International Whaling Commission

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RESEARCH PROPOSAL

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

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:

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Associate Investigators:

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iv) *Name* JUSTIN COOKE
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v) *Name* TREVOR BRANCH
Address *School of Aquatic and Fishery Sciences*
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University of Washington
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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 in the reconstruction of population trajectories, outlined as a goal in the Comprehensive Assessment of the IWC. Recent developments of population dynamic models have used the number of surviving mtDNA haplotypes (maternal lineages) to help set a lower bound on this value, referred to as N_{min}. To improve estimates of the number of surviving mtDNA haplotypes in the Antarctic blue whale, we are requesting access to biopsy samples of blue whales collected on Antarctic feeding grounds during IDCR and SOWER cruises from 1989 to 2009. The improved estimate of mtDNA haplotypes will be used in efforts for the improved modelling of N_{min} and the population trajectory of blue whales.

(i) BACKGROUND: Antarctic blue whales (*Balaenoptera musculus intermedia*) were reduced to less than 1% of their original abundance of 202,000-322,000 by commercial whaling between 1928-1972 (Branch et al. 2004). Bayesian models have indicated a rate of increase of 7.3% for Antarctic blue whale (Branch et al. 2004); however, the 1997 abundance estimate is still at only 0.9% of the original population size with an abundance estimate of 2,280 (95% confidence interval 1160-4500) (Branch 2007). The blue whales found in this region are assumed to be the Antarctic (or 'true') blue whale subspecies (*Balaenoptera musculus intermedia*), based on genetics (LeDuc et al. 2007), acoustics (McDonald et al. 2006) and biological characteristics (Branch and Mikhalev 2008, Branch et al. 2007a, 2007b, 2009). This model incorporated an estimate of N_{min} = 214 for the Antarctic blue whales based on the mitochondrial control region of 26 haplotypes from 47 Antarctic samples collected between 1993-2002 (sequences by LeDuc et al. 2007).

(ii) SPECIFIC OBJECTIVE:

1) To improve the estimation of N_{min} for the Antarctic blue whale from mitochondrial haplotype diversity (Jackson et al. 2007; Branch and Jackson 2008) through the addition of 185 samples collected from the 1989-2009 IWCR-SOWR cruises.
2) To identify replicate samples and evaluate subspecies identity of the 185 samples using microsatellite genotyping, following LeDuc et al. (2007).
3) To incorporate results into a revised Bayesian model of Antarctic blue whales following the methods of Branch (2008).

(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 Olavarría *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). We will also investigate the potential for using whole mitochondrial DNA sequencing (mitogenomes), to resolve further unique lineages defined by variable sites outside the standard control region fragment.

(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 4700*xl*) and bioinformatics facilities.

The laboratory work and analysis of data will be completed 12 months after receiving the IDCR/SOWER samples. Associate investigators, Jackson, Branch, and Cooke, have agreed to incorporate the empirical results of the proposed analysis of mtDNA diversity into further modelling 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 modelling 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 (Jackson et al. 2007). 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 those observed for the real population. Branch and Jackson (2008) 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). The minimum population size of a given population is an essential component for constructing population trajectories (e.g. Branch 2008). 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 minimum 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, 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

Baker, C. S. and Clapham, P. J. 2004. Modelling the past and future of whales and whaling. Trends . Evol. 19(7): 365-371.

Baker, C. S. Slade, R. W., Bannister, J. L., Abernmethy, M. T., Weinrich, M. T., Lien, J., Urban, J., Corkeron, P., Calambokidis, J., Vasquex, O., and Palumbi, S. R. 1994. Hierarchial structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. Molecular Ecology. 3:313-327.

Branch, T.A. 2007. Abundance of Antarctic blue whales south of 60°S from three complete circumpolar sets of surveys. J. Cetacean Res. Manage. 9(3): 87-96.

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.Branch, T. A., Abubaker, E. M. N., Mkango, S., and Butterworth, D. S. 2007b. Separating southern blue whale subspecies based on length frequencies of sexually mature females. Mar. Mammal Sci. 23:803-833.

Branch, T. A. and Jackson, J. A. Minimum bottleneck abundance of Antarctic blue whales based on current mtDNA diversity. Report SC/60/SH10 to the Scientific Committee of the International Whaling Commission.

Branch, T. A., Matsuoka, K., and Miyashita, T. 2004. Evidence for increase in Antarctic blue whales based on Bayesian modelling. Mar. Mammal Sci. 20(4) 726-754.

Branch, T. A., and Mikhalev, Y.A. 2008. Regional differences in length at sexual maturity for female blue whales based on recovered Soviet whaling data. Mar. Mammal Sci. 24(3):690-703.

Branch, T.A., Mikhalev, Y.A., and Kato, H.. 2009. Separating pygmy and Antarctic blue whales using long-forgotten ovarian data. Mar. Mammal Sci. doi: 10.1111/j.1748-7692.2009.00292.x

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., Hucke-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.

Jackson, J. A., Patenaude N. J., Carroll E. L., and Baker, C. S. 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.

McDonald, M. A., Hildebrand, J.A. and Mesnick, S.L. 2006. Biogeographic characterization of blue whale song worldwide: using song to identify populations. J. Cetacean Res. Manage. 8(1):55-65.

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)
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):

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*.
Associate editor, *Canadian Journal of Zoology*.
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
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 1009.

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. Oregon State University is a recognised CITES institution allowing exchange of samples with other institutions.

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.