**International Whaling Commission**

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**RESEARCH PROPOSAL**

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

**SINGLE NUCLEAR POLYMORPHISMS (SNPS) AND DIVERSITY IN BONES FROM SOUTH GEORGIA ISLAND AND CONTEMPORARY ANTARCTIC BLUE WHALES: a request for access to IDCR-SOWER biopsy samples**

**2. DETAILS OF NAMED INVESTIGATORS** (principal investigator first)

**Primary Investigators:**

| | |
|---|---|
| (i) *Name* | CHARLES SCOTT BAKER |
| *Address* | *Marine Mammal Institute*<br>*Oregon State University*<br>*Newport, Oregon 97365* |
| *Nationality* | *USA* |
| *Domicile* | *USA* |
| (ii) *Name* | ANGIE SREMBA |
| *Address* | *Marine Mammal Institute*<br>*Oregon State University*<br>*Newport, Oregon 97365* |
| *Nationality* | *USA* |
| *Domicile* | *USA* |

**Collaborating Investigators:**

| | |
|---|---|
| (ii) *Name* | ANOTHONY R. MARTIN |
| *Address* | *University of Dundee*<br>*Nethergate, Dundee DD1 4 HN*<br>*Scotland* |
| *Nationality* | *UK* |
| *Domicile* | *UK* |

**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**: The Antarctic blue whale population was reduced to less than 1% of its original abundance, to an estimated 400 individuals, due to intense exploitation by the commercial whaling industry. The impact of this bottleneck on genetic diversity remains unknown. The contemporary Antarctic blue whale has been described by a relatively high mitochondrial DNA (mtDNA) haplotype diversity, and may have escaped a greater loss of genetic diversity due to its long life span, overlapping generations and the brief period of the bottleneck. The impact of 20$^{th}$ century commercial whaling on genetic diversity can be explored through a comparison of historic and contemporary genetic diversity. Here, we request continued access to biopsy samples of blue whales collected on Antarctic feeding grounds during IDCR and SOWER cruises and funding to assist in marker development for bone samples collected from whaling stations from South Georgia. This will require transfer of additional DNA from SWFSC as all previous DNA has been expended in completing published project (Sremba et al. 2012). We propose to develop and target single nuclear polymorphisms (SNPs) within the contemporary

Antarctic blue whale. This will allow us to target SNPs in the contemporary Antarctic blue whale population and historic South Georgia Antarctic blue whale population to gauge a loss of genetic diversity.

**(i) BACKGROUND:** Commercial whaling during the 20th century reduced the Antarctic blue whale (*Balaenoptera musculus intermedia*) population to an estimated 400 individuals in 1972, which is less than 1% of its original abundance of 202,000-322,000 (Branch 2008). The population is believed to be recovering with the most recent abundance estimated at 2,280 individuals (95% CI 1,160-4,500; Branch, 2008). Population bottlenecks can leave signatures in contemporary genetic diversity. However, the Antarctic blue whale population has a relatively high haplotype diversity (Sremba et al. 2012), potentially due to the longevity and long life spans of Antarctic blue whales relative to the brief period of the population bottleneck. With a loss of over 99% of the population it is plausible that this loss impacted the genetic diversity of the population. To explore this potential loss, we propose to compare nuclear genetic diversity of the historical and contemporary population.

The first commercial whaling station in the Southern Ocean was established at the South Atlantic island of South Georgia. Throughout the 61 years commercial whaling industry, over 175,000 whales were killed, including over 40,000 blue whales (Headland 1984). Species identification of whale bones from South Georgia have identified 18 blue whales among 289 whale bones (Sremba et al. 2010). Initial comparisons of mitochondrial DNA haplotypes between the South Georgia and contemporary Antarctic blue whale population identified 11 unshared mtDNA haplotypes, or maternal lineages, out of the 16 identified within the historic population. To further assess the impact of commercial whaling on the genetic diversity of the Antarctic blue whale population, we propose to design and target a nuclear marker, SNPs, within the Antarctic blue whale, as other commonly used markers, microsatellites, can be problematic when amplified from samples of variable or poor quality, such as 'ancient' DNA (Taberlet et al. 1996).

**(ii) SPECIFIC OBJECTIVES**:

1) To develop single nuclear polymorphisms (SNPs) assays within the Antarctic blue whale population based on nearly 4,000 contigs available from RAD tagging of a North Pacific blue whale. From this we have identified a minimum of 307 likely SNPs.
2) To genotype SNPs in the contemporary Antarctic blue whale population (n=218) biopsy samples collected on IDCR/SOWER cruises from 1990-2009 as well as the historic South Georgia blue whale population (n=18).
3) To compare the historic and contemporary Antarctic blue whale SNP frequencies to assess the impact of the commercial whaling industry on nuclear DNA within the Antarctic blue whale.

**(iii) METHODS:** We will screen a subset of contemporary Antarctic blue whales for SNPs identified in a North Pacific blue whale using RAD tagging data following methods of Baird et al. 2008. If the observed diversity is low, we will screen for SNPs in the Antarctic blue whale using data from the 4,000 contigs available from the North Pacific blue whale and next generation sequencing, i.e. Illumina HiSeq 2000 or GS 454 Jr. SNPs will be and targeted in the historic South Georgia population and the contemporary population of Antarctic blue whales using next generation sequencing or methods similar to Morin and McCarthy (2007).

**(iv) PROGRAMME:** The analysis of the samples will be in the Cetacean Conservation and Genetics Laboratory (CCGL) 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 sequencing (ABI 3730*xl* and GS 454 Jr.) and bioinformatics facilities.

**(v) RESOURCES:** We request continued access to biopsy samples of blue whales collected on Antarctic feeding grounds during IDCR and SOWER cruises between 1990 and 2009. This will require transfer of additional DNA from SWFSC as all previous DNA has been expended in completing published project (Sremba et al. 2012). For comparison to historic diversity we have access to a collection of whale bones from early 20th century whaling stations on the sub-Atlantic island of South Georgia. These samples will extend the knowledge base of genetic variation in Antarctic blue whales and explore the impact of the 20th century commercial whaling industry on the genetic diversity of the Antarctic blue whale.

**(vi) WIDER JUSTIFICATION:** Over 350,000 Antarctic blue whales were killed by the commercial whaling industry during the 20th century. The effects of this drastic reduction in population abundance on the genetic diversity of the population remains unknown. This research will utilize a previously unexplored resource to assess the impact of the 20th century commercial whaling industry on the genetic diversity of the Antarctic blue whale. Although the contemporary population has been described by a relatively high haplotype diversity and appear to be increasing, the population still remains at less than 1% of its original abundance. A comparison of

nuclear diversity (SNPs) between a historic and contemporary Antarctic blue whale population will provide insight as to genetic diversity lost due to 20th century commercial whaling.

**Table 1.** Summary of blue whale biopsy samples from IWC IDCR/SOWER cruises 1990-2009.

| Area | I | II | III | IV | V | VI | Total |
|---|---|---|---|---|---|---|---|
| 1990 | 1 | | | | | | 1 |
| 1991 | | | | | | 0 | 0 |
| 1992 | | | | | 0 | | 0 |
| 1993 | | | 2 | | | | 2 |
| 1994 | 0 | | | | | | 0 |
| 1995 | | | 0 | 2 | | | 2 |
| 1996 | | | | | | 0 | 0 |
| 1997 | | 4 | | | | | 4 |
| 1998 | | 6 | | | | | 6 |
| 1999 | | | | 10 | | | 10 |
| 2000 | 0 | | | | | | 0 |
| 2001 | 4 | | | | | 18 | 22 |
| 2002 | | | | | 16 | | 16 |
| 2003 | | | | | 5 | | 5 |
| 2004 | | | | | 14 | | 14 |
| 2005 | | | 4 | 1 | | | 5 |
| 2006 | | | 36 | | | | 36 |
| 2007 | | | 85 | | | | 85 |
| 2008 | | | | 0 | | | 0 |
| 2009 | | | | 9 | | | 9 |
| Total n | 5 | 11 | 127 | 22 | 35 | 18 | 218 |

**REFERENCES**


Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A., & Johnson, E. A. (2008). Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLos One, 3*(10), e3376. doi: 10.1371/journal.pone.0003376

Branch, T. A. (2008). Current status of Antarctic blue whales based on Bayesian modeling. *Report (SC/60/SH7) to the Scientific Committee of the International Whaling Commission, available on request from the Secretariat International Whaling Commission, The Red House, 135 Station Road, Impington, Cambridge, Cambridgeshire CB24 9NP, UK*

Headland, R. (1984). *The Island of South Georgia*. New York: Cambridge University Press.

Morin, P. A., & McCarthy, M. (2007). Highly accurate SNP genotyping from historical and low-quality samples. *Molecular Ecology Notes, 7*(6), 937-946. doi: 10.1111/j.1471-8286.2007.01804.x

Sremba, A., Martin, A. R., & Baker, C. S. (2010). Genetic Approach to Species Identification of Whale Bones from South Georgia Island Whaling Stations. *Report to the International Whaling Commission SC/62/SH19.*

Sremba, A. L., Hancock-Hanser, B., Branch, T. A., LeDuc, R. G., & Baker, C. S. (2012). Circumpolar diversity and geographic differentiation of mtDNA in the critically endangered Antarctic blue whale (*Balaenoptera musculus intermedia*). *PLos One, 7*(3), e32579. doi:32510.31371/journal.pone.0032579.

Taberlet, P., Griffin, S., Boossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L. P., & Bouvet, J. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research, 24*(16), 3189-3194.


**4. CURRICULUM VITAE OF NAMED INVESTIGATORS** (1 page per investigator)
For PI only, others available on request


**NAME**: Charles Scott Baker

**CURRENT POSITION**: Associate Director and Professor, Marine Mammal Program, Oregon State University, and Professor, School of Biological Sciences, University of Auckland

**DEPARTMENT**: Department of Fish and Wildlife, Oregon State University


**EDUCATIONAL QUALIFICATIONS: [Tertiary only]**

University of Hawaii, Manoa (1978 - 1985)
Degree: Ph.D. in Zoology
Concentration: Animal behaviour and ecology.
Dissertation Title: The population structure and social organization of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific.

**DISTINCTIONS AND HONOURS:**
Royal Society of New Zealand, Science and Technology Medal (2001) for research in applied molecular systematics for conservation of whales and dolphins.

**APPOINTMENTS (2005 to present):**

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|---|---|
| 2006-present | Associate Director, Marine Mammal Institute, Professor, Department of Fisheries and Wildlife, Oregon State University |
| 2005-present | Professor (Personal Chair), Molecular Ecology and Evolution, School of Biological Sciences, University of Auckland |

**RESEARCH SPECIALTIES / CAREER:**
**Summary Statement:** My research interests are directed toward understanding the molecular mechanisms and demographic forces, including human exploitation, influencing the diversity and distribution of natural populations, particularly endangered and commercially valuable marine species. This interest includes a range of disciplines across the hierarchy of the biological sciences from molecular evolution to molecular systematics, taxonomy, population ecology and conservation biology.

**PROFESSIONAL SOCIETIES AND SERVICE:**
Member, Cetacean Specialist Group, IUCN- The World Conservation Union
Member, American Genetic Association
Member, Society for Marine Mammal Science
Member, New Zealand Royal Society

**JOURNAL EDITORIAL SERVICE:**
Editor in Chief (from 2007), *Journal of Heredity*.
Former Associate editor (tenure 2001 - 2004), *Marine Mammal Science*

**REFEREED JOURNAL ARTICLES (91 total, 33 as senior author)**

**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 through Graduate Resident Assistantship |
| 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*
SNP development
$2,000 for oligionucleotide primer development and amplification
+ $4,000 454 GS Jr. Sequencing ($2,000 per run)
+ $1,200 SNP assay development kit (i.e. Amplifluor kit, Taqman kit)
+ 20% Oregon State University overhead
= $8,640 total

**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)

Lab work of participating researchers have been funded primarily from the following sources:
US $9,800 Mamie Markham Award, Hatfield Marine Science Center, Oregon State University. May 2012.

## 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 will be completed at the Conservation and Genetics Laboratory (CCGL), Marine Mammal Institute, Oregon State University.

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

No permits for collection of samples are required. The CCGL is permitted to hold cetacean tissue and DNA under a Letter of authority from the National Marine Mammal Laboratory of the U.S. National Marine Fisheries Service.

## 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 24 months after samples are received. A report on SNP development will be presented at IWC 2013.