attached!	250-500	humpback	
WH 174	1	DfSi 16	S14-16/W25-27	20	C		3.00-3.10	small vertbral disc frag		500-1000	humpback	
WH 176	2	DfSi 16	S14-16/W25-27	13	A		2.30-2.40	2 undet frags..		500-1000	humpback	
WH 37	1	DfSi 17	S5-7/W11-13	14			4.10-4.20	likely a whale but….	might be a sea lion	250-500	humpback	A1
WH 66	1	DfSi 17	N43-44/W53-55	2	A		4.85-4.95	1 small fragment		250-500	humpback	E8
WH 83	1	DfSi 17	N43-44/W53-55	7	C		5.35-5.45	2 vert frags (in separate bags)		250-1000	xx	
WH 87	1	DfSi 17	S5-7/W11-13	4	A		3.10-3.20	1 fragment		250-500	grey whale	
WH 104	1	DfSi 17	S5-7/W11-13	14			4.1-4.2	scapula?	1999	500-1000	finback	
WH 109	1	DfSi 17	S5-7/W11-13	16			4.30-4.40	rib frag	1999	500-1000	finback	
WH 115	2	DfSi 17	S5-7/W11-13	15			4.20-4.30	two large rib frags	1999	500-1000	finback/right whale	
WH 170	1	DfSi 17	S5-7/W11-13	20	B		4.70-4.80	unfused phalange		500-1000	xx	
WH 175	1	DfSi 17	N43-44/W53-55	2	A		4.85-4.95	1 fragment		250-1000	humpback	
WH 169	1	DfSi17	S5-7/W11-13	6			3.30-3.40	whale? Large fragment in paperbag, verebrae frag?	excavated July 1999	250-500	humpback	E8

Appendix B: Sample extraction and PCR amplifications

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH026	23/01/06	12/14(86)	C09 F	C09 F	C21 F	C37 F	G13 F/R	G136F324	PT026
	12/12/08				C36 F,C35 R	G56F*	G57R*		
WH027	23/01/06	10/16(63)	C09 F	C09 F	C35 R	C18 R,C36 R	G57 R		PT027
				C96 R	C18 R	C21F, C35F	E99R		
WH028	23/01/06	7/18(39)	C09 F	C09 F	C18R	C18R	E90R		PT028
	23/03/09			C15 F/R	C21 F	C37R			
WH031	23/01/06	13/17(76)	C09 F	C09 F	C21F	G33F,G56F	G34R		PT031
	23/03/09			C15 R	C98R,G60F	C18R,C37F			
WH033	23/01/06	14/18(78)	C09 F	C09 F	C21 F,R	E97 F	E98 R		PT033
			C51 F	C52 F	C109 R	C18R,C15F	G61F		
WH037	23/01/06	11/15(73)	C14F	C14F	C21 R	G7 F	E75F		PT037
				C15 R	*C37R,C98R*	C37R,G59F	G57R		
WH039	23/01/06	11/15(73)	C14F/R	C15R	C21 F/R	E73 F	E75 F/R		PT039
					G55F, C98R	G59F	D64F,R		
WH042	31/01/06	11/21(52)	C51 F	C15F/R	G36 R	E73 F,G56F	G13 F/R	G136,F324	PT042
					C21 F,R	E98F	D64 F/R,E90R		
WH044	31/01/06	21/22(55)	C51 F	C96F	G6 F/R,C35F	E73 F	G13 F/R,G57R		PT044
				C15R	E88 F,C109F	E89 F	D64 F/R,D71R		
WH046	31/01/06	12/12(100)	C14F	C14F	C21 F/R	C18 R	G13 F/R		PT046
				C15R	C18R, C98R	G56 F	G57 R		
WH050	28/02/06	11/13(85)	C29F	C32R	C56 F	E73 F	G34 R		PT050
	12/03/06		C32R	C30F	C98 R	G33 F	G57R		
WH055	28/02/06	7/14(50)	C32R	C26F	C98F	E89 F	D64F/F		PT055
	12/03/06		C26F	C96R	G55F	G59F	G13 F/R		
WH057	28/02/06	9/11(82)	C26F	C26F	C98 R,E73F	G33 F	G35R		PT057
	12/03/06		C32R	C30F	G55F	G56F	G61F		
WH058	28/02/06	14/23(61)	C26F	C52F	G6 F/R	G33F,E89	D71F, D64F	G139,F294	PT058
				C96R	G36 R	G56F	G13, G35R		
WH059	28/02/06	11/15(73)	C29F	C30F	C56 F, C98R	D62F	G13F/R		PT059
				C96R	G55F	G59F	G35R		
WH060	28/02/06	9/10(90)	C32R	C26F	C98R	D62F	G13 F/R		PT060

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
	12/03/06		C26R	C96R	C56 F	G56 F	G35R		
WH064	28/02/06	8/25(32)	C51 F	C96R	C123 F	G33 F	G61 F,D71F	G139 F294	PT064
				C52F			E98R		
WH065	28/02/06	13/23(65)		C30R	G36 R,E91F	G7F,G33 F	E75 F/R		PT065
				C96F	G6 F/R,C56F	E89F	G36F,D64F		
WH066	28/02/06	8/12(67)		C30R	C123 F	G56 F	E75 F/R		PT066
				C96F		D62F	G57 R,D64F		
WH068	28/02/06	9/11(82)		C96F	C123 F	E73 F	G57 R		PT068
	12/03/06			C30R	G55F	D60F	E75 F/R		
WH069	22/04/06	9/10(90)	C53F	C53F	C98 R	D62F	D71 R	G137 R409	PT069
				C97R	C56 F	G56 F	G57 R		
WH070	22/04/06	9/9(100)	C53F	C97R	C56 F	D62F	E75 F/R		PT070
				C53F		G56 F	G57 R, D71R		
WH071	22/04/06	9/10(90)	C53F	C53F	G55F	E73 F	E75 F/R	G136F324	PT071
				C97R	C56F	G56 F			
WH072	22/04/06	9/9(100)	C53F	C53F	G55F	G56F	E75 F/R		PT072
				C97R	C56F	D62F	G61 F		
WH073	22/04/06	8/10(80)	C53F	C53F	G55F	E73 F	E75 F/R		PT073
				C97R	C123 F	D62F	G61 F		
WH074	22/04/06	11/15(73)	C53F	C124F	G55F	E93F	G13F/R		PT074
	04/01/08			C53F	C56F	G56F	G61F		
WH076	22/04/06	11/11(100)	C53F	C53F	G55F	D62F	G13F/R		PT076
	04/01/08			C97R	C56F	G56F/E73F	E75F	G139 F294	
WH077	22/04/06	10/10(100)	C53F	C53F	G6 F/R	E74 F/R	E75 F/R		PT077
				C97R	C56 F	D62F	G32F,G57R		
WH078	22/04/06	8/9(89)	C53F	C53F	C56F	E74F/F	E75F/R		PT078
				C97R	G55F		G57R		
WH079	22/04/06	7/8(88)	C53F	C53F	C123F	D62F	E75F/R		PT079
				C97R					
WH081	22/04/06	8/11(73)	C53F	C53F	C56F	E74F/R	E98R	G137 R409	PT081
					G55F	E97F	G57R		

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH082	22/04/06	9/12(75)		C124F	C123F	G56F	G13F/R		PT082
					G60F	D62F			
WH085	22/04/06	10/11(91)		C124F	C55F	G56F	G13F/R		PT085
					E71F	D62F	G61F		
WH086	29/04/06	12/14(86)		C57F	C123F	E74F/R	G13F/R		PT086
					G55F		G57R		
WH088	29/04/06	12/13(92)		C87F	C69F	C69R	G13F/R		PT088
					G55F	E2R	E76R		
WH089-1	29/04/06	9/10(90)		C57F	G55F,C123F	G56F	G13F/R		PT089-1
					C69F/R	E74R	G35R		
WH092	29/04/06	10/11(91)		C57F	C69 F	C69 R	G13 F/R		PT092
					E1 F	G7 F,E2R	G57R		
WH096	29/04/06	8/10(80)		C57F	E71F	C69R	G13F/R		PT096
						G59F,G56F	G61F		
WH097	29/04/06	10/12(83)		C57F	C69F	C69R	G13F/R		PT097
					E1F,G55F	E2F	G57R		
WH101	29/04/06	11/11(100)		C83F	C69F	C69R	E76R	G139 F294	PT101
					E1R	E02F	G57R		
WH102	12/05/06	7/14(50)	C64F	C83F	C123F	G07F	G13F/R		PT102
			C80F/R				E76R		
WH106	12/05/06	7/8(88)	C64F/R	C83F	C123F	E74F/R	G13F/R		PT106
			C80R			G7 F	E76R		
WH107	12/05/06	10/12(83)	C64F	C83F	G55F	E74R	E76R		PT107
					G123F	G33F	G35R,G32F		
WH112	12/05/06	6/12(50)	C64R	C83F	G123F	E97F	E98R		PT112
	23/03/09				G60F		G57R		
WH113	12/05/06	7/15(47)		C83F	G123F	G7 F	E98R		PT113
							G61F		
WH125	19/05/06	7/9(78)	C74F/R		E92F	E93F	E98F		PT125
	23/03/09				D59F				
WH126	19/05/06	7/10(70)	C74F	C84F	G55F,E92F	E93F	E98R		PT126

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
					D59F		G57R		
WH127	19/05/06	8/10(80)	C74F	C84F	E92F	E93F	E98R		PT127
					D59F	G56F	G57R		
WH129	19/05/06	9/10(90)	C74F	C84F	E72R	E97F	E98R		PT129
					G55F,D59F		G61F,G57R		
WH131	19/05/06	7/9(78)	C74F	C74F	G58R	E97F	E98R		PT131
					D59F				
WH132	19/05/06	6/10(60)	C82R	C84F	G55F	E99F	E105R	G139 F294	PT132
					E104F				
WH133	19/05/06	10/10(100)	C74R		E72R, D59F	E97F	E98R		PT133
					G60F,G55F	G56F			
WH136	19/05/06	7/7(100)	C74R		E72R	E97F	G13F/R		PT136
					D59F		E98R		
WH138	19/05/06	8/8(100)	C74R		G60F,D59F	E99F	E105R		PT138
	23/03/09				E104F,E72R				
WH141	25/05/06	8/8(100)	C75F		E104F,E72R	E99F	E105R		PT141
					G60F,D60F				
WH142-1	25/05/06	10/12(83)	C75R		E104F	E99F	E105R		PT142
						G63F	G64R		
WH143	25/05/06	5/8(63)	C75R		E104F	E99F	E105R		PT143
	23/03/09				D60F				
WH145	25/05/06	9/9(100)	C75R		D60F	G63F	G64R		PT145
					E104F	E99F	E105R,G61F		
WH146	25/05/06	10/11(91)	C75F		G32R,E104F	E99F	E105R		PT146
					G62F,D60F	G63F	G64R,G61F		
WH150	25/05/06	8/8(100)	C75F		E104F	E99F	E105R		PT150
					D60F	G63F	G64R		
WH151-2	25/05/06	9/10(90)	C75F		G6R/F	E99F	E105R	G138 R432	PT151
					E104F	G63F	G64R		
WH156	25/05/06	8/8(100)	C75F		E104F	E99F	E105R		PT156
					G62F,D60F	G63F	G64R		

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH163	27/05/06	9/9(100)	C79F		E104F	E99F	E105R		PT163
					G62F,D60F	G63F	G64R		
WH165	27/05/06	9/9(100)	C79F		E104F	E99F	E105R		PT165
					G62F,D61F	G63F	G64R		
WH169	27/05/06	9/11(82)	C79F		E104F	E100F	G13F/R		PT169
			C90F		G62F,D61F	G63F	E105R		
WH185	17/11/06	10/10(100)	D125F		G62F,E61F	E100F	E105R	G138 R432	PH185
			D154R		E104F	G63F	G64R		
WH186	17/11/06	7/8(88)	D125F		E104F	E100F	E105R		PH186
						G63F	G64R		
WH193	17/11/06	8/10(80)	D146F		G62F	E100F	E105R		PH193
			D154R		E104F	G63F	G64R		
WH196	06/01/08	8/8(100)	E5R		E104F	E100F	E105R		PH196
					E61F	G63F	G64R		
WH198	06/01/08	7/8(88)	E5R		E104F	E100F	E105R		PH198
					G62F	G63F	G64R		
WH199	06/01/08	9/9(100)	E5R		E104F,E61F	E100F	G13F/R	G138 R432	PH199
					G62F	G63F	E105R,G35R		
WH200	06/01/08	6/10(60)	E5F		G32R	E100F	G64R		PH200
					E104F	G63F	E105R		
WH203	06/01/08	7/7(100)	E5F		E104F	E100F	E105R		PH203
					G62F	G63F	G64R		
WH208	12/02/08	6/6(100)	E34R		E168 F/R	E169 F	E170 F/R	G138 R432	PH208
							G61F		
WH209	12/02/08	7/8(88)	E34R		E168F/R	E169F/R	G35R		PH209
					G58R	G59F	E170F/R		
WH210	12/02/08	9/9(100)	E34R		G58R,E168F/R	E169F/R	E170F/R		
	23/03/09				G60F	G7F	G61F		
WH212	12/02/08	6/9(67)	E34R		G60F	E217F	E218F/R		PH212
	23/03/09				E168F/R	E169F/R			
WH213	12/02/08	7/7(100)	E34R		E168F/R	E169F/R	E170F/R	G139 F294	

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
					G58R		G61F		
WH214	12/02/08	6/6(100)	E34R		E168F/R	E169F/R	E170F/R		PH214
	23/03/09				G60F				
WH216	12/02/08	7/11(64)	E34R		G32R	E217F	E218F/R		PH216
					G36R	E169F/R			
WH217	12/02/08	8/10(80)	E34R		E216 F/R	E217 F	E218 F/R		PH217
					E168R	E169F/R,G59F			
WH218	12/02/08	9/11(82)	E34R		E168F/R	E169F/R	E218F/R	G139 F294	PH218
	23/03/09					E217F	G61R,G13R		
WH219	12/02/08	7/7(100)	E34R		E168F/R	E169F/R	E170F/R		PH219
					G58R		G61R		
WH220	12/02/08	7/8(88)	E34R		E168 F/R	E169 F/R	E170 F/R		PH220
	23/03/09				G60F		G61R		
WH221	12/02/08	6/6(100)	E34R		E168F/R	E169F/R	E170F/R		PH221
							G61R		
WH222	25/02/08	10/10(100)	E52F		G32 R	E169 F/R	E170 F/R		PH222
					E168 F	G7 F	E170R		
WH223	25/02/08	6/6(100)	E52F		E168F/R	E169F/R	E170F/R		PH223
					G60F				
WH225	25/02/08	7/8(100)	E52F		E168F/R	E169F/R	E170F/R		PH225
			E60F			G59F	G61R		
WH227	25/02/08	6/7(86)	E60F		E168F/R	E169F/R	E170F/R		PH227
			E63F				G61R		
WH229	25/02/08	8/9(89)	E60F		E168F/R	E169F/R	E170F/R	G139 F294	PH229
	23/03/09		E63F		G60F		G61R		
WH230	25/02/08	11/12(92)	E60F		E216F/R	E217F	G13,E170,E218		PH230
					E168F/R	E169F/R	G35R,G32F		
WH233	25/02/08	7/8(88)	E60F		E168F/R	E169F/R	E170F/R		PH233
						G59F	G61R		
WH241	02/03/08	9/9(100)	E52F		E173 F/R	E174 F	E175 F/R	G139 F294	PH241
					G25 F	G26 F	G34F/R,G61F		

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH246	02/03/08	7/7(100)	E52F		E173F/R	E174F/R	E175F/R		PH246
					G25 F	G26 F			
WH247	02/03/08	7/7(100)	E52F		E173F/R	E174 F/R	E175F/R		PH247
					G25 F	G26 F	G27F		
WH249	02/03/08	6/7(86)	E52F		E173 F/R	E174 F/R	G13F		PH249
							G35R,G61R		
WH250	02/03/08	7/7(100)	E52F		G6F/R	G7F	G61R		PH250
					E173F/R	E174F/R	E175F/R		
WH251	02/03/08	7/7(100)	E52F		E173F/R	G26 F	E175F/R		PH251
					G25 F	E174 F/R	G27 F		
WH252	02/03/08	5/5(100)	E52F		E173 F/R	E174 F	E175 F/R		PH252
					G25 F	G26 F	G27 F		
WH253	02/03/08	5/5(100)	E52F		E173 F/R	E174 F	E175 F/R		PH253
WH254	02/03/08	10/10(100)	E52F		E173 R	G26 F	E175 R		PH254
					G25 F	G7 F	G13 F		
WH256	02/03/08	7/7(100)	E52F		E173 F/R	E174 F	E175 F/R		PH256
					G25 F	G26 F	G27 F		
WH257	02/03/08	7/7(100)	E52F		E173 F/R	G26 F	E175 F/R		PH257
					G25 F	E174 F	G27 F		
WH261	02/03/08	7/7(100)	E53R		E173 F/R	G26 F	E175 F/R		PH261
					G25 F	E174 F	G27 F		
WH265	03/03/08	7/7(100)	E63F		E173 F/R	E174 F	E175 F		PH265
			E53R		G25F	G26 F	G27F/R		
WH268	03/03/08	7/7(100)	E53R		E173 F/R	E174 F	E175 F/R		PH268
					G25 F	G26 F	G27F/R		
WH271	03/03/08	8/8(100)	E53R		E173 F/R	E174 F	E175 F	G138 R432	PH271
	23/03/09				G25 F	G26 F	G27F/R		
WH275	03/03/08	5/5(100)	E53R		G60F	E174F	E175 F/R		PH275
	23/03/09				E173 F/R				
WH276	03/03/08	9/9(100)	E53R		G6R,E173F/R	E174F	E175 F/R		PH276

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
					G25 F	G26 F,G7F	G27 F		
WH277	03/03/08	8/8(100)	E53R		E173 F/R	E174 F	E175 F/R		PH277
					G25 F,G6F/R	G26 F	G27 F		
WH278	03/03/08	7/7(100)	E53R		E173F/R	G26 F	E175F/R		PH278
					G25F	E174 F/R	G27F		
WH279	03/03/08	6/6(100)	E53R		E173F/R	E174 F	E175F/R		PH279
						G59	G61R		
WH034	23/01/06	6/13(46)	C9F	C96F,C15R		C37R			
WH035	23/01/06	11/11(100)	C09F	C09F	C21F/R,C98R	C18R			
WH036	23/01/06	4/7(57)	C09F	C15F/R, C9f					
WH038	31/01/06	3/7(43)	C14	C96F,C15R					
WH041	31/01/06	1/8(13)	C51F						
WH043	31/01/06	5/19(26)	C51F	C107F	C107F				
WH045	31/01/06	2/6(33)	C14F	C15R,C96F		C18R			
WH047	31/01/06	3/4(75)	C14F	C96F					
WH048	31/01/06	8/9(89)	C14F	C14F	C96R,C21F/R	E73F,C18R			
WH049	28/02/06	5/11(45)	C29F,C51F	C95R,C52F					
WH051	28/02/06	9/18(50)		C96R,C52F	E91F,C98F	E89F	D71F,D64F		
WH053	28/02/06	4/5(80)	C29F,C32R	C30F,C26F	C56F,C98R	E73F			
WH054	28/02/06	4/7(57)	C26F	C52F,C54F,C96R					
WH056	28/02/06	4/5(80)	C26F,C32R	C26F,C30F					
WH061	28/02/06	5/17(29)	C26R,C32R	C26R,C52F			D64F		
WH062	28/02/06	3/9(33)	C51F	C52F					
WH063	28/02/06	7/20(35)	C51F	C52F,C96R			D64F		
WH079	28/02/06	6/7(86)	C53F	C97R,C53F	C123F	D62F	E75F/R		
WH080	22/04/06	1/3(33)							
WH084	22/04/06	0/4(0)	C14/F						
WH087	22/04/06	1/3(33)	C82R						
WH094	29/04/06	2/5(40)		C57F		C57F/R			

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH095	29/04/06	3/4(75)		C61F	C69F				
WH098	29/04/06	2/6(33)	C57R						
WH099	29/04/06	2/3(67)	C57R						
WH100	29/04/06	2/9(22)		C57F	C69R	E74F/R			
WH104	12/05/06	3/8(38)	C64R	C83F					
WH105	12/05/06	2/7(29)	C64F/R,C80F			E93F			
WH108	12/05/06	3/16(19)	C71F	C83F					
WH109	12/05/06	2/3(67)	C64R	C83F					
WH110	12/05/06	3/5(60)	C64F	C83F					
WH111	12/05/06	4/6(67)	C64F	C83F					
WH114	12/05/06	2/4(50)	C83F						
WH115-1	12/05/06	3/4(75)	C64F,C80F						
WH115-3	12/05/06	1/5(20)	C64R				D64F		
WH116-1	12/05/06	1/3(33)	C64F						
WH116-2	12/05/06	2/4(50)	C82F						
WH117	19/05/06	8/11(73)	C64R,C80R		D59F,E76F,E92F	E93F,E74F/R,	E76R		
WH118	19/05/06	3/8(38)	C64F,C90F	C90R		E74F/R			
WH119	19/05/06	3/8(38)	C74R	C83F	C123F	E74R			
WH120	19/05/06	3/6(50)	C74F	C83F	E71F,D59F	E74F/R	E76R		
WH121	19/05/06	6/7(86)	C74R	C83F	E92F	E93F			
WH122	19/05/06	6/7(86)	C74R	C83F	D59F/E71F	E93F	E98R		
WH123	19/05/06	1/3(33)	C74F/R						
WH124-1	19/05/06	7/8(88)	C74R			E93F	E98R		
WH124-2	19/05/06	2/4(50)	C74F		E92F,D59F				
WH128	19/05/06	4/6(67)	C82R	C84F		E97F			
WH130	19/05/06	1/3(33)	C74R						
WH134	19/05/06	5/7(71)	C74F	C74F	D59F				
WH137	19/05/06	7/7(100)	C74F		E104F,D59F,E72R	E99F	E105R		
WH139	19/05/06	4/4(100)	C74F		D59F				
WH140-1	25/05/06	2/2(100)	C75F						
WH140-2	25/05/06	1/2(50)	C75F						

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH141	25/05/06	8/8(100)	C75F		G60F,E72R,D60F.E104	E99F	E105R		
WH142-1	25/05/06	10/12(83)	C75F		E104F	G63F,E99F	G645,E105R		
WH142-2	25/05/06	4/6(67)	C75F		E104F,D60F	E99F	E105R		
WH144	25/05/06	1/5(20)	C75F						
WH147	25/05/06	1/3(33)	C82R						
WH148	25/05/06	2/3(67)	C82R						
WH149	25/05/06	1/2(50)	C75F/R						
WH151-1	25/05/06	3/4(75)	C75R						
WH152	25/05/06	4/4(100)	C75R		D60F	E99F			
WH153	25/05/06	4/5(80)	C75F		D60F	E99F			
WH154	25/05/06	1/3(33)	C82R						
WH155	25/05/06	2/4(50)	C75R						
WH157	25/05/06	1/4(25)	C75R						
WH158	25/05/06	1/4(25)	C90F						
WH159	25/05/06	3/6(50)	C79F		E104F	E99F	E105R		
WH160	25/05/06	1/2(50)	C79R						
WH161	25/05/06	2/2(100)	C79F						
WH162	25/05/06	0/7(0)	C79R,C90F						
WH164	27/05/06	2/5(40)	C79R			E99F	E105R		
WH166	27/05/06	0/4(0)	C79R						
WH168	27/05/06	2/9(22)	C90R		E100F				
WH171	27/05/06	1/5(20)	C74R,C79R						
WH172	27/05/06	1/2(50)	C79F						
WH173	27/05/06	2/3(67)	C79R						
WH174	27/05/06	2/3(67)	C79F		D61F				
WH175	27/05/06	1/4(25)	C79R	D73F					
WH176-1	27/05/06	2/3(67)	C79F						
WH176-2	27/05/06	2/3(67)	C79R						
WH180	17/11/06	3/7(43)	D146F		E71F				
WH181	17/11/06	1/4(25)	D146F						
WH183	17/11/06	2/3(67)	D125F		E61F				

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH184	17/11/06	5/7(71)	D146F,D154R						
WH188	17/11/06	2/5(40)	D125F			E100F	E105R		
WH189	17/11/06	2/5(40)	D125F			E100F			
WH190	17/11/06	5/6(83)	D146F		E104F	E100F	E105R		
WH191	17/11/06	2/2(100)	D154R						
WH192	17/11/06	1/1(100)	D154R						
WH194	17/11/06	2/3(67)	D146F,D154R						
WH197	06/01/08	1/6(17)	E5R			E100F			
WH205	06/01/08	1/1(100)	E5F						
WH211	12/02/08	2/8(25)	E34R			E217F,E169F/R	E218F/R		
WH215	12/02/08	2/2(100)	E34R						
WH221	12/02/08	6/6(100)	E34R		E168F/R	E169F/R	E170F/R,G61		
WH222	25/02/08	10/10(100)	E52F		E168F/R	E169F/R,G7F,G33F	E170F/R,G32F,G35R	G61R588	
WH223	25/02/08	6/6(100)	E52F		E168F/R	E169F/R	E170F/R	G137R409	
WH224	25/02/08	2/2(100)	E52F						
WH226	25/02/08	2/3(67)				G59F			
WH228	25/02/08	3/3(100)	E60F,E63F						
WH231	25/02/08	2/3(67)	E60F						
WH232	25/02/08	3/3(100)	E60F,E63F						
WH234	25/02/08	4/9(44)	E60F			E217F/R	E218F/R		
WH235	25/02/08	3/3(100)	E60F,E63F						
WH236	25/02/08	1/5(20)	E60F						
WH237	25/02/08	2/2(100)	E52F						
WH238	25/02/08	3/6(50)	E60F,E63F			E169F/R			
WH240	02/03/08	1/3(33)	E63F			E169F/R			
WH245	02/03/08	2/3(67)	E60F,E63F						
WH255	02/03/08	0/3(0)	E63F						
WH258	02/03/08	1/1(100)	E53R						
WH262	02/03/08	1/1(100)	E53R						
WH263	02/03/08	1/1(100)	E53R						
WH264	02/03/08	1/1(100)	E53R						

Table 14:Extraction and PCR overview

				sequences included in allignment					
lab ID	**extraction**	**PCR setup**	**cytB**	**F22/R258**	**F101/R308**	**F360/R569**	**F322/R588**		**RECODED**
		success/all(%)			**F86/R308**	**F167/R569**			**STATISTIC**
WH266	02/03/08	1/1(100)	E53R						
WH267	02/03/08	1/1(100)	E53R						
WH269	02/03/08	1/1(100)	E53R						
WH270	02/03/08	1/1(100)	E53R						
WH272	02/03/08	1/1(100)	E53R		E173F/R				
WH274	02/03/08	1/1(100)	E53R						