

AN ABSTRACT OF THE THESIS OF

Angie Sremba for the degree of Master of Science in Fisheries Science presented on January 5, 2011.

Title: A Whale's Tale of mtDNA Diversity and Differentiation: The Antarctic Blue Whale.

Abstract approved:

C. Scott Baker

Twentieth century commercial whaling drastically reduced the abundance of great whale populations in the Southern Ocean. Exploitation began on the south Atlantic island of South Georgia, where catch records account for over 175,000 whales killed. Modern whaling within the Southern Ocean depleted populations rapidly, and by 1966, hunting blue whales south of 40°S was prohibited by the International Whaling Commission (IWC). After 40 years of protection, this species has shown little recovery. A current abundance estimate of 2,280 (CV=0.036) individuals from sighting data (1991/92-2003/04) represents less than 1% the original abundance. With such an intensive demographic 'bottleneck,' it is likely that genetic diversity has been lost from some or all components of the Southern Ocean population. Here I describe historical and contemporary Antarctic blue whale mtDNA diversity and report the first circumpolar analyses of contemporary population structure. In Chapter 2, historical mtDNA diversity is described from whale bones collected from the first Southern Hemisphere whaling stations established in 1904 on the island of South Georgia. A total of 281 whale bones were representative of three prominent species hunted in South Georgian waters. Using ancient DNA methods and

sequencing of the mtDNA control region, bone samples were first identified to species, identifying 153 humpback, 49 fin, 18 blue, 2 sei, 1 southern right whale and 1 elephant seal. Within each of the three prominent historic species populations, mtDNA haplotypes were described resulting in 64 humpback, 34 fin, and 16 blue whale haplotypes. Haplotype and nucleotide diversity within each of the three historic species populations ranged from 0.980-0.987 and 1.87-3.16%, respectively. In chapter 3, I update the previous estimate of contemporary Antarctic blue whale mtDNA diversity with biopsy samples of living whales collected during research cruises conducted with IWC oversight from 1990-2009 (n=218) for comparison to historical blue whale mtDNA diversity. After the removal of replicate samples based on 15 microsatellite loci, the dataset described 167 individuals. This dataset was combined with additional published Antarctic blue whale mtDNA control region sequences (LeDuc et al. 2007; n=20) to represent the most comprehensive dataset available for Antarctic blue whale mtDNA diversity (n=187). A high haplotype diversity was described within this contemporary population (0.968). With this dataset, I report the first evidence of population structure within the IWC Southern Ocean management Areas I-VI through an analysis of genetic differentiation. The identification of recaptures within the dataset through microsatellite genotyping, allows for the first inference of movement of six individuals with the Southern Ocean since the end of the Discovery marking program 50 years ago. In the final chapter of this thesis, I explore the impact of the 20th century commercial whaling industry on the Antarctic blue whale population through a comparison of historical and contemporary Antarctic blue

whale mtDNA diversity. The comparison showed that only 6 of the 16 haplotypes from the South Georgian population were found in contemporary worldwide blue whale populations, indicating a potential loss of mtDNA lineages. The loss of mtDNA haplotypes suggests two hypotheses; either a low predicted loss of widespread Antarctic blue whale mtDNA diversity or the loss of a South Georgia local Antarctic blue whale population driven to commercial extinction. The impact of commercial whaling is also assessed through a prediction of the minimum number of maternal lineages, or haplotypes, to have survived the exploitation bottleneck. The number of 51 mtDNA haplotypes identified within the contemporary Antarctic blue whale population is used to update the estimate of haplotypes within the unsampled contemporary population. We predict 69 mtDNA lineages within the contemporary population from the current abundance estimate of 2,280 (1,160-4,500) individuals (Branch 2008). This prediction will increase the lower bound of population abundance used in population dynamic modeling and may reduce an upward bias in population increase estimates used to assess the recovery of this species.

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A Whale's Tale of mtDNA Diversity and Differentiation: The Antarctic Blue Whale

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Angie Sremba

A THESIS

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Angie Sremba, Author

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A Whale's Tale of mtDNA Diversity and Differentiation: The Antarctic Blue Whale

CHAPTER ONE: INTRODUCTION

“I see them in hundreds and thousands” Norwegian captain C. F. Larsen in reference to the prolific great baleen whales of the Southern Ocean –TONNESSEN AND JOHNSON 1982

OVERVIEW

At the beginning of the 20th century, advancements in whaling technology enabled the expansion of commercial whaling into the Southern Hemisphere. Now equipped with explosive harpoons, air compressors and steam ships, commercial whalers were able to exploit the faster and larger baleen whales: the humpback (*Megaptera novaeangliae*), fin (*Balaenoptera physalus*), sei (*Balaenoptera borealis*) and blue whale (*Balaenoptera musculus*) (Tonnessen and Johnsen, 1982). Prior to 1900, commercial whalers targeted slow coastal species of baleen whales including the gray whale (*Eschrichtidae robustus*) and two species of right whales (*Eubalaena glacialis* and *Eubalaena australis*) (Tonnessen and Johnsen, 1982). With these populations driven to commercial extinction, commercial whalers ventured into the Southern Hemisphere and discovered the abundant populations of the Southern Ocean. The first prominent Southern Ocean commercial whaling station was established on the south Atlantic island of South Georgia in 1904. After 60 years of hunting, the great whales from the surrounding waters of South Georgia had vanished (Headland 1984).

The Antarctic blue whale was one of the great baleen whale species hunt to near extinction. Over 20% of the recorded catches at South Georgia are blue whales (Headland 1984). As the commercial whaling industry grew within the Southern

Ocean, there was a shift from shore-based factories to open water factory ships, which resulted in an explosion in the annual catch of the Antarctic blue whale. Catches increased from 2,000-6,000 whales in 1914-1924 to 12,734 whales in 1928-1929 and escalated to 29,410 whales in 1930-1931 (Mizroch et al., 1984). In 1966, as Antarctic blue whales had nearly vanished, the International Whaling Commission (IWC) prohibited whaling of blue whales south of 40°S (IWC, 1966). Despite this attempt to protect the species, it is estimated the Antarctic blue whale had been reduced to a population abundance of a mere 395 individuals in 1972 (Branch, 2008).

The severity of the exploitation reduced the once abundant population to less than 1% of its original abundance (Branch et al., 2004). Now, almost fifty years after the IWC declared protection of this species, the extent of the recovery of the Antarctic blue whale population is still debated.

DISTRIBUTION AND TAXONOMY

The blue whale, *Balaenoptera musculus*, is the largest of the baleen whales and largest animal known to have lived; reaching over 30 meters in length and weighing up to 160 metric tons (Mackintosh, 1942). Blue whales feed primarily on krill during summer months in high-latitude polar feeding areas. During winter months, blue whales migrate north to low-latitude regions to breed (Mizroch et al., 1984), although exact locations of breeding areas remain unknown. Social structure has not been observed in blue whale populations. From Southern Hemisphere sighting data, 65% of

sightings were solitary animals with almost 25% observed in groups of 2 and the remaining 10% were observed in groups of 3 or more whales (Branch et al., 2007c).

Blue whales are distributed throughout the northern and southern hemisphere, spanning five major ocean basins. Within this wide global distribution, four subspecies have been described (Rice, 1998) (Fig. 1.1).

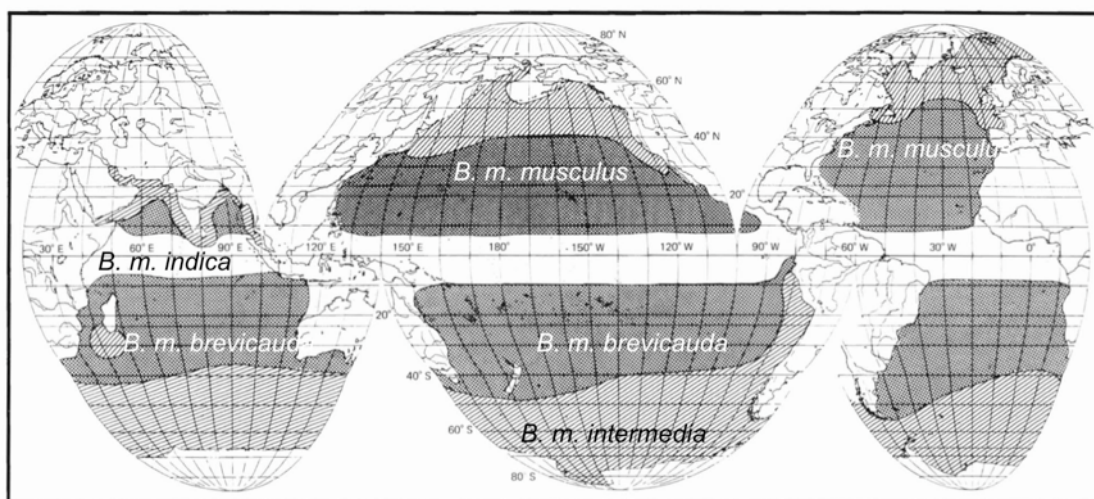


Fig. 1.1 Global distribution of the four described blue whale subspecies: *B. m. musculus* in the North Pacific and North Atlantic, *B. m. indica* in the northern Indian Ocean, *B. m. brevipcauda* in the South Pacific and southern Indian Ocean and *B. m. intermedia* in the Southern Ocean. Dark shaded areas represent tropical breeding grounds and light shaded areas represent polar feeding areas. Figure adapted from Mizroch et al. 1984.

One subspecies has been described within the Northern Hemisphere, found in both the North Pacific and North Atlantic Oceans, *B. m. musculus*. Another subspecies, *B. m. indica*, was once found in the northern Indian Ocean but is believed to have been completely extirpated by the commercial whaling industry (Rice, 1998); although evidence of a distinct blue whale acoustic call has recently been recorded in this region suggesting some portion of the population survived (McDonald et al., 2006).

Within the Southern Hemisphere, two recognized subspecies co-exist, the pygmy blue whale, *B. m. breviceauda*, and the ‘true’ or Antarctic blue whale, *B. m. intermedia*. These two subspecies were first differentiated based on morphological characteristics of catches described from the commercial whaling era (Ichihara, 1966). A sexually mature female Antarctic blue whale average length reaches 25.4-26 m and a sexually mature female pygmy blue whale only reaches an average of 21 m in length (Branch et al., 2007a). The pygmy blue whale also has different body proportions, with a shorter posterior to anus region relative to total body length (Ichihara, 1966).

The pygmy and Antarctic blue whale have relatively segregated distributions, although migration patterns result in some overlap within the sub-Antarctic region. During the austral summer, the Antarctic blue whale congregates near the pack ice south of 55°S, while the pygmy blue whale is found primarily north of 54°S (between 40°S and 54°S) and between 0°E and 80° E. This region is known as the “pygmy box” (Branch et al., 2004; Kato et al., 1995). Only 7% of blue whales south of 54°S are believed to be pygmy blue whales (IWC, 2003). Based on these observations, it has been assumed in previous literature that the whales found ‘south of 54°S’ are Antarctic blue whales (Branch et al., 2004)

The subspecies status of the pygmy and Antarctic blue whale is also supported by differences in signature acoustical calls (McDonald et al., 2006) and patterns of genetic differentiation (Conway 2005; LeDuc et al. 2007). Within the Southern Hemisphere, five distinct acoustic calls have been described; two are found in the South Pacific, two are found in the southern Indian Ocean and one is found throughout

the circumpolar Southern Ocean (McDonald et al., 2006). These acoustic data support two separate pygmy blue whale populations, one in the southeast Pacific and one in the Indian Ocean, as well as a separate Antarctic blue whale population. Acoustic data also suggest Antarctic blue whales remain within the Southern Ocean year round (Sirovic et al., 2004).

Evidence of genetic differentiation between these three geographic Southern Hemisphere blue whale populations is based on nuclear DNA introns, mitochondrial DNA and microsatellite allele frequencies (Conway, 2005; LeDuc et al., 2007). Within the Southern Hemisphere, frequencies of nuclear DNA introns, mtDNA haplotypes and microsatellite loci differentiated all three geographic populations, the Southern Ocean, the South Pacific Ocean and the Indian Ocean (Conway, 2005, LeDuc et al. 2007). The lowest levels of genetic diversity were described in the Indian Ocean population (LeDuc et al., 2007). A STRUCTURE analysis based on 7 microsatellite loci identified three genetic clusters corresponding to the South Pacific Ocean, Indian Ocean, and Southern Ocean basins and only identified a single individual within the Southern Ocean population as a potential pygmy blue whale (LeDuc et al., 2007). A neighbor-joining reconstruction based of mtDNA control region haplotypes showed little resolution of the three Southern Hemisphere oceans. Reciprocal monophyly of the two subspecies or three genetically differentiated populations was not supported.

20TH CENTURY COMMERCIAL WHALING AT SOUTH GEORGIA

The Southern Ocean commercial whaling industry was first established on the south Atlantic island of South Georgia. It was ‘one of the most important places’ in the commercial whaling industry during its years of operation, 1904-1965 (Headland, 1984). Throughout the 61-year commercial whaling industry, 175,250 whales were killed and processed at South Georgia, including over 40,000 blue whales before the surrounding water great baleen whale populations were driven to commercial extinction (Headland, 1984). Humpback, fin, sei, and sperm whales account for the remainder of the catch record at South Georgia. The whale populations killed from the surrounding waters of South Georgia were the first Southern Hemisphere populations targeted by the commercial whaling industry. With over 2 million whales killed within the Southern Hemisphere (Baker and Clapham, 2002), the commercial whaling industry at South Georgia was responsible for slightly less than 10% of the total Southern Hemisphere catches. The high catches of blue whales at South Georgia account for 10% of the total Southern Hemisphere blue whale catch (Headland 1984, Branch et al. 2008).

THE END OF 20TH CENTURY COMMERCIAL WHALING

Of all the blue whale subspecies, the Antarctic blue whale was the most drastically impacted by the 20th century commercial whaling industry. Modern whaling vessels (operating after 1904) caught an order of magnitude more Antarctic blue whales than the totals of all the other blue whale subspecies combined (Branch et al., 2008). It is

estimated that 382,595 blue whales were killed worldwide, of which 90% were estimated to be Antarctic blue whales (Branch et al., 2008). In 1947, the International Whaling Commission (IWC) was established in ‘the interest of nations of the world in safeguarding for future generations the great natural resources represented by the whales’ (Baker and Clapham, 2002). Whaling of blue whales south of 40°S was banned in 1966 (IWC 1966). However, it was not until 1986, when worldwide whale populations had been driven to near extinction, that the IWC imposed a moratorium on all commercial whaling to allow recovery of exploited populations (Baker and Clapham, 2002).

CONTEMPORARY ANTARCTIC BLUE WHALE POPULATION ABUNDANCE

The current abundance of the Antarctic blue whale population is monitored through data collected from the International Decade of Cetacean Research and the Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) cruises. These cruises are conducted aboard Japanese research ships with oversight by the IWC and international scientists. Although the primary purpose of these research cruises is to collect sighting data on the abundance of Antarctic minke whales (*Balaenoptera bonaerensis*), sighting data, biopsy samples and acoustical data are collected from opportunistic sightings of other whales. Three circumpolar surveys (CP) of Antarctic waters south of 60°S have been completed over 26 years (CPI: 1978/79-1983/84; CPII 1985/86-1990/91; CPIII 1991/92-2003/04) during the austral summer (December-March). Antarctic blue whale abundance has been estimated from sighting data from

each of the three surveys and ranged from 440 (CV=0.41; CPI) to 550 (CV=0.48; CPII), to the most recent abundance estimate of 2,280 (CV= 0.36; CPIII) (Branch, 2007). These three estimates are not strictly comparable due to the range of percent survey coverage of waters south of 60°S during the three surveys and the change in survey design (Branch, 2007; Branch and Butterworth, 2001).

Throughout the 20th century, catch records account for over 345,000 Antarctic blue whales killed (Branch et al., 2008). Population dynamic models extrapolated from current abundance and past catch records estimate pre-exploitation Antarctic blue whale population abundance between 235,000 and 307,000 individuals (Branch 2008). The model used by Branch (2008) shows a steep decline in population abundance to a minimum of 395 individuals in the year 1972 (Fig. 1.2). Using the methods developed by Jackson et al. (2008), the model was limited by a lower bound minimum population abundance of 214 individuals (Branch and Jackson, 2008) which was estimated from the number of surviving mtDNA lineages found in the previous genetic study (LeDuc et al. 2007). Despite the evidence of a slow recovery, the most recent abundance estimate of 2,280 individuals still remains at less than 1% of the pre-exploitation abundance (Branch, 2008). The population is believed to be increasing at 1.4-11.6% per annum, an estimate based on the minimum population abundance and current abundance (Branch, 2008).

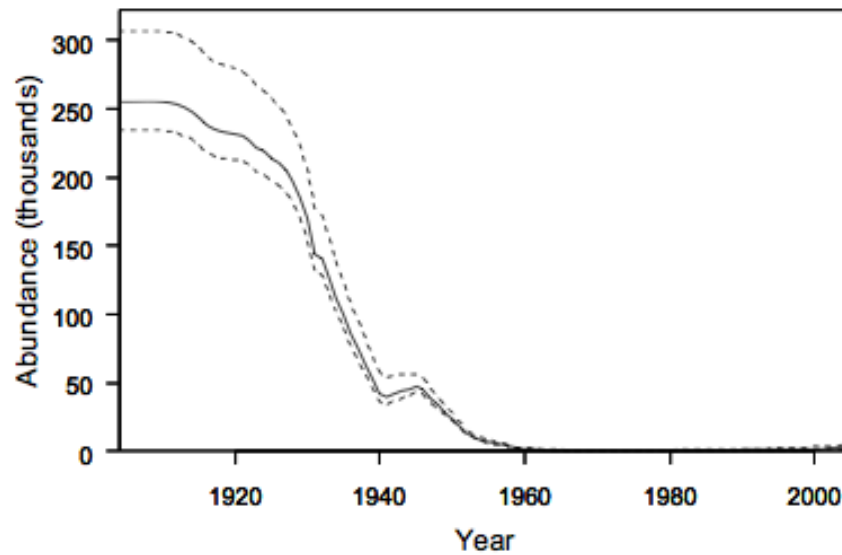


Fig.1.2. Population trajectory of the Antarctic blue whale estimating the decline of population abundance from 235,000-307,000 whales to 395 individuals in 1972 and increasing to the current population abundance of 2,280 (1,160-4,500) individuals from CIII IWC IDCR/SOWER sighting surveys (1991-2004). Figure from Branch 2008.

CONTEMPORARY ANTARCTIC BLUE WHALE POPULATION STRUCTURE

Our understanding of Antarctic blue whale population structure was established during the commercial whaling era. The circumpolar distributed Antarctic blue whale population is currently managed in separate subpopulations, or ‘stocks,’ based on IWC management Areas I-VI (Fig. 1.3).

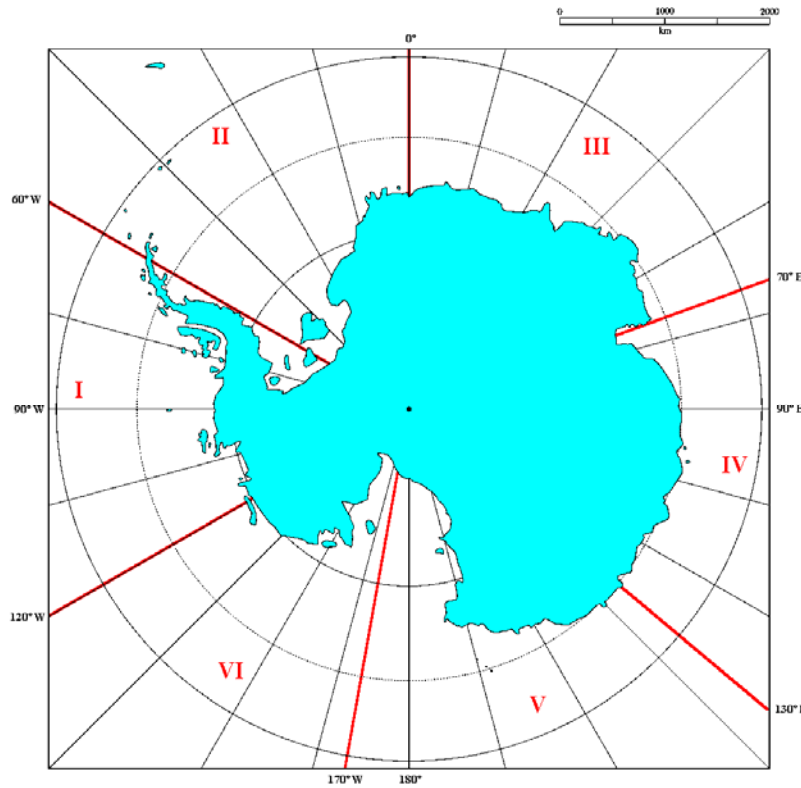


Fig. 1.3. IWC management Areas I-VI in the Southern Ocean.

These boundaries were originally established based on reports of fin and blue whale concentrations in catch records during the 1930s (Donovan, 1991) and are assumed to reflect biological ‘stocks.’ However, the extent to which these reflect true population structure is unknown. The only evidence of Antarctic blue whale population structure is from identification of individual movements. Movement of Antarctic blue whales between Areas has been inferred from mark-recaptures of tagged whales in the ‘Discovery’ marking program (Branch et al., 2007b). ‘Discovery’ marks were steel darts stamped with a unique serial number that was fired into the muscle of the whale with a modified shotgun. The mark was recovered if the whale was killed and flensed

(Hardy, 1940). The location of implantation and recovery of the 'Discovery' marks were recorded in the catch records. A total of 2,295 'Discovery' marks were implanted in Antarctic blue whales (locations in upper panel of Fig. 1.4) but only 104 were recovered from 95 individual whales (some were double marked). 'Discovery' marks from 54 whales were recovered within the same season and only fifteen were recovered over a duration of more than two seasons (Branch et al., 2007b). Fig. 1.4 illustrates the inferred movements of Antarctic blue whales from the recovery of 'Discovery' marks. The majority was recovered within one season (54 whales). Most of these marks were recovered no further than 60° longitude of the implantation location. Fifteen 'Discovery' marks were recovered after a prolonged period of more than two seasons. The longitudinal range of inferred movement increased with longer elapsed time periods, where the longest longitudinal range (180°) was recorded in an elapsed time of at least two seasons (Branch et al. 2007b) (Fig. 1.5). The distance of longitudinal movements evident in these marking records suggests no limitations to gene flow and therefore no opportunity for local population differentiation within the Antarctic blue whale population.

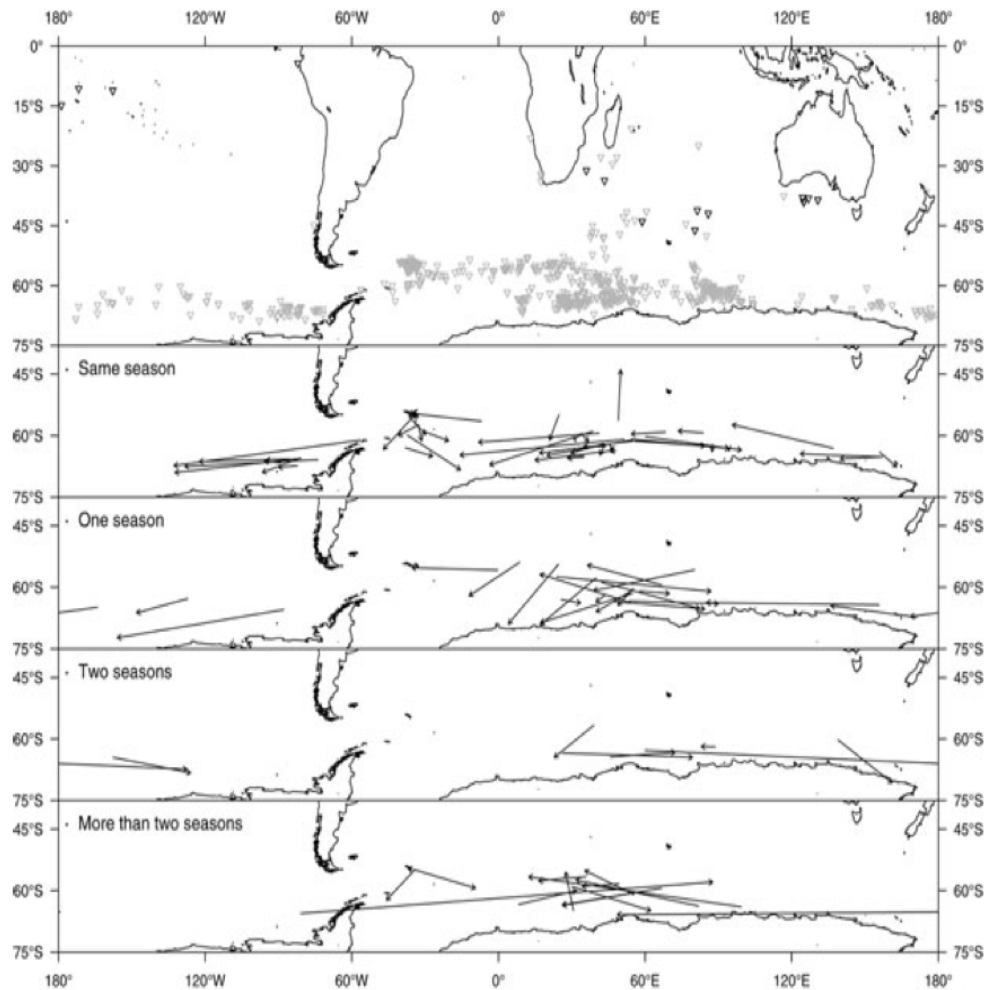


Fig.1.4. Movements of Antarctic blue whales inferred from 'Discovery' mark recaptures. The top panel illustrates the implantation location of 'Discovery' marks. Movements are inferred from recovery of 'Discovery' marks and are depicted within the 4 lower panels for recoveries within the same season, one season, two seasons, and more than two seasons. Figure from Branch et al. 2007b.

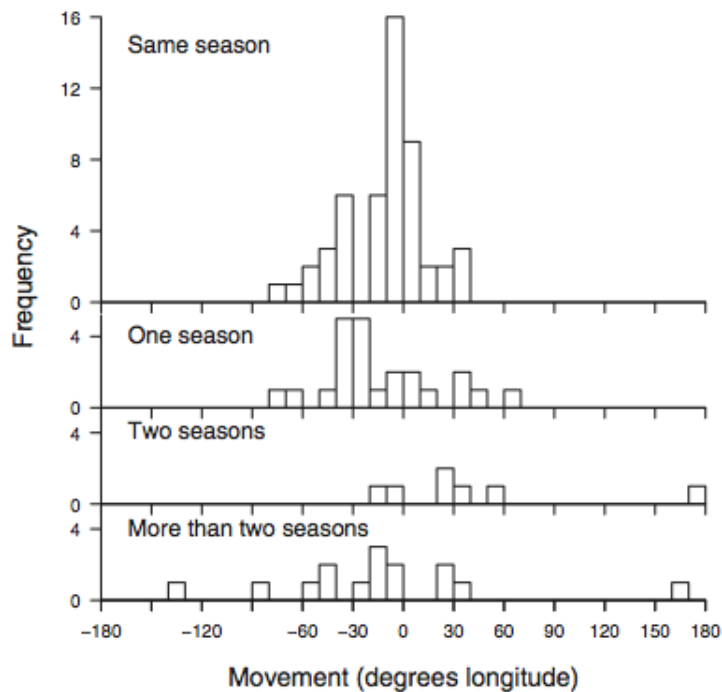


Fig.1.5. The frequency of longitudinal movement of Antarctic blue whales as described from inferred movements from ‘Discovery’ marks. Frequency is shown for recovered marks within the same season, one season, two seasons, and more than two seasons. Figure from Branch et al. 2007b.

SCOPE OF THESIS

This thesis aims to evaluate the impact of the 20th century commercial whaling industry on the Antarctic blue whale population through an analysis of mtDNA diversity. As the extent of recovery of this population from 20th century commercial whaling exploitation is still debated, contemporary Antarctic blue whale mtDNA diversity is used to assess a potential loss of diversity during the demographic ‘bottleneck’ caused by commercial whaling. Comparisons of historical mtDNA diversity, as described in Chapter 2, to contemporary Antarctic blue whale mtDNA diversity, as described in Chapter 3, and an estimation of the minimum female

population abundance are used to gauge this potential loss within Chapter 4. In Chapter 3, we also report the first analysis of genetic differentiation within the contemporary Antarctic blue whale population. Below I describe the genetic tools and sources of samples used within analyses in this thesis.

GENETIC TOOLS: MITOCHONDRIAL DNA (MTDNA)

Mitochondrial DNA (mtDNA) is an ideal tool used in a variety of genetic analyses of baleen whales: including species identification (Baker and Palumbi, 1994) the examination of contemporary population structure (Baker et al., 1993) and the description of genetic diversity within historical populations (Lindqvist et al., 2009). The mitochondrial genome represents a single genetic locus as it is maternally inherited with no recombination (Avice, 1994). The mitogenome is circular, ranges from 15,000 to 20,000 base pairs long and encodes 37 genes. The mtDNA control region, approximately 1,000 base pairs, is believed to be non-coding and selectively neutral. The control region evolves at a rate 5-10 times higher than single-copy nuclear genes (Brown et al., 1979). The rapid mutation rate of mtDNA, due to a lack of repair enzymes during DNA replication (Wilson et al., 1985), makes the control region an ideal marker to study population structure, both between and within species.

The mtDNA control region is recognized for its power in species identification of cetaceans where samples cannot be identified through morphology, i.e. meat market samples (Baker and Palumbi, 1994) or historic remains (Rastogi et al., 2004).

Mitochondrial DNA is found in high copy number in the cell; each mitochondrion contains 2-10 mtDNA copies (Wiesner et al., 1992). This results in a total of 100-100,000 copies of mtDNA per cell, making mtDNA ideal for the amplification of ancient DNA. Primers targeting short DNA fragments allow for amplification of fragmented and degraded DNA. The mtDNA control region is conserved between species and can be used for species identification in the web-based program DNA Surveillance (www.cebl.auckland.ac.nz:9000), which implements a phylogenetic approach for species identification through the use of a curated database of all known cetacean species (Ross et al., 2003).

As mtDNA is representative of maternal lineages, it can be used to investigate population structure influenced by female gene flow (Olavarria et al., 2007). MtDNA diversity is described by the number and frequency of haplotypes within a population. In this thesis, a haplotype is defined as a mtDNA control region sequence differentiated from other sequences by one or more substitutions. Haplotype diversity is compared between populations or subpopulations to test for genetic differentiation. For these analyses, replicates are removed from the dataset to ensure haplotype frequencies are not biased (see *genetic tools: microsatellites* below). Comparison of haplotype diversity between populations is measured by a fixation index (F_{ST}) (Wright, 1931) which can be used to estimate gene flow or genetic differentiation between populations. For bivalent allelic loci, F_{ST} indices range from 0 to 1. Under Wright's island model, where subpopulations are assumed of equal size and alleles have equal probability of exchange (Avise, 1994), values closer to 0 result when allele

frequencies are similar between the two populations and indicate low differentiation. Values closer to 1 result when allele frequencies are different between two populations and indicate high genetic differentiation. F_{ST} indices of genetic differentiation are indicative of gene flow between the sampled regions where two strongly differentiated populations will have minimal gene flow between them. For perspective, a F_{ST} index of 0.2 for nuclear loci is equivalent to 1 migrant per generation (Avice, 1994).

The impact of a demographic ‘bottleneck’ can be explored through analysis of contemporary mtDNA diversity. Within a previously exploited contemporary population, the number of haplotypes is representative of the minimum number of females to have survived the bottleneck. This number of surviving maternal lineages can be extrapolated to estimate the minimum population abundance at the point of the bottleneck (Jackson et al., 2008). A minimum population abundance is an essential parameter in Bayesian logistic models used to construct population trajectories (Branch and Jackson, 2008; Jackson et al., 2008). This parameter creates a lower bound for the estimation of population abundance at the point of the demographic ‘bottleneck’. This population abundance is used to estimate rates of recovery, and can lead to inflated estimates if the minimum population abundance is too low.

A comparison between historic and contemporary mtDNA diversity is the most direct approach to gauge the impact of exploitation. A loss of individuals from within a population has been shown to reduce population mtDNA diversity in commercially exploited whale populations such as in the North Atlantic right whale population (Rosenbaum et al., 2000). This loss of mtDNA diversity is evident through

comparisons of shared and unshared haplotypes between the historic and contemporary populations. This approach is often limited by access to representative samples of sufficient quality for amplification of DNA.

GENETIC TOOLS: MICROSATELLITE GENOTYPING

Variation in the biparentally inherited nuclear marker microsatellites enables the identification of individuals. Microsatellites are non-encoding tandem repeats within the nuclear DNA genome (Awise, 1994). Primers are used to amplify specific microsatellite loci within the nuclear genome. The high variability of alleles at each locus between individuals within a population can be used to create a DNA profile or 'genotype' for each individual. Based on variability of alleles, a probability of identity (pID) can be calculated, which is the probability that two individuals drawn at random from a population would share the same genotype by chance. The removal of replicate individuals reduces bias in mtDNA studies. The identification of replicate individuals (i.e. recaptures) within a dataset allows inference of individual movement throughout the sampled region.

SAMPLE AND DATA AVAILABILITY

Bone samples used for analyses in Chapter 2 were collected between 2006 and 2007 by collaborator Tony Martin in association with the British Antarctic Survey (BAS). Bones were scattered around abandoned whaling stations from the island of South Georgia and have been preserved in permafrost in the sub-Antarctic for the past

40 years. Samples of bone powder were stored at BAS and sent to Hatfield Marine Science Center (HMSC).

In Chapter 3, contemporary Antarctic blue whale mtDNA diversity is described from biopsy samples collected on the IWC IDCR/SOWER cruises from 1990 to 2009 (n=218). A loan request was submitted in June 2009 to the IWC for access to the IWC IDCR/SOWER Antarctic blue whale biopsy samples (Appendix A). Due to an increasing concern of depletion of IWC IDCR/SOWER biopsy samples, a ‘working group’ was established at the 62nd meeting of the IWC. The ‘working group’ allowed for access to the biopsy samples contingent on receiving whole genome amplifications (WGA) (Lasken and Egholm, 2003) in place of genomic DNA (gDNA) for a subset of samples (n=154). The use of WGA as replacement for limited gDNA for future loan requests was to be evaluated in a test study of 40 samples for both gDNA and WGA. For the analyses within this thesis, we received a total of 64 gDNA and 154 WGA samples. This analysis of replicate samples is ongoing and initial results are discussed in Appendix F.

Analyses of contemporary Antarctic blue whale mtDNA diversity were supplemented by published sequences and supplementary material of Southern Hemisphere blue whale mtDNA diversity provided by Ric LeDuc from his 2007 publication (n=46). A subset of samples analyzed in LeDuc et al. (2007) (n=26) were re-sequenced and re-analyzed within this study (Chapter 3). However, 20 samples analyzed within LeDuc et al. (2007), were collected during Japan’s scientific whaling

program (Japanese Whale Research Program under Special Permit in the Antarctic; JARPA) and were not available to be re-analyzed in this study.

In Chapter 4, worldwide blue whale mtDNA diversity is described by both published and unpublished sequences. The Southern Hemisphere mtDNA diversity is described by three geographically distributed populations: the Southern Ocean (Chapter 3), and published sequences from the Indian Ocean and South Pacific from LeDuc et al. (2007). An additional 4 samples from New Zealand were used to supplement the South Pacific population. Sequences for the New Zealand samples were processed by Debbie Steel at the Conservation Cetacean Genetics Laboratory (CCGL) at Hatfield Marine Science Center (HMSC).

The Northern Hemisphere mtDNA diversity is represented by biopsy samples collected from the eastern North Pacific Ocean (n=46) through satellite tagging efforts of the Marine Mammal Institute (MMI) at Oregon State University (OSU) and were available for this study through Bruce Mate. A subset of the samples were collected in collaboration with John Calamabokidis in association with Cascadia Research Collective (n=4). In addition, extracted DNA from the North Atlantic (n=3) was loaned via Carole Conway. The North Pacific biopsy samples and North Atlantic extracted DNA were analyzed in the CCGL at HMSC (Appendix G).

STRUCTURE OF THESIS AND COLLABORATORS

Chapter Two

In Chapter 2, the sequenced mtDNA control region is used to identify to species the largest collection of whale bones (n=281) from the first whaling stations established on the island of South Georgia in the Southern Ocean. The mtDNA diversity is described for the three prominent species found within the dataset. These bones capture the genetic diversity of pre-exploitation great whale populations. Initial results of this study have been submitted as a report to the scientific committee at the 62nd meeting of the International Whaling Commission in Agadir, Morocco (SC/62/SH19). For these analyses, I processed all ‘ancient’ DNA bone samples, performed subsequent laboratory analyses, completed all data and statistical analysis and prepared the manuscript. Tony Martin, formerly of BAS, initiated the study, collected the samples and arranged for permits to transfer the bone powder to SWFSC. My advisor, Scott Baker, negotiated the collaboration, supervised the laboratory and data analyses and helped edit the chapter.

Chapter Three

Chapter 3 updates the previous estimate of contemporary Antarctic blue whale mtDNA diversity. Descriptions of contemporary genetic diversity provide a means to gauge the impact of 20th century commercial whaling on the Antarctic blue whale population in Chapter 4. These data also provide the opportunity for the first analysis of Antarctic blue whale population structure and maternal gene flow. For these

analyses, Kelly Robertson and Brittany Hancock extracted genomic DNA, performed whole genome amplifications (WGA) and curated samples. Rick LeDuc generously provided information as supplementary material in LeDuc et al. (2007). Debbie Steel reviewed mtDNA sequences and DNA genotypes for data quality. My advisor, Scott Baker, initiated the project through the application to the IWC, supervised lab analyses and assisted with statistical analyses.

Chapter Four

Chapter 4 assesses the impact of 20th century whaling on the Antarctic blue whale population. New information from the Southern, North Pacific, and North Atlantic Ocean are integrated to the previous description of worldwide blue whale mtDNA diversity to describe global population structure. The historic South Georgia blue whale mtDNA diversity is compared to contemporary worldwide blue whale mtDNA diversity. In a final analysis, the minimum number of females to survive the demographic ‘bottleneck’ caused by commercial whaling is predicted from current Antarctic blue whale mtDNA diversity following methods of Jackson et al. (2008) in collaboration with Trevor Branch. For these analyses, I extracted gDNA for the majority of the North Pacific biopsy samples and performed lab data analysis for Northern Hemisphere samples (Appendix G). Debbie Steel performed laboratory analyses on a subset of the samples. The updated estimate of mtDNA haplotypes within the contemporary Antarctic blue whale population will be submitted as a manuscript with listed co-author Trevor Branch. North Pacific blue whales samples

were made available primarily through the efforts of the Marine Mammal Institute (MMI) satellite tagging efforts as directed by Bruce Mate and also John Calambokidis in association with Cascadia Research Collective. My advisor, Scott Baker, Bruce Mate and John Calambokidis set up the collaboration for this project. Scott Baker supervised laboratory and data analyses and initiated collaboration with Trevor Branch.

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CHAPTER TWO: BEACHED BONES: GENETIC APPROACH TO SPECIES IDENTIFICATION OF WHALE BONES FROM THE ISLAND OF SOUTH GEORGIA WHALING STATIONS

Indicative co-authors: Tony Martin¹ and C. Scott Baker²

1. The University of Dundee, Nethergate, Dundee, Scotland, UK

2. Marine Mammal Institute, Oregon State University, Newport, OR, USA

ABSTRACT

Skeletal remains of baleen whales, killed during the onset of 20th century commercial whaling, lie scattered across the shores and abandoned whaling stations of the sub-Antarctic island of South Georgia. These bones provide testament to the once abundant whale populations exploited at the first Southern Ocean commercial whaling stations. Here we present species identification of whale bones collected from whaling stations at South Georgia. Using standard ‘ancient’ DNA protocols, we amplified and sequenced the maternally inherited mitochondrial DNA (mtDNA) control region to identify bone samples to species following methods implemented in the web-based program DNA Surveillance. Of the 281 available bone samples, 223 provided DNA of sufficient quality for species identification: 153 bones were identified as humpback whale (*Megaptera novaeangliae*), 49 bones were identified as fin whale (*Balaenoptera physalus*), 18 bones were identified as blue whale (*Balaenoptera musculus*), 2 were identified as sei whale (*Balaenoptera borealis*), 1 was identified as a southern right whale (*Eubalaena australis*) and 1 was identified as a southern elephant seal (*Mirounga leonina*). Haplotype diversity of mtDNA sequences was high ($h \geq 0.98$) in the humpback, fin and blue whale populations, and a

high number of haplotypes were described within the historic populations of these species. The prominence of humpback, fin and blue whale bones in the collection correspond to the catch record of the early years of the whaling industry from the island of South Georgia and suggest the genetic diversity found in the bones reflects that of the relatively pristine whale populations killed during early exploitation between 1904 and the 1920s.

INTRODUCTION

At the beginning of the 20th century, pristine great whale populations surrounded the south Atlantic island of South Georgia (Fig. 2.1). In 1903, Norwegian Captain C. F. Larsen recognized the potential for the commercial whaling industry on the island due to the flat shorelines, safe harbors and access to fresh water (Tonnessen and Johnsen, 1982). The first Southern Hemisphere commercial whaling station was established at Grytviken, South Georgia, in 1904 (Headland, 1984). The initial whaling operations established at South Georgia were floating factories. These were large converted ships anchored in harbors to process whales caught and towed in from the surrounding waters. After processing, remains of whale carcasses were discarded into the harbors, broken up by harbor currents and some of the bones drifted on shore. Between 1904 and 1965, 13 floating factories and 6 land-based whaling stations operated at South Georgia (Headland, 1984) (Fig. 2.1).

By the end of the 61 year commercial whaling industry, 175,250 whales had been caught and processed (Tonnessen and Johnsen, 1982). The relative frequency of

species catch fluctuated throughout the 61 years (Fig. 2.2). During the first 10 years of the whaling industry, 1904-1914, humpback whales were the prominent species caught, accounting for 80% of the total catch (Tonnessen and Johnsen, 1982). However, by 1915, humpback whales in the surrounding waters of South Georgia had been hunted to commercial extinction (Tonnessen and Johnsen, 1982). Blue whales were quick to follow the same fate and by 1936, had disappeared from South Georgian waters. Fin whales were heavily exploited throughout the entirety of the commercial whaling industry and were commercially extinct from surrounding waters at the close of the industry in 1961. The first sei whales were taken from South Georgian waters during the 1913-1914 whaling season. Catches of sei whales were higher towards the end of the commercial whaling industry. A total of 87,555 fin whales, 41,515 blue whales, 26,754 humpback whales and 15,128 sei whales were processed at South Georgia (Headland, 1984). Sperm whales (3,716) and southern right whales (577) account for the rest of the catch record (Headland 1984) (Table 2.1). By 1965, all great baleen whale populations in the surrounding waters of South Georgia Island had been driven to commercial extinction (Headland, 1984).

To date, great baleen whales have not returned to the South Georgian waters, despite the high productivity as a potential feeding area (Clapham et al., 2007). Among the previously exploited species, southern right whales are the most frequently reported whale sighted within the region (Moore et al., 1999; Rossi-Santos et al., 2007). Of the most heavily exploited species, humpback whales have been reported at the highest frequency, primarily in waters northwest of South Georgia (Moore et al.,

1999; Rossi-Santos et al., 2007). Southern right whales and humpback whales have been reported in shallow near-shore waters (Sirovic et al., 2006). Fin whales also have been reported within the region, with a sighting of a potential feeding aggregation of 20 whales in waters northwest of South Georgia (Rossi-Santos et al., 2007). Acoustic data have also identified fin whales within the central Scotia Sea, south of South Georgia, displaying a more pelagic off-shore distribution (Sirovic et al., 2006). A single reporting of blue whales was recorded from each of three independent surveys from 1979-1998; all sighted in waters north of South Georgia (Moore et al., 1999). A single blue whale mother and calf were reported around Shag Rock (120 nautical miles west of South Georgia), with two additional sightings reported from the Bird Island and Mariners reports off the northwest waters of South Georgia (Moore et al., 1999). Blue whale acoustic calls were recorded in northern South Georgia waters and within the Scotia Sea, south of South Georgia at latitudes south of 60°S (Sirovic et al., 2006). The great baleen whale populations exploited by the South Georgia commercial whaling industry have not returned to the surrounding waters. Potentially the exploited populations were local populations that were completely extirpated by whaling (see Chapter 4).

The success of the South Georgian commercial whaling industry drove the abundant great baleen whale populations from the surrounding waters to extinction in 61 years. This exploitation was even more rapid for both the humpback (~10 years) and blue whale (~30 years). From records of the first years of oil production in the South Georgia whaling industry, there is evidence that a large percentage of the whale

was wasted during processing at the floating factories; an average of only a third of the possible oil yield was obtained, resulting in a large percentage of the whale carcass discarded into the harbor waters (Tonnessen and Johnsen, 1982). These high catches and high percentage of waste within early years of whaling on the island have resulted in bones scattered across the shores of the now abandoned whaling stations.

Previous research has attempted to relate historical whaling records to bones through DNA species identification and genetic analyses of whale bones preserved from the whaling era. These data have been used to estimate not only pre-exploitation population abundances but to measure historical genetic diversity (Lindqvist et al., 2009; McLeod et al., 2010; Rastogi et al., 2004; Roman and Palumbi, 2003; Rosenbaum et al., 1997; Rosenbaum et al., 2000). Successful DNA extraction and species identification from the mtDNA *cytochrome b* (*cyt b*) region has been demonstrated for South Georgian bone samples (Lindqvist et al., 2009). Genetic diversity within the *cyt b* region was described and identified to species over 70% of bone samples, revealing 19 humpback, 8 sei, 4 fin, and 1 Bryde's whale within the bone collection (n=44) (Lindqvist et al. 2009). A description of genetic diversity from a more variable molecular marker would allow for comparison with published sequences from contemporary species populations.

Objectives

Here, I extracted DNA from bone and amplified mitochondrial DNA (mtDNA) due to its high prevalence within the cell which allowed efficient amplification from

degraded DNA (Lindqvist et al., 2009). The mtDNA control region is recognized for its power in species identification (Ross et al., 2003) and high level of variability makes it ideal for population genetic studies and for judging loss of haplotype diversity through comparisons to contemporary mtDNA diversity (LeDuc et al., 2007; Olavarria et al., 2007). Using the mtDNA control region I identify to species whale bones collected from shorelines of South Georgia (n=281) and assessed mtDNA diversity within each historic species population.

As the largest collection of whale bones remnant of Southern Ocean commercial whaling, this dataset (n=281) offers the most in depth assessment of whale populations that were exploited in South Georgian waters. South Georgia was a prominent location for the commercial whaling industry in the Southern Hemisphere between 1904 and 1965 (Headland, 1984) and these data offer one of the few opportunities to study pre-exploitation Southern Ocean whale populations.

METHODS

Sample collection

Bones samples (n=281) were collected during fieldwork by the British Antarctic Survey (BAS) from several whaling stations on the island of South Georgia (Fig. 2.1) between 2006 and 2007. Bones scattered along the shores were beach-worn and fragmented, and lacked osteological characteristics to distinguish species or bone anatomy (i.e femur, skull). Among the thousands of fragments, selection was based on denser bones, as the less dense bones were honey-combed and appeared more

susceptible to environmental contamination. Each sample ranged from 0.1-5 kg in mass and 50 mm to 1.5 m in size. No specific precaution was taken to avoid the collection of replicate bone samples. However, this possibility was considered unlikely given the thousands of whale carcasses discarded into the harbors and broken up by harbor currents, potentially moving the individual bones far distances. Following collection, the bone samples were stored in separate plastic bags at 4°C and transferred to BAS, Cambridge, UK, for DNA extraction preparation.

'Ancient' DNA extraction

Standard 'ancient' DNA protocols were followed in order to minimize contamination either between bone samples or from modern cetacean DNA. Bone samples were drilled at BAS in a fume hood at a facility that had never been exposed to cetacean DNA. Prior to drilling, the surface of each bone was cleaned with 70% ethanol, and the fume hood run to remove any bone particles from the air. Twist drill bits were used to create a powder for extraction. Drill bits (2-8 mm) were washed in bleach, autoclaved and UV treated before each sampling. Drilling was performed at the slowest practicable speed per sample. The drilling procedure followed Dalebout et al. (2004) and the powder from the first 2-5 mm of drilling was discarded to minimize contamination. Bone powder was shipped to Hatfield Marine Science Center (HMSC) of Oregon State University (OSU) in Newport, OR and a subsample will be archived at SWFSC. An isolated ancient DNA laboratory was established for processing the bones, in a quarantine unit for the study of fish disease, inside a restricted access

building, separate from the modern cetacean DNA laboratory. Within the ancient DNA laboratory, neither the extraction materials nor the equipment had been exposed to modern cetacean DNA.

Approximately 0.5 cm of bone powder of each sample was transferred to a sterile 1.7 ml eppendorf tube for DNA extraction. DNA extraction was performed in a UV hood in the dedicated ancient DNA laboratory and followed a modified silica-column based procedure (Qiagen DNeasy Blood and Tissue Kit). A separate aliquot of reagents was used for each extraction to avoid contamination of stock reagents. The Qiagen Tissue DNA extraction was modified to include an extended digestion in a 40µl proteinase K digest for 5 hours at 37°C with rotation. Sample tubes were wrapped in parafilm to ensure tubes were adequately sealed. Samples were processed in sets of 11 with a negative control. Due to the dilute DNA concentration yields, DNA was concentrated by evaporation at 37°C and resuspended in 50µl 1X TE. The laboratory surfaces used for extractions were cleaned with a 50% bleach solution between extractions.

mitochondrial DNA (mtDNA) control region sequencing

PCR (polymerase chain reaction) reagents and set-up were performed in a separate laboratory that had never been exposed to cetacean DNA. The mtDNA control region was amplified in reaction conditions consisting of 1X buffer (Invitrogen), 2.5 mM MgCl₂, BSA (Bovine Serum Albumin), 0.4 µM of both the forward and reverse primer, 0.1 mM dNTPs, Platinum *Taq* DNA Polymerase

(Invitrogen) and 5 μ L of template DNA, made up to a total 25 μ l reaction volume with nuclease-free and DNAase-free ultrapure water. Forward primer M13Dlp1.5 and either reverse primer Dlp4 or Dlp5 were used to amplify up to 500 bp of the 5' end of the mtDNA control region (Fig. 2.3). As DNA extracted from bone can be degraded to short fragments and difficult to amplify, the mtDNA control region was amplified in two sequential PCR reactions (Fig. 2.4). After the first PCR reaction, a second amplification was run with a dilution of the initial PCR product as the template. Amplification was most successful with nested primers: an initial amplification using reverse primer Dlp5 followed by a secondary amplification from the product of the first reaction using reverse primer Dlp4. For quality control, both amplifications were run with negative controls and a subsequent dilution negative control during the second amplification. For a subset of the reverse sequenced samples, a PCR product was obtained with only one round of PCR (n=14). Fig. 2.4 gives a visual representation of the different pathways and lists the nested primers used in the primary path of double amplification (e.g. single round/double-round PCR).

PCR reactions were run at a standard thermocycle profile on ABI GeneAmp PCR System 9700: denaturing temperature of 94°C for 3 minutes and 30 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 60 seconds followed by a final extension step of 72°C for 10 minutes. PCR products were electrophoresed on a 1.6% agarose gel, stained with ethidium bromide and exposed to ultraviolet light to visually verify amplification before sequencing.

In preparation for sequencing, excess dNTPs and primers were removed from amplified mtDNA control region products using shrimp alkaline phosphatase and exonuclease I (SAPEX - Amersham Biosciences), and a dye termination sequencing reaction was carried out using a 1/8 dilution of BigDye Dye Terminator Chemistry v3.1 (Applied Biosystems Inc.), following the manufacturer's protocol. Unincorporated bases and dyes were removed using CleanSEQ (Agencourt) and the product was run on an ABI 3730xl. Sequences were visually inspected, edited and aligned using Sequencher v 4.9 (Gene Codes Corporation).

mtDNA Quality control

Quality of each sequence was assessed using ABI Phred scores based on the peaks in the electropherograms as analyzed by Sequencher v 4.9. Using this method, base calls are given a quality score based on the probability they have been miscalled. Quality scores are binned in categories. A score of 20 indicates an error rate of 1 in 100, a score of 30 indicates an error rate of 1 in 1,000 and a score of 40 indicates an error rate of 1 in 10,000. Only sequences with 90-100% of base pair Phred scores at >20 were included in the final dataset (Ewing et al., 1998). Sequences with quality below this threshold were re-sequenced or removed from the dataset. All variable sites were visually inspected to confirm correct base calls. See Appendix B for example electropherograms at quality scores <20, 20-40, >40 to depict the threshold of quality scores in this analysis.

Species identification

Species were identified from the mtDNA control region consensus sequence using the web-based program DNA Surveillance, Witness for the Whale v4.3 (<http://www.cebl.auckland.ac.nz:9000/>). The submitted mtDNA control region sequence was identified to species using a phylogenetic approach and a curated database of all 88 recognized cetacean species, represented by 399 mtDNA control region sequences (Ross et al., 2003). The DNA Surveillance identity of the sequence was validated for a subset of the samples through submission of the sequence to the NCBI database BLAST (Basic Local Alignment Search Tool) of GenBank to confirm species identity. After species identification, all sequences obtained from the bone samples were divided into datasets according to species.

mtDNA haplotype definition

Within each species dataset, sequences were aligned in MACLADE v.4 (Maddison and Maddison, 1992) to a database of worldwide sequences of the species to identify haplotypes. Novel haplotypes were defined based on variable sites within the mtDNA control region sequence that differentiated the sequence from all other mtDNA control region sequences for the species. All variable sites were visually inspected on electropherograms for verification. Unique haplotypes, those found in only one sample, were sequenced using the reverse primer from a second independent amplification for verification (n=77) (see Fig. 2.5). Haplotype (*h*), nucleotide (π)

diversity and the number of variable sites were computed in Arlequin (Excoffier et al., 2005) for each species represented in the bone collection by more than 2 sequences.

Genetic Sex identification

In a preliminary analysis to amplify nuclear DNA from ‘ancient’ DNA, two molecular markers X chromosome P1-5EZ and P2-3EZ, (Gilson et al., 1998) and Y chromosome Y53-3C and Y53-3D, (Aasen and Medrano, 1990) were used to identify sex. A multiplex PCR was set up consisting of 1X buffer (Invitrogen), 2.5 mM MgCl₂, BSA (Bovine Serum Albumin), 0.4 μM of both the forward and reverse sex primers, 0.1 mM dNTPs, Platinum *Taq* DNA Polymerase (Invitrogen) and 5 μL of template DNA to make made up to a total 25 μl reaction volume with nuclease-free and DNAase-free ultrapure water. After the first amplification, a second amplification was run with a dilution of the initial PCR product under the same PCR conditions as the first round, following a similar protocol to *mtDNA control region sequencing*. Amplification following a temperature profile of 3 minutes denaturing at 94°C followed by 30 cycles of denaturing at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 60 seconds with a final extension of 10 minutes at 72°C. PCR products were visualized on 1.6% agarose gels and stained with ethidium bromide. Males are expected to show two bands, an SRY band of 224 bp sand a ZFX band of 443-445 bp. Females are expected to show a single ZFX band of approximate double intensity (Appendix E).

RESULTS

Species identification

The mtDNA control region was amplified and sequenced for 223 of the 281 bone samples (80% success rate). Sequences ranged from 300 to 500 bp in length. Of these samples, 153 were identified as humpback, 49 as fin, 18 as blue, two as sei and one as a southern right using DNA Surveillance. See Appendix C for species identification of bone samples. The one elephant seal sequence was identified in a BLAST search of the NCBI database (max identity score of 99% and 98% query coverage). Species identification was validated for a subset of sequences through reverse sequencing from an independent PCR amplification (n=77; Fig. 2.4). Of these reverse sequenced samples, one sample was initially identified as a fin whale, with two subsequent independent reverse sequences identified as a humpback whale (E-003).

Approximately 20 DNA extraction negative controls provided no evidence of contamination during subsequent amplification. Internal contamination was only evident in two negative PCR controls used in the second re-amplification (i.e. DNA smears). These negative controls were sequenced and but there was no evidence of cross-contamination within sequencing tray (i.e. identical sequences to the blank). The large number of novel haplotypes within the three species population also indicated a low probability of internal contamination.

mtDNA control region haplotype diversity

Sequences from each species were trimmed to a consensus region (347 bp for blue whales; 287 bp for humpbacks; 288 bp for fin whales) to define haplotypes. Haplotype (h) and nucleotide (π) diversity was measured for each of the species (Table 2.2). All three species had high haplotype diversities, ranging from 0.980 to 0.987.

The greatest number of haplotypes (64) was observed in the historic humpback whale population, which had the largest sample size ($n=153$) but was also trimmed to the shortest consensus length (278 bp). The humpback haplotypes were described by a high number of variable sites (47), and the sequences have a high nucleotide diversity (3.16%) in comparison to the other three species. Within the large sample size for the humpback ($n=153$), there were fewer haplotypes proportionate to sample size, in relation to the blue and fin whale population. The blue whale had the smallest sample size ($n=18$) and the longest the consensus mtDNA control region (347 bp). There were significantly fewer variable sites (23 sites) relative to sequence length found within this region compared to the consensus control region sequences of the humpback ($n = 47$ sites, 278bp consensus) and fin whale ($n = 23$ sites, 288bp consensus).

Genetic Sex identification

The amplification of nuclear sex markers verified the potential for amplification of nuclear DNA from 'ancient' DNA, but at a low rate of success. In a trial of 31 samples, we were able to amplify nuclear sex markers and identified sex of

only two individuals. One was identified as a male and one was identified as a female. The low rate of success is consistent with the expected degradation of DNA within the bones.

DISCUSSION

Species identification

To date, this study represents the most comprehensive investigation of mtDNA diversity in populations of great whales in the Southern Ocean prior to their decline due to commercial whaling. A high success rate of DNA extraction enabled the amplification and sequencing of mtDNA (300 to 500 bp) of 223 whale bones, which resulted in a positive species identification of 80% of the bone collection. No evidence of external contamination was detected and only one internal error was revealed by re-sequencing experiments.

The proportion of bone samples attributed to each of the 3 species was consistent with South Georgia whaling records. From the species identification, approximately 70% of the bones collected were identified as humpback. The majority of the remaining 40% of the bones were primarily identified as blue or fin whale. During the onset of the commercial whaling industry on the island of South Georgia, the first species to be heavily exploited was the humpback whale which reached commercial extinction in the South Georgian waters by 1915 (Headland, 1984).

In the early whaling years (1904-1913), whales were processed primarily in the floating factories. After 1913, there was an increase in the number of land-based

whaling stations on the island (Headland, 1984). At these land based stations, the entirety of the whale was processed, even the bone. The frequency of species composition in the bone collection corresponds with the early catch history of the floating factories (Fig. 2.5). As the first sei whale was reported in the catch record during the 1913/1914 whaling season and blue and fin whales were caught at higher intensities after 1914, the presence of two sei whales, 18 blue whales and 49 fin whales with the bone samples indicate that these samples include remains of whales processed during the later years of floating factory whaling.

Historic mtDNA genetic diversity

As the species composition of the bone samples appears to be representative of the great whale populations exploited during the early period of commercial whaling, this indicates that the bone samples have the potential to capture the highest genetic diversity that existed within these pre-exploited populations. The genetic diversity within the ‘early pre-exploited’ populations might have been in comparison to the whales that were caught towards the end of the whaling era when genetic diversity might already have been lost.

The high haplotype diversities seen in the fin, blue and humpback whale indicate a high genetic variability in the South Georgia pre-exploitation cetacean populations. The high haplotype diversities within the three species populations (Table 2.1) are illustrative of large unexploited populations.

Population recovery

There is little evidence of a return of great whale populations to the surrounding waters of South Georgia. South Georgia has been identified as the breeding area for the Brazilian humpback stock A (Stevick et al., 2004; A. Zerbini et al., 2006) despite discrepancy between the low densities of humpbacks sighted around South Georgia and estimated population sizes from Brazilian waters (Andriolo et al., 2006; Rossi-Santos et al., 2007; A. Zerbini et al., 2006; A. Zerbini et al., 2004). There have been only few sightings of fin or blue whales in the area as well, indicating these species have not returned to the area. The most prominent baleen whale found in the surrounding waters is the southern right whale. This species was not heavily exploited during the South Georgia commercial whaling industry and only 522 were recorded in the catch record (Headland 1984).

This study validates not only the utility of ancient DNA, but also the methods for extraction and amplification used in this study, as 80% of the bone samples yielded DNA for positive species identification. We were also able to amplify nuclear DNA in a genetic sex identification trial study but at much lower rates of success. This work employs methods to utilize a previously untouched resource for the study of pre-whaling cetacean populations. Assessments of contemporary cetacean populations will be enhanced with a better comprehension of historical population abundances and genetic diversity. This is assessed in Chapter 4 where historical and contemporary mtDNA haplotype diversities of the Antarctic blue whale are compared to indicate a potential loss of diversity due to exploitation (see Chapter 4).

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Fig. 2.1. Map of the locations of seven whaling stations that operated on the island of South Georgia between 1904 and 1965. Inset illustrates the location of South Georgia in the south Atlantic Ocean (Headland 1984).

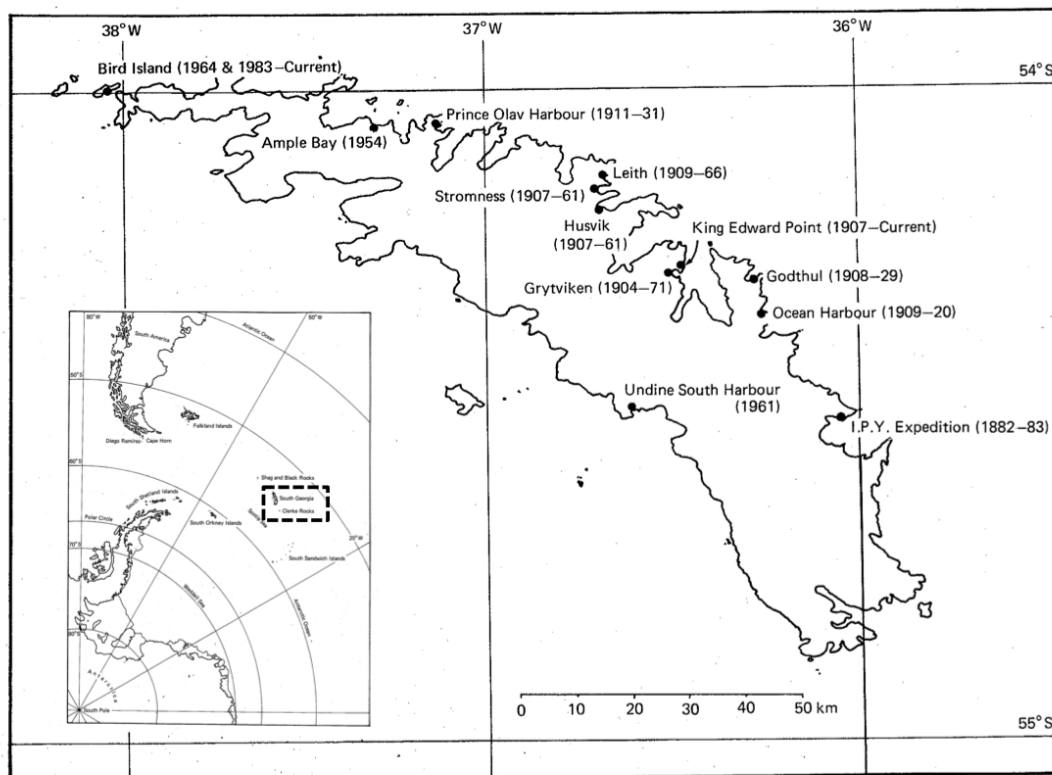


Fig. 2.2. The South Georgia catch record illustrates the total catch of the prominent species killed during whaling seasons beginning with the 1904/05 whaling season (Headland 1984).

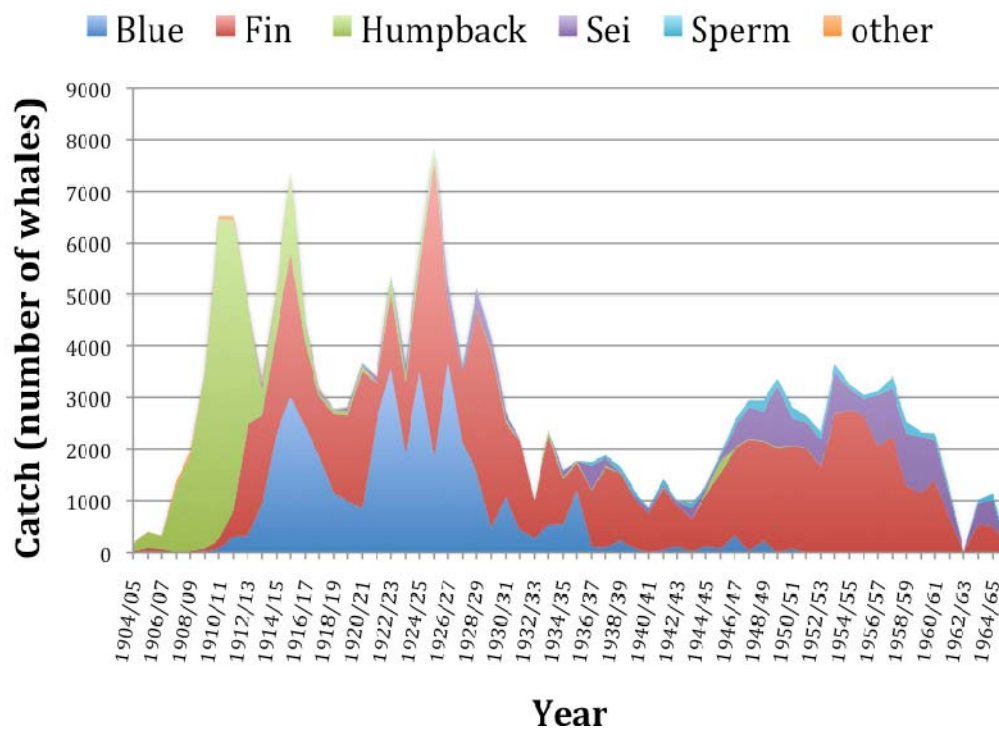


Table 2.1. Total species catch of the five prominent species killed at South Georgia whaling stations (Headland 1984). Catches for whale seasons are categorized in 10-year intervals. The percentage catch per species is listed in bold beneath the total species catch for each time interval.

	Year	Blue	Fin	Humpback	Sei	Sperm	TOTAL
Total Catch	1904-1914	1738	4776	21,894	94	39	28,541
Percentage		6.09	16.73	76.71	0.33	0.14	
	1914-1924	20,719	16,813	3,548	467	202	41,749
		49.63	40.27	8.50	1.12	0.48	
	1924-1934	15,555	22,361	723	12,48	224	40,111
		38.78	55.75	1.80	3.11	0.56	
	1934-1944	2,534	9602	155	1,260	599	14,150
		17.91	67.86	1.10	8.90	4.23	
	1944-1954	926	18,508	432	5,196	1,426	26,488
		3.50	69.87	1.63	19.62	5.38	
	1954-1964	43	14,774	2	6,353	1,068	22,240
		0.19	66.43	0.01	28.57	4.80	
	1964-1966	0	721	0	510	158	1,389
		0.00	51.91	0.00	36.72	11.38	

Fig. 2.3. The targeted DNA sequence by primers used to amplify the mtDNA control region in this study. DlpM13d1p1.5 was used as the forward primer in all reactions, with either Dlp4 or Dlp5 used as the reverse primer. The figure illustrates the replication initiation site by the forward and reverse primers within the mtDNA control region.

M13d1p1.5	5' TGTA AACGACAGCCAGTTCACCCAAAGCTGRARTTCTA 3'
Dlp4	5' GCGGGWTRYTGRTTTCACG 3'
Dlp5	5' CCATCGWGATGTCTTATTTAAGRGGAA 3'

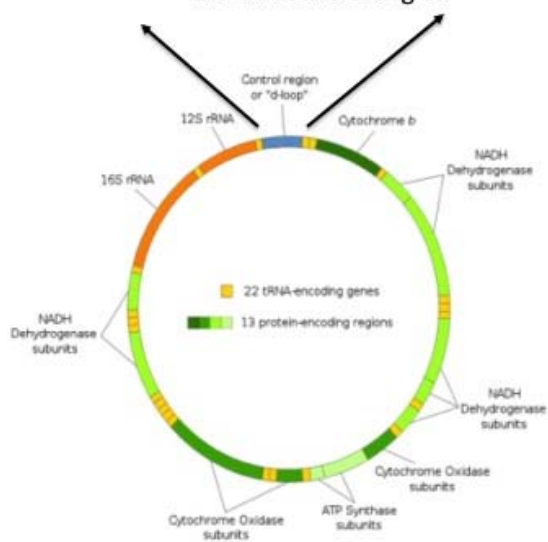
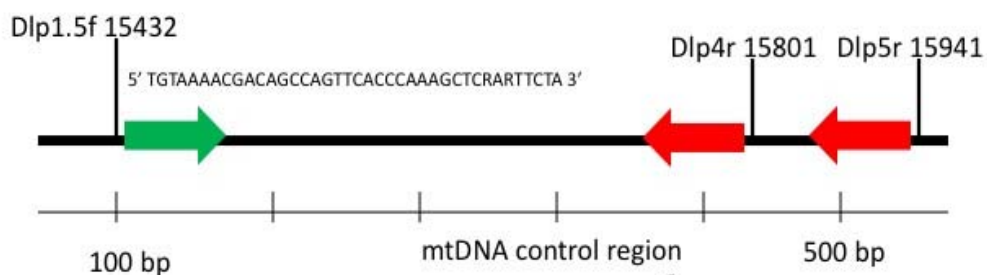


Fig.2.4. The PCR flowchart illustrates the different pathways for mtDNA amplification used in this study. Extracted samples were amplified and forward sequenced using two rounds of PCR. A nested approach was used for the majority of the PCR amplifications for the forward and reverse sequencing. Primers used for each round of PCR are listed within the figure. For a subset of the samples, a nested primer approach was used for the second amplification (see Fig. 2.3 nested primers). The total number of species and haplotypes identified for each species are listed.

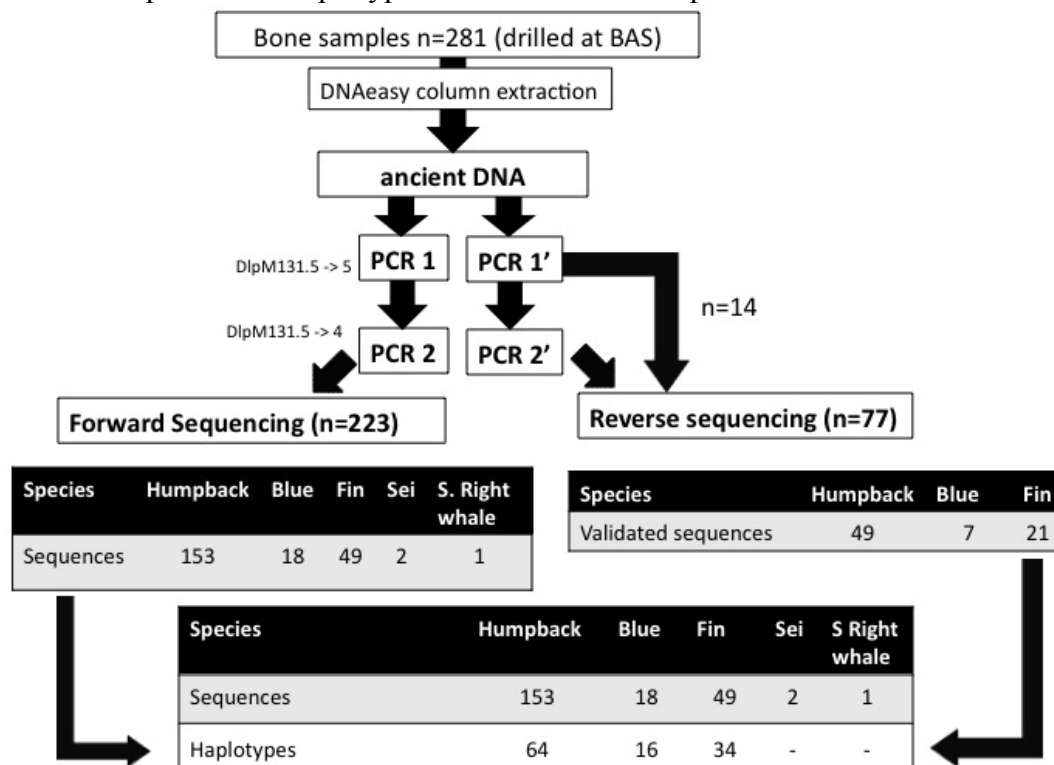
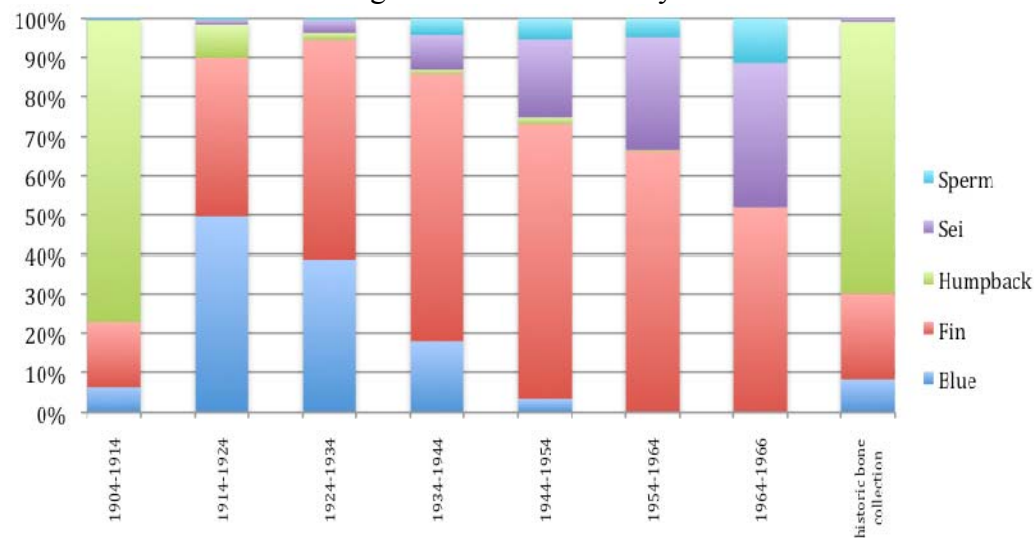


Table 2.2. The number of sequences, consensus sequence length, maximum sequenced region (bp), number of described haplotypes identified for each historic species population identified within the South Georgia bone samples. The number of variable sites and haplotype (h) and nucleotide (π) diversity are listed for each species population. See Tables 2.3, 2.4, and 2.5 for listing of variable sites and haplotype frequencies for the historic humpback, fin, and blue whale populations.

Species	Sequences	Consensus sequence length (bp)	Max sequenced region (bp)	Haplotypes	Variable Sites	H (SE)	π (%)	Reference
<i>M. novaeangliae</i>	153	278	489	64	47	0.9800 (0.0031)	3.16 (1.62)	this study
<i>B. physalus</i>	49	288	474	34	23	0.9821 (0.0080)	1.87 (1.02)	this study
<i>B. musculus</i>	18	347	480	16	23	0.9869 (0.0029)	1.90 (1.05)	this study

Fig. 2.5. Comparison of the species composition of the South Georgia bone sample collection to the South Georgia catch record in 10-year intervals.



CHAPTER THREE: CIRCUMPOLAR mtDNA DIVERSITY AND GEOGRAPHIC DIFFERENTIATION OF THE CONTEMPORARY ANTARCTIC BLUE WHALE

Indicative co-authors: Brittany Hancock¹, Debbie Steel², C. Scott Baker²

1. Southwest Fisheries Science Center, Population Identity Program, 3333 North Torrey Pines Court, La Jolla, CA

2. Marine Mammal Institute, Oregon State University, Newport, OR

ABSTRACT

The 20th century commercial whaling industry killed over 99% of the Antarctic blue whale population between 1904 and 1972. Despite this demographic ‘bottleneck,’ published estimates of mtDNA diversity are high compared to other worldwide blue whale populations (LeDuc et al. 2007). Here we update the previous estimate of Antarctic blue whale mtDNA diversity with an extended dataset of biopsy samples collected from 1990-2009 on IWC IDCR/SOWER research cruises throughout the Southern Ocean (n=218). From microsatellite genotypes, we identify 167 individuals within these samples, describing 49 haplotypes. This dataset is combined with an additional 20 published sequences (LeDuc et al. 2007) to create the most comprehensive dataset of contemporary Antarctic blue whale mtDNA diversity, described by 187 individuals and 51 haplotypes. The Antarctic blue whale population was characterized by a relatively high haplotype diversity (0.968) in comparison to other baleen whales, despite a dramatic loss of Antarctic blue whales during the 20th century. In addition, we perform the first analysis of circumpolar population structure within the Antarctic blue whale feeding grounds. We find significant population structure based on the *a priori* IWC management Areas I-VI ($F_{ST}=0.017-0.09$,

$p < 0.005$). We also identified 6 genotype mark-recapture events, providing the first description of movements of individual Antarctic blue whales within the Southern Ocean since the 'Discovery' mark program over 50 years ago.

INTRODUCTION

Antarctic blue whale exploitation and current abundance

Antarctic blue whale exploitation began in the 20th century at land-based stations in the Southern Ocean. However, the development of open water pelagic factory ships dramatically increased Antarctic blue whale catch, with a peak of 29,420 whales caught in 1931 (Mizroch et al., 1984). By 1966, the pre-exploitation abundance, estimated between 235,000 and 307,000 individuals (Branch, 2008), had declined to less than 1,000 individuals (IWC 1966). With such a decline in population abundance, the IWC banned killing of blue whales south of 40°S to protect the species (IWC, 1966). However, by 1972, the population is estimated to have declined to 395 (235-804; 95% Bayesian intervals) individuals (Branch, 2008). In total, catch records throughout the 20th century account for over 345,000 Antarctic blue whales killed (Branch et al., 2008).

As a part of the IWC Comprehensive Assessment, the recovery of the Antarctic blue whale population has been assessed through efforts of the IWC International Decade of Cetacean Research (IDCR) and Southern Ocean Whale and Ecosystem Research (SOWER) surveys (see Chapter 1 for more detail). However, with a vast distribution and reduced population abundance, it has proven difficult to

gauge the recovery and status of the Antarctic blue whale population. Population abundance estimates have been based on sighting surveys from three circumpolar surveys (CP) initiated in 1978 and completed in 2004. Sighting data was used to estimate abundance based on opportunistic sightings of Antarctic blue whales during CPI (1978/79-1983/84), CPII (1985/86-1990/91) and CPIII (1991/92-2003/04). Cruise dates for each year span the calendar year, as the cruises begin in early austral summer November/December and are completed in March of the following year. Abundance estimates varied from 440 (CV=0.41) individuals during CPI to 550 (CV=0.48) individuals from CPII with wide confidence intervals. The final circumpolar cruise (CPIII), completed in 2004, provided the current abundance at 2,280 (CV=0.36) individuals. Taken together, the 26 years of surveys indicate a sign of recovery (Branch, 2007). Despite this, the population is still recognized as “one of the most depleted populations of whales in the world” (Branch, 2008). Since 2004, IWC IDCR/SOWER annual research cruises have been conducted with independent research aims but have continued to obtain data on the Antarctic blue whale.

Genetic analyses: mtDNA diversity

Genetic analyses offer a powerful tool to study the Antarctic blue whale population through biopsy samples collected during the IWC IDCR/SOWER cruises. Genetic tools have helped differentiate four geographic populations of the three blue whale subspecies; *B. m. musculus* in the Northern Hemisphere; *B. m. breviceauda* in the South Pacific and Indian Ocean; and the Antarctic blue whale, *B. m. intermedia* in the

Southern Ocean. These populations have been genetically differentiated based on analyses of nuclear DNA introns, mtDNA and microsatellite allele frequencies (Conway, 2005; LeDuc et al., 2007).

Within the Southern Hemisphere, few mtDNA haplotypes are shared between the three blue whale populations (South Pacific, Indian and Southern Ocean) supporting distinct genetic population units. The published estimate of Antarctic blue whale mtDNA diversity is high (0.969) in comparison to the South Pacific and Indian Ocean pygmy blue whale populations (LeDuc et al. 2007). This estimate was based on 46 biopsy samples of 46 individuals collected between 1997 and 2002 on IWC IDCR/SOWER research cruises (n=26) and JARPA cruises (n=20) (see Chapter 1 for more information). A total of 26 haplotypes were reported within the population. Further mtDNA analyses also can be used to explore the potential population structure regionally throughout the circumpolar Southern Ocean.

Genetic analyses: population structure and individual movement

Movements of Antarctic blue whales in the Southern Ocean provide insight into population structure within feeding grounds. The only individual movement information for Antarctic blue whales has been recorded in the mark-recaptures of ‘Discovery’ marks (see Chapter 1 for more detail). A total of 2,295 ‘Discovery’ marks were implanted and only 104 were recovered from 95 individual whales (Branch et al., 2007b). The majority of ‘Discovery’ marks were recovered within one season (54 whales), and most were recovered no further than 60° longitude of the implantation

location. Fifteen ‘Discovery’ marks were recovered after a prolonged period of more than two seasons. The longitudinal range of inferred movement increased with longer elapsed time periods, where the longest longitudinal range (180°) was recorded in an elapsed time of at least two seasons.

Objectives

This study aims to update the previous estimate of mtDNA diversity of the Antarctic blue whale through analysis of an extended dataset collected on IWC IDCR/SOWER cruises from 1990-2009 (n=218). This new estimate of contemporary diversity is compared to ‘early’ whaling diversity and used to revise estimates of surviving mtDNA lineages following methods of Jackson et al. (2008) in Chapter 4. As a second objective, we describe the distribution of mtDNA haplotype diversity within the Southern Ocean and explore potential structure based on the *a priori* geographic regions established by the IWC management Areas I-VI. For these mtDNA analyses, replicate individuals are removed from the dataset using microsatellite allele frequencies genotyped up to 15 loci. Through the identification of replicate individuals, we infer individual movement of Antarctic blue whales throughout the circumpolar Southern Ocean in a genotype mark-recapture. Further detailed analyses of population structure of nuclear loci was limited by quality of genomic DNA (gDNA) and whole genome amplifications (WGA) made available through a loan request to the IWC (see Chapter 1).

METHODS

Antarctic blue whale samples

Antarctic blue whale biopsy samples were collected during the IWC IDCR/SOWER cruises from 1990-2009 (n=218) as described in annual cruise reports. Sampling locations spanned Southern Ocean IWC management Areas I-VI throughout 19 years (Fig 3.1; Table 3.1). Antarctic blue whale samples were defined in this study as biopsy samples collected below the Antarctic convergence (54°-55°S). A subset of these samples (n=26) were included in the previous analysis of Antarctic blue whale mtDNA diversity (LeDuc et al., 2007) and re-analyzed in this study (i.e. mtDNA sequencing and microsatellites were repeated). The remainder of samples analyzed in LeDuc et al. (2007) (n=20) were collected from JARPA cruises and were not available for re-analysis in this study; however, published sequences were available in supplementary material provided by Ric LeDuc. IWC IDCR/SOWER biopsy samples are archived at Southwest Fisheries Science Center (SWFSC) in La Jolla, CA, where genomic DNA (gDNA) was extracted and whole genome amplifications (WGA) completed. For these analyses, we received 64 gDNA samples and 154 WGA samples. See Appendix D for biopsy sample collection locations, tissue storage and whether gDNA or WGA was provided for the sample. Genomic DNA extractions were performed at SWFSC following a variety of methods, namely lithium chloride extraction (Gemmell and Akiyama, 1996), sodium chloride protein precipitation (Miller et al., 1988), silica-based filter purification (DNeasy kit, Qiagen, Valenica, CA, USA) and (Xtractor gene, Corbett Robotics, San Francisco, CA, USA) or a

standard phenol/chloroform extraction (Sambrook et al., 1989). The WGA were prepared using the REPLI-g UltraFast Minikit (Qiagen).

mtDNA sequencing and resolution of mtDNA haplotypes

The mtDNA control region was amplified in reaction conditions consisting of 1X buffer (Invitrogen), 2.5 mM MgCl₂, BSA (Bovine Serum Albumin), 0.4 μM of both the forward and reverse primer, 0.1 mM dNTPs, Platinum *Taq* DNA Polymerase (Invitrogen) and 1 μL of template gDNA or WGA, made up to a total 10 μl reaction volume with nuclease-free and DNAase-free ultrapure water. Forward primer M13Dlp1.5 and reverse primer Dlp8 were used to amplify 560 bp of the 5' end of the mtDNA control region. PCR reactions were run at a standard thermocycle profile on the ABI GeneAmp PCR System 9700: denaturing temperature of 94°C for 3 minutes and 30 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 60 seconds, followed by a final extension step of 72°C for 10 minutes. PCR products were electrophoresed on a 1.6% agarose gel, stained with ethidium bromide and exposed to ultraviolet light to visually verify amplification before sequencing.

In preparation for sequencing, excess dNTPs and primers were removed from amplified mtDNA control region products using shrimp alkaline phosphatase and exonuclease I (SAPEX - Amersham Biosciences), and a dye termination sequencing reaction was carried out using a 1/8 dilution of BigDye Dye Terminator Chemistry v3.1 (Applied Biosystems Inc.) following the manufacturer's protocol. Unincorporated

bases and dyes were removed using CleanSEQ (Beckman Coulter Genomics) and the product was run on an ABI 3730xl. Sequences were visually inspected, edited and aligned using Sequencher v 4.9 (Gene Codes Corporation).

mtDNA Quality control

Quality of each sequence was assessed using ABI Phred scores based on the peaks in the electropherograms as analyzed by Sequencher v.4.9. Using this method, base calls are given a quality score expressed as the probability of an error at each base; categorized from 20 (error rate of 1 in 100), 30 (error rate of 1 in 1,000) and 40 (error rate of <1 in 10,000). Only sequences with 90-100% of Phred scores at >20 were included in the final dataset (Ewing et al., 1998). Sequences with quality below this threshold were re-sequenced or removed from the dataset. All variable sites were visually inspected to confirm correct base calls. See Appendix B for example electropherograms with quality scores <20, 20-40, >40.

mtDNA haplotype definition

MtDNA haplotypes were described based on one or more substitutions within the 560 bp control region sequence and compared to a database of worldwide blue whale mtDNA control region sequences which included published sequences from the Indian and South Pacific (LeDuc et al., 2007), and unpublished sequences from the North Pacific (n=46; Appendix G), North Atlantic (n=3; Appendix G) and New Zealand (n=4). Unique haplotypes that were not found in the worldwide database were

reverse sequenced from an independent amplification for verification of unique polymorphic sites. Previously undescribed haplotypes were named according to the lab code of the first sample found to have that haplotype. Haplotypes derived from samples used previously by LeDuc et al. 2007 (n=24) were sequenced and extended to 560 bp.

Genetic Sex identification

A PCR targeting sites on the nuclear X and Y chromosome was used to identify sex: primers P1-5EZ and P2-3EZ on the X chromosome (Gilson et al., 1998) and primers Y53-3C and Y53-3D on the Y chromosome (Aasen and Medrano, 1990). A multiplex PCR was run under reaction conditions of 1X buffer (Invitrogen), 2.5 mM MgCl₂, BSA (Bovine Serum Albumin), 0.4 μM of both X and Y sex primers, 0.1 mM dNTPs, Platinum *Taq* DNA Polymerase (Invitrogen) and 1 μL of template gDNA or WGA, made up to a total 10 μl reaction volume with nuclease-free and DNAase-free ultrapure water. Amplification following a temperature profile of 3 minutes denaturing at 94°C, followed by 30 cycles of denaturing at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and an extension at 72°C for 60 seconds, with a final extension of 10 minutes at 72°C. PCR products were visualized on 1.6% agarose gels and stained with ethidium bromide. Males are expected to show two bands, an SRY band of 224 bp and a ZFX band of 443-445 bp. Females are expected to show a single ZFX band of approximate double intensity (Appendix E).

Microsatellite genotyping and matching

To identify replicates within the dataset, samples (n=218) were genotyped for up to 17 microsatellite loci (Table 3.2). These microsatellite markers were chosen based on LeDuc et al. (2007) and a trial study of other published loci on 19 North Pacific blue whale samples (D. Steel) (see Table 3.1, where asterisks note loci used in LeDuc et al. 2007). All microsatellites were amplified individually (i.e. no PCR multiplexes) using the following thermocycle profile: denaturing for 3 minutes at 94°C, and 30 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 60 seconds, followed by a final extension step of 72°C for 10-30 minutes depending on the locus. Products were run out on 1.6% agarose gels and stained with ethidium bromide to visually confirm amplification. Microsatellite products were co-loaded for genotyping in 4 sets of up to 5 loci (see Table 3.2). Two µl of co-load in addition to size standard GS500 LIZ (Applied Biosystems) were heated to 95°C for 5 minutes and genotyped on ABI 3730 Genetic Analyzer (Applied Biosystems) at Hatfield Marine Science Center (HMSC). Each co-load was run with negative controls to detect contamination. Genotypes were assigned after visually checking the automated calling of alleles by GENEMAPPER v.4.0 (Applied Biosystems). For quality control purposes, peaks were required to have signal strength above 100. All allele calls were double checked by an independent researcher (D. Steel) and the identification of blind replicates was used as check for errors (see Appendix F). Microsatellite genotypes were reviewed using the program CERVUS v.3 (Kalinowski et al., 2007) to identify likely replicates. Individual

movement was inferred from the identification of replicates. The sampling locations and dates, probability of identity (pID) and approximate distance traveled (km), as calculated by Google Earth, are reported for each individual.

mtDNA haplotype and nucleotide diversity and differentiation

Measurements of haplotype (h) and nucleotide (π) diversity were calculated in Arlequin v. 3.1 (Excoffier et al., 2005). Antarctic blue whale diversity was compared to haplotype (h) and nucleotide (π) diversity of other worldwide cetacean populations that have undergone a demographic ‘bottleneck’ due to commercial whaling. Neutrality was tested in a Tajima’s D and Fu’s F test performed in Arlequin.

Population structure was measured in an Analysis of Molecular Variance (AMOVA) as implemented in Arlequin (Excoffier et al., 2005). Differentiation between the IWC management Areas I-VI was measured for both F_{ST} and ϕ_{ST} . All individuals identified within an Area were included in calculation of haplotype and nucleotide diversity for that Area. The single recapture found in two Areas was included in both Areas (III and V), resulting in a total sample size of 188. The significance of genetic differentiation was tested using 5,040 random permutations of the data matrix as well as the test of differentiation or modified exact test in Arlequin. Temporal heterogeneity was also tested through a pairwise F_{ST} comparison of mtDNA haplotype between individuals within Area III in 2006 ($n=33$) and 2007 ($n=65$).

RESULTS

mtDNA quality control

Of the total of 218 samples amplified, sequences with 90% of base pair ABI phred scores >20 were included in the dataset, resulting in a final dataset of 204 mtDNA control region sequences (94% success). A total of 49 unique haplotypes were found, of which 24 matched to LeDuc et al. (2007). The frequencies of haplotypes ranged from 1 to 13 samples. All unique haplotypes (n=6) were validated through reverse sequencing from an independent PCR.

Microsatellite quality control

Amplification was attempted for 17 loci with variable success. Two loci (Ev14 and Ev104) were excluded from analyses due to evidence of allelic dropout and excessive stutter patterns. Genotypes from a subset of loci (14) were checked by a second researcher to validate identification (D. Steel). A subset of samples, for which there was adequate amounts of WGA or gDNA, was rerun to increase the number of loci within the genotype for the replicate analysis. Following Quality Control (QC) review, an average of 11.7 loci out of 15 was genotyped for each sample and was considered suitable for identification of replicate samples. However, given amplification failures for many WGA samples, genotypes were incomplete.

Identification of genetic sex

Genetic sex was determined for 110 out of the 218 samples (50% success rate); identifying 69 males and 41 females. The relatively poor success of sex identification was attributed to low gDNA and WGA concentrations and variability in WGA (see Appendix F). As genetic sex identification for the dataset was incomplete it was used to supplement replicate identification through microsatellite genotypes and not for further analyses of sex-specific patterns in mtDNA.

Identification of replicates

As the primary purpose of microsatellite genotyping was identification of replicates for further mtDNA analyses, we first sorted and compared incomplete genotypes by haplotypes. All samples were divided into haplotype groups and replicates were identified using CERVUS v.3 and through visual comparisons between genotypes. Sex information was used to support identification of replicates where available. Samples which amplified for only 3-5 loci, but alleles but did not match other samples within their haplotype group, were included in remaining analyses. Four samples that provided haplotype did not amplify for enough loci to verify as individuals, were removed from further analyses, as they were potential replicates (see 'pseudo' replicates 21-24 Table 3.3).

Replicates were further evaluated according to whether they were considered to be 'pseudo' replicates or recaptures. 'Pseudo' replicates were replicate samples of individuals that had been collected on the same day in the same location. A 'recapture'

was considered to be an individual that was sampled on different dates. Incomplete genotypes were used for identification of 'pseudo' replicates but more loci were required for identification of recaptures. A minimum of 4 matching loci were used to calculate probability of identity (pID) for 'pseudo' replicates and a higher threshold of a minimum of 8 matching loci was used to calculate the pID for recaptures.

Genotype matching at 4-15 loci resolved 20 'pseudo' replicates with a probability of identity (pID) range of 1.03×10^{-03} to 2.38×10^{-15} (Table 3.3). Genotyping matching also revealed 6 recaptures of individuals on different dates and in some cases, different Areas. Four within-year recaptures were identified (Table 3.4). All within-year recaptures were captured and recaptured within Area III, one event occurring in 2006 and the other three in 2007. Probability of identity for within year recaptures ranged from 3.50×10^{-14} to 1.89×10^{-09} . Two individuals were recaptured between years. Capture 51452, a female, was originally captured in Area V in 2002 and was re-captured in Area III in 2006 (pID= 6.13×10^{-19}). Capture 62489, a male, was captured in Area III in 2006 and again in Area III in 2007 (pID= 2.94×10^{-14}). Inferred movements of individuals from the recaptures ranged from approximately 100 to 6,650 kilometers and can be seen in Fig. 3.2.

mtDNA haplotype resolution at 560 bp

The mtDNA control region haplotypes sequenced were 560 bp compared to the 414 bp sequenced in LeDuc et al. (2007). However, the extended mtDNA control region sequences only differentiated one previously undescribed haplotype (noted in

Table 3.5 by *) based on one substitution at site 491. Because the majority of the variation of mtDNA control region diversity is found within the first 347 bp, this shorter length of sequence was used for consistency and comparison to the published sequences.

mtDNA haplotype resolution at 347 bp

After removal of replicate samples, 167 individuals remained within the dataset. A total of 49 haplotypes were found, based on 46 variable sites: 45 transitions and 3 transversions (Table 3.5). Of the 49 haplotypes, 23 had not been found previously in any of the worldwide blue whale populations. Six of the 23 previously undescribed haplotypes were found in only one individual and denoted by an asterisk in Table 3.5. Two of the previously found haplotypes had been reported in the Indian Ocean and South Pacific blue whale populations (LeDuc et al., 2007) but had not previously been reported in the Southern Ocean. Three haplotypes were relatively common within the Antarctic blue whale population: haplotype l, haplotype m, and haplotype 72956 together accounting for 21% of all individuals (Fig. 3.3).

Sequence information from an additional 20 samples from the Southern Ocean collected during JARPA surveys (LeDuc et al., 2007) were added to the dataset for a cumulative database of 187 individuals, the most comprehensive dataset of Antarctic blue whales. Within these 20 sequences added from LeDuc et al. (2007), 13 haplotypes were found, 11 of which were also found within the 49 haplotypes in the

IWC IDCR/SOWER dataset (n=167). Thus, in total, the final dataset represented 51 haplotypes from a database of 187 individuals (Fig. 3.4).

Haplotype and nucleotide diversity and test of neutrality

Based on the final total of 187 individuals, haplotype diversity within the Antarctic blue whale was high (0.968), with a nucleotide diversity of 1.63%. With an increase in sample size, Antarctic blue whale haplotype diversity remained similar to the previous estimate (0.969) (LeDuc et al., 2007) (Table 3.6).

Haplotype diversities for the IWC management Areas I-VI ranged from 0.8727 in Area II to 1.00 in Area I. Nucleotide diversity ranged from 1.63% in Area II to 2.35% in Area I (Table 3.7). Seven haplopytes were shared between 3 or more Areas (Table 3.8).

Both Tajima's D and Fu's F test did not provide evidence of a recent bottleneck. Based on 5,000 simulations, Fu's F test was significant ($F=-24.74$, $p=0.002$) supporting population growth or genetic hitchhiking. These results were consistent by Tajima's D test, although not significant (-0.874670 , $p=0.209$).

Geographic differentiation and temporal heterogeneity

The AMOVA revealed significant overall differentiation among the 6 feeding Areas ($F_{ST}=0.03$, $\phi=0.03$ $p=0.0057$). Significant F_{ST} pairwise comparisons of haplotype diversity within each Area ranged from 0.017 to 0.090 ($p<0.05$) and significant ϕ_{ST} comparisons of nucleotide diversity ranged from 0.053 to 0.080

($p < 0.05$) (Table 3.9). The sample size for Area I was considered too small for statistical analysis but F_{ST} and ϕ_{ST} are reported for clarity in the table. Significant differentiation in F_{ST} values was observed in all pairwise Area comparisons except between Areas IV and VI and Areas V and VI. Area VI had a small sample size ($n=8$, 6 haplotypes) and shared 3 haplotypes with Area V and 2 haplotypes with Area IV. Area IV and V shared 7 haplotypes, but this was a low percentage of shared haplotypes compared to the total haplotypes within each area (Area V, 22 haplotypes; Area IV, 15 haplotypes).

A test of temporal heterogeneity of mtDNA diversity was only possible for Area III but revealed a small but significant difference between 2006 ($n=36$) and 2007 ($n=84$) ($F_{ST}=0.019$, $p=0.029$).

DISCUSSION

Identification of replicates

We identified 167 individual Antarctic blue whales within the IWC IDCR/SOWER dataset from 218 samples, based on mtDNA haplotypes and partial genotypes described from up to 15 microsatellite loci. With the addition of 20 individuals from JARPA as reported in LeDuc et al. (2007), the dataset was representative of 187 individuals or approximately 8% of the current population, based on the most recent abundance estimate of 2,280 individuals (Branch, 2008).

The identification of genotype recaptures provided evidence of Antarctic blue whale movement within the Southern Ocean. We identified two recapture events

between years and infer movement of a female between Area III and Area V, a distance of approximately 6,650 kilometers. The remaining four recapture events occurred in 2006 or 2007 and were recaptured within the same Area (Area III). Although the number of recapture events is small, the trends of longitudinal movement and time elapsed between re-sampling events are consistent with trends in longitudinal movement reported from the 'Discovery' marks (Branch et al., 2007a). The majority of longitudinal movement as inferred from the 'Discovery' marks remained within 60° of their implantation location. As the IWC management areas span 60° longitude, five of the six identified recapture events follow this trend. The one recapture event that documented movement of 130° longitude was over an elapsed time of over 2 years is also consistent with 'Discovery' mark records showing movement of up to 180° longitude in an elapsed time of more than two years (see Chapter 1).

mtDNA haplotype resolution

This study represents the first circumpolar assessment of mtDNA diversity and population structure for the Antarctic blue whale to date (n=187). The 414 bp haplotypes published by LeDuc et al. (2007) were extended to 560 bp which differentiated only one previously undescribed haplotype further. Based on these results, we conclude that the majority of mtDNA control region diversity is found within the first 347 bp for the Antarctic blue whale, allowing us to compare to other sequences, including Chapter 2.

This study updates the previous census of mtDNA diversity of 26 haplotypes (n=46) to 51 haplotypes (n=187). Of the 46 samples included in LeDuc et al., 26 samples were re-sequenced in our analysis (n=167). The re-sequencing of these 26 samples confirmed 24 of the previously described haplotypes by LeDuc et al. (2007). Our study identified 23 previously undescribed mtDNA haplotypes within the contemporary sample (n=167). Two additional haplotypes that had been described in the pygmy blue whale populations (one in the southeast Pacific and one from the Indian Ocean) (LeDuc et al., 2007) also were found in our extended contemporary Antarctic blue whale dataset. This result may indicate shared haplotypes between the two populations or a potential pygmy blue whale migrant into the Southern Ocean.

From the previous census of 26 haplotypes, Branch and Jackson (Branch and Jackson, 2008) predicted a total of 51 surviving haplotypes within the contemporary population. Our discovery of 23 additional haplotypes in addition to the previously described 28 haplotypes equals this previous prediction of haplotypes, suggesting that this prediction might be too low. The number of mtDNA haplotypes within the contemporary population described here is used to update their prediction of the number of haplotypes within the population in Chapter 4.

Haplotype and nucleotide diversity

The Antarctic blue whale population appears to have retained high levels of mtDNA diversity despite an estimated decline to less than 1% of former abundance (Branch, 2008). Haplotype diversity within the Antarctic blue whale population

($n=187$) was 0.968, similar to the previously reported value by LeDuc et al. (2007) (0.969). However, this haplotype diversity is higher than other blue whale populations in the North Pacific, South Pacific, and Indian Ocean (Table 3.6) and other worldwide baleen whale populations reduced to mere hundreds of individuals by the commercial whaling industry (Clapham et al., 1999). Antarctic blue whale mtDNA diversity may not have lost due to the short period of intensive exploitation. The longevity of blue whales and subsequent long generation time (Taylor et al., 2007) has lessened the impact of exploitation on mtDNA diversity within the population (Amos, 1996) (see Chapter 4).

Test of neutrality

Tajima's D and Fu's F test of neutrality did not provide evidence that the population has recently undergone a bottleneck, but rather provide evidence of a recent population expansion or genetic hitchhiking. Whaling of the Antarctic blue whale was prohibited in 1966 and the population has shown signs of recovery over the past 44 years. This elapsed time is equivalent to approximately two generations for this long-lived species but given estimated longevity, probably includes individuals that lived through the demographic 'bottleneck' (Taylor et al., 2007). Within this time the population is estimated to have increased four to five fold, although not sufficient time for new mtDNA mutations to have arisen (Roman and Palumbi, 2003). The absence of evidence of a genetic bottleneck, as seen in results of the neutrality test, is also consistent with the high mtDNA diversity.

Population structure

Here, we report the first evidence of population structure for mtDNA in the Antarctic blue whale based on the *a priori* Southern Ocean IWC management Areas I-VI. The levels of differentiation in the Antarctic blue whale population observed between Area pairwise comparisons are similar to the differentiation observed between pairwise comparisons of the breeding areas for the South Pacific humpback whale ($F_{ST} = 0.009$ - $F_{ST} = 0.079$) (Olavarria et al., 2007). These levels of differentiation are lower than the levels observed between breeding areas of the Indo-Pacific compared to the breeding areas of the South Atlantic southern right whale subpopulations ($F_{ST} = 0.147$ - $F_{ST} = 0.235$). The lower haplotype diversity observed in right whales in the Indo-Pacific basin (0.701) and low number of haplotypes (7) may be influencing the effect size between the two isolated southern right whale basin subpopulations. The humpback and southern right whale samples for these analyses were collected from the breeding grounds, where these subpopulations are believed to be ‘source’ or breeding stocks. There is no evidence yet for how the Antarctic blue whale population is structured on breeding grounds.

The only comparison for feeding Areas of the Southern Ocean is reported for the Antarctic minke whale. Population structure within the Southern Ocean feeding Areas (I-VI) has been reviewed through further division of the IWC management Areas I-VI. Significant differentiation between Antarctic minke ‘stocks’ within the feeding Areas IV and V have been identified by dividing the areas into 3 geographical sectors, 70°-100°E, 110°-150°E and 150°-180°E (Pastene, 2009). Analyzing alternative divisions

within the Antarctic blue whale population could uncover stronger population structure. However, the finer scale area divisions would decrease sample sizes and reduce the power of statistical analyses.

Antarctic blue whale population structure is continuing to be explored through an analysis of movement in ongoing photo-identification studies. As a part of the in-depth assessment of Southern Hemisphere blue whales (IWC, 2007), the photographs from the 1987-1988 to 2007-2008 IWC IDCR/SOWER cruises are being reconciled. Preliminary photo-identification studies reconciling over 21,000 photos collected over 19 years have identified 203 individual whales (Olson, 2008). To date, only within Area comparisons have been made. Photo-identification comparisons between Areas may identify more individual movement within the Southern Ocean and provide information about population structure.

Test of heterogeneity

Temporal heterogeneity in mtDNA haplotype diversity was observed between years in Area III. Comparisons of samples from Area III collected in 2006 and in 2007 were statistically differentiated, providing evidence that although population structure was observed in the Area comparisons, population mtDNA diversity within Areas are not necessarily consistent from year to year. However, the level of differentiation found between 2006 and 2007 within Area III is similar to the lower range of F_{ST} values seen in the Area I-VI pairwise comparisons. Although some differentiation is seen between years, this differentiation is not as strong as the differentiation observed

between the majority of IWC management Area comparisons. These results suggest Antarctic blue whales show both maternal fidelity to specific regions within the Southern Ocean as well as some degree of movement. This degree of movement is consistent with the results from the identification of recaptures and from inferred movements from the 'Discovery' marks.

Conclusion

In this study, we found high levels of mtDNA genetic diversity (0.968) and some level of genetic differentiation between feeding areas among the circumpolar distribution of Antarctic blue whales. Antarctic blue whale breeding grounds have not been identified, but there is acoustic data indicating that a portion of the population remains within the Southern Ocean year round (Sirovic et al., 2004). If Antarctic blue whales remain within the Southern Ocean year round, movement throughout the entire ocean may have helped retain genetic diversity during the exploitation by commercial whalers.

The relatively high Antarctic blue whale haplotype diversity indicates the population abundance may not have been reduced to as low a level as previously estimated. Our observation of 51 haplotypes is equal to the predicted value estimated by Branch and Jackson (2008). This revised census of haplotypes within the contemporary population will be used to update the estimate of the minimum number of female lineages to have survived the bottleneck in Chapter 4, a parameter that will be used in future population trajectories to estimate population rates of increase.

Acknowledgements

We thank the Secretariat of the IWC for access to the IDCR/SOWER samples and to H. Kato and P. Ensor for efforts in coordination sampling. We also thank Brittany Hancock, Kelly Robertson and Barb Taylor for the extractions of the IWC IDCR/SOWER biopsy samples at SWFSC and Ric LeDuc for access to supplementary data from the 2007 study. We appreciate access to eastern North Pacific *B. m. musuculus* samples from Bruce Mate and the Marine Mammal Institute tagging efforts and John Calambokidis in association with Cascadia Research Collective and access to three North Atlantic samples courtesy of Carole Conway. Finally, thanks to Tomas Follet for the construction of maps used in this manuscript and Alana Alexander and Debbie Steel for assistance with microsatellite checking.

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Table 3.1. IWC IDCR/SOWER cruise biopsy samples collected between 1990-2009 (n=218). Frequency of samples collected per Area each year are listed and the total number of samples collected within each year and each Area.

Area	1990	1993	1995	1997	1998	1999	2001	2002	2003	2004	2005	2006	2007	2009	Total n
I	1						4								5
II				5	6										11
III		2									4	36	85		127
IV			2			10					1			9	22
V							5	16	5	14					40
VI							13								13
Total	1	2	2	4	6	10	22	16	5	14	5	36	85	9	218

Fig. 3.1. Map of the Southern Ocean illustrating sample locations for IWC IDCR/SOWER Antarctic blue whale biopsy samples collected between 1990-2009 (n=218).

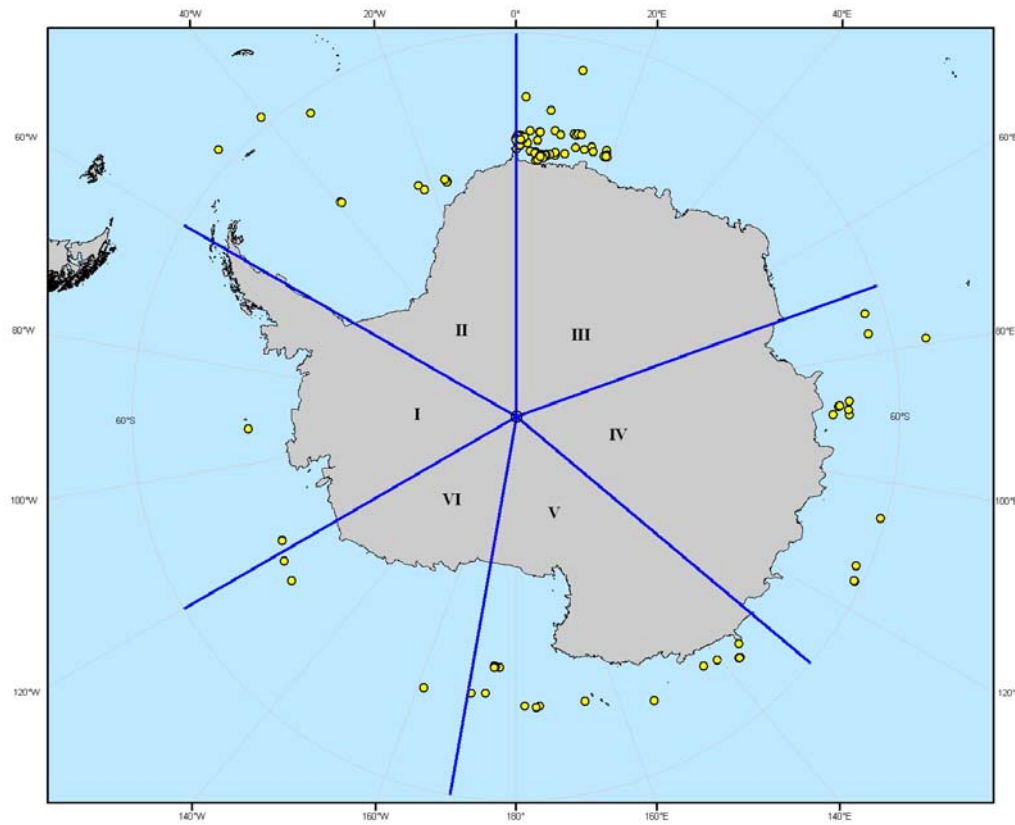


Table 3.2. List of the fifteen microsatellite loci used to identify replicates, including locus name, fluorescent dye label, size range (bp), number of alleles, number of individuals typed, probability of identity (pID) and the reference for each locus. The median number of alleles per locus and median number of individuals types per locus are listed at the base of the table.

Locus and co-load	Dye	Allele range	No. of alleles	no. of individuals typed	pID	Reference
Co-load 1						
*GATA417	FAM	179-231	19	120	0.0228	Palsboll et al. 1997
*GATA28	NED	145-190	9	125	0.0461	Palsboll et al. 1997
GT211	FAM	87-103	10	153	0.082	Berube et al. 2000
GT575	FAM	142-162	9	172	0.1933	Berube et al. 2000
Rw4-10	VIC	190-223	19	173	0.0344	Waldick et al 1999
Co-load 2						
*Ev37	NED	171-197	13	154	0.2333	Valsecchi and Amos 1996
Ev94	FAM	191-203	4	139	0.7097	Valsecchi and Amos 1996
Ev96	FAM	157-180	12	174	0.0612	Valsecchi and Amos 1996
Rw31	FAM	119-130	4	152	0.8766	Waldick et al. 1999
Rw48	NED	114-146	13	126	0.1915	Waldick et al. 1999
Co-load 3						
464.465	FAM	115-154	12	176	0.093	Schlotterer et al. 1991
Rw26	NED	157-191	27	113	0.0176	Waldick et al. 1999
*GT23	VIC	105-132	13	154	0.0708	Berube et al. 2000
Co-load 4						
Ev1	NED	129-167	18	151	0.0292	Valsecchi and Amos 1996
Ev104	FAM	135-156	11	142	0.0992	Valsecchi and Amos 1996
Total loci = 15		Median	12.87	148.27		

Table 3.3. Identification of ‘Pseudo’ replicates within the IWC IDCR/SOWER dataset (n=218). Within each ‘Replicate’ the first listed sample was included in analyses and subsequent ‘pseudo’ replicates were removed from the dataset. The number of matching microsatellite loci matching the first listed individual within each ‘Replicate’ are reported. For each ‘Replicate’, lab ID code, haplotype, identified sex if available, Area, date of sampling event and sample location (latitude and longitude) are listed. ‘Replicate’ 20 through 23 were removed as potential replicates as they did not amplify for sufficient loci to be excluded as a replicate (Not Enough Data).

Replicate	ID	Hap	Sex	Area	Date	Lat	Long	Matching loci
1	26592	z	x	I	3-Feb-2001	-69.25	-117.83	8
	26591	z	m	I	3-Feb-2001	-69.25	-117.83	
2	26588	v	f	VI	27-Jan-2001	-68.22	-126.02	9
	26586	v	f	VI	27-Jan-2001	-68.22	-126.02	
3	72923	62482	x	III	30-Jan-2007	-62.38	10.85	16
	72924	62482	x	III	30-Jan-2007	-62.38	10.85	
4	13951	13951	m	IV	28-Jan-1999	-63.95	89.62	6
	13953	13951	m	IV	28-Jan-1999	-63.95	89.62	
5	88256	88257	m	IV	5-Feb-2009	-64.67	88.10	15
	88257	88257	m	IV	5-Feb-2009	-64.67	88.10	
6	72959	i	m	III	7-Feb-2007	-69.60	5.83	13
	72957	i	m	III	7-Feb-2007	-69.60	5.83	
7	72939	hh	m	III	6-Feb-2007	-69.32	7.22	13
	72942	hh	m	III	6-Feb-2007	-69.45	6.63	
8	51449	m	m	V	6-Jan-2002	-64.27	137.07	11
	51450	m	m	V	6-Jan-2002	-64.28	137.13	
9	72954	72956	f	III	7-Feb-2007	-69.60	5.83	12
	72956	72956	f	III	7-Feb-2007	-69.60	5.83	
10	72909	72910	x	III	9-Jan-2007	-68.78	0.75	10
	72910	72910	x	III	9-Jan-2007	-68.78	0.75	
11	72950	72943	x	III	7-Feb-2007	-69.40	5.42	5
	72953	72943	x	III	7-Feb-2007	-69.40	5.42	
12	26581	bb	f	VI	14-Jan-2001	-67.58	-161.12	9
	26580	bb	x	VI	14-Jan-2001	-67.58	-161.12	
13	26585	m	m	VI	27-Jan-2001	-68.22	-126.02	10
	26587	m	m	VI	27-Jan-2001	-68.22	-126.02	
14	51457	72956	m	III	21-Jan-2002	-65.12	135.57	4
	51459	72956	x	III	21-Jan-2002	-65.12	135.57	
15	72945	aa	x	III	5-Feb-2007	-69.08	8.33	10
	72946	aa	m	III	6-Feb-2007	-69.37	6.23	
16	26582	oo	m	VI	14-Jan-2001	-67.58	-161.12	13
	26583	oo	m	VI	14-Jan-2001	-67.58	-161.12	
	26578	oo	m	VI	14-Jan-2001	-67.58	-161.12	
17	13945	gg	x	IV	3-Feb-1999	-60.42	105.58	8
	13946	gg	m	IV	3-Feb-1999	-60.42	105.58	
	13947	gg	m	IV	3-Feb-1999	-60.42	105.58	
18	26574	nn	x	VI	12-Jan-2001	-68.10	-170.70	8
	26575	nn	x	VI	12-Jan-2001	-68.10	-170.70	
	26576	nn	f	VI	12-Jan-2001	-68.10	-170.70	
	26577	nn	f	VI	12-Jan-2001	-68.10	-170.70	

Table 3.3 continued.

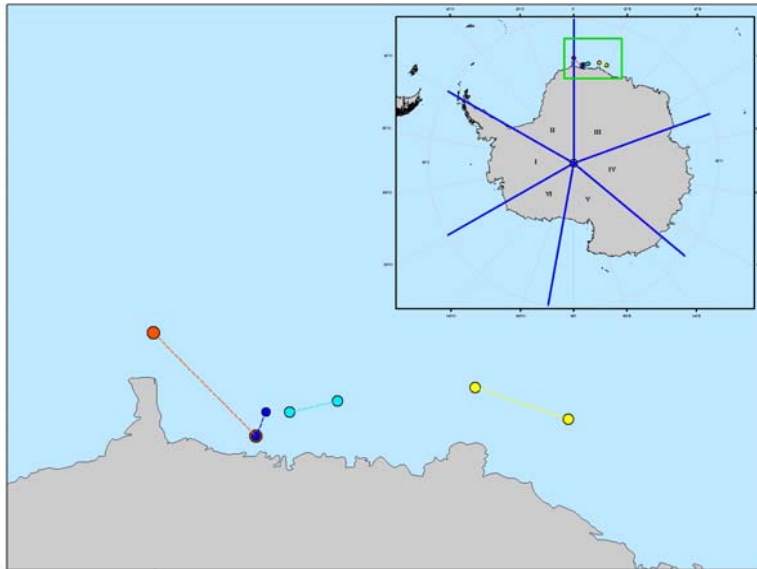
19	51468	bb	x	V	4-Feb-2003	-65.32	154.07	
	51466	bb	x	V	4-Feb-2003	-65.32	154.07	3
	51467	bb	x	V	4-Feb-2003	-65.32	154.07	7
20	88258	l	m	IV	7-Feb-2009	-63.88	87.35	
	88260	l	m?	IV	7-Feb-2009	-63.88	87.35	12
	88261	l	x	IV	7-Feb-2009	-63.88	87.35	11
21	72894	ff	x	III	3-Jan-2003	-68.13	0.43	NED
22	26579	nn	f	VI	13-Jan-1997	-67.58	-161.12	NED
23	72948	hh	m	III	5-Feb-2003	-69.37	6.23	NED
24	72934	72934	x	III	4-Feb-2003	-69.08	8.33	NED

Table 3.4. Recaptures of individual blue whales identified from the replicate analysis of microsatellite genotypes. The first sampling event is listed as the capture and the second sampling event as the recapture event. The lab ID code, sex identification if available, date of capture and re-capture, and latitude and longitude location are reported for each sampling event. For each capture-recapture, the time elapsed between in sampling events (days), longitudinal difference between the two sampling events (degrees) and approximate minimum distance between sampling locations (km) along with the probability of identity (pID) of the two individuals from CERVUS v.3 are listed.

Individual	ID code	Sex	Area	Date	Lat. (Degrees)	Long. (Degrees)	Day(s) elapsed	Long. Difference	Minimum distance (km)	pID																																																																																												
1	62501	F	III	9-Feb-2006	-68.48	18.55	4 days	4.32	200	3.50E-14																																																																																												
	62508		III	13-Feb-2006	-68.42	14.23					2	72906	F	III	8-Jan-2007	-68.17	-0.03	30 days	4.82	600	6.48E-10	72971		III	8-Feb-2007	-69.82	4.78	3	72930	M	III	5-Feb-2007	-69.08	8.33	1 day	2.10	100	1.80E-11	72946		III	6-Feb-2007	-69.37	6.23	4	72949	M	III	7-Feb-2007	-69.40	5.15	1 day	0.37	35	1.89E-09	72973		III	8-Feb-2007	-69.82	4.78												5	51452	F	V	6-Jan-2002	-64.32	137.27	4 years	131.83	6,650	6.13E-19	62484		III	26-Jan-2006	-69.38	5.43	20 days	6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14	72957
2	72906	F	III	8-Jan-2007	-68.17	-0.03	30 days	4.82	600	6.48E-10																																																																																												
	72971		III	8-Feb-2007	-69.82	4.78					3	72930	M	III	5-Feb-2007	-69.08	8.33	1 day	2.10	100	1.80E-11	72946		III	6-Feb-2007	-69.37	6.23	4	72949	M	III	7-Feb-2007	-69.40	5.15	1 day	0.37	35	1.89E-09	72973		III	8-Feb-2007	-69.82	4.78												5	51452	F	V	6-Jan-2002	-64.32	137.27	4 years	131.83	6,650	6.13E-19	62484		III	26-Jan-2006	-69.38	5.43	20 days	6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14	72957		III	7-Feb-2007	-69.60	5.83												
3	72930	M	III	5-Feb-2007	-69.08	8.33	1 day	2.10	100	1.80E-11																																																																																												
	72946		III	6-Feb-2007	-69.37	6.23					4	72949	M	III	7-Feb-2007	-69.40	5.15	1 day	0.37	35	1.89E-09	72973		III	8-Feb-2007	-69.82	4.78												5	51452	F	V	6-Jan-2002	-64.32	137.27	4 years	131.83	6,650	6.13E-19	62484		III	26-Jan-2006	-69.38	5.43	20 days	6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14	72957		III	7-Feb-2007	-69.60	5.83																													
4	72949	M	III	7-Feb-2007	-69.40	5.15	1 day	0.37	35	1.89E-09																																																																																												
	72973		III	8-Feb-2007	-69.82	4.78																5	51452	F	V	6-Jan-2002	-64.32	137.27	4 years	131.83	6,650	6.13E-19	62484		III	26-Jan-2006	-69.38	5.43	20 days	6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14	72957		III	7-Feb-2007	-69.60	5.83																																														
5	51452	F	V	6-Jan-2002	-64.32	137.27	4 years	131.83	6,650	6.13E-19																																																																																												
	62484		III	26-Jan-2006	-69.38	5.43					20 days	6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14	72957		III	7-Feb-2007	-69.60	5.83																																																																										
6	62489	M	III	29-Jan-2006	-67.32	12.32	374 days	6.48	400	2.94E-14																																																																																												
	72957		III	7-Feb-2007	-69.60	5.83																																																																																																

Fig. 3.2. Maps illustrating capture-recapture locations and inferred movements of individuals. Panel a.) illustrates inferred movement of individuals that were recaptured within a year and panel b.) illustrates inferred movement of individuals recaptured between year.

a.



b.

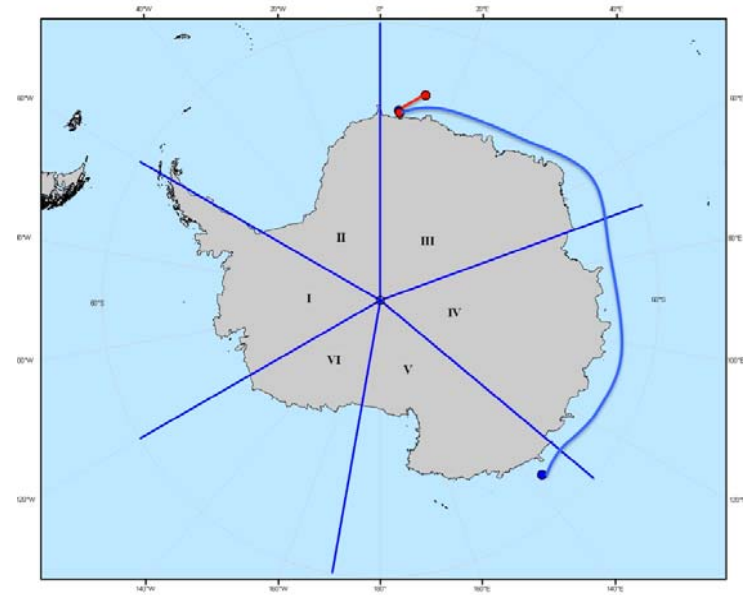
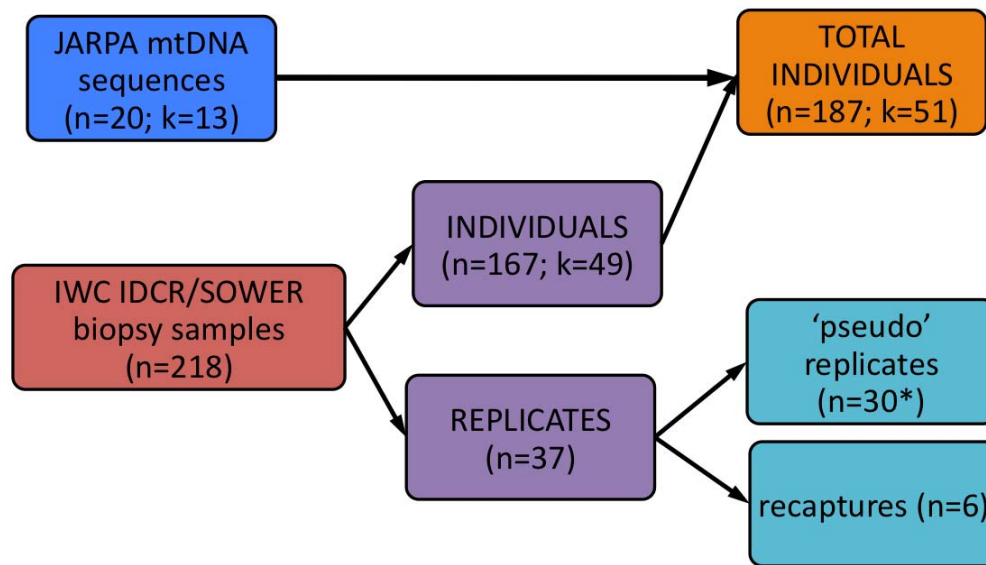


Fig. 3.3. Flow chart illustrating samples and data available for analysis of Antarctic blue whale mtDNA diversity. A total of 218 biopsy samples collected from the IWC IDCR/SOWER cruises identified 167 individuals (k=49) and 37 replicates were removed. Among the replicates, 6 recaptures were identified and 30 'pseudo' replicates (*One 'pseudo' replicate was also identified as a recapture). The 167 individuals were combined with sequences of 20 individuals (k=13) identified in LeDuc et al. (2007) collected from JARPA cruises resulting in a total dataset of 187 individuals and 51 haplotypes.



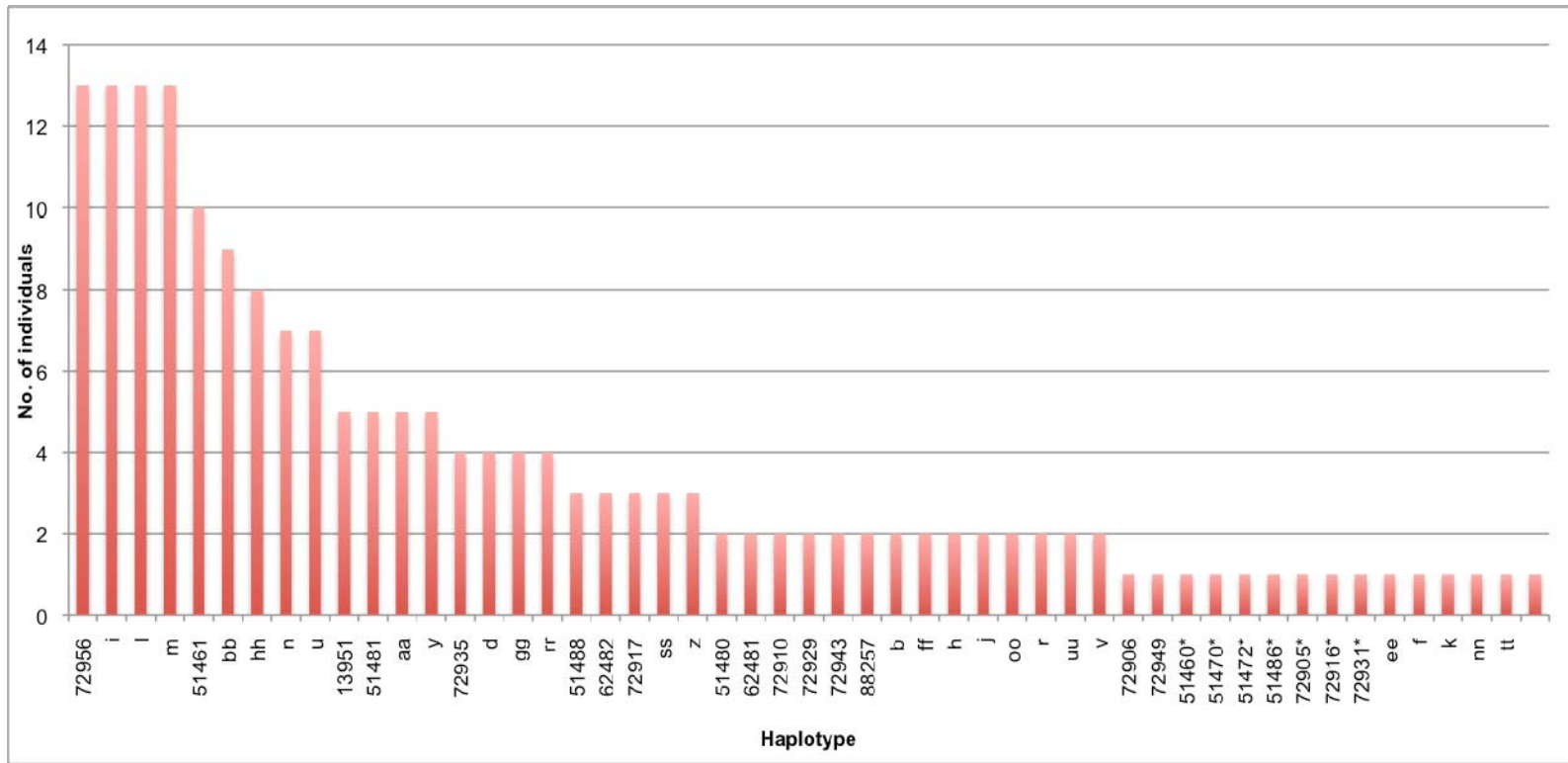


Fig. 3.4. Frequency of the 51 haplotypes described within the contemporary Antarctic blue whale population (n=187) as described from IWC IDCR/SOWER biopsy samples (n=167) and additional samples in LeDuc et al (2007) (n=20).

Table 3.6. Haplotype (h) and nucleotide (π) diversities (and standard deviations) are reported for commercially exploited whale species distributed throughout the Northern and Southern Hemisphere. Regional population, mtDNA sequenced region (bp), sample size (n) and number of haplotypes (k) are reported.

Species	Region	bp	n	k	h (SD)	π (%) (SD)	Reference
Blue whale							
<i>B. musculus</i>							
<i>B. m. musculus</i>	North Pacific	347	45	12	0.839 (0.042)	0.815 (0.486)	Appendix G
<i>B. m. breviceauda</i>	Indian	414	36	12	0.765 (0.069)		LeDuc et al. 2007
<i>B. m. breviceauda</i>	South Pacific	414	28	10	0.851 (0.042)		LeDuc et al. 2007
<i>B. m. intermedia</i>	Southern Ocean	414	47	26	0.969 (0.009)		LeDuc et al. 2007
	Southern Ocean	347	187	51	0.968 (0.003)	1.63 (0.87)	this study
Southern Right Whale							
<i>E. australis</i>	South Atlantic basin	275	69	28	0.948 (0.013)	2.90 (1.51)	Patenaude et al. 2007
	Indo-Pacific basin	275	67	7	0.701 (0.037)	2.03 (1.09)	Patenaude et al. 2007
North Atlantic Right Whale							
<i>E. glacialis</i>	Western North Atlantic	275	269	5	0.698 (0.016)	0.60 (0.30)	Patenaude et al. 2007
North Pacific Right Whale							
<i>E. japonica</i>	North Pacific	275	5	2	0.600 (0.129)	1.89 (1.22)	Patenaude et al. 2007
Gray Whale							
<i>E. robustus</i>	western Pacific	NA	45	10	0.7 (0.05)	1.7	LeDuc et al. 2002
	eastern Pacific	NA	120	33	0.95 (0.01)	1.6	LeDuc et al. 2002
Humpback Whale							
<i>M. novaeangliae</i>	South Pacific	470	1,112	115	0.975 (0.001)	2.04 (1.03)	Olavarria et al. 2007
	North Atlantic	283	246	NA	0.881 (0.015)	2.4 (1.0)	Baker and Medrano-Gonzales 2002
	North Pacific	283	109	NA	0.772 (0.024)	4.6 (0.80)	Baker and Medrano-Gonzales 2002

Table 3.7. Haplotype (h) and nucleotide (π) diversities (and standard deviations) are listed for Southern Ocean IWC management Areas I-VI with the estimated population abundance of each Area (Branch, 2007), length of mtDNA control region sequence (bp), sample size (n) and number of haplotypes (k).

Blue whale	Area	Abundance (CV)	bp	n	k	h (SD)	π (%) (SD)
<i>B. m. intermedia</i>							
	I	88 (0.85)	347	4	4	1.000 (0.176)	2.35 (0.02)
	II	298 (0.55)	347	11	7	0.873 (0.089)	1.63 (0.96)
	III	166 (0.60)	347	104	33	0.955 (0.007)	1.48 (0.80)
	IV	419 (0.51)	347	21	15	0.942 (0.039)	1.81 (0.10)
	V	765 (0.43)	347	40	22	0.959 (0.014)	1.63 (0.89)
	VI	500 (0.68)	347	8	6	0.929 (0.084)	2.26 (1.34)
	Southern Ocean	2,280 (0.36)	347	187	51	0.968 (0.003)	1.63 (0.87)

Table 3.8. Frequencies of 51 haplotypes from Antarctic blue whales in IWC management Areas I-VI. Total number of individuals (n) identified for each haplotype (k) are listed for each Area including the total number of samples (total n) for each Area and total number of individuals identified with each haplotype (k total n). Haplotypes only described by one sequence are noted by an asterisk (*). The two additional haplotypes described from the JARPA samples (n=20) are noted by a double asterisk (**). For the analysis of genetic differentiation between Areas I-VI, haplotypes of all individuals sampled within the Area were included resulting in one individual accounted for twice (Area III and V) (Hap 72956) and a total of 188 individuals.

Haplotype (k)	I	II	III	IV	V	VI	k total n
13951			4	1			5
51461			9		1		10
51480			1		1		2
51481			3		2		5
51488				1	2		3
62481			2				2
62482			3				3
72906			1				1
72910			2				2
72917			2	1			3
72929			2				2
72935			3	1			4
72943			2				2
72949			1				1
72956			11		3		14
88257				1	1		2
51460*					1		1
51470*					1		1
51472*					1		1
51486*					1		1
72905*			1				1
72916*			1				1
72931*			1				1
aa			5				5
b			2				2
bb				2	5	2	9
d			4				4
ee**				1			1
f			1				1
ff			1	1			2
gg			3	1			4
h		1	1				2
hh			7	1			8
i		4	7	1	1		13
j		2					2
k	1						1
l		1	10	1	1		13
m			7	1	4	1	13
n			1	5	1		7

Table 3.8 continued.

nn					1		1
oo						2	2
r		1	1				2
rr	1				2	1	4
ss				2		1	3
tt	1						1
u		1	3		3		7
uu					2		2
v		1				1	2
x**					1		1
y			1		4		5
z	1		1		1		3
total n	4	11	104	21	40	8	188

Table 3.9. Pairwise F_{ST} and ϕ_{ST} values for the comparison of blue whale haplotype diversities in IWC management Areas I-VI. Sample sizes are listed for each Area and p-values are listed under the ϕ_{ST} or F_{ST} values in italics. ϕ_{ST} values are reported above the diagonal and F_{ST} values are below. P-values from 5040 permutations and an exact test of differentiation are bolded if significant.

		I (n=4)	II (n=11)	III (n=104)	IV (n=40)	V (n=21)	VI (n=8)
F_{ST}	I	*	0.049	0.092	-0.043	0.032	0.001
	Permutation		<i>0.235</i>	<i>0.063</i>	<i>0.658</i>	<i>0.249</i>	<i>0.410</i>
	Exact test						
II		0.076	*	-0.006	0.0468	0.005	0.060
	Permutation	<i>0.155</i>		<i>0.527</i>	<i>0.0925</i>	<i>0.335</i>	<i>0.167</i>
	Exact test	<i>0.110</i>					
III		0.026	0.046	*	0.058	0.002	0.080
	Permutation	<i>0.196</i>	<i>0.008</i>		<i>0.002</i>	<i>0.322</i>	<i>0.0169</i>
	Exact test	0.002	0.050				
IV		0.035	0.070	0.029	*	0.053	0.056
	Permutation	<i>0.201</i>	<i>0.007</i>	<i>0.003</i>		<i>0.010</i>	<i>0.097</i>
	Exact test	<i>0.149</i>	0.038	0.000			
V		0.007	0.063	0.017	0.021	*	0.047
	Permutation	<i>0.344</i>	<i>0.001</i>	<i>0.003</i>	<i>0.036</i>		<i>0.086</i>
	Exact test	<i>0.188</i>	0.017	0.000	0.041		
VI		0.010	0.090	0.048	0.023	0.005	*
	Permutation	<i>0.538</i>	<i>0.031</i>	<i>0.014</i>	<i>0.180</i>	<i>0.397</i>	
	Exact test	<i>0.525</i>	0.038	0.000	<i>0.339</i>	<i>0.425</i>	

CHAPTER 4: ANTARCTIC BLUE WHALE mtDNA DIVERSITY: WHAT WAS LOST?

Indicative co-authors: Trevor Branch¹, C. Scott Baker², Bruce Mate²

- 1. School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle WA*
- 2. Marine Mammal Institute, Oregon State University, Newport OR*

ABSTRACT

Exploitation of the Antarctic blue whale reduced the population to less than 1% of its original abundance. Access to bone samples collected from whaling stations on the island of South Georgia have captured the mtDNA diversity from blue whale populations prior to their decline within the Southern Ocean. Here we assess the potential loss of Antarctic blue whale genetic diversity due to exploitation through a comparison of contemporary mtDNA diversity to diversity in a historic South Georgia population. Of the 16 mtDNA haplotypes found in the South Georgia bone samples, only 5 were shared with the 51 haplotypes found in a survey of 187 individuals in the contemporary circumpolar Antarctic blue whale population. A ‘loss’ of mtDNA diversity is assessed through tests of genetic differentiation and a comparison of haplotype frequencies between the historic and contemporary Antarctic blue whale population. Evidence of a loss of mtDNA diversity suggests either a widespread loss within the Antarctic blue whale population or a loss of a local South Georgia Antarctic blue whale population extirpated by commercial whaling. We also estimate the minimum number of females to have survived the demographic ‘bottleneck’ from contemporary Antarctic blue whale mtDNA diversity and update the previous estimate to 69 maternal lineages. This value can be extrapolated to estimate the lower bound of

the minimum population abundance surviving the bottleneck for a more accurate prediction of rates of increase.

INTRODUCTION

Blue whales were targeted by the commercial whaling industry throughout the 19th and 20th centuries. Catch records (1868-1978) account for over 380,000 blue whales killed worldwide (Branch et al., 2008). This exploitation impacted three geographically defined blue whales subspecies; *B. m. musculus* in the northern hemisphere North Pacific and North Atlantic Oceans, the pygmy blue whale (*B. m. breviceauda*) in the southern Indian and southeast Pacific Oceans and the Antarctic blue whale (*B. m. intermedia*) in the Southern Ocean (Rice, 1998). Over 94% of worldwide blue whale catches were killed within the Southern Hemisphere. Of these, 2.7% were assumed to be Indian Ocean pygmy blue whales, 1.4% assumed to be southeast Pacific pygmy blue whales and 90.4% assumed to be Antarctic blue whales, based on geographic location and morphological data (Branch et al., 2008).

The exploitation of Southern Hemisphere blue whales began at the beginning of the 20th century. Southern Hemisphere commercial whaling stations were first established on the south Atlantic island of South Georgia (54°-55°S, 36°-38°W). A total of 13 floating factories and 6 land-based whaling stations operated on South Georgia (Headland, 1984). Over 40,000 blue whales are recorded in the South Georgia catch record (Headland, 1984), of which 90% are assumed to be Antarctic blue whales based on catch record length data (Branch 2008). Exploitation of blue whales began

during the first South Georgia whaling season (1904/05) and increased to a maximum catch of 3,689 blue whales recorded during the 1928/29 whaling season. By 1936, blue whales had been hunted to commercial extinction within the surrounding area (Headland, 1984).

This intensive exploitation of the Antarctic blue whale accounts for over 90% of worldwide blue whale catches, reducing this population to less than 1% of its original abundance of 256,000 (235,000-307,000) individuals (Branch 2008). Population trajectories have estimated the impact of exploitation on Antarctic blue whale population abundance (Branch 2008). The Bayesian logistic models estimate the population abundance at the point of the bottleneck in 1972 to have declined to 395 individuals (Branch, 2008). These models are fitted to a lower bound of the minimum population abundance set at 214 individuals (Branch and Jackson, 2008). This number is based on the previous number of mtDNA haplotypes (k), or maternal lineages, identified within the contemporary population ($k=26$; $n=47$) by LeDuc et al. (2007).

Prolonged and severe exploitation has reduced mtDNA diversity in some species of great whales. Surveys of populations heavily exploited by the 19th century commercial whaling industry have found few mtDNA haplotypes (k) in contemporary populations, such as the western gray whale ($k=10$), (LeDuc et al., 2002; Rastogi et al., 2004) and North Atlantic right whale ($k=5$), (Rastogi et al., 2004). Loss of mtDNA diversity has also been described through a comparison of mtDNA haplotypes within the contemporary and the historic population in the North Atlantic right whale

(Rastogi et al., 2004). For this type of analysis, historic genetic diversity has been preserved in pre-exploitation whale remains, such as bone or baleen. In the North Atlantic right whale population, the one haplotype described from a historic baleen sample has not been identified within the contemporary population and is assumed to be a maternal lineage lost from the population.

Objectives

Here, we assemble the largest database of blue whale mtDNA control region sequences representative of the North Pacific, North Atlantic, South Pacific, Indian and Southern Oceans to assess worldwide blue whale mtDNA diversity and to compare to diversity in a historic blue whale population. The latter was estimated from DNA extracted from whale bones collected from the south Atlantic island of South Georgia whaling stations (Chapter 2). We gauge the potential impact of a bottleneck on the contemporary Antarctic blue whale population through comparisons of historic and contemporary haplotype frequencies and mtDNA diversity. We also predict the number of maternal lineages, or haplotypes, estimated to have survived exploitation and likely to be present in the contemporary Antarctic blue whale population from contemporary mtDNA diversity.

METHODS

Historic and contemporary population datasets

This historic Antarctic blue whale population is represented by 18 bone samples collected from early 20th century whaling stations from the south Atlantic island of South Georgia. DNA extracted from bone samples was amplified and 347 bp of the mtDNA control region was sequenced, describing 16 haplotypes (see Chapter 2 for more detail). For comparison to the historic database, the contemporary mtDNA control region sequences were trimmed to the same region of 347 bp to define haplotypes. A total of 78 haplotypes were described from 324 published and unpublished mtDNA control region sequences represented by 5 geographic blue whale populations including sequences from the North Pacific (n=46, k=13; Appendix G), the North Atlantic (n=3, k=3; Appendix G) in addition to individuals identified from sequences within the Indian Ocean (n=36, k=12) (LeDuc et al., 2007), South Pacific (n=28, k=10) (LeDuc et al. 2007), and the Southern Ocean (n=187, k=51) (Chapter 3) (see Table 4.1). Sequences from New Zealand (n=4, k=3; unpublished) were added to the published South Pacific sequences for these analyses (total South Pacific n=32, k=12). For further comparison with the Southern Ocean Antarctic blue whale population, the historic population was compared to IWC management Areas I-VI.

WORLDWIDE BLUE WHALE COMPARISON

Worldwide blue whale mtDNA phylogenetic reconstruction and genetic differentiation

The evolutionary relationship between worldwide blue whale mtDNA haplotypes (contemporary and historic; $k=88$) was illustrated in a neighbor-joining phylogenetic reconstruction using pairwise differences among the mtDNA control region haplotype sequences in PAUP* (Swofford, 2003). We update the previous Southern Hemisphere phylogenetic tree (LeDuc et al., 2007) with additional haplotypes described from the North Pacific ($k=13$), North Atlantic ($k=2$), New Zealand ($k=4$), and Southern Ocean ($k=51$) and historic South Georgia haplotypes ($k=16$). The model of evolution used to calculate distance between the mtDNA haplotype sequences used in the analysis was determined in Modeltest 0.1.1 (Posada, 2008). Bootstrap support for the tree was based on 1,000 replicates.

Genetic differentiation between contemporary worldwide blue whale populations was tested through pairwise F_{ST} and ϕ_{ST} comparisons implemented in Arlequin (Excoffier et al., 2005). Significance of comparisons was based on 5,000 permutations of the data matrix and an exact test of differentiation in Arlequin.

Haplotype identity comparison

The 16 haplotypes identified within the historic South Georgia population ($n=18$) were compared to haplotypes from 5 contemporary worldwide regional populations including 78 haplotypes ($n=324$). Shared haplotypes were identified

between the historic and contemporary worldwide blue whale populations, as well between the historic South Georgia population and IWC management Areas I-VI.

ANTARCTIC BLUE WHALE COMPARISON

Historical and contemporary haplotype diversity

Given the small sample size of the historic South Georgia population, a resampling method was used to compare historical ($n=18$; $k=16$) and contemporary ($n=187$; $k=51$) haplotype frequencies of the Southern Ocean. Subsamples (x) of the historical sample size were randomly drawn with replacement from the haplotype distribution of the contemporary Antarctic blue whale population. The probability of observing the historical haplotype distribution in the contemporary Antarctic blue whale population was calculated from the average number of subsamples drawn from the contemporary population with an equivalent number of historic haplotypes calculated from 10,000 iterations.

Historical and contemporary genetic differentiation

Genetic differentiation between the contemporary Antarctic blue whale and historic South Georgia haplotype diversity was tested through pairwise F_{ST} comparisons implemented in Arlequin v.3.11 (Excoffier et al., 2005). The differentiation between the Southern Ocean IWC management Areas I-VI and the historic South Georgia haplotype diversity was tested as well. F_{ST} values were

calculated from pairwise comparisons and significance was calculated from 5,000 permutations of the data matrix and an exact test of differentiation in Arlequin.

PREDICTION OF CONTEMPORARY ANTARCTIC BLUE WHALE

HAPLOTYPES

Discovery Curve Analysis

A rarefaction analysis and Discovery curve were used to predict the number of unsampled haplotypes within the contemporary Antarctic blue whale population following the methods of Jackson et al. (2008). A Discovery Curve was generated from the haplotype frequency and diversity of the contemporary Antarctic blue whale sampled population ($n=187$, $k=51$). The haplotype distribution of the sampled population was re-sampled without replacement to generate a Discovery Curve from the number of unique haplotypes after sampling n whales. The Discovery Curve is fit to a 'Clench equation' where parameters a and b are estimated to minimize the sum of squares between the Clench function and the Discovery Curve. The method developed by T. Branch also incorporates information on contemporary population abundance. This is derived from a mean lognormal distribution based on the current Antarctic blue whale abundance estimate of 2,280 (1,160-4,500) individual to estimate a total abundance (N). Parameters a , b and N are used to predict the number of haplotypes for a population of size N . A total of 10,000 Discovery Curves was generated to predict the number of haplotypes within the contemporary population. The Clench equation is expressed as follows:

$$k = \frac{a * n}{1 + b * n}$$

where k is the number of haplotypes within the population, a and b are parameters estimated in each simulation and n or N is the population abundance.

RESULTS AND DISCUSSION

WORLDWIDE BLUE WHALE COMPARISON

Worldwide blue whale mtDNA phylogenetic reconstruction and genetic differentiation

To evaluate worldwide blue whale phylogeography, an alignment of 88 historical and contemporary worldwide blue whale mtDNA haplotypes was used to construct a neighbor-joining (NJ) phylogenetic tree using the Jukes and Cantor model to calculate pairwise distances (equal substitutions and mutation rates for all bases) as recommended in Modeltest 1.1.0. The tree was rooted with a fin whale (*Balaenoptera physalus*) outgroup. In the NJ tree, few clades were supported with over 50% bootstrap support and there was little clustering of the historic of contemporary geographic populations (Fig. 4.1). This lack of phylogenetic structure is similar to the previous phylogenetic reconstruction of Southern Hemisphere populations (LeDuc et al. 2007). Reciprocal monophyly of subspecies or ocean basins was not supported in the tree but cannot be rejected due to low bootstrap support.

Few haplotypes were shared between two or more geographic populations or subspecies. Three haplotypes were widely distributed; Q, D, and R were found in 3 out of the 6 geographic populations. The identification of few haplotypes shared between

regions was also supported by the significant genetic differentiation observed between the contemporary worldwide blue whale populations ($F_{ST}= 0.05 - 0.15$, $p<0.05$). The highest levels of differentiation were observed between the Indian Ocean and all other worldwide populations (Table 4.2). The North and South Pacific Ocean showed the lowest levels of differentiation ($F_{ST}= 0.05$, $p=0.006$); however, samples included in the analysis had a wide geographic range within the Pacific Ocean, from coastal Chilean waters, the eastern tropical Pacific including both Ecuadorian and Costa Rican waters, and North Pacific Californian waters.

The addition of 25 haplotypes within the Southern Ocean to the LeDuc et al. analysis (2007) uncovered two additional haplotypes shared between the pygmy blue whale and Antarctic blue whale. The three blue whale geographic populations in the Southern Hemisphere blue are genetically differentiated (Table 4.2). Two of these populations are thought to represent the pygmy blue whale (*B. m. brevicauda*). In this updated comparison, a greater number of haplotypes were shared between the Antarctic blue whale population and the South Pacific or Indian Ocean pygmy blue whale populations than between the two geographically separated pygmy blue whale populations. This sharing of haplotypes between the populations may be evidence of the overlapping distribution and migration of pygmy blue whales into waters south of 60° S or incomplete lineage sorting between the two subspecies.

Haplotype identity comparison

Of the haplotypes found in the South Georgia bones, ten were not found in any contemporary worldwide blue whale population. Of the six shared haplotypes, five of were shared between South Georgia and the contemporary Southern Ocean. Within the Southern Ocean, at least one shared haplotype was found in each of the IWC management Areas I-VI (Fig 4.2). The highest frequency of shared haplotypes (4) was identified in Area III, which also had the largest sample size (n=104).

The sixth shared haplotype was found within the South Georgia population and both the contemporary Indian Ocean and North Pacific population (q) (Fig. 4.1). From catch record length frequency data, Antarctic blue whales were caught primarily south of 54°S, while pygmy blue whales were primarily caught in waters north of 54°S, although the pygmy blue whale does migrate south of 54°S at a low frequency and have been identified in the catch record south of 54°S at 3.4% (Branch et al., 2008). This haplotype could represent pygmy blue whales caught in the early whaling period that had migrated south of 54°S or alternatively, as seen in shared haplotypes between the contemporary Antarctic blue whale and pygmy blue whale populations, could be evidence of lack of lineage sorting between subspecies.

ANTARCTIC BLUE WHALE

Historical and contemporary haplotype frequency comparison

To evaluate the loss of haplotypes between the historic and contemporary Antarctic blue whale population, we compared haplotype frequencies within both

populations. Subsampling simulations were run with and without the potential pygmy blue whale haplotype. The contemporary Antarctic blue whale population was defined as both the circumpolar region ($n=187$; $k=51$) and IWC management Areas II and III ($n=114$; $k=36$) due to the proximity of these regions to South Georgia. With these sensitivity analyses, four subsample simulations were run. The frequencies of haplotypes found within the historic and contemporary Antarctic blue whale population can be observed in Fig. 4.3. The frequency of haplotypes observed in the historic South Georgia population was not significantly different ($p\text{-value}=0.07\text{-}0.22$) from the frequency of haplotypes observed in the contemporary Antarctic blue whale population defined as Area II and III ($n=114$) or the circumpolar region ($n=187$) (Table 4.3). The mode and median number of haplotypes (k) observed in each subsampling simulation were equivalent; these numbers are listed in Table 4.3 and ranged from 12 to 13 for $x=17$ (historic $k=15$) subsampling events and 13 to 14 for $x=18$ (historic $k=16$) subsampling events. Haplotype frequency distributions for the 4 simulations are illustrated in Fig 4.4.

Historical and contemporary genetic differentiation

Although the resampling simulations did not show significantly greater haplotype diversity in the historic sample, there was a significant genetic differentiation between the historic South Georgia population and the contemporary Antarctic blue whale population. Overall differences were significant based on an exact test, although marginal for the F_{ST} permutations ($F_{ST}=0.0119$, exact test $p<0.05$).

Historic haplotype diversity was significantly differentiated from 5 of the IWC management Areas I-VI ($F_{ST}=0.02-0.05$, $p<0.05$) (Table 4.4), indicating genetic differentiation between the historic and contemporary Antarctic blue whale population.

PREDICTION OF CONTEMPORARY ANTARCTIC BLUE WHALE HAPLOTYPES

Discovery Curve Analysis

Following methods of Jackson et al. (2008), a Clench equation was fit to Discovery Curves generated from contemporary Antarctic blue whale mtDNA diversity. The average estimates of parameters a and b were 0.9377 ± 0.1414 and 0.0131 ± 0.0029 respectively based on 10,000 Discovery Curves (Fig. 4.5a). The Clench Curve predicted 69 (95% CI, 61-82) haplotypes surviving in the estimated contemporary population of 2,280 (1,160-4,500) individuals (Fig 4.5b).

This represents a substantial increase of the previous estimate of 51 (53-55) haplotypes, increasing the prediction of contemporary haplotypes by 35%. From this prediction and the previous estimate of mtDNA diversity (Chapter 3), 74 % of mtDNA haplotype diversity has been described for the Antarctic blue whale with potentially 18 undescribed haplotypes remaining within the population. This haplotype prediction is representative of the absolute minimum number of females to have survived the bottleneck and can be used to update the previous estimate of minimum population abundance of the Antarctic blue whale population (Branch and Jackson, 2008). With

an increased estimate of the number of females to have survived the bottleneck, predictions of minimum population abundance will increase and raise the lower bound within population trajectories and reduce the estimated population rate of increase.

Loss of mtDNA diversity?

Given the small historic blue whale sample ($n=18$) and the high haplotype diversity described in the contemporary Antarctic blue whale population (0.968) the power for a test of a loss of mtDNA diversity was limited. A potential loss of mtDNA haplotypes is evident in 11 unshared haplotypes between the historic South Georgia population and the contemporary Antarctic blue whale population. Here, we explore two hypotheses to explain this evidence for loss: 1.) the observed loss of mtDNA haplotypes could be lost from the widespread circumpolar Antarctic blue whale population or 2.) the loss of mtDNA haplotypes could be representative of a loss of a local South Georgia Antarctic blue whale population.

An overall loss of mtDNA diversity (h) for the Antarctic blue whale population can be estimated from the female effective population size (N_{ef}) where $h_{t+t} = h_t(1-1/N_{ef})$ where t is time in generations of the bottleneck (Hedrick, 2005). To approximate the predicted loss we can assume female effective population size (N_{ef}) is equal to half the census female abundance (N_f) (Nunney, 1993) and a 50:50 sex ratio of the census minimum population abundance of 395 individuals in 1972 (Branch, 2008). This suggests a bottleneck of ~ 100 effective females at the time of the bottleneck in 1972. From the start of commercial whaling in the Southern Ocean in 1904 to today

approximately four generations (31 years; Taylor et al. 2007) of the Antarctic blue whale have elapsed, and approximately two generations have passed since the bottleneck in 1972. This equates to a low estimated loss of 1% of mtDNA diversity per generation. This low estimated loss of mtDNA diversity is consistent with the high haplotype diversity described in the Antarctic blue whale population (Chapter 3).

However, these data suggest the historic South Georgia blue whale population is representative of a local Antarctic blue whale population driven to extinction by the commercial whaling industry. This hypothesis is supported by the observed similar haplotype frequencies but differentiated mtDNA diversities in comparisons of the two populations. There is little evidence of a return to blue whales to South Georgian waters (Moore et al. 1999). This is potentially due to a loss of “cultural memory” of this particular habitat (Clapham et al. 2007). As South Georgia is a productive feeding area, a local blue whale population may have utilized this area as a feeding ground and did not migrate into the Southern Ocean. With the loss of this potential local population and the overall adjacent circumpolar population loss of abundance, there is little opportunity for repopulation of the area (Clapham et al. 2007).

Conclusion

Although commercial whaling of this long-lived species was severe and diminished the population abundance to low levels within 60 years (Branch 2008), the high level of diversity (0.968) and a prediction of 18 unsampled haplotypes within the contemporary Antarctic blue whale population reveals that the population may not

have been driven to levels as low as previously thought. The 10 unshared haplotypes are evidence of either a widespread or local population loss of mtDNA diversity. As the small South Georgia historic sample (n=18) may have only captured a glimpse of the historic Antarctic blue whale mtDNA genetic diversity, the power in testing this 'loss of diversity' is limited. The commercial whaling industry recorded over 40,000 blue whales killed at the South Georgia (Tonnessen and Johnsen, 1982) and further sampling will provide a greater understand of Antarctic blue whale mtDNA loss.

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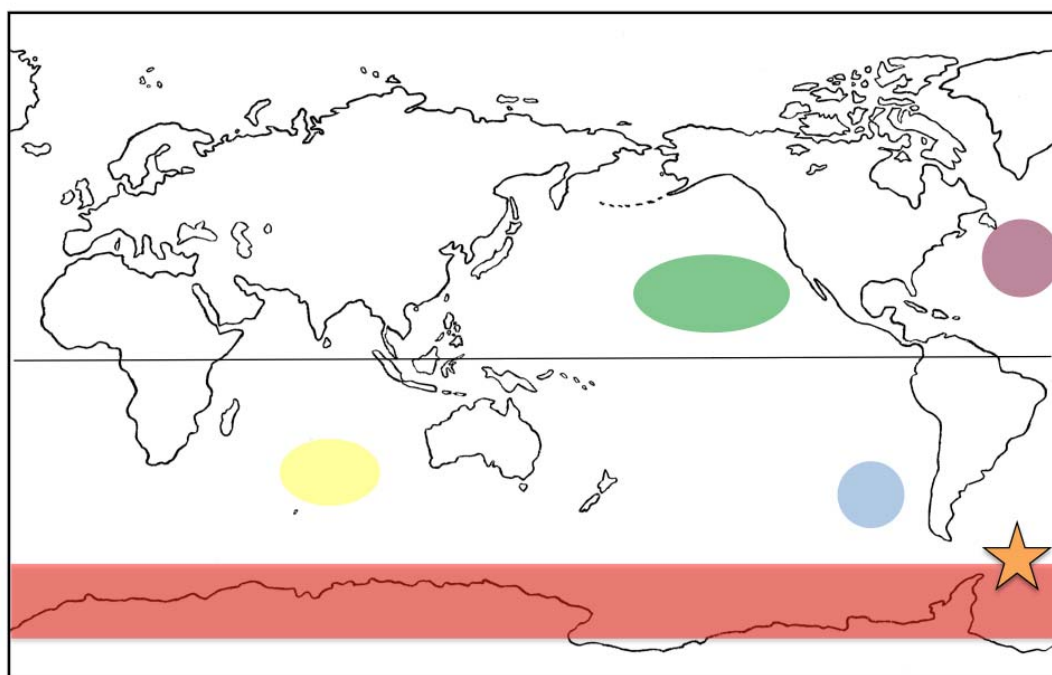
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Table 4.1. Sample sizes (n) and number of mtDNA haplotypes (k) analyzed within the study of worldwide blue whale populations. South Georgia represents the historic population and all other worldwide populations represent contemporary populations. The Southern Ocean population is divided into IWC management Areas I-VI for comparison to the historic South Georgia population. See source for more information sample processing and mtDNA analysis of each population. Asterisk (*) denotes that replicates have not been removed from the dataset. The South Pacific population is supplemented by 4 New Zealand samples (**).

Subspecies	Population	n	k	Source
<i>B. m. intermedia</i>	South Georgia* (historic)	18	16	Chapter 2
<i>B. m. intermedia</i>	Southern Ocean	187	51	Chapter 3
	I	4	4	Chapter 3
	II	11	7	Chapter 3
	III	104	33	Chapter 3
	IV	21	15	Chapter 3
	V	40	22	Chapter 3
	VI	8	6	Chapter 3
<i>B. m. musculus</i>	North Atlantic	2	2	Appendix G
<i>B. m. musculus</i>	North Pacific*	46*	13	Appendix G
<i>B. m. intermedia</i>	Indian Ocean	36	12	LeDuc et al. 2007
<i>B. m. intermedia</i>	South Pacific	32	12	LeDuc et al. 2007 **

Fig.4.1. Map of approximate locations for sample collection locations of blue whale populations analyzed in the neighbor-joining (NJ) reconstruction. The NJ phylogenetic tree illustrates the evolutionary relationship between all contemporary worldwide blue whale haplotypes and historic South Georgia (SG; orange star) haplotypes. Contemporary worldwide populations represent 3 blue whale subspecies; *B. m. intermedia* in the Southern Ocean (SO; red), *B. m. intermedia* in the Indian Ocean (IO; yellow) and South Pacific (SP; blue); and *B. m. musculus* in the North Pacific (NP; green) and North Atlantic (NA; maroon). Colors of geographic populations on map correspond to NJ tree geographic haplotype frequency table. Asterisks (*) denote clades with over 50% bootstrap support.



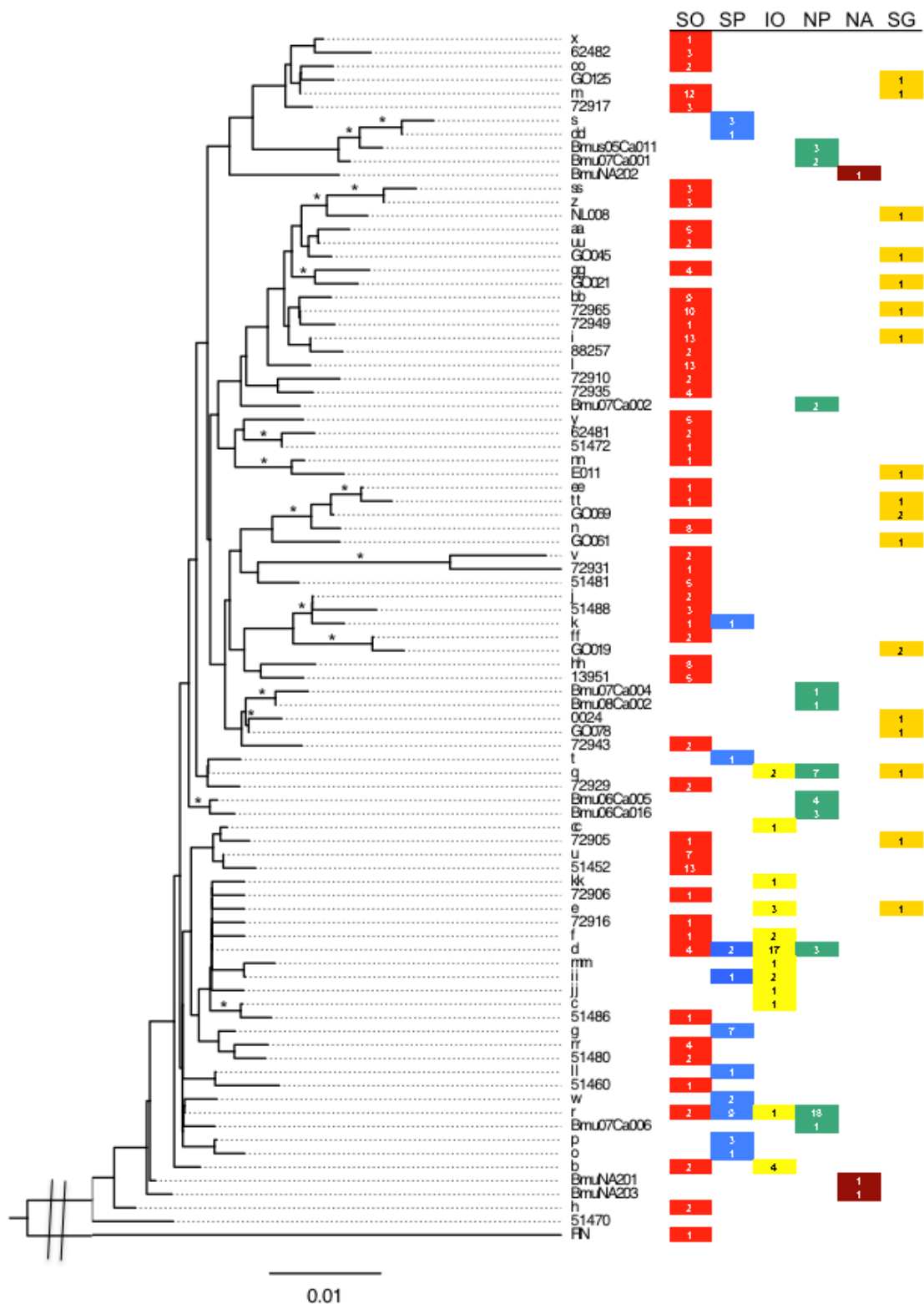


Table 4.2. Differentiation of mtDNA haplotype diversities between contemporary worldwide blue whale populations representing the three subspecies. Tests of differentiation based on haplotype and nucleotide diversity within the 347 bp of the mtDNA control region. ϕ_{ST} values are listed above the diagonal and F_{ST} values are listed below. Significant F_{ST} and ϕ_{ST} values are listed in bold where p-values are based on 5,040 permutations of the data matrix.

	SO (n=187) <i>B. m. intermedia</i>	NP (n=46) <i>B. m. musculus</i>	SP (n=32) <i>B. m. breviceauda</i>	IO (n=36) <i>B. m. breviceauda</i>
SO	*	0.12791 <i>p</i> <0.0001	0.16424 <i>p</i> <0.0001	0.27381 <i>p</i> <0.0001
NP	0.0871 <i>p</i> <0.0001	*	0.04567 <i>0.023</i>	0.24956 <i>p</i> <0.0001
SP	0.0735 <i>p</i> <0.0001	0.0468 <i>0.0062</i>	*	0.17081 <i>p</i> <0.0001
IO	0.1130 <i>p</i> <0.0001	0.1547 <i>p</i> <0.0001	0.1512 <i>p</i> <0.0001	*

Fig. 4.2 Sharing of the 5 mtDNA haplotypes found in both the historic South Georgia population and the contemporary subpopulations for each of the IWC management Areas I-VI. For each subpopulation the total number of haplotypes are listed out of the number of samples available for the study.

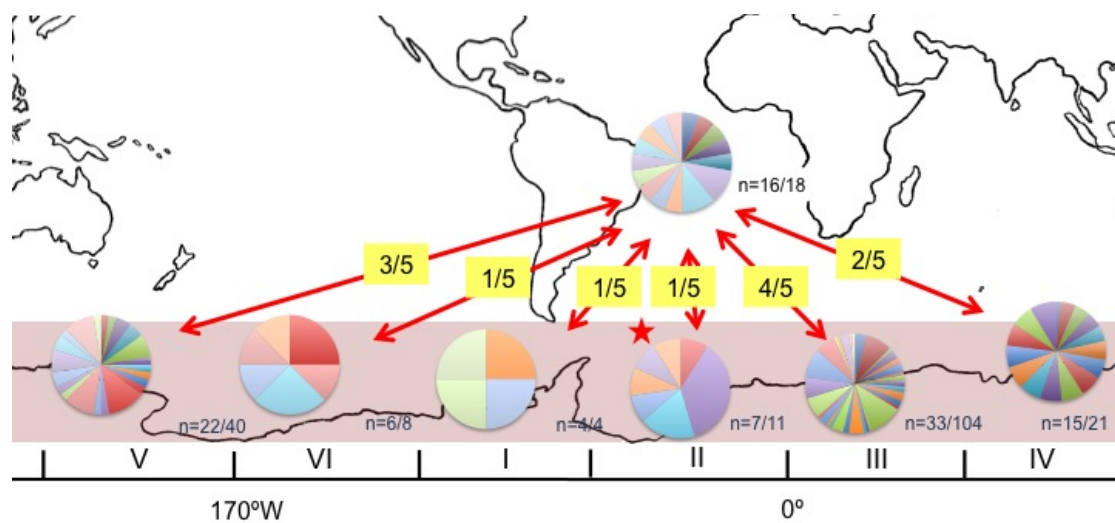
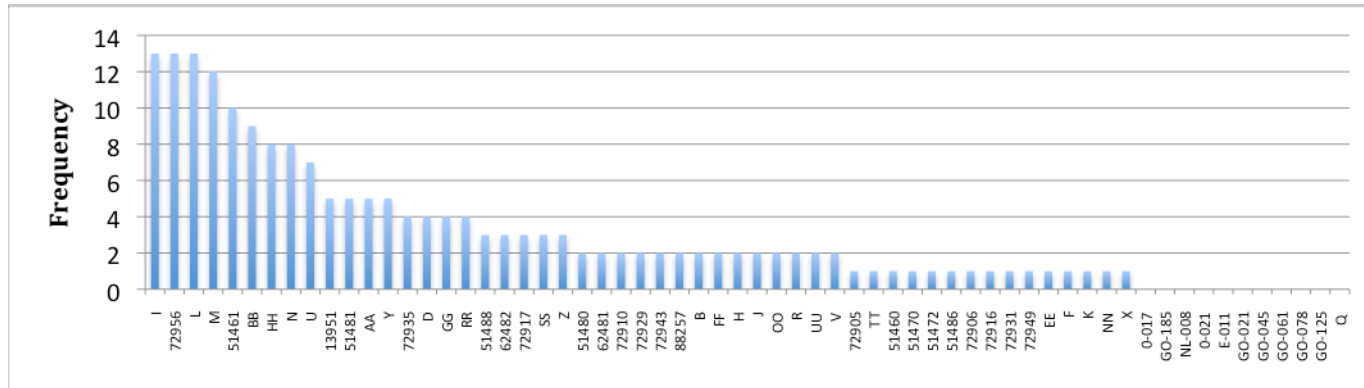


Fig. 4.3. Frequency distribution of mtDNA haplotypes for the (a) contemporary Antarctic blue whale population and (b) the historic South Georgia population.

a.



b.

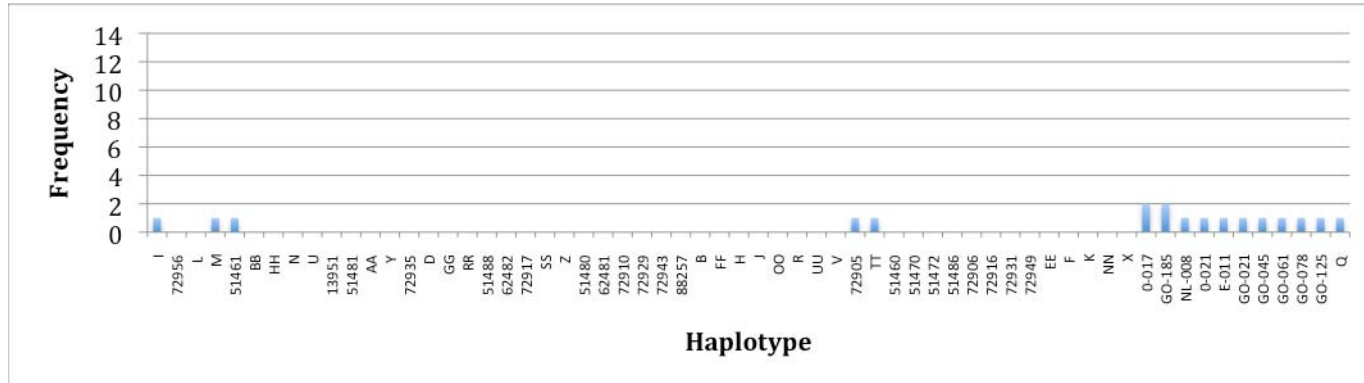


Table 4.3. Results from subsampling simulations of haplotype frequency comparison between historical and contemporary Antarctic blue whale populations. For each simulation (1-4), the sampled contemporary population and historic population, sample size (n) and haplotypes (k) used for the analysis are listed. The subsample (x), median and mode number of haplotypes found within each subsample and significance are reported.

	Contemporary	N	k	Historic	n	K	x	Median k in x	Mean k in x	p-value
1	Area II and III	114	36	South Georgia	17	15	17	13	12.95	0.1
2	Southern Ocean	187	51	South Georgia	17	15	17	14	13.87	0.22
3	Area II and III	114	36	South Georgia	18	16	18	14	13.49	0.07
4	Southern Ocean	187	51	South Georgia	18	16	18	15	14.48	0.17

Fig. 4.4. Haplotype frequency distributions for the 4 subsampling simulations described in Table 4.3.

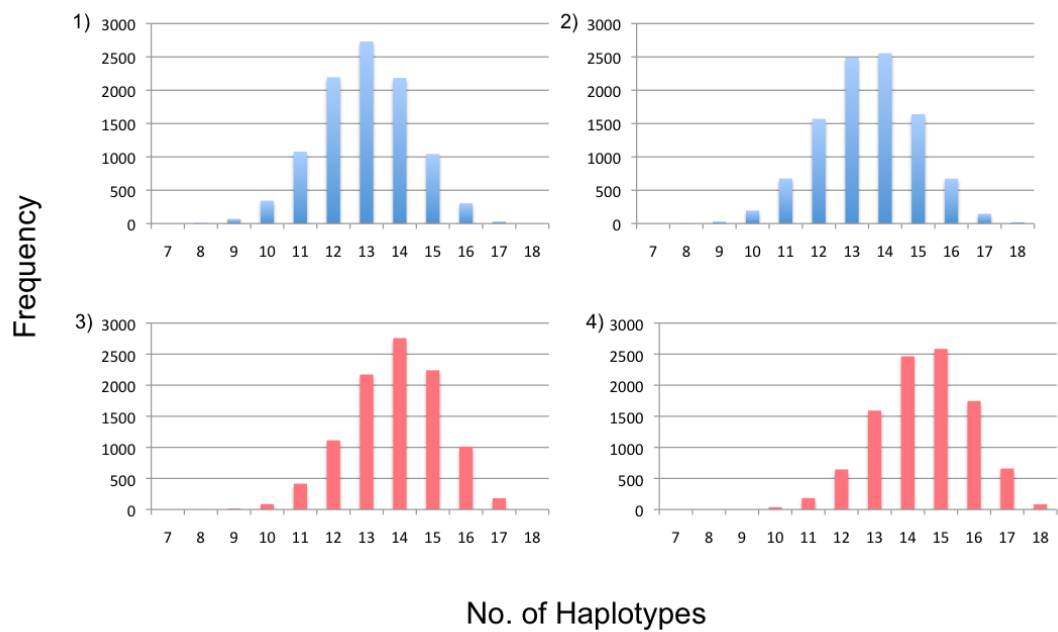
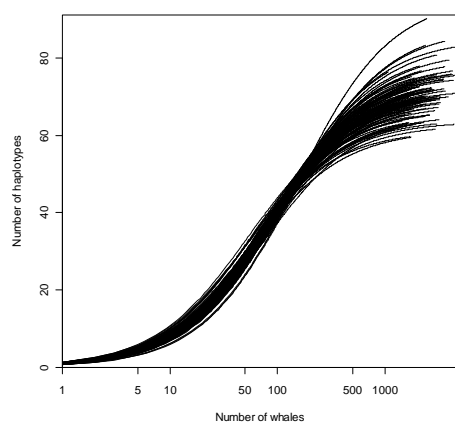


Table 4.4. Genetic differentiation found in the comparison of the historic South Georgia population to contemporary Antarctic blue whale IWC management Areas I-VI and the Southern Ocean population. P-values are listed below F_{ST} values in italics based on a 5,000 permutations and an exact test of differentiation. Significant values are listed in bold.

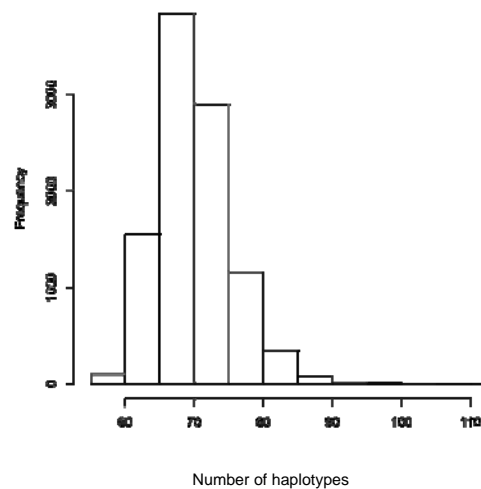
South Georgia (n=18)	I (n=4)	II (n=11)	III (n=104)	IV (n=21)	V (n=40)	VI (n=8)	Southern Ocean (n=187)
F_{ST}	-0.00612	0.04824	0.01746	0.0302	0.01941	0.03309	0.01193
p-value (permutation test)	<i>0.78596</i>	<i>0.01726</i>	<i>0.03432</i>	<i>0.0242</i>	<i>0.02678</i>	<i>0.0363</i>	<i>0.06257</i>
p-value (Exact test)	<i>0.79955</i>	<i>0.09340</i>	<i>0.0000</i>	<i>0.01215</i>	<i>0.00085</i>	<i>0.26775</i>	<i>0.00000</i>

Fig. 4.5. Prediction of the number of haplotypes within the contemporary Antarctic blue whale population: (a) a total of 10,000 discovery curves were plotted and fit to individual Clench equations to predict the average number of haplotypes in the contemporary population with an abundance estimate of 2,280 individuals. (b) The frequency of predicted haplotypes from 10,000 Clench equations fit to Discovery Curves from the mtDNA haplotype diversity described from the contemporary Antarctic blue whale population. Predicted haplotypes are based on the contemporary abundance estimate of 2,280 (1,160-4,500) individuals.

a.



b.



APPENDICES

Appendix A. Loan request to the IWC for access to Antarctic blue whale IDCR/SOWER biopsy samples (June 2009).

International Whaling Commission

The Red House, Station Road, Impington
Cambridge, United Kingdom, CB4 9NP

Telephone: Cambridge (01223) 233971 Fax: Cambridge (01223) 232876
e-mail: secretariat@iwcoffice.org

R E S E A R C H P R O P O S A L

1. TITLE OF PROJECT (do not exceed 30 words)

**IMPROVED ESTIMATION OF THE MINIMUM HISTORICAL
ABUNDANCE (N_{\min}) OF ANTARCTIC BLUE WHALES:
a no-cost request for access to IDCR-SOWER biopsy samples (1989-2009)**

2. DETAILS OF NAMED INVESTIGATORS (principal investigator first)

Primary Investigators:

(i) *Name* ANGIE SREMBIA

Address *Marine Mammal Institute*
Oregon State University
Newport, Oregon 97365

Nationality USA

Domicile USA

(ii) *Name* CHARLES SCOTT BAKER

Address *Marine Mammal Institute*
Oregon State University
Newport, Oregon 97365
School of Biological Sciences
University of Auckland
Auckland, New Zealand

Nationality USA/NZ

Domicile USA

Associate Investigators:

(iii) *Name* JEN JACKSON
Address 11 Spencer Road
 Southsea, Portsmouth
 Hants, PO4 9RN
 England
Nationality UK
Domicile UK

iv) *Name* JUSTIN COOKE
Address CEMS
 Höllenbergstr. 7
 79312 Windenreute
Nationality UK
Domicile Germany

v) *Name* TREVOR BRANCH
Address School of Aquatic and Fishery Sciences
 Box 355020
 University of Washington
 Seattle, WA 98195
Nationality USA
Domicile USA

3. DESCRIPTION OF PROJECT (do not exceed 3000 words)

This should explain adequately the following aspects:

- (i) Background to the proposal, underlying rationale **and relevance to IWC needs**.
- (ii) Specific objectives.
- (iii) Scientific methodology and approach.
- (iv) Programme or plan of research.
- (v) Requirement for resources sought in this application.
- (vi) Any wider justification for the project.

SUMMARY: An estimate of the minimum historical abundance of the Antarctic blue whales at the point of the ‘bottleneck’ after the era of commercial whaling is an important component for the projection of population trajectories, outlined as a goal in the Comprehensive Assessment of the IWC. Recent developments of assessment models have used the number of surviving mtDNA haplotypes to help set a lower bound on this value, referred to as N_{\min} . Here we request access to biopsy samples of blue whales collected on Antarctic feeding grounds during IDCR and SOWER cruises from 1989 to 2009 to assess mtDNA diversity within the region to estimate N_{\min} .

(i) BACKGROUND:

Antarctic blue whales were depleted to less than 1% of their original abundance of 202,000-322,000 between 1928-1972 (Branch et al., 2004). Bayesian models have indicated an increasing rate of 7.3% within this region; however, the 1997 abundance estimate is at 0.9% of the original population size with an abundance estimate of 2,280 (95% Confidence Interval 1160-4500) (SC/60/SH7)(Branch et al., 2004). The blue whales found in this region are assumed to be the 'true' blue whale subspecies (*Balaenoptera musculus intermedia*) (Branch et al. 2007). An estimation of the size of the reduced population (N_{\min}) that occurred during the whaling era will be estimated from the current mtDNA diversity of an increased sample size to update the previous lower bound estimate of 214 by Branch and Jackson (2008).

This project will update the previous published estimates of N_{\min} of the Antarctic blue whales (lower bound at 214) proposed by Jackson and Branch (2008) from the mitochondrial control region of 26 haplotypes from 47 Antarctic samples collected between 1993-2002 (sequences by LeDuc et al. 2007).

(ii) SPECIFIC OBJECTIVE: To improve the estimation of N_{\min} for the Antarctic blue whale from mitochondrial haplotype diversity (Branch and Jackson 2008 SC/60/SH10) through the addition of 185 samples collected from the 1989-2009 IWCR-SOWR cruises. We propose to analyze mitochondrial and nuclear (microsatellite) DNA diversity from these samples.

(iii) METHODS: The analysis of the samples will follow steps of extraction of genomic DNA, amplification of mtDNA control region by PCR as detailed by Baker et al. (2004) and sequencing as detailed in Olavarria *et al.* (2007). The mtDNA haplotypes will be compared to the previously sequenced 420 bp of 47 Antarctic samples (26 unique haplotypes) collected between 1993-2002 (LeDuc et al 2007). Replicate samples will be identified by microsatellite genotypes. The total sample size will be used to estimate N_{\min} from the haplotype diversity using methods as published by Branch and Jackson (2008).

(iv) PROGRAMME: The analysis of the samples will be in the molecular laboratory at the Marine Mammal Institute at Hatfield Marine Science Center, as part of Oregon State University. This laboratory is equipped advanced facilities for automated DNA sequencer (ABI 377 and 3100) and bioinformatics facilities.

The laboratory work and analysis of data will be completed 12 months following the receiving of the IDCR/SOWER samples.

Associate investigators, Jackson, Branch, and Cooke, have agreed to incorporate the empirical results of the proposed analysis into further modeling efforts.

(v) RESOURCES: The resources required in this application correspond to samples of blue whales collected during the IDCR and SOWER cruises from 1989-2009. These samples will extend the knowledge base of the analysis of genetic variation in Antarctic blue whales. The N_{\min} estimate is essential for modeling historical population trajectories and will be used as an *a posteriori* constraint for the Bayesian logistic population dynamic models based on the IWC Comprehensive Assessment.

(vi) WIDER JUSTIFICATION:

The mitochondrial DNA (haplotype) diversity has been used to estimate the point of minimum abundance or 'bottleneck' in cetacean population that occurred during the whaling era (Baker and Clapham 2004). The point of minimum abundance or effective population size refers to the number of individuals in an idealized population that have same genetic properties as the observed for the real population. Branch and Jackson estimated the lower bound minimum population size of the Antarctic blue whales at 214 individuals from 26 unique haplotypes determined from the sequenced 420 base pairs of the mitochondrial control region of 47 by LeDuc et al. 2007 (2008). The minimum population size of a given population is an essential component for constructing population trajectories. To reconstruct the history of the Antarctic blue whale, a trajectory must be fit to three abundance estimates: prior to exploitation, the point of the 'bottleneck', and the current population abundance (Jackson et al. 2008). An increased estimation of the lower abundance would have a great impact on the recovery assessment of Antarctic blue whales. The estimated rate of increase of the species would be reduced and the estimated pre-exploitation abundance would be inflated and therefore the population would be more depleted than the assessment assumed (Branch and Jackson 2008) and result in a premature 'down-listing' (Jackson et al. 2008). An estimation of N_{\min} would provide a baseline assessing the recovery of the Antarctic blue whale (Jackson et al. 2008).

Table 2: Summary of blue whale biopsy samples from IWC IDCR/SOWER cruises 1989-2009.

Year	Area	Antarctic blue whales
2008-09	IV	6
2007-08	IV	0
2006-07	III W	72
2005-06	III W	36
2004-05	III W	4
	IV (transit)	1
2003-04	V	12
2002-03	V	4
2001-02	V	16
2000-01	V, VI, I	14
1999-2000	I, II	0
1998-99	III, IV	7
1997-98	II	3
1996-97	II	4
1995-96	VI	1
1994-95	III IV	2
1993-94	I	0
1992-93	III w	2
1991-92	V	0
1990-91	VI	0
1989-90	I	1
Total Antarctic blue whales sampled		185

References

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- Branch, T. A., Matsuoka, K., and Miyashita, T. 2004. Evidence for increase in Antarctic blue whales based on Bayesian Modelling. *Marine Mammal Science*. 20(4) 726-754.
- Branch, T. A., Stafford, K. M., Palacios, D. M., Allison, C., Bannister, J. L., Burton, C. L. K., Cabrera, E., Carlson, C. A., Galletti Vernazzani, B., Gill, P. C., Huckle-Gaete, R., Jenner, C., Jenner, M., Matsuoka, K., Mikhaliev, Y. A., Miyashita, T., Morrice, M. G., Nishiwaki, S., Sturrock, V. J., Tormosov, D. D., Anderson, R. C., Baker, A. N., Best, P. B., Borsa, P. Brownell Jr., R. L., Childerhouse, S., Findlay, K. P. Gerrodette, T., Llangakoon, A. D., Joergensens, M., Kahn, B., Ljunblad, D. K., Maughan, B., McCauley, R. D., McKay, S., Norris, T. F., Oman Whale and Dolphin Research Group, Rankin, S., Samaran, F., Thiele, D., Van Waerebeek, K. and Warneke, R. M. 2007 Past and present distribution, densities and movements of blue whales *Balaenoptera musculus* in the Southern Hemisphere and northern Indian Ocean. *Mammal Review*. 37(2) 116-175.
- Branch, T. A. and Jackson, J. A. Minimum bottleneck abundance of Antarctic blue whales based on current mtDNA diversity. SC/60/SH10.
- Jackson, J. A., N. J. Patenaude, E. L. Carroll, and C. S. Baker. 2007. How few whales were there after whaling? Inference from contemporary mtDNA diversity. *Molecular Ecology* 17: 236-251.
- LeDuc, R. Dizon, A. E., Goto, M., Pastene, L. A., Kato, H., Nishiwaki, S., LeDuc, C. A. and Brownell, R. L. 2007. Patterns of genetic variation in Southern Hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds. *J. Cetacean Res. Manage*. 9(1): 73-80.
- Olavarria, C.B., Baker, C.S., Medrano, L.G., Aguayo, A.L., Caballero, S.G., Florez-Gonzalez, L., Capella, J.A., Rosenbaum, H.C., Garrigue, C., Greaves, J., Bannister, J.L., Jenner, M. and Jenner, C. 2000. Stock identity of Antarctic Peninsula Humpback Whales inferred from MtDNA variation. SC/52/IA15.

4. CURRICULUM VITAE OF NAMED INVESTIGATORS (1 page per
investigator)
others available on request

Name CHARLES SCOTT BAKER

Degrees and posts held, with dates and any other relevant information.

1993-present: Senior Lecture, Ecology and Evolution Group, School of Biological Sciences,
University of Auckland, New Zealand

1992-93: Research Associate, Pacific Biomedical Research Center, University of Hawaii,
Honolulu, Hawaii, USA

1990-91: Postdoctoral fellow at the School of Biological Sciences, Victoria University of
Wellington, Wellington, New Zealand

1987-89: Postdoctoral fellow at the National Cancer Institute, Frederick, Maryland, and the
Smithsonian Institution, Washington, D.C., USA

Summers 1985-88: Marine Mammal Biologist of Glacier Bay National Park, Alaska, USA

1985: Ph.D. Zoology, University of Hawaii, Manoa (Thesis: The population structure and
social organization of humpback whales (*Megaptera novaeangliae*) in the central and
eastern North Pacific)

1977: B.A. Environmental Studies, New College, University of South Florida

5. BUDGET

(if proposal is for more than one year, present budget for each year of study)

(i) Salaries and wages (include name or position of each individual and time involved)

Angie Sremba (50% for 12 months)	contributed
C. Scott Baker (10% for 6 months)	contributed

(ii) Travel

none

(iii) Services (e.g. computer, aircraft or ship time, consultant fees)

none

(iv) Non-expendable capital equipment (this becomes IWC property on completion of
project)

None

(v) Expendable capital equipment

None

**6. OTHER GRANTS HELD FOR THIS OR OTHER RESEARCH,
OBTAINED OR SOUGHT WITHIN THE PRECEDING THREE YEARS**
(give amount, title of project and completion date)

Field and lab work conducted in the Antarctic and previous working meetings of participating researchers have been funded primarily from the following sources:

US\$330,000 Regional Natural Heritage Program, Department of Environment and Heritage, Government of Australia, completion date 2007.

US\$10,000 Maime Markham Award, Hatfield Marine Science Center, Oregon State University. May 2009.

7. WHERE PROPOSED WORK IS TO BE CARRIED OUT; PERMITS

(i) Geographical location for field work and/or institutions where research (and subsequent analysis) is to be carried out

Laboratory analysis: Laboratory analysis will be completed at the Marine Mammal Institute, Oregon State University. This Laboratory is fully equipped for the molecular genetic analysis proposed here.

(ii) If a permit is required to carry out work, has one been obtained? (If yes, please enclose copy)

To import the samples it is necessary to obtain a CITES permit from the country of origin, where the samples are stored.

8. SCHEDULE OF WORK

(i) Expected completion of field work (if appropriate)

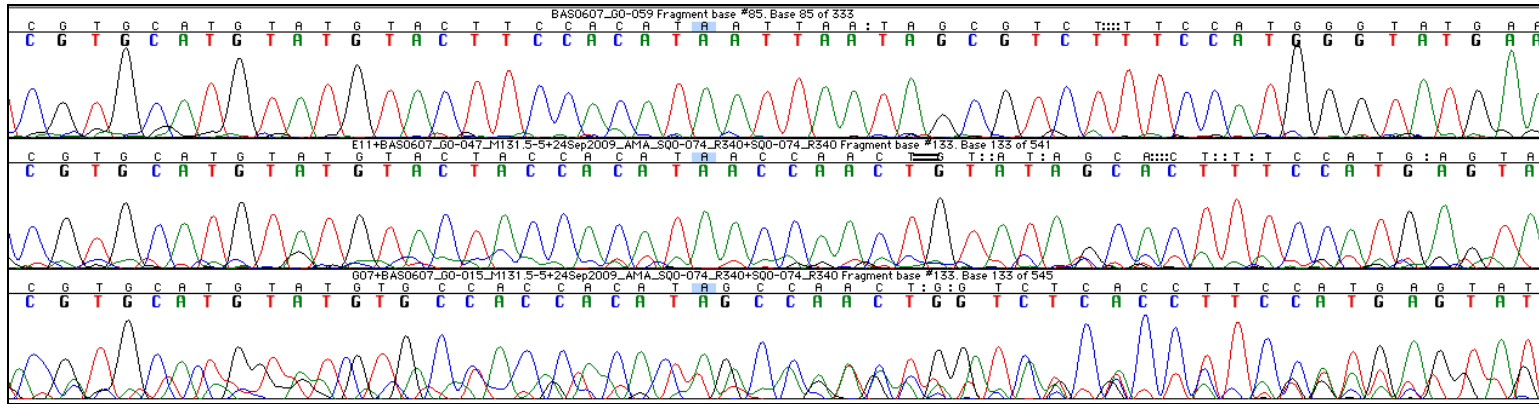
none

(ii) Expected completion of final report (note that an annual progress report is required)

The analysis and final report will be completed 12 months after samples are received.

Appendix B. Electropherogram of mtDNA control region sequences to illustrate quality control Phred scores.

Base calls are given a quality score based on the probability they have been miscalled base: categorized from <20 (error rate of 1 in 100; bottom sequence), 20-40 (error rate of 1 in 1,000; middle sequence) and >40 (error rate of 1 in 10,000; top sequence).



Appendix C. Spreadsheet of South Georgia bone samples species identification.

Field ID, lab ID, and species identification for the 281 South Georgia bone samples are listed. Samples that did not amplify for the mtDNA control region sequence are denoted by 'x.' Asterisks (*) note which samples were analyzed for genetic sex identification. Sample lab ID 0-025 was identified as a female and lab ID 0-027 was identified as a male.

Field ID	Lab ID	Species ID
BAS 06/07 T104876 Mnov Z77826	0-001*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104877 Mnov Z77827	0-002*	<i>Balaenoptera physalus</i>
BAS 06/07 T104878 Mnov Z77838	0-003	<i>Balaenoptera physalus</i>
BAS 06/07 T104879 Mnov Z77829	0-004*	<i>Balaenoptera physalus</i>
BAS 06/07 T104880 Mnov Z77830	0-005*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104881 Mnov Z77831	0-006*	<i>Balaenoptera physalus</i>
BAS 06/07 T104882 Mnov Z77832	0-007	X
BAS 06/07 T104883 Mnov Z77833	0-008*	<i>Balaenoptera physalus</i>
BAS 06/07 T104884 Mnov Z77834	0-009*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104885 Mnov Z77835	0-010*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104886 Mnov Z77836	0-011	X
BAS 06/07 T104887 Mnov Z77837	0-012	<i>Balaenoptera physalus</i>
BAS 06/07 T104888 Mnov Z77838	0-013*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104889 Mnov Z77839	0-014*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104890 Mnov Z77840	0-015*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104891 Mnov Z77841	0-016*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104892 Mnov Z77842	0-017*	<i>Balaenoptera musculus</i>
BAS 06/07 T104893 Mnov Z77843	0-018*	<i>Balaenoptera physalus</i>
BAS 06/07 T104894 Mnov Z77844	0-019*	X
BAS 06/07 T104895 Mnov Z77845	0-020*	<i>Balaenoptera musculus</i>
BAS 06/07 T104896 Mnov Z77846	0-021*	<i>Balaenoptera musculus</i>
BAS 06/07 T104897 Mnov Z77847	0-022*	<i>Balaenoptera physalus</i>
BAS 06/07 T104898 Mnov Z77848	0-023	<i>Balaenoptera physalus</i>
BAS 06/07 T104899 Mnov Z77849	0-024*	X
BAS 06/07 T104900 Mnov Z77850	0-025*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104901 Mnov Z77851	0-026*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104902 Mnov Z77852	0-027*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104903 Mnov Z77853	0-028*	<i>Balaenoptera physalus</i>
BAS 06/07 T104904 Mnov Z77854	0-029*	X
BAS 06/07 T104905 Mnov Z77855	0-030	<i>Megaptera novaeangliae</i>
BAS06/07 T104946 Mnov Z77896	E-001	<i>Megaptera novaeangliae</i>
BAS06/07 T104947 Mnov Z77897	E-002	<i>Balaenoptera physalus</i>
BAS06/07 T104948 Mnov Z77898	E-003	<i>Balaenoptera physalus</i>

BAS06/07 T104949 Mnov Z77899	E-004	<i>Megaptera novaeangliae</i>
BAS06/07 T104950 Mnov Z77900	E-005	<i>Megaptera novaeangliae</i>
BAS06/07 T104951 Mnov Z77901	E-006	<i>Balaenoptera physalus</i>
BAS06/07 T104952 Mnov Z77902	E-007	<i>Megaptera novaeangliae</i>
BAS06/07 T104953 Mnov Z77903	E-008	<i>Megaptera novaeangliae</i>
BAS06/07 T104954 Mnov Z77904	E-009	X
BAS06/07 T104955 Mnov Z77905	E-010	<i>Balaenoptera physalus</i>
BAS06/07 T104956 Mnov Z77906	E-011	<i>Balaenoptera musculus</i>
BAS 06/07 T104669 Mnov Z77619	GO-001*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104670 Mnov Z77620	GO-002*	<i>Balaenoptera physalus</i>
BAS 06/07 T104671 Mnov Z77621	GO-003*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104672 Mnov Z77622	GO-004*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104673 Mnov Z77623	GO-005*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104674 Mnov Z77624	GO-006*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104675 Mnov Z77625	GO-007*	<i>Megaptera novaeangliae</i>
BAS 06/07 T104676 Mnov Z77626	GO-008	<i>Balaenoptera musculus</i>
BAS 06/07 T104677 Mnov Z77627	GO-009	<i>Megaptera novaeangliae</i>
BAS 06/07 T104678 Mnov Z77628	GO-010	<i>Megaptera novaeangliae</i>
BAS 06/07 T104679 Mnov Z77629	GO-011	<i>Megaptera novaeangliae</i>
BAS 06/07 T104680 Mnov Z77630	GO-012	<i>Megaptera novaeangliae</i>
BAS 06/07 T104681 Mnov Z77631	GO-013	<i>Megaptera novaeangliae</i>
BAS 06/07 T104682 Mnov Z77632	GO-014	<i>Megaptera novaeangliae</i>
BAS 06/07 T104683 Mnov Z77633	GO-015	<i>Megaptera novaeangliae</i>
BAS 06/07 T104684 Mnov Z77634	GO-016	x
BAS 06/07 T104685 Mnov Z77635	GO-017	<i>Megaptera novaeangliae</i>
BAS 06/07 T104686 Mnov Z77636	GO-018	<i>Megaptera novaeangliae</i>
BAS 06/07 T104687 Mnov Z77637	GO-019	<i>Balaenoptera musculus</i>
BAS 06/07 T104688 Mnov Z77638	GO-020	<i>Balaenoptera physalus</i>
BAS 06/07 T104689 Mnov Z77639	GO-021	<i>Balaenoptera musculus</i>
BAS 06/07 T104690 Mnov Z77640	GO-022	<i>Balaenoptera physalus</i>
BAS 06/07 T104691 Mnov Z77641	GO-023	<i>Balaenoptera physalus</i>
BAS 06/07 T104692 Mnov Z77642	GO-024	<i>Megaptera novaeangliae</i>
BAS 06/07 T104693 Mnov Z77643	GO-025	<i>Megaptera novaeangliae</i>
BAS 06/07 T104694 Mnov Z77644	GO-026	<i>Megaptera novaeangliae</i>
BAS 06/07 T104695 Mnov Z77645	GO-027	<i>Megaptera novaeangliae</i>
BAS 06/07 T104696 Mnov Z77646	GO-028	x
BAS06/07 T104697 Mnov Z77647	GO-029	<i>Balaenoptera physalus</i>
BAS 06/07 T104698 Mnov Z77648	GO-030	<i>Megaptera novaeangliae</i>
BAS 06/07 T104699 Mnov Z77649	GO-031	<i>Balaenoptera musculus</i>
BAS 06/07 T104700 Mnov Z77650	GO-032	<i>Megaptera novaeangliae</i>
BAS 06/07 T104701 Mnov Z77651	GO-033	<i>Megaptera novaeangliae</i>
BAS 06/07 T104702 Mov Z77652	GO-034	<i>Megaptera novaeangliae</i>

BAS 06/07 T104703 Mnov Z77653	GO-035	<i>Megaptera novaeangliae</i>
BAS 06/07 T104704 Mnov Z77654	GO-036	<i>Megaptera novaeangliae</i>
BAS 06/07 T104705 Mnov Z77655	GO-037	<i>Megaptera novaeangliae</i>
BAS 06/07 T104706 Mnov Z77656	GO-038	<i>Megaptera novaeangliae</i>
BAS 06/07 T104707 Mnov Z77657	GO-039	<i>Megaptera novaeangliae</i>
BAS 06/07 T104708 Mnov Z77658	GO-040	<i>Megaptera novaeangliae</i>
BAS 06/07 T104709 Mnov Z77659	GO-041	<i>Megaptera novaeangliae</i>
BAS 06/07 T104710 Mnov Z77660	GO-042	<i>Megaptera novaeangliae</i>
BAS 06/07 T104711 Mnov Z77661	GO-043	<i>Megaptera novaeangliae</i>
BAS 06/07 T104712 Mnov Z77662	GO-044	<i>Balaenoptera physalus</i>
BAS 06/07 T104713 Mnov Z77663	GO-045	<i>Balaenoptera musculus</i>
BAS 06/07 T104714 Mnov Z77664	GO-046	<i>Balaenoptera physalus</i>
BAS 06/07 T104715 Mnov Z77665	GO-047	<i>Megaptera novaeangliae</i>
BAS 06/07 T104716 Mnov Z77666	GO-048	<i>Megaptera novaeangliae</i>
BAS 06/07 T104717 Mnov Z77667	GO-049	<i>Megaptera novaeangliae</i>
BAS 06/07 T104718 Mnov Z77668	GO-050	<i>Megaptera novaeangliae</i>
BAS 06/07 T104719 Mnov Z77669	GO-051	<i>Balaenoptera musculus</i>
BAS 06/07 T104720 Mnov Z77670	GO-052	<i>Balaenoptera physalus</i>
BAS 06/07 T104721 Mnov Z77671	GO-053	<i>Megaptera novaeangliae</i>
BAS 06/07 T104722 Mnov Z77672	GO-054	<i>Balaenoptera physalus</i>
BAS 06/07 T104723 Mnov Z77673	GO-055	<i>Megaptera novaeangliae</i>
BAS 06/07 T104724 Mnov Z77674	GO-056	<i>Balaenoptera physalus</i>
BAS 06/07 T104725 Mnov Z77675	GO-057	<i>Megaptera novaeangliae</i>
BAS 06/07 T104726 Mnov Z77676	GO-058	<i>Megaptera novaeangliae</i>
BAS 06/07 T104727 Mnov Z77677	GO-059	<i>Balaenoptera physalus</i>
BAS 06/07 T104728 Mnov Z77678	GO-060	<i>Megaptera novaeangliae</i>
BAS 06/07 T104729 Mnov Z77679	GO-061	<i>Balaenoptera musculus</i>
BAS 06/07 T104730 Mnov Z77680	GO-062	<i>Megaptera novaeangliae</i>
BAS 06/07 T104731 Mnov Z77681	GO-063	<i>Megaptera novaeangliae</i>
BAS 06/07 T104732 Mnov Z77682	GO-064	<i>Megaptera novaeangliae</i>
BAS 06/07 T104733 Mnov Z77683	GO-065	<i>Balaenoptera physalus</i>
BAS 06/07 T104734 Mnov Z77684	GO-066	x
BAS 06/07 T104735 Mnov Z77685	GO-067	<i>Megaptera novaeangliae</i>
BAS06/07 T104736 Mnov Z77686	GO-068	<i>Megaptera novaeangliae</i>
BAS 06/07 T104737 Mnov Z77687	GO-069	<i>Balaenoptera musculus</i>
BAS 06/07 T104738 Mnov Z77688	GO-070	<i>Balaenoptera physalus</i>
BAS 06/07 T104739 Mnov Z77689	GO-071	<i>Balaenoptera physalus</i>
BAS 06/07 T104740 Mnov Z77690	GO-072	<i>Megaptera novaeangliae</i>
BAS 06/07 T104741 Mnov Z77691	GO-073	<i>Megaptera novaeangliae</i>
BAS 06/07 T104742 Mnov Z77692	GO-074	<i>Megaptera novaeangliae</i>
BAS 06/07 T104743 Mnov Z77693	GO-075	<i>Megaptera novaeangliae</i>
BAS 06/07 T104744 Mnov Z77694	GO-076	<i>Megaptera novaeangliae</i>

BAS 06/07 T104745 Mnov Z77695	GO-077	<i>Balaenoptera physalus</i>
BAS 06/07 T104746 Mnov Z77696	GO-078	<i>Balaenoptera musculus</i>
BAS 06/07 T104747 Mnov Z77697	GO-079	<i>Megaptera novaeangliae</i>
BAS 06/07 T104748 Mnov Z77698	GO-080	<i>Balaenoptera physalus</i>
BAS 06/07 T104749 Mnov Z77699	GO-081	<i>Megaptera novaeangliae</i>
BAS 06/07 T104750 Mnov Z77700	GO-082	<i>Balaenoptera physalus</i>
BAS 06/07 T104751 Mnov Z77701	GO-083	<i>Megaptera novaeangliae</i>
BAS 06/07 T104752 Mnov Z77702	GO-084	<i>Megaptera novaeangliae</i>
BAS 06/07 T104753 Mnov Z77703	GO-085	<i>Megaptera novaeangliae</i>
BAS 06/07 T104754 Mnov Z77704	GO-086	x
BAS 06/07 T104755 Mnov Z77705	GO-087	x
BAS 06/07 T104756 Mnov Z77706	GO-088	<i>Megaptera novaeangliae</i>
BAS 06/07 T104757 Mnov Z77707	GO-089	<i>Megaptera novaeangliae</i>
BAS 06/07 T104758 Mnov Z77708	GO-090	<i>Megaptera novaeangliae</i>
BAS 06/07 T104759 Mnov Z77709	GO-091	x
BAS 06/07 T104760 Mnov Z77710	GO-092	x
BAS 06/07 T104761 Mnov Z77711	GO-093	x
BAS 06/07 T104762 Mnov Z77712	GO-094	<i>Megaptera novaeangliae</i>
BAS 06/07 T104763 Mnov Z77713	GO-095	<i>Megaptera novaeangliae</i>
BAS 06/07 T104764 Mnov Z77714	GO-096	x
BAS 06/07 T104765 Mnov Z77715	GO-097	<i>Megaptera novaeangliae</i>
BAS 06/07 T104766 Mnov Z77716	GO-098	<i>Megaptera novaeangliae</i>
BAS 06/07 T104767 Mnov Z77717	GO-099	x
BAS 06/07 T104768 Mnov Z77718	GO-100	x
BAS 06/07 T104769 Mnov Z77719	GO-101	<i>Megaptera novaeangliae</i>
BAS 06/07 T104770 Mnov Z77720	GO-102	<i>Megaptera novaeangliae</i>
BAS 06/07 T104771 Mnov Z77721	GO-103	<i>Megaptera novaeangliae</i>
BAS 06/07 T104772 Mnov Z77722	GO-104	x
BAS 06/07 T104773 Mnov Z77723	GO-105	<i>Megaptera novaeangliae</i>
BAS 06/07 T104774 Mnov Z77724	GO-106	<i>Megaptera novaeangliae</i>
BAS 06/07 T104775 Mnov Z77725	GO-107	<i>Megaptera novaeangliae</i>
BAS 06/07 T104776 Mnov Z77726	GO-108	<i>Megaptera novaeangliae</i>
BAS 06/07 T104777 Mnov Z77727	GO-109	<i>Megaptera novaeangliae</i>
BAS 06/07 T104778 Mnov Z77728	GO-110	<i>Balaenoptera physalus</i>
BAS 06/07 T104779 Mnov Z77729	GO-111	<i>Balaenoptera physalus</i>
BAS 06/07 T104780 Mnov Z77730	GO-112	<i>Balaenoptera physalus</i>
BAS 06/07 T104781 Mnov Z77731	GO-113	<i>Megaptera novaeangliae</i>
BAS 06/07 T104782 Mnov Z77732	GO-115	x
BAS 06/07 T104783 Mnov Z77733	GO-116	<i>Megaptera novaeangliae</i>
BAS 06/07 T104784 Mnov Z77734	GO-117	x
BAS 06/07 T104785 Mnov Z77735	GO-118	<i>Megaptera novaeangliae</i>
BAS 06/07 T104786 Mnov Z77736	GO-119	<i>Balaenoptera physalus</i>

BAS 06/07 T104787 Mnov Z77737	GO-120	x
BAS 06/07 T104788 Mnov Z77738	GO-121	x
BAS 06/07 T104789 Mnov Z77739	GO-122	<i>Megaptera novaeangliae</i>
BAS 06/07 T104790 Mnov Z77740	GO-123	<i>Megaptera novaeangliae</i>
BAS 06/07 T104791 Mnov Z77741	GO-124	<i>Balaenoptera musculus</i>
BAS 06/07 T104792 Mnov Z77742	GO-125	<i>Balaenoptera musculus</i>
BAS 06/07 T104793 Mnov Z77743	GO-126	<i>Megaptera novaeangliae</i>
BAS 06/07 T104794 Mnov Z77744	GO-127	X
BAS 06/07 T104795 Mnov Z77745	GO-128	X
BAS 06/07 T104796 Mnov Z77746	GO-129	<i>Megaptera novaeangliae</i>
BAS 06/07 T104797 Mnov Z77747	GO-130	<i>Balaenoptera physalus</i>
BAS 06/07 T104798 Mnov Z77748	GO-131	<i>Megaptera novaeangliae</i>
BAS 06/07 T104799 Mnov Z77749	GO-132	X
BAS 06/07 T104800 Mnov Z77750	GO-133	<i>Megaptera novaeangliae</i>
BAS 06/07 T104801 Mnov Z77751	GO-134	<i>Megaptera novaeangliae</i>
BAS 06/07 T104802 Mnov Z77752	GO-135	<i>Megaptera novaeangliae</i>
BAS 06/07 T104803 Mnov Z77753	GO-136	X
BAS 06/07 T104804 Mnov Z77754	GO-137	<i>Megaptera novaeangliae</i>
BAS 06/07 T104805 Mnov Z77755	GO-138	X
BAS 06/07 T104806 Mnov Z77756	GO-139	X
BAS 06/07 T104807 Mnov Z77757	GO-140	<i>Megaptera novaeangliae</i>
BAS 06/07 T104808 Mnov Z77758	GO-141	X
BAS 06/07 T104809 Mnov Z77759	GO-142	<i>Megaptera novaeangliae</i>
BAS 06/07 T104810 Mnov Z77760	GO-143	<i>Megaptera novaeangliae</i>
BAS 06/07 T104811 Mnov Z77761	GO-144	<i>Megaptera novaeangliae</i>
BAS 06/07 T104812 Mnov Z77762	GO-145	X
BAS 06/07 T104813 Mnov Z77763	GO-146	<i>Balaenoptera physalus</i>
BAS 06/07 T104814 Mnov Z77764	GO-147	<i>Megaptera novaeangliae</i>
BAS 06/07 T104815 Mnov Z77765	GO-148	<i>Balaenoptera physalus</i>
BAS 06/07 T104816 Mnov Z77766	GO-149	X
BAS 06/07 T104817 Mnov Z77767	GO-150	<i>Megaptera novaeangliae</i>
BAS 06/07 T104818 Mnov Z77768	GO-151	X
BAS 06/07 T104819 Mnov Z77769	GO-152	<i>Megaptera novaeangliae</i>
BAS 06/07 T104820 Mnov Z77770	GO-153	<i>Megaptera novaeangliae</i>
BAS 06/07 T104821 Mnov Z77771	GO-154	<i>Megaptera novaeangliae</i>
BAS 06/07 T104822 Mnov Z77772	GO-155	<i>Megaptera novaeangliae</i>
BAS 06/07 T104823 Mnov Z77773	GO-156	<i>Megaptera novaeangliae</i>
BAS 06/07 T104824 Mnov Z77774	GO-157	<i>Megaptera novaeangliae</i>
BAS 06/07 T104825 Mnov Z77775	GO-158	<i>Megaptera novaeangliae</i>
BAS 06/07 T104826 Mnov Z77776	GO-159	<i>Megaptera novaeangliae</i>
BAS 06/07 T104827 Mnov Z77777	GO-160	<i>Megaptera novaeangliae</i>
BAS 06/07 T104828 Mnov Z77778	GO-161	<i>Mirounga leonina</i>

BAS 06/07 T104829 Mnov Z77779	GO-162	X
BAS 06/07 T104830 Mnov Z77780	GO-163	X
BAS 06/07 T104831 Mnov Z77781	GO-164	X
BAS 06/07 T104832 Mnov Z77782	GO-165	X
BAS 06/07 T104833 Mnov Z77783	GO-166	<i>Megaptera novaeangliae</i>
BAS 06/07 T104834 Mnov Z77784	GO-167	<i>Megaptera novaeangliae</i>
BAS 06/07 T104835 Mnov Z77785	GO-168	<i>Megaptera novaeangliae</i>
BAS 06/07 T104836 Mnov Z77786	GO-168A	<i>Megaptera novaeangliae</i>
BAS 06/07 T104837 Mnov Z77787	GO-169	<i>Megaptera novaeangliae</i>
BAS 06/07 T104838 Mnov Z77788	GO-170	<i>Megaptera novaeangliae</i>
BAS 06/07 T104839 Mnov Z77789	GO-171	<i>Megaptera novaeangliae</i>
BAS 06/07 T104840 Mnov Z77790	GO-172	<i>Megaptera novaeangliae</i>
BAS 06/07 T104841 Mnov Z77791	GO-173	<i>Balaenoptera physalus</i>
BAS 06/07 T104842 Mnov Z77792	GO-174	<i>Megaptera novaeangliae</i>
BAS 06/07 T104843 Mnov Z77793	GO-175	<i>Megaptera novaeangliae</i>
BAS 06/07 T104844 Mnov Z77794	GO-178	<i>Megaptera novaeangliae</i>
BAS 06/07 T105845 Mnov Z77795	GO-179	<i>Megaptera novaeangliae</i>
BAS 06/07 T104846 Mnov Z77796	GO-180	<i>Megaptera novaeangliae</i>
BAS 06/07 T104847 Mnov Z77797	GO-181	<i>Megaptera novaeangliae</i>
BAS 06/07 T104848 Mnov Z77798	GO-182	X
BAS 06/07 T104849 Mnov Z77799	GO-183	X
BAS 06/07 T104850 Mnov Z77800	GO-184	<i>Megaptera novaeangliae</i>
BAS 06/07 T104851 Mnov Z77801	GO-185	<i>Balaenoptera musculus</i>
BAS 06/07 T104852 Mnov Z77802	GO-186	<i>Balaenoptera physalus</i>
BAS 06/07 T104853 Mnov Z77803	GO-187	<i>Balaenoptera physalus</i>
BAS 06/07 T104854 Mnov Z77804	GO-188	X
BAS 06/07 T104855 Mnov Z77805	GO-189	<i>Megaptera novaeangliae</i>
BAS 06/07 T104856 Mnov Z77806	GO-191	X
BAS 06/07 T104857 Mnov Z77807	GO-192	<i>Balaenoptera physalus</i>
BAS 06/07 T104858 Mnov Z77808	GO-193	<i>Megaptera novaeangliae</i>
BAS 06/07 T1048559 Mnov Z77809	GO-194	<i>Megaptera novaeangliae</i>
BAS 06/07 T104860 Mnov Z77810	GO-195	<i>Megaptera novaeangliae</i>
BAS 06/07 T104861 Mnov Z77811	GO-196	<i>Megaptera novaeangliae</i>
BAS 06/07 T104862 Mnov Z77812	GO-197	<i>Megaptera novaeangliae</i>
BAS 06/07 T104863 Mnov Z77813	GO-198	<i>Balaenoptera physalus</i>
BAS 06/07 T104864 Mnov Z77814	GO-199	<i>Megaptera novaeangliae</i>
BAS 06/07 T104865 Mnov Z77815	GO-200	<i>Megaptera novaeangliae</i>
BAS 06/07 T104866 Mnov Z77816	GO-201	<i>Balaenoptera musculus</i>
BAS 06/07 T104867 Mnov Z77817	GO-202	<i>Megaptera novaeangliae</i>
BAS 06/07 T104868 Mnov Z77818	GO-203	X
BAS 06/07 T104869 Mnov Z77819	GO-204	<i>Balaenoptera physalus</i>
BAS 06/07 T104870 Mnov Z77820	GO-205	X

BAS 06/07 T104871 Mnov Z77821	GO-206	<i>Balaenoptera physalus</i>
BAS 06/07 T104872 Mnov Z77822	GO-207	<i>Balaenoptera physalus</i>
BAS 06/07 T104873 Mnov Z77823	GO-208	<i>Megaptera novaeangliae</i>
BAS 06/07 T104874 Mnov Z77824	GO-209	X
BAS 06/07 T104875 Mnov Z77825	GO-210	<i>Megaptera novaeangliae</i>
BAS 06/07 T104906 Mnov Z77856	NG-001	<i>Megaptera novaeangliae</i>
BAS 06/07 T104907 Mnov Z77857	NG-002	X
BAS 06/07 T104908 Mnov Z77858	NG-003	<i>Megaptera novaeangliae</i>
BAS06/07 T104909 Mnov Z77859	NG-004	<i>Megaptera novaeangliae</i>
BAS06/07 T104910 Mnov Z77860	NG-005	<i>Megaptera novaeangliae</i>
BAS06/07 T104911 Mnov Z77861	NG-006	<i>Megaptera novaeangliae</i>
BAS06/07 T104912 Mnov Z77862	NG-007	<i>Balaenoptera physalus</i>
BAS06/07 T104913 Mnov Z77863	NG-008	X
BAS06/07 T104914 Mnov Z77864	NG-009	<i>Megaptera novaeangliae</i>
BAS06/07 T104915 Mnov Z77865	NG-010	<i>Megaptera novaeangliae</i>
BAS06/07 T104916 Mnov Z77866	NG-011	<i>Megaptera novaeangliae</i>
BAS06/07 T104917 Mnov Z77867	NG-012	<i>Megaptera novaeangliae</i>
BAS06/07 T104918 Mnov Z77868	NG-013	<i>Megaptera novaeangliae</i>
BAS06/07 T104919 Mnov Z77869	NG-014	<i>Megaptera novaeangliae</i>
BAS06/07 T104920 Mnov Z77870	NG-015	<i>Balaenoptera physalus</i>
BAS06/07 T104921 Mnov Z77871	NG-016	X
BAS06/07 T104922 Mnov Z77872	NG-017	<i>Megaptera novaeangliae</i>
BAS06/07 T104923 Mnov Z77873	NG-018	<i>Megaptera novaeangliae</i>
BAS06/07 T104924 Mnov Z77874	NG-019	X
BAS06/07 T104925 Mnov Z77875	NG-020	<i>Megaptera novaeangliae</i>
BAS06/07 T104926 Mnov Z77876	NG-021	<i>Megaptera novaeangliae</i>
BAS 06/07 T104928 Mnov Z77878	NL-001	<i>Balaenoptera physalus</i>
BAS 06/07 T104927 Mnov Z77877	NL-002	X
BAS 06/07 T104929 Mnov Z77879	NL-003	<i>Balaenoptera borealis</i>
BAS 06/07 T104930 Mnov Z77880	NL-004	<i>Balaenoptera physalus</i>
BAS 06/07 T104931 Mnov Z77881	NL-005	X
BAS 06/07 T104932 Mnov Z77882	NL-006	<i>Balaenoptera borealis</i>
BAS 06/07 T104933 Mnov Z77883	NL-007	X
BAS 06/07 T104934 Mnov Z77884	NL-008	<i>Balaenoptera musculus</i>
BAS06/07 T104942 Mnov Z77892	NOH-001	X
BAS06/07 T104943 Mnov Z77893	NU-001	<i>Megaptera novaeangliae</i>
BAS06/07 T104944 Mnov Z77894	NU-002	<i>Megaptera novaeangliae</i>
BAS06/07 T104945 Mnov Z77895	NU-003	<i>Balaenoptera physalus</i>

Appendix D. Spreadsheet of contemporary IWC IDCR/SOWER Antarctic blue whale samples analyzed in Chapter 2.

For each sample, SWFSC lab ID, whether gDNA or WGA was supplied, concentration as measured by Picogreen, the number of microsatellite loci that failed (out of 17), haplotype, genetic sex identification, date and location of biopsy sample collection and how the tissue was stored at SWFSC is listed. Previously undescribed unique haplotypes were named according the name of the first sample ID code. Haplotypes which were only identified within one sequence are noted by an asterisk (*). Samples where haplotype was not identified are noted by (-). Within the sex column, question marks indicate a probable male or female based on light bands due to low quantity or quality of gDNA or WGA.

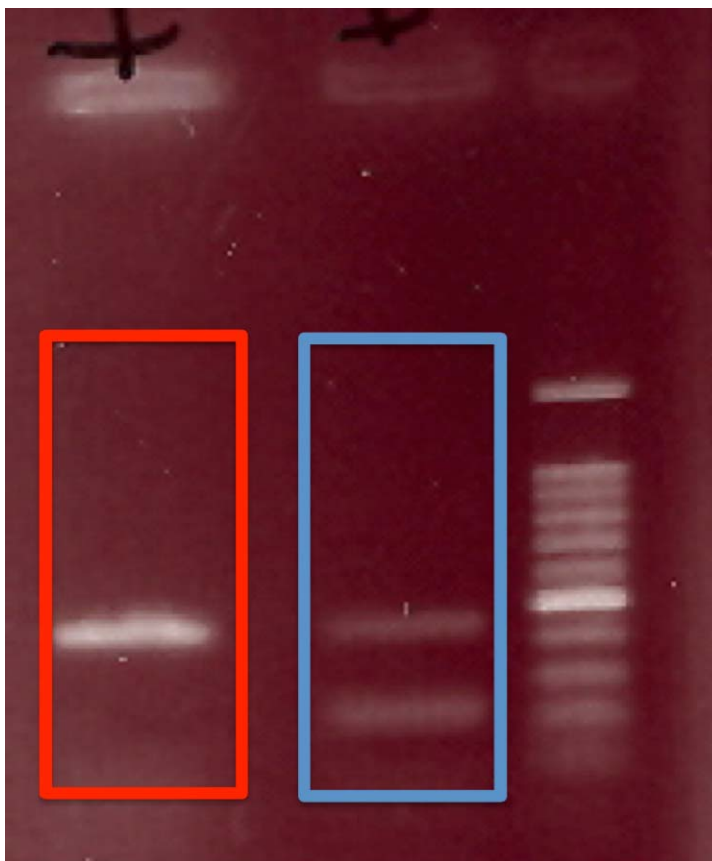
SWFSC C lab ID	gDNA or WGA	ng/ul	# usat failure s	k	sex	Area	Date	LAT	LONG	T Store
7336	gDNA	0.35	12	h	M	II	2-Sep-1997	-70.80	-22.00	DMSO
7337	gDNA	7.80	9	l	F	II	2-Dec-1997	-70.37	-22.95	DMSO
7340	gDNA	-0.53	7	i	M	II	29-Jan-1997	-70.80	-16.25	DMSO
7341	gDNA	16.91	8	j	M	II	29-Jan-1997	-70.83	-16.52	DMSO
7342	gDNA	7.94	9	j	M	II	29-Jan-1997	-70.58	-16.75	DMSO
7619	gDNA	1.62	17	k	X	I	21-Jan-1990	-68.98	-92.50	DMSO
7620	gDNA	4.00	8	d	M	III	2-Jan-1993	-65.83	6.45	DMSO
7621	gDNA	4.05	6	l	F	III	10-Jan-1993	-67.97	18.65	DMSO
7622	gDNA	-0.64	11	m	X	IV	15-Feb-1995	-61.53	73.50	DMSO
7623	gDNA	5.07	9	n	M?	IV	19-Feb-1995	-61.68	76.78	DMSO
11162	gDNA	1.14	6	i	M?	II	30-Jan-1998	-61.25	-34.15	DMSO
11163	gDNA	3.61	7	i	X	II	30-Jan-1998	-61.25	-34.15	DMSO
11164	gDNA	12.07	8	u	M	II	25-Jan-1998	-58.65	-48.12	DMSO
11165	gDNA	5.47	9	r	M?	II	27-Jan-1998	-59.18	-40.43	DMSO
11166	gDNA	-0.10	14		X	II	7-Feb-1998	-68.27	-39.28	DMSO
11167	gDNA	-0.88	9		X	II	7-Feb-1998	-68.35	-39.22	DMSO
13944	gDNA	3.53	9	ff	X	IV	25-Jan-1999	-65.18	89.62	DMSO
13945	gDNA	-0.50	9	gg	X	IV	3-Feb-1999	-60.42	105.58	DMSO
13946	gDNA	2.48	6	gg	M	IV	3-Feb-1999	-60.42	105.58	DMSO
13947	gDNA	3.06	7	gg	M	IV	3-Feb-1999	-60.42	105.58	DMSO
13948	gDNA	1.74	9	hh	F	IV	8-Feb-1999	-60.93	113.70	DMSO
13949	gDNA	-0.13	7	n	F	IV	10-Feb-1999	-60.53	115.92	DMSO
13950	gDNA	1.44	8	n	F	IV	10-Feb-1999	-60.62	115.92	DMSO
13951	gDNA	-0.80	6	13951	M	IV	28-Jan-1999	-63.95	89.62	DMSO
13952	gDNA	-0.95	10	72917	F	IV	28-Jan-1999	-63.95	89.62	DMSO
13953	gDNA	3.58	10	13951	M	IV	28-Jan-1999	-63.95	89.62	DMSO
26573	gDNA	1.01	17	-	X	V	1-Dec-2001	-68.10	-170.70	DMSO
26574	WGA	-1.27	5	nn	X	V	12-Jan-2001	-68.10	-170.70	DMSO
26575	WGA	-1.28	2	nn	X	V	12-Jan-2001	-68.10	-170.70	DMSO
26576	gDNA	7.74	8	nn	F	V	12-Jan-2001	-68.10	-170.70	DMSO
26577	gDNA	2.77	7	nn	F	V	12-Jan-2001	-68.10	-170.70	DMSO
26578	gDNA	0.20	8	oo	M	VI	14-Jan-2001	-67.58	-161.12	DMSO
26579	gDNA	-0.77	14	nn	X	VI	14-Jan-2001	-67.58	-161.12	DMSO
26580	gDNA	-0.31	9	bb	X	VI	14-Jan-2001	-67.58	-161.12	DMSO
26581	gDNA	-0.88	9	bb	F	VI	14-Jan-2001	-67.58	-161.12	DMSO
26582	gDNA	1.04	6	oo	M	VI	14-Jan-2001	-67.58	-161.12	DMSO
26583	gDNA	0.45	8	oo	M?	VI	14-Jan-2001	-67.58	-161.12	DMSO
26584	gDNA	0.36	5	oo	F	VI	14-Jan-2001	-67.58	-161.12	DMSO
26585	WGA	-1.20	4	m	X	VI	27-Jan-2001	-68.22	-126.02	DMSO
26586	gDNA	-0.21	7	v	F?	VI	27-Jan-2001	-68.22	-126.02	DMSO
26587	gDNA	1.73	8	m	M	VI	27-Jan-2001	-68.22	-126.02	DMSO

26588	gDNA	-0.58	5	v	F	VI	27-Jan-2001	-68.22	-126.02	DMSO
26589	gDNA	3.94	5	rr	F	VI	27-Jan-2001	-68.22	-126.02	DMSO
26590	gDNA	1.58	11	ss	X	VI	30-Jan-2001	-68.60	-121.78	DMSO
26591	gDNA	3.26	8	z	M	I	3-Feb-2001	-69.25	-117.83	DMSO
26592	gDNA	0.79	8	z	X	I	3-Feb-2001	-69.25	-117.83	DMSO
26593	gDNA	0.09	7	rr	F	I	3-Feb-2001	-69.28	-117.80	DMSO
26594	gDNA	2.97	9	tt	X	I	3-Feb-2001	-69.25	-117.83	DMSO
51449	WGA	-1.23	5	m	X	V	6-Jan-2002	-64.27	137.07	DMSO
51450	WGA	-1.04	5	m	X	V	6-Jan-2002	-64.28	137.13	DMSO
51451	WGA	-1.19	2	y	M	V	6-Jan-2002	-64.28	137.13	DMSO
51452	WGA	-1.21	1	72956	M	V	6-Jan-2002	-64.32	137.27	DMSO
51453	WGA	-1.20	5	bb	X	V	6-Jan-2002	-64.33	137.30	DMSO
51454	gDNA	0.69	0	m	F	V	7-Jan-2002	-64.27	137.20	DMSO
51455	gDNA	-1.13	3	m	F	V	8-Jan-2002	-64.28	137.23	DMSO
51456	WGA	-1.11	1	72956	M	V	21-Jan-2002	-65.12	135.57	DMSO
51457	WGA	-1.28	4	72956	X	V	21-Jan-2002	-65.12	135.57	DMSO
51458	WGA	-0.89	1	m	F	V	21-Jan-2002	-65.12	135.57	DMSO
51459	gDNA	0.71	10	72956	M?	V	21-Jan-2002	-65.12	135.57	DMSO
51460	WGA	-1.14	0	51460*	M	V	29-Jan-2002	-65.33	140.42	DMSO
51461	WGA	-0.97	3	51461	M	V	29-Jan-2002	-65.33	140.42	DMSO
51462	gDNA	3.03	8	i	M	V	29-Jan-2002	-65.33	140.42	DMSO
51463	gDNA	-0.79	1	n	F	V	31-Jan-2002	-65.58	143.13	DMSO
51464	WGA	-0.70	0	u	F	V	30-12-2002	-67.35	178.37	DMSO
51465	gDNA	1.90	9	bb	X	V	4-Feb-2003	-65.32	154.07	DMSO
51466	gDNA	0.85	11	bb	X	V	4-Feb-2003	-65.32	154.07	DMSO
51467	gDNA	-0.30	10	bb	X	V	4-Feb-2003	-65.32	154.07	DMSO
51468	gDNA	-0.18	8	bb	X	V	4-Feb-2003	-65.32	154.07	DMSO
51469	gDNA	-0.10	11	bb	X	V	23-Jan-2003	-67.08	166.48	DMSO
51470	gDNA	0.36	10	51470*	X	V	19-Jan-2004	-67.28	175.47	DMSO
51471	gDNA	2.00	0	y	F	V	19-Jan-2004	-67.18	176.03	DMSO
51472	gDNA	-0.69	1	51472*	F	V	17-Feb-2004	-70.33	-176.17	DMSO
51473	WGA	-0.94	6	51488	F	V	17-Feb-2004	-70.33	-176.17	DMSO
51474	gDNA	1.80	8	-	X	V	17-Feb-2004	-70.33	-176.17	DMSO
51475	gDNA	-0.21	12	uu	X	V	17-Feb-2004	-70.33	-176.18	DMSO
51476	WGA	0.00	2	rr	M	V	17-Feb-2004	-70.38	-175.20	DMSO
51477	gDNA	-0.09	8	rr	X	V	17-Feb-2004	-70.40	-175.00	DMSO
51478	gDNA	0.44	12	51488	X	V	17-Feb-2004	-70.40	-175.00	DMSO
51479	gDNA	2.47	9	88257	X	V	17-Feb-2004	-70.40	-175.00	DMSO
51480	gDNA	0.01	9	51480	X	V	21-Feb-2004	-70.32	-175.02	DMSO
51481	gDNA	-0.13	9	51481	X	V	26-Feb-2004	-68.25	-173.57	DMSO
51482	WGA	-0.79	0	51481	F	V	26-Feb-2004	-68.25	-173.57	DMSO
51483	WGA	-0.55	4	51481	F	V	26-Feb-2004	-68.25	-173.57	DMSO
51484	WGA	-0.63	6	72910	X	III	23-Jan-2005	-69.07	10.48	DMSO
51485	gDNA	0.01	8	hh	X	III	4-Feb-2005	-68.32	19.17	DMSO
51486	gDNA	0.25	13	51486*	X	III	4-Feb-2005	-68.32	19.17	DMSO
51487	gDNA	0.53	10	72956	X	III	4-Feb-2005	-68.32	19.17	DMSO
51488	gDNA	0.34	9	51488	X	IV	28-Feb-2005	-57.33	79.07	DMSO
62474	WGA	-1.10	1	m	M	III	19-Jan-2006	-68.30	4.38	DMSO
62475	gDNA	-1.20	1	h	X	III	20-Jan-2006	-69.17	4.13	DMSO
62476	gDNA	-1.21	1	m	M	III	20-Jan-2006	-69.17	4.13	DMSO
62477	WGA	-1.19	1	72956	F	III	24-Jan-2006	-69.02	0.07	DMSO
62478	WGA	-0.92	2	13951	F	III	24-Jan-2006	-68.55	2.28	DMSO
62479	WGA	-1.22	0	72956	M	III	24-Jan-2006	-68.55	2.28	DMSO
62480	WGA	-1.11	2	51461	F	III	25-Jan-2006	-69.17	3.02	DMSO
62481	WGA	-0.95	0	62481	M	III	25-Jan-2006	-69.32	4.07	DMSO
62482	WGA	-1.25	0	62482	M	III	26-Jan-2006	-69.28	4.03	DMSO
62483	WGA	1.90	0	l	F	III	26-Jan-2006	-69.28	4.03	DMSO
62484	WGA	-0.26	0	72956	F	III	26-Jan-2006	-69.38	5.43	DMSO
62485	WGA	-1.12	0	13951	X	III	26-Jan-2006	-69.33	6.55	DMSO
62486	WGA	-1.07	1	l	F	III	28-Jan-2006	-67.42	11.53	DMSO
62487	WGA	-1.24	4		X	III	29-Jan-2006	-67.48	12.07	DMSO

62488	WGA	-0.97	2	72956	F	III	29-Jan-2006	-67.48	12.07	DMSO
62489	WGA	-0.76	0	i	M	III	29-Jan-2006	-67.32	12.32	DMSO
62490	WGA	-1.23	3	m	X	III	29-Jan-2006	-67.32	12.32	DMSO
62491	gDNA	-0.96	1	m	F	III	29-Jan-2006	-67.32	12.32	DMSO
62492	gDNA	-1.29	3	i	X	III	30-Jan-2006	-67.32	13.07	DMSO
62493	gDNA	-1.23	5	m	X	III	30-Jan-2006	-68.08	15.57	DMSO
62494	gDNA	-1.16	1	hh	M	III	30-Jan-2006	-68.08	15.57	DMSO
62495	gDNA	-1.30	0	72917	M	III	9-Jan-2006	-68.47	19.28	DMSO
62496	gDNA	-1.24	2	l	X	III	9-Feb-2006	-68.47	19.28	DMSO
62497	gDNA	-0.62	0	51480	M	III	9-Feb-2006	-68.47	19.28	DMSO
62498	gDNA	-0.89	0	13951	M	III	9-Feb-2006	-68.50	19.17	DMSO
62499	gDNA	-0.91	0	72956	X	III	9-Feb-2006	-68.50	19.17	DMSO
62500	gDNA	-1.22	4	72956	X	III	9-Feb-2006	-68.48	19.05	DMSO
62501	WGA	-0.84	2	51461	X	III	9-Feb-2006	-68.48	18.55	DMSO
62502	WGA	4.24	0	l	X	III	9-Feb-2006	-68.48	18.53	DMSO
62503	WGA	-1.25	7	y	X	III	9-Feb-2006	-68.48	18.53	DMSO
62504	WGA	-0.57	0	13951	X	III	9-Feb-2006	-68.42	19.03	DMSO
62505	WGA	-1.04	0	l	X	III	9-Feb-2006	-68.42	19.03	DMSO
62506	WGA	-1.14	0	d	M	III	12-Feb-2006	-68.42	16.13	DMSO
62507	WGA	-1.24	1	l	M	III	13-Feb-2006	-68.42	14.23	DMSO
62508	WGA	-1.12	2	51461	M	III	13-Feb-2006	-68.42	14.23	DMSO
62509	WGA	-0.87	1	i	M	III	13-Feb-2006	-68.40	12.35	DMSO
72893	WGA	-1.03	13		X	III	3-Jan-2007	-64.93	1.73	ETOH
72894	WGA	-1.08	16	ff	X	III	4-Jan-2007	-68.13	0.43	ETOH
72895	gDNA	-1.24	13	aa	X	III	5-Jan-2007	-67.98	0.70	ETOH
72896	gDNA	-1.00	12	b	X	III	5-Jan-2007	-67.98	0.70	ETOH
72897	gDNA	-1.12	14	u	X	III	5-Jan-2007	-67.98	0.70	ETOH
72898	gDNA	-1.20	6	51481	X	III	5-Jan-2007	-68.02	1.25	ETOH
72899	gDNA	-1.17	7	gg	X	III	5-Jan-2007	-68.02	1.25	ETOH
72900	WGA	-0.63	14	72956	X	III	5-Jan-2007	-68.22	1.43	ETOH
72901	WGA	-0.98	9	72956	X	III	5-Jan-2007	-68.22	1.43	ETOH
72902	WGA	0.75	8	n	X	III	6-Jan-2007	-68.05	1.72	ETOH
72903	WGA	-0.99	3	51481	X	III	7-Jan-2007	-67.58	2.75	ETOH
72904	WGA	-0.05	11	hh	X	III	7-Jan-2007	-67.58	2.75	ETOH
72905	WGA	-0.90	6	hh	M	III	8-Jan-2007	-67.98	0.17	ETOH
72906	WGA	-0.84	3	72906	X	III	8-Jan-2007	-68.17	-0.03	ETOH
72907	WGA	-0.08	0	f	X	III	8-Jan-2007	-68.32	-0.03	ETOH
72908	WGA	-0.45	3	51481	M	III	8-Jan-2007	-68.70	0.45	ETOH
72909	WGA	-0.80	4	72910	X	III	9-Jan-2007	-68.78	0.75	ETOH
72910	WGA	-1.18	6	72910	X	III	9-Jan-2007	-68.78	0.75	ETOH
72911	WGA	-1.13	5	62481	X	III	9-Jan-2007	-68.78	0.75	ETOH
72912	WGA	-0.14	11	-	X	III	9-Jan-2007	-68.78	0.75	ETOH
72913	WGA	-1.06	8	hh	X	III	9-Jan-2007	-68.78	0.75	ETOH
72914	WGA	-0.98	3	i	X	III	9-Jan-2007	-68.70	0.70	ETOH
72915	WGA	-0.99	11	51461	X	III	9-Jan-2007	-68.70	0.70	ETOH
72916	WGA	-1.17	2	72916*	X	III	10-Jan-2007	-68.28	0.82	ETOH
72917	WGA	-0.23	2	72917	X	III	12-Jan-2007	-67.65	4.65	ETOH
72918	WGA	-1.30	13	-	X	III	12-Jan-2007	-67.62	4.68	ETOH
72919	WGA	-0.90	12	r	X	III	13-Jan-2007	-67.38	7.73	ETOH
72920	WGA	-0.41	1	d	X	III	13-Jan-2007	-67.65	8.87	ETOH
72921	WGA	-0.89	5	m	X	III	12-Jan-2007	-67.62	4.87	ETOH
72922	WGA	-0.95	2	62482	X	III	30-Jan-2007	-62.38	10.85	ETOH
72923	WGA	-0.89	1	62482	X	III	30-Jan-2007	-62.38	10.85	ETOH
72924	gDNA	0.48	0	62482	X	III	30-Jan-2007	-62.38	10.85	ETOH
72925	gDNA	-0.40	2	-	X	III	31-Jan-2007	-69.28	8.37	ETOH
72926	gDNA	-0.66	2	51461	X	III	31-Jan-2007	-69.28	8.37	ETOH
72927	gDNA	0.04	1	-	X	III	31-Jan-2007	-69.28	8.37	ETOH
72928	gDNA	-0.75	1	51461	M	III	31-Jan-2007	-69.28	8.37	ETOH
72929	gDNA	-0.73	4	72929	F	III	31-Jan-2007	-69.28	8.37	ETOH
72930	gDNA	-0.65	5	aa	M	III	5-Feb-2007	-69.08	8.33	ETOH
72931	gDNA	-0.78	4	72931*	M	III	5-Feb-2007	-69.08	8.33	ETOH

72932	WGA	-0.83	3	l	M	III	5-Feb-2007	-69.08	8.33	ETOH
72933	WGA	-0.21	6	l	M	III	5-Feb-2007	-69.08	8.33	ETOH
72934	WGA	-0.81	15	51461	X	III	5-Feb-2007	-69.08	8.33	ETOH
72935	WGA	-0.60	15	72935	X	III	5-Feb-2007	-69.08	8.33	ETOH
72936	WGA	-0.90	1	i	M	III	5-Feb-2007	-69.08	8.33	ETOH
72937	WGA	-0.92	7	gg	M	III	6-Feb-2007	-69.32	7.22	ETOH
72938	WGA	-1.10	13	gg	X	III	6-Feb-2007	-69.32	7.22	ETOH
72939	WGA	-0.85	3	hh	M	III	6-Feb-2007	-69.32	7.22	ETOH
72940	WGA	0.35	1	72956	X	III	6-Feb-2007	-69.32	7.22	ETOH
72941	WGA	-0.67	6	72935	M	III	6-Feb-2007	-69.32	7.22	ETOH
72942	WGA	-1.05	0	hh	M	III	6-Feb-2007	-69.45	6.63	ETOH
72943	WGA	-0.95	12	72943	X	III	6-Feb-2007	-69.37	6.23	ETOH
72944	WGA	-1.09	2	-	X	III	6-Feb-2007	-69.37	6.23	ETOH
72945	WGA	-0.56	2	aa	X	III	6-Feb-2007	-69.37	6.23	ETOH
72946	WGA	-0.93	4	aa	M	III	6-Feb-2007	-69.37	6.23	ETOH
72947	WGA	-1.12	2	hh	M	III	6-Feb-2007	-69.37	6.23	ETOH
72948	WGA	-0.86	12	hh	M	III	6-Feb-2007	-69.37	6.23	ETOH
72949	WGA	-1.08	2	72949	M	III	7-Feb-2007	-69.40	5.15	ETOH
72950	WGA	-0.82	2	72943	X	III	7-Feb-2007	-69.40	5.42	ETOH
72951	WGA	-0.98	12	i	X	III	7-Feb-2007	-69.40	5.42	ETOH
72952	WGA	-0.78	4	i	M	III	7-Feb-2007	-69.40	5.42	ETOH
72953	WGA	0.16	9	72943	X	III	7-Feb-2007	-69.40	5.42	ETOH
72954	WGA	-0.06	0	72956	F	III	7-Feb-2007	-69.60	5.83	ETOH
72955	WGA	-1.02	4	72935	M	III	7-Feb-2007	-69.60	5.83	ETOH
72956	WGA	-0.79	2	72956	F	III	7-Feb-2007	-69.60	5.83	ETOH
72957	gDNA	-0.36	3	i	M	III	7-Feb-2007	-69.60	5.83	ETOH
72958	gDNA	-0.60	13	-	F	III	7-Feb-2007	-69.60	5.83	ETOH
72959	gDNA	-1.16	2	i	M	III	7-Feb-2007	-69.60	5.83	ETOH
72960	gDNA	0.03	8	d	M	III	8-Feb-2007	-69.85	4.38	ETOH
72961	gDNA	-1.12	8	51461	X	III	8-Feb-2007	-69.85	4.38	ETOH
72962	WGA	1.27	8	m	X	III	8-Feb-2007	-69.87	4.27	ETOH
72963	WGA	1.16	1	aa	F	III	8-Feb-2007	-69.67	4.88	ETOH
72964	WGA	-1.00	16	u	X	III	8-Feb-2007	-69.67	4.88	ETOH
72965	WGA	-0.64	5	51461	F?	III	8-Feb-2007	-69.67	4.88	ETOH
72966	WGA	-1.13	5	ff	X	III	8-Feb-2007	-69.67	4.88	ETOH
72967	WGA	-1.17	4	u	M	III	8-Feb-2007	-69.67	4.88	ETOH
72968	WGA	-0.96	6	51461	F	III	8-Feb-2007	-69.67	4.88	ETOH
72969	WGA	-0.62	4	b	X	III	8-Feb-2007	-69.67	4.88	ETOH
72970	WGA	-1.03	2	aa	F?	III	8-Feb-2007	-69.82	4.78	ETOH
72971	WGA	-0.72	3	72906	F?	III	8-Feb-2007	-69.82	4.78	ETOH
72972	WGA	-0.51	2	l	F	III	8-Feb-2007	-69.82	4.78	ETOH
72973	WGA	-0.92	2	72949	X	III	8-Feb-2007	-69.82	4.78	ETOH
72974	WGA	-0.61	12	72929	X	III	8-Feb-2007	-69.82	4.78	ETOH
72975	WGA	0.19	11	-	F	III	8-Feb-2007	-69.82	4.78	ETOH
72976	WGA	-1.15	11	-	X	III	8-Feb-2007	-69.82	4.78	ETOH
72977	WGA	-0.16	13	51461	X	III	9-Feb-2007	-69.55	5.32	ETOH
88253	WGA	-1.15	7	72935	X	IV	30-01-2009	-63.98	88.82	DMSO
88254	WGA	-0.31	1	ss	M	IV	5-Feb-2009	-64.75	88.13	DMSO
88255	WGA	-0.99	8	ss	M	IV	5-Feb-2009	-64.75	88.13	DMSO
88256	WGA	-1.04	2	88257	M	IV	5-Feb-2009	-64.67	88.10	DMSO
88257	gDNA	-0.18	1	88257	M	IV	5-Feb-2009	-64.67	88.10	DMSO
88258	gDNA	-0.77	1	l	M	IV	7-Feb-2009	-63.88	87.35	DMSO
88259	gDNA	-0.91	1	bb	M	IV	7-Feb-2009	-63.88	87.35	DMSO
88260	gDNA	-0.50	1	l	M?	IV	7-Feb-2009	-63.88	87.35	DMSO
88261	gDNA	-0.77	6	l	X	IV	7-Feb-2009	-63.88	87.35	DMSO

Appendix E. Genetic identification of sex. Image of a sex gel indicating the 223 ZFX band and 442-445 SRY band used to identify males (two bands; blue) and females (single band; red).



Appendix F. Report of IWC IDCR/SOWER Whole Genome Amplification (WGA) and genomic DNA (gDNA) microsatellite analysis

This appendix provides an overview of methods and results of the WGA and gDNA microsatellite and sex analysis.

DATASET

Genomic DNA (gDNA) and WGA preparation

Access to Antarctic blue whale biopsy samples collected during the IWC IDCR/SOWER research cruises (1990-2009; n=218) were provided for this study via a loan request to the IWC (June 2009; Appendix A). Samples were archived at Southwest Fisheries Science Center (SWFSC) in La Jolla, CA where the biopsy samples were extracted and genomic DNA (gDNA) archived. Due to a concern of the depletion of Antarctic blue whale gDNA, a subset of the samples were provided for the study as whole genome amplifications (WGA) (n=154). Combined with 64 gDNA samples, the total dataset accounted for 218 samples. We were given both gDNA and WGA for a subset of 40 samples to test the use of WGA in replacement of gDNA for genetic analyses. Thus, there was a total of 154 WGA and 104 gDNA samples.

Approximately 20-25 μ l of gDNA or WGA were received for each sample. Genomic DNA extractions were performed at SWFSC following a variety of methods, namely lithium chloride extraction (Gemmell and Akiyama 1996), sodium chloride protein precipitation (modified from Miller et al., 1988), silica-based filter purification (DNeasy kit, Qiagen, Valenica, CA, USA and Xtractor gene, Corbett Robotics, San Francisco, CA, USA) or a standard phenol/chloroform extraction (Sambrook et al.,

1989). The WGA were prepared using the REPLI-g UltraFast Minikit (Qiagen).

Prior to analyses, gDNA and WGA samples were quantified using the Picogreen (Molecular Probes, Eugene, OR) (see Appendix D for information regarding sample collection location, tissue storage, whether the sample provided was gDNA or WGA and Picogreen concentration for each sample within the dataset).

METHODS

see Chapter Three for mtDNA sequencing, microsatellite and genetic sex methods

RESULTS

Picogreen concentration readings were low ranging from -1.3 to 16.91 ng/ μ l. Only 7 samples had concentrations greater than 5 ng/ μ l (Appendix D). Despite this, initial amplification of the mtDNA control region was successful for 204 samples, a 94% success rate. However, success was much reduced for nuclear microsatellites and sex identification. The subset of 40 gDNA and WGA replicates amplified for 90% and 98% for 8 out of the 17 loci, respectively. This resulted in many incomplete sample genotypes and this provided little overlap of loci between sample genotypes to successfully identify replicates in an automated program such as CERVUS v.3 (Kalinowski et al., 2007). Further indication of low quantity and possibly quality of the gDNA and WGA was evident in the identification of genetic sex. Only 63% of samples were successfully identified to sex. For the replicate 40 samples, we had a lower success rate for the WGA (45%) in comparison to the gDNA (75%). We were

unable to determine if failure to amplify for microsatellites and genetic sex identification was unable to be determined if due to the low quantity gDNA or WGA or both quantity and quality of the samples.

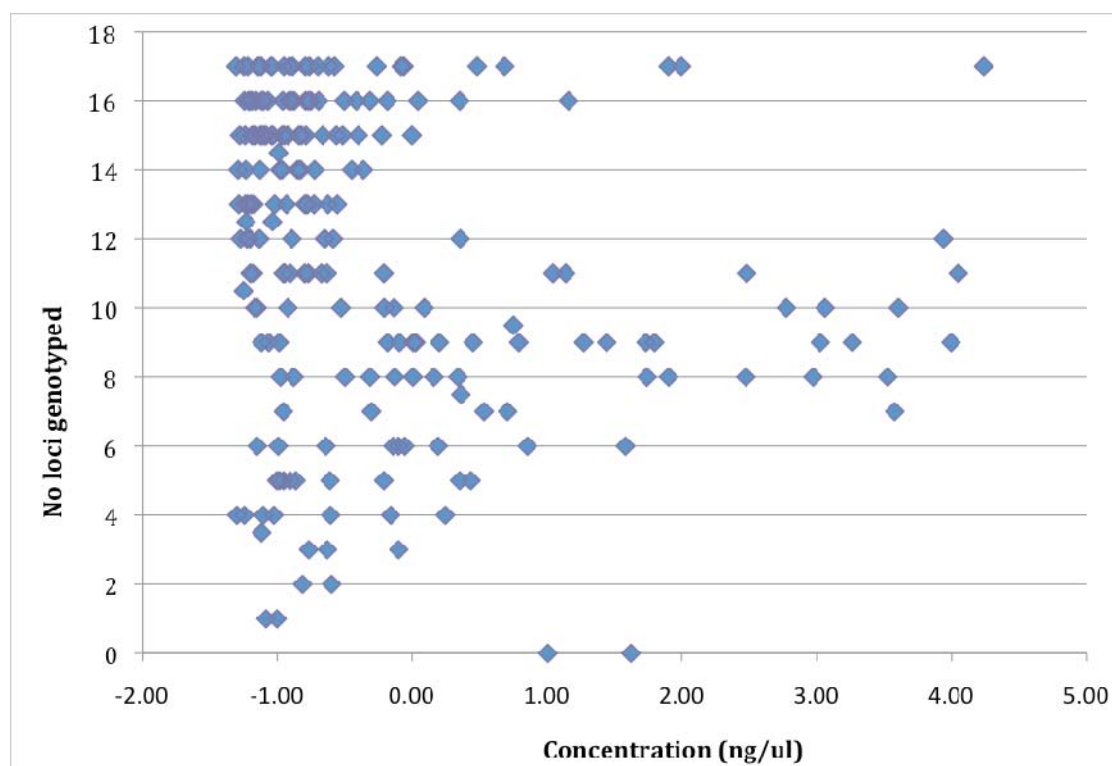


Fig. F-1. Concentration of gDNA and WGA samples as measured by Picogreen assay compared to the number of microsatellite loci that failed per sample. Graph excludes five samples with concentration >5 ng/ μ l.

Protocol for the identification of replicates

Due to the initial results, we modified protocols for the identification of replicates. To identify replicates within the dataset, we used mtDNA haplotype data to establish *a priori* haplotype groups to search for likely replicates. Only samples that successfully sequenced for the mtDNA control region were included in this analysis

(n=204). Replicates were identified within haplotype groups using CERVUS v.3 and through visual comparisons between genotypes. As genotypes were incomplete for many samples due to variable quantity or quality of gDNA and WGA, samples which only amplified for 3-5 loci but alleles did match other samples in their haplotype group were included in remaining analyses as they were excluded as a likely replicate. Evidence of allelic dropout was prevalent and all potential replicate genotypes were visually checked.

See Chapter Three for identification of replicates results

CONCLUSION

From these analyses, the genotype data was considered sufficient for identification of replicates but due to the low quantity of WGA and gDNA and failure to amplify for a sufficient number of loci for, the dataset was not sufficient for further microsatellite analyses. The data were considered sufficient to identify probable replicates within the dataset to remove bias in the mtDNA diversity analysis.

We were unable to determine if the high rate of microsatellite failure was due to low quantity or quality of gDNA or WGA. At the end of data analysis, depletion of gDNA and WGA disabled further attempts to obtain further microsatellite and sex information. Requests have been made to SWFSC and we will be receiving gDNA for all WGA samples, and additional aliquots of gDNA for all depleted gDNA samples to rerun microsatellite and sex identification analyses. A complete dataset will allow for

population structure to be investigated by nuclear markers to further study

Antarctic blue whale population structure.

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Appendix G. Northern Hemisphere blue whale (*B. m. musculus*) mtDNA diversity as described from the North Pacific and North Atlantic

DATASET

Biopsy samples from tagging efforts Bruce Mate and the Marine Mammal Institute (n=64) and John Calambokidis in association with Cascadia Research Collective (n=5) were collected from the North Pacific between 2000 and 2007. Samples were primarily collected off the coast of California, while two samples (2008) were collected from the Costa Rica Dome. In addition, three DNA samples from the North Atlantic (1998-1999) were provided for analyses courtesy of Carole Conway.

METHODS

DNA was extracted from the North Pacific biopsy samples following a phenol-chloroform procedure as described in (Sambrook et al., 1989) and modified by Baker et al. (1994). DNA concentrations from extracted North Pacific biopsy samples and loaned North Atlantic samples were quantified using Picogreen and normalized prior to mtDNA amplification. Primers DlpM131.5 and Dlp8 along with 1ul of template DNA were used to amplify up to 700 bp of the mtDNA control region sequence following the protocol listed in Chapter 2.

RESULTS

Haplotypes, as described from unique mutations within the mtDNA control region sequence, were identified through comparison to the worldwide blue whale mtDNA haplotypes. Quality control of mtDNA sequences followed the protocol in Chapter 2. Within the North Pacific, 46 samples amplified for the mtDNA control region sequence to 560 bp, describing 12 haplotypes. Three previously undescribed haplotypes were only identified by one sequence and validated through reverse sequencing of an independent amplification. Nine haplotypes were not found within the worldwide database of known haplotypes. Three haplotypes (D, Q, R) were shared the worldwide population of blue whales.

The three North Atlantic samples were sequenced for 560 bp of the mtDNA control region sequence and described three unique haplotypes. All identified unique haplotypes were independently reverse sequenced to validate unique mutations. All mtDNA control region sequences were truncated to 347 bp for comparison to the historic South Georgia blue whale sequences.

REFERENCES

- Baker, C. S., Slade, R. W., et al. (1994). Hierarchical structure of mitochondrial DNA gene flow among humpback whales, *Megaptera novaeangliae*, world-wide. *Molecular Ecology*, 3, 313-327.
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Table G-1. Lab ID, sample collection date and location, collaborator are reported in addition to haplotype and genetic sex identification for the North Pacific and North Atlantic blue whales. Asterisk (*) haplotypes were represented in only sequence but validated through reverse sequencing. An (x) notes that genetic sex was not identified.

Country	Location	Date	Lat	Long	Collaborator	Lab ID	Haplotype	lab sex
USA	California	4-Oct-00	37.62N	123.03W	Bruce Mate; MMI	Bmus00Ca001	r	X
USA	California	5-Oct-00	37.61N	123.02W	Bruce Mate; MMI	Bmus00Ca002	q	X
USA	California	12-Aug-04	34.20N	120.18W	Bruce Mate; MMI	Bmus04Ca008	Bmu04Ca016	X
USA	California	12-Aug-04	34.22N	120.23W	Bruce Mate; MMI	Bmus04Ca011	Bmu06Ca005	X
USA	California	12-Aug-04	34.22N	120.22W	Bruce Mate; MMI	Bmus04Ca012	r	X
USA	California	13-Aug-04	34.16N	120.01W	Bruce Mate; MMI	Bmus04Ca013	r	X
USA	California	20-Aug-04	37.69N	123.13W	Bruce Mate; MMI	Bmu04Ca017	Bmu05Ca011	X
USA	California	15-Aug-05	36.68N	122.01W	Bruce Mate; MMI	Bmus05Ca006	d	male
USA	California	15-Aug-05	36.67N	122.00W	Bruce Mate; MMI	Bmus05Ca007	Bmu07Ca001	male
USA	California	15-Aug-05	36.67N	122.00W	Bruce Mate; MMI	Bmus05Ca008	q	female
USA	California	18-Aug-05	36.67N	122.01W	Bruce Mate; MMI	Bmus05Ca009	r	male
USA	California	19-Aug-05	36.66N	122.02W	Bruce Mate; MMI	Bmus05Ca010	d	female
USA	California	19-Aug-05	36.71N	121.98W	Bruce Mate; MMI	Bmus05Ca011	Bmu05Ca011	X
USA	California	12-Sep-06	34.05N	120.60W	Bruce Mate; MMI	Bmu06Ca001	r	male
USA	California	12-Sep-06	34.07N	120.61W	Bruce Mate; MMI	Bmu06Ca002	Bmu06Ca002	male
USA	California	13-Sep-06	34.10N	120.62W	Bruce Mate; MMI	Bmu06Ca003	r	male
USA	California	13-Sep-06	34.10N	120.61W	Bruce Mate; MMI	Bmu06Ca004	r	female
USA	California	14-Sep-06	34.12N	120.61W	Bruce Mate; MMI	Bmu06Ca005	Bmu06Ca005	male
USA	California	14-Sep-06	34.10N	120.61W	Bruce Mate; MMI	Bmu06Ca006	q	female
USA	California	26-Sep-06	34.52N	120.98W	Bruce Mate; MMI	Bmu06Ca008	q	male
USA	California	19-Aug-04	37.67N	123.10W	Bruce Mate; MMI	Bmu04Ca016	Bmu04Ca016	X
USA	California	6-Sep-07	34.15N	120.02W	Bruce Mate; MMI	Bmu07Ca001	Bmu07Ca001	female
USA	California	6-Sep-07	34.15N	119.93W	Bruce Mate; MMI	Bmu07Ca002	Bmu07Ca002	male
USA	California	6-Sep-07	34.13N	120.09W	Bruce Mate; MMI	Bmu07Ca003	q	female

USA	California	6-Sep-07	34.15N	119.97W	Bruce Mate; MMI	Bmu07Ca004	Bmu07Ca004*	male
USA	California	6-Sep-07	34.14N	120.04W	Bruce Mate; MMI	Bmu07Ca005	Bmu05Ca011	male
USA	California	7-Sep-07	34.14N	120.03W	Bruce Mate; MMI	Bmu07Ca006	Bmu07Ca006*	female
USA	California	7-Sep-07	34.14N	120.05W	Bruce Mate; MMI	Bmu07Ca007	Bmu07Ca002	female
USA	California	7-Sep-07	34.13N	120.02W	Bruce Mate; MMI	Bmu07Ca008	r	male
USA	California	9-Sep-07	34.13N	120.09W	Bruce Mate; MMI	Bmu07Ca009	R	female
USA	California	9-Sep-07	34.12N	120.08W	Bruce Mate; MMI	Bmu07Ca010	Bmu06Ca005	female
USA	California	9-Sep-07	34.12N	120.05W	Bruce Mate; MMI	Bmu07Ca011	R	female
USA	California	11-Sep-07	34.20N	119.9W	Bruce Mate; MMI	Bmu07Ca012	Q	female
USA	California	8-Sep-07	34.12N	120.07W	John Calambokidis; CRC	Bmu07Ca013	R	X
USA	California	8-Sep-07	34.12N	120.03W	John Calambokidis; CRC	Bmu07Ca014	R	X
USA	California	8-Sep-07	34.13N	120.05W	John Calambokidis; CRC	Bmu07Ca015	Q	X
USA	California	8-Sep-07	34.12N	120.03W	John Calambokidis; CRC	Bmu07Ca016	Bmu06Ca016	female
USA	Costa Rica	1/13/08	9.99N	97.07W	Bruce Mate; MMI	Bmus08CA001	R	male
USA	Costa Rica	1/14/08	8.77N	96.61W	Bruce Mate; MMI	Bmus08Ca002	Bmu08Ca002*	male
USA	California	23-Jul-08	34.18N	119.99W	Bruce Mate; MMI	Bmus08CA003	Bmu06Ca005	female
USA	California	25-Jul-08	34.12N	120.07W	Bruce Mate; MMI	Bmus08Ca004	R	male
USA	California	27-Jul-08	34.11N	120.14W	Bruce Mate; MMI	Bmus08Ca005	D	male
USA	California	27-Jul-08	34.13N	120.04W	Bruce Mate; MMI	Bmus08Ca006	R	female
USA	California	27-Jul-08	34.12N	120.04W	Bruce Mate; MMI	Bmus08Ca007	r	X
USA	California	28-Jul-08	34.13N	120.02W	Bruce Mate; MMI	Bmus08Ca008	Bmu06Ca002	male
NA	N. Atlantic	29-Jul-99	42.83N	62.19W	Carole Conway	BmuNA201	BmuNA201	X
NA	N. Atlantic	26-Aug-99	44.23N	62.34W	Carole Conway	BmuNA202	BmuNA202	X
NA	N. Atlantic	16-Aug-99	43.51N	63.00W	Carole Conway	BmuNA203	BmuNA203	X

