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INTERNATIONAL WHALING COMMISSION

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**Abstract**
The 20th century commercial whaling industry reduced great whale populations to low abundances in the Southern Hemisphere. The Antarctic blue whale population was probably reduced to less than 1% of its original abundance. The effect of this exploitation on this population has been explored through comparisons of historical and contemporary genetic diversity from bones collected on South Georgia Island (Sremba et al. 2012, Sremba et al. 2015). Here we report on progress with a project funded by the Southern Ocean Research Partnership (SORP), to extend our previous sampling with the species identification of additional whale bones from the Antarctic Peninsula and South Georgia collected in February 2016. Using conventional PCR and mtDNA control region sequencing, we identified 37 humpback whales, 14 blue whales, 16 fin whales, 1 minke whale and 1 sperm whale. The 14 blue whale bones will be used for Next Generation Sequencing to further characterize pre-whaling genomic diversity and a subsample of whale bone for stable isotope analysis to characterize stability or heterogeneity of foraging ecology.

**Background**
Over 2 million whales were killed by the commercial whaling industry during the 20th century (Rocha et al. 2014). The Antarctic blue whale was one of the most heavily exploited species, where over 360,000 were killed during the 20th century (Branch et al. 2008). The population is estimated to have been reduced to less than 1% of its original abundance in ~70 years (Branch 2008).

This exploitation began at commercial whaling stations established on the South Atlantic island of South Georgia and near the Antarctic Peninsula. Throughout the 20th century, over 175,000 whales were killed at South Georgia between 1904-1961 (Headland 1984) and 84,741 whales were killed in the South Shetland islands between 1904-1931 (Hart 2006). In total, 69,787 blue whales were killed in South Georgia and the South Shetland islands between 1904 and 1931 (Hart 2006). Many whaling stations operated in this region during the first half of the 20th century. In 1931, the pelagic factory ship was introduced, resulting in the abandonment of Norwegian whaling stations in the Antarctic (Hart 2006).

During the early years of hunting in the Antarctic, whales killed by the commercial whaling industry were processed close to shore. The remains of these whales remain along the shores and abandoned whaling stations in this region. Previous research has successfully extracted DNA and identified to species whale bones from South Georgia island (Sremba et al. 2015). Of the 281 whale bones, 231 were identified to species. Among these bones, we identified 158 humpback whales, 18 blue whales, 51 fin whales, 1 southern right whale, 2 sei whales and 1 elephant seal. As these whales were likely killed during the first decade of hunting, we can assume that the bones have preserved the genetic diversity of the pre-exploitation populations.

From these data, our previous research suggests that, although the Antarctic blue whale population may have retained considerable mitochondrial (mtDNA) diversity as a species, there has been a significant loss

of regional diversity (Sremba et al. *in prep*). To further explore this hypothesis, we have adopted next-generation sequencing methods to assemble the complete miotgenome of the 18 historical blue whales samples and compare these with mitogenome of contemporary Antarctic blue whale population in collaboration with Aimee Lang at Southwest Fisheries Science Center (SWFSC). We have also collected additional whale bones from South Georgia and the Antarctic Peninsula to increase the historical population size in this comparison. Here, we present results of genetic species identification of 94 whale bones collected from South Georgia island and the Antarctic Peninsula in February-March 2016.

**Methods**

*Sample Collection.* Whale bone samples were collected from the Antarctic Peninsula and South Georgia by coauthors RP and PW. Larger bone fragments were targeted for collection and subsamples of bone were taken using a small saw. Caution was taken to minimize visual impact of the bones. Whale bones were targeted that appeared to be large baleen whale skulls or vertebrae to avoid multiple bone collection of the same individual.

*DNA extraction.* Whale bones transferred to OSU and drilled in a facility that has not been exposed to modern cetacean DNA. Drilling was conducted at the slowest speed possible and drill bits were sterilized with bleached between samples. DNA was extracted using a modified silica column based procedure (Qiagen DNeasy). Inhibitors were removed from DNA extraction using a Zymo clean up column (Zymo).

*mitochondrial DNA sequencing and species identification*. The mtDNA control region was amplified using a standard sequencing protocol described in Sremba et al. 2015. Species were identified from the mtDNA control region sequence using the web-based program DNA Surveillance (http://www.cebl.auckland.ac.nz:9000/) (Ross et al. 2003). The submitted mtDNA control region sequence is identified to species using a phylogenetic approach involving a curated database of all recognized cetacean species (Witness for the Whale, version 4.3).

**Results**

A total of 94 whale bones were collected from historic whaling stations on the Antarctic Peninsula and South Georgia in February and March 2016. Thirty-seven bones were collected from eight locations on the Antarctic Peninsula (Figure 1; Table 1) and 57 whale bones were collected from four locations on South Georgia island (Figure 2; Table 1). Photographs were taken of all whale bones that were sampled (Figure 3). Whale bones were collected from various locations on the Antarctic Peninsula and South Georgia February-March 2016 (Figure 1 and 2; Table 1).

Using methods for ancient DNA extraction and mtDNA control region sequencing described in Sremba et al. (2015), we have identified 69 of the 94 whale bones to species (74%). Among the collection, we identified 37 humpback whales, 14 blue whales, 16 fin whales, 1 minke whale and 1 sperm whale. Twenty-eight of the 37 (76%) whale bones collected from the Antarctic Peninsula and 41 of the 57 (72%) whale bones collected from South Georgia were identified to species. As seen in Table 2, whale bones were successfully identified to species from most regions. Humpback whales were the predominant species identified within the bone collection, similar to reported by Sremba et al. 2015. The majority of the humpback whales were identified among bones collected from Grytviken, South Georgia (Table 2). The highest reported number of blue whales (6) was identified among the whale bone collected from Mikkelsen Harbor on the Antarctic Peninsula.

**Discussion**

The species identification of ~74% of the whale bones collected from South Georgia and the Antarctic Peninsula indicate the extensive potential of this 'molecular' archive. These 12 locations are just a small representation of the potential of this research to archive pre-exploitation populations of South Atlantic whales. Between 1905 and 1931, 8 whaling harbours operated on South Georgia, 36 whaling harbours in

Graham's Land and the Antarctic Peninsula and 17 whaling harbours in the South Shetland Islands (Hart 2006). This study has indicated potential areas where a higher number of blue whale bones may be preserved, e.g. Mikkelson Harbour, for future whale bone collection.

For the next phase of this project, we are preparing the 14 blue whale bones for NGS shotgun sequencing. We will assemble mitogenomes from the shotgun sequencing reads. The historical Antarctic blue whale mitogenome diversity will be compared to the contemporary Antarctic blue whale population in collaboration with Aimee Lang at Southwest Fisheries Science Center.

In the next phase of this project, we will also provide sub-samples of whale bone for stable isotope analysis at British Antarctic Survey. Analysis of stable isotopes of Antarctic blue whales foraging in the vicinity of South Georgia will enable us to address two questions regarding regional population structure: (1) is there any heterogeneity in feeding area preferences within the South Atlantic blue whale population (for example, whales caught in South Georgia and those caught in the South Orkney Islands), and (2) if detected, are feeding area preferences driven by long-term, inherited fidelity to foraging areas? For this, we will use principal component analysis of mtDNA lineages and C/N values to determine whether long-term maternal fidelity to sites is a driver explaining foraging ecology differences within the blue whale population.

*Acknowledgements.* The 37 whale bone samples were collected from the Antarctic Peninsula under Antarctic Conservation Act permit ACA 2016-006 permit holder Angela Sremba and under the National Marine Fisheries Science permit no. 17030-02 permit holder C. Scott Baker and 57 whale bone samples were collected from South Georgia under South Georgia and South Sandwich Islands Regulated Activity Permit 2016/008. We thank the crew and officers of the National Geographic Explorer for their logistical support in obtaining these samples. Funding for this research is from an IWC-SORP research award to ALS, JAJ and CSB.

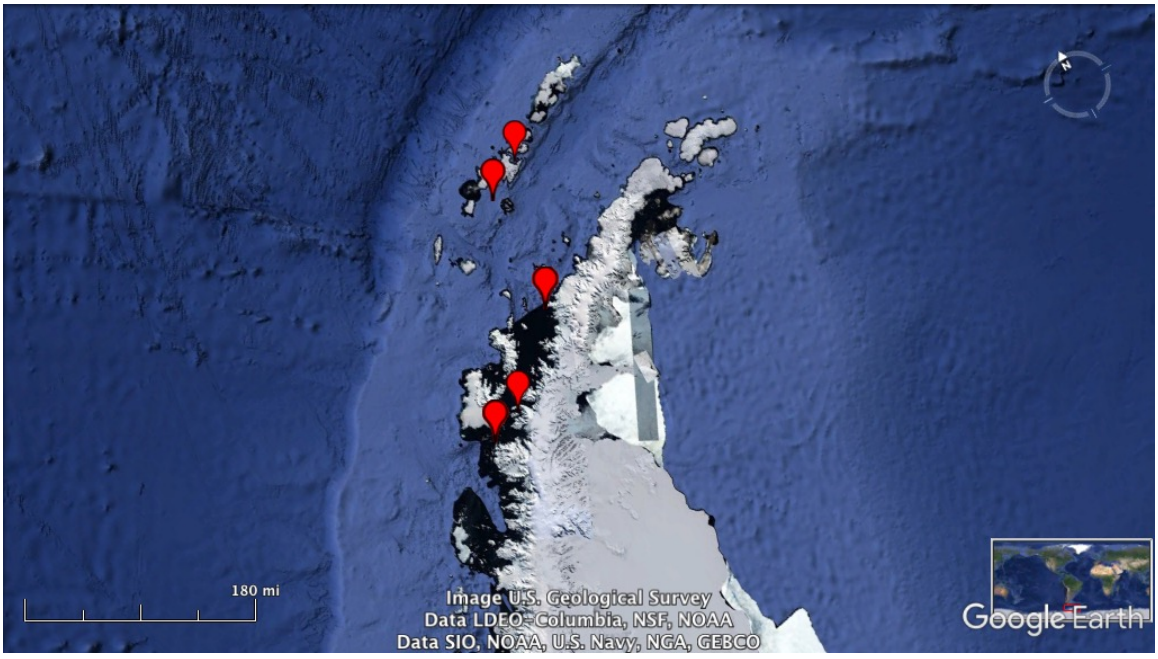


Figure 1. Whale bone sample locations from the Antarctic Peninsula.

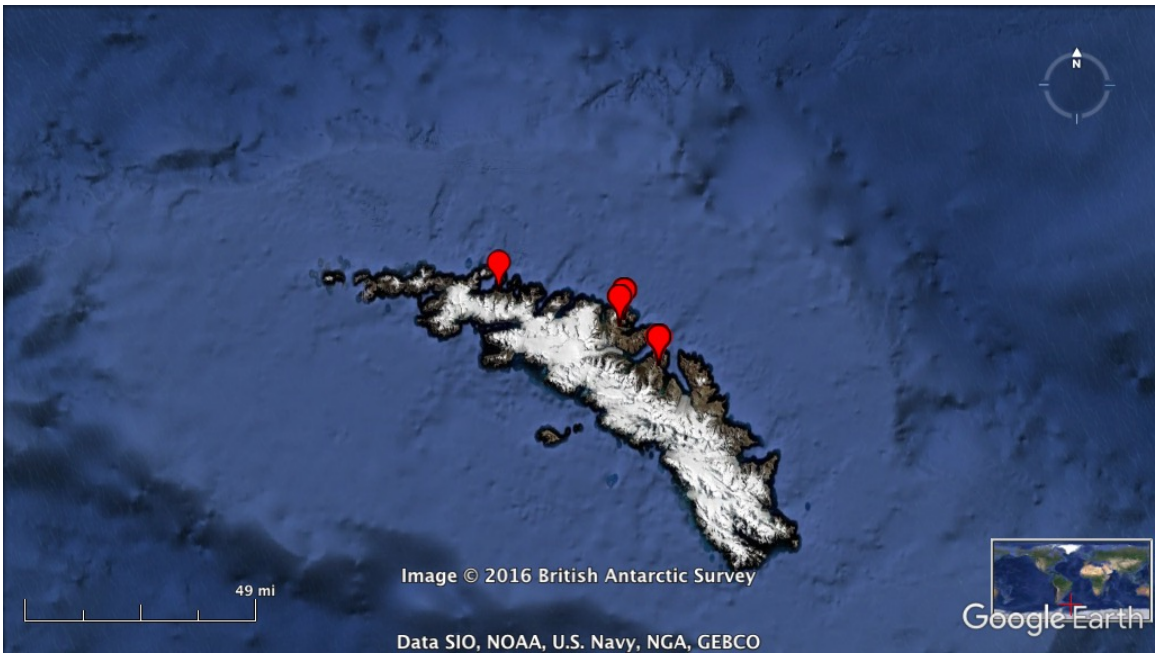


Figure 2. Whale bone sample locations from South Georgia.

Figure 3. Example of whale bone sampled for genetic species identification.

Table 1. Collection location of 94 whale bone samples from whaling stations in the Antarctic Peninsula (AP) and South Georgia (SG) in February 2016.

| **Location** | Date | Bones collected |
|---|---|---|
| Walker Bay, Livingstone Island, AP | 9-Feb-16 | 3 |
| Mikkelsen Harbour, AP | 11-Feb-16 | 20 |
| Bill's Island, Port Lockroy, AP | 14-Feb-16 | 1 |
| Goudier Island, Port Lockroy, AP | 14-Feb-16 | 2 |
| Jougla Point, AP | 14-Feb-16 | 8 |
| Port Lockroy Seabed, AP | 14-Feb-16 | 1 |
| Barrientos Island, AP | 19-Feb-16 | 1 |
| Cuverville Island, AP | 20-Feb-16 | 1 |
| **AP subtotal** | | **37** |
| Stromness, SG | 29-Feb-16 | 4 |
| Grytviken, SG | 29-Feb-16 | 42 |
| Leith, SG | 29-Feb-16 | 10 |
| Salisbury Plain, SG | 1-Mar-16 | 1 |
| **SG subtotal** | | **57** |
| **Total** | | **94** |

Table 2. Species identification of 94 whale bones collected from various locations on the Antarctic Peninsula and South Georgia February-March 2016. Species identified include *Balaenoptera musculus* (Bmu), *Balaenoptera physalus* (Bph), *Balaenoptera bonaerensis* (Bbo), *Megaptera novaeangliae* (Mno) and *Physeter macrocephalus* (Pma).

| | **Bmu** | **Bph** | **Bbo** | **Mno** | **Pma** | **Total** |
|---|---|---|---|---|---|---|
| Walker Bay, Livingstone Island, AP | | | 1 | 2 | | 3 |
| Mikkelsen Harbour, AP | 6 | 4 | | 4 | | 14 |
| Bill's Island, Port Lockroy, AP | | 1 | | | | 1 |
| Goudier Island, Port Lockroy, AP | | | | | | 0 |
| Jougla Point, AP | | 6 | | 2 | | 8 |
| Port Lockroy Seabed, AP | 1 | | | | | 1 |
| Barrientos Island, AP | | | | | | 0 |
| Cuverville Island, AP | | | | 1 | | 1 |
| Grytviken, SG | 3 | 5 | | 24 | | 32 |
| Leith Harbor, SG | 3 | | | 2 | 1 | 6 |
| Stromness, SG | | | | 2 | | 2 |
| Salisbury Plain, SG | 1 | | | | | 1 |
| **Total** | 14 | 16 | 1 | 37 | 1 | 69 |

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