

# SC/67B/SDDNA/05 Rev1

---

## Historical and contemporary population structure and the impact of whaling on sei whales

Maria Jose Perez-Alvarez, Francisca Rodriguez,  
Sebastian Kraft, Carlos Olavarria, Camilo Naretto,  
Elie Poulin



INTERNATIONAL  
WHALING COMMISSION

# Historical and contemporary population structure and the impact of whaling on sei whales

María José Pérez-Álvarez<sup>1,2,3</sup>, Francisca Rodríguez<sup>2</sup>, Sebastián Kraft<sup>2</sup>, Carlos Olavarría<sup>3,4</sup>, Camilo Naretto<sup>2</sup>, Elie Poulin<sup>2</sup>

<sup>1</sup> Escuela de Medicina Veterinaria, Facultad de Ciencias, Universidad Mayor, Santiago, Chile

<sup>2</sup> Instituto de Ecología y Biodiversidad (IEB), Facultad de Ciencias, Universidad de Chile, Santiago, Chile

<sup>3</sup> Centro de Investigación EUTROPIA, Santiago, Chile

<sup>4</sup> Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile

*mjose.perez@gmail.com*

## ABSTRACT

The largest whale mass mortality event ever recorded took place recently in Southern Chile, where at least 340 dead rorquals were reported (Häussermann et al. 2017). In this event, all the positively identified whales were sei whales, *Balaenoptera borealis*. Population studies of sei whale are very limited in the Southern Hemisphere, even though this species was one of the major targets of commercial whaling worldwide, having an important exploitation history, particularly in Chilean waters. In this context, the whale mortality event has become the largest source of samples for the species in the Southern Hemisphere and a unique opportunity to address questions regarding genetic diversity and population structure of the species as well as dynamic of population demography considering historical and contemporary temporal frameworks. We are currently undertaken the analyses of skin and bone samples collected in the area (n = 160). Preliminary analyses of a set of those samples (n = 50) include that (1) the forensic technique we are using has proven successful in amplifying mt and nuDNA (sex), (2) 14 mtDNA control region sequences from skin samples (699 bp) revealed 13 haplotypes (h = 0.989,  $\pi$  = 0.94%), (3) 36 mtDNA control region sequences from bones (650 bp) revealed 23 haplotypes (h = 0.971,  $\pi$  = 0.99%), (4) all sequences corresponded to sei whales when compared with BLAST and DNA Surveillance data bases, and (5) sexing bones samples identified 22 males, 6 females and 8 unknown. Future work will include the collection of more samples from the same area, other areas along Chilean coast where sei whales are observed and other geographic regions in the Southern Hemisphere. We expect to include data from other Northern Hemisphere sei whales through collaboration.

## **SPECIES BACKGROUND**

The sei whale, *Balaenoptera borealis*, is one of the least known mysticete species and is considered by IUCN as an endangered species (Reilly et al. 2008). It has a mainly offshore distribution, occurring in disjoint populations in the North Atlantic, North Pacific and Southern Hemisphere (Rice 1998). This antitropical distribution, reported for many marine species including other species of cetaceans, has been generally considered the result of paleogeographic and paleoceanographic changes such as closure of major seaways (Steeman et al. 2009). In the Southern Hemisphere, sei whales occur mainly between the Subtropical Convergence (40°S) and the Antarctic Convergence (50°S), although some adults have been reported south of the Antarctic Convergence during the austral summer (Gambell, 1974). Tomilin (1957) proposed two subspecies, *B. b. borealis* in the Northern Hemisphere and *B. b. schlegelii* in the Southern Hemisphere. This classification has not been widely recognized, however, more recently it has been considered (Committee of Taxonomy 2017). Sei whale is thought to undertake seasonal migration between high latitude summer grounds and low latitude winter grounds as other whale species do; however, the locations of the winter and the summer grounds are still unknown. This migration pattern, in conjunction with other factors, such as geographical barriers existing between and within oceanic regions, seasonal differences between hemispheres, and water depth and temperature differences between areas, may result in spatial separation of sei whale populations (Wada & Numachi 1991, Kanda et al. 2006).

## **OUR GOALS AND TOOLS**

This study is part of a recently funded project (Pérez-Álvarez 2018) that aims at: (1) evaluate genetic differentiation and (2) assess divergence between inter-hemispheric populations of sei whales, evaluating the existence of two Evolutionary Significant Units (ESUs, North and South Pacific) and (3) to analyze the population demography of this endangered species over time considering the past commercial whaling.

Genetic population differentiation will be evaluated analyzing its population structure, contemporary gene flow and first generation migrants between Northern Hemisphere (NH) and Southern Hemisphere (SH) sei whale populations. Divergence between inter-hemispheric populations (ESUs) will be evaluated through phylogeographic and phylogenetic approaches. If applicable, divergence time between ESUs will be estimated and potential biogeographic scenarios involved in the current population structure of the species will be proposed and evaluated. The impact of the historic commercial whaling in the SH whale populations will be

evaluated through the identification of population bottleneck signal through the analysis of genetic diversity. The effective population size ( $N_e$ ) for SH sei whale populations will be estimated and compared with its current population size ( $N$ ) in SH and the  $N_e$  of NH populations.

We expect a marked genetic differentiation between NH and SH sei whale populations and consequently the existence of two ESUs. Additionally, we expect that SH population exhibit a genetic signal of population bottleneck, finding  $N_e > N$ . This project will contribute to the study of historical and contemporary population structure of sei whale and the impact of whaling, which should have implications in its systematics and conservation. Finally, it represents an important contribution to the biogeography of marine mammals in the SH, particularly for species with high dispersal capabilities in an environment with no obvious barriers.

Population genetic studies of the sei whale are very limited and as far as we know, there are no studies in Southern Hemisphere. In the Northern Hemisphere, a pioneer study of Wada and Numachi (1991), analyzed the stock structure of sei whales in the North Pacific based on three polymorphic allozyme loci, reporting no evidence of temporal and spatial genetic heterogeneity, suggesting the existence of a single stock in the area. Later, Kanda et al. (2006) confirmed the results of the previous study demonstrating no evidence of genetic population differences with microsatellite DNA analyses, indicating that a single stock of sei whales exists in the offshore open water of the North Pacific.

## **THE MME**

The recent whale mass mortality event (MME) occurred at Gulf of Penas (ca. 46°30'S), southern Chile, has become the largest source of information for the species in the Southern Hemisphere. Marine and aerial surveys documented the presence of at least 340 dead rorquals (baleen whales, Häussermann et al. 2017). Necropsies conducted *In situ* and taphonomic analyses indicated a synchronous death of the whales. Häussermann et al. (2017) suggested that the high number of dead whales, concentrated in a relatively small coastal area, would have resulted from oceanographic currents that carried them, once dead, from a much larger area, corresponding to feeding grounds of these rorquals. Although the cause of this mortality has not been definitively identified, the presence of paralytic shellfish toxin in mytilids from the area and in some whale carcasses suggest harmful toxic algal bloom (HABs) as the most probable cause. However, as recently reported by Starr et al. (2017), HABs should have caused a multi-specific mortality. In any case, all whale carcasses examined until now correspond to sei whales *Balaenoptera borealis*, however, the taxonomic identification of more whale carcasses is necessary in order to explore the multi-specificity of this MME.

## **WORK DONE SO FAR**

Between 2015 and to date, we have collected a total of 160 samples (100 bone samples, 60 skin samples) of dead whales from the MME at Gulf of Penas. To date we have been able to analyze 50 samples (36 bone samples, 14 skin samples). The skin samples were collected during two expeditions undertaken by the Chilean Navy during several weeks after the stranded whales were found. Most bone samples have been collected during private expeditions to the area afterwards (vessel Saoirse, Keri Lee Pashuk and Greg Landreth).

DNA from skin samples has been successfully extracted following the salt extraction methods (Aljanabi & Martínez 1997). DNA from bone samples has been extracted using a forensic protocol (Tebbutt et al. 2000), which has proven successful to amplify mtDNA and nuDNA (see below).

A fragment of the mitochondrial DNA control region (Dloop) has been amplified using the primers reported by Dalebout et al. (2005). The taxonomic identity (species level) of sequences have been assessed by comparing with the data bases (1) “Basic Local Alignment Search Tool (BLAST, [www.blast.ncbi.nlm.nih.gov](http://www.blast.ncbi.nlm.nih.gov)) and (2) DNA Surveillance (Ross et al. 2003, <http://www.dna-surveillance.auckland.ac.nz/>). The totality of the samples analyzed to date have been identified as sei whales.

The 14 sequences from skin samples (699 bp) revealed 13 unique sequences (haplotypes), defined by 20 polymorphic sites. Overall, mtDNA haplotype diversity (h) was 0.989 and nucleotide diversity ( $\pi$ ) was 0.94%. From bones samples it was possible to identify sex (males: 22, females 6, without identification: 8)

The 36 sequences from bones (650 bp) revealed 23 unique sequences (haplotypes), defined by 21 polymorphic sites. Overall, mtDNA haplotype diversity (h) was 0.971 and nucleotide diversity ( $\pi$ ) was 0.99%. From bones samples it was possible to identify sex (males: 22, females 6, without identification: 8).

## **FUTURE WORK**

We are exploring the collection of more samples from (1) Gulf of Penas area (more bone samples from the MME and live whales using a biopsy darting system (Krutzen et al. 2002)) and (2) other localities along the Chilean coast where the species has been reported around Cobquecura (36°S), Chiloé (42°S) and Punta Arenas (53°S). Additionally, we have contacted UK scientists in order to collaborate and obtain sei whales biopsy samples from the Falkland Islands, to get a better representation from Southern Hemisphere whales.

Further analyses will include nuclear introns sequences such as ACT, CAT, CHRNA1

(described in Jackson et al 2009) and microsatellites genetic markers (at least 15 loci used in Kanda et al. 2006).

To get access to Northern Hemisphere samples, mtDNA and/or microsatellite data we have contacted (1) our colleague Dr. Scott Baker, Professor & Associated Director of the Marine Mammal Institute of Oregon State University, (2) the Southwestern Fisheries Science Center, NOAA, La Jolla, California, USA and (3) the London Natural History Museum. We will be used published and public sequences available at Genbank.

## ACKNOWLEDGMENTS

We thank Mauricio Ulloa (SERNAPESCA) for assisting with the collection of skin samples during Chilean Navy surveys to Gulf of Penas, as well as to all the colleagues that participated on those expeditions. We thank Keri Lee Pashuk, Greg Landreth and all the crew from vessel Saoirse for kindly collecting bone samples during their expeditions to Gulf of Penas, and Vreni Häussermann for assisting with the coordination and collection of samples, as well as supporting the genetic analysis of the MME. Biopsy samples were collected under permit from Undersecretariat of Fisheries and Aquaculture, Ministry of Economy of Chile Permits 1502/2013, 1803/2015 and 2982/2015. This study was financially supported by CONICYT Postdoctoral FONDECYT Program 3140513, CONICYT Iniciación 11170182, FONDECYT 1140548, Projects ICM P05-002 and PFB 023 and CONICYT III Fortalecimiento Puente R16A10003.

## REFERENCES

- Aljanabi S, I Martinez.1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR based techniques. *Nucleic Acids Res.* 25: 4692–4693.
- Committee on Taxonomy. 2017. List of marine mammal species and subspecies. Society for Marine Mammalogy, [www.marinemammalscience.org](http://www.marinemammalscience.org).
- Dalebout, M., K Robertson, A Frantzis, D Engehaupt, A Mignucci, R Rosario & S Baker. 2005. Worldwide structure of mtDNA diversity among Cuvier's beaked whales (*Ziphius cavirostris*): implications for threatened populations. *Mol. Ecol.* 14, 3353–3371.
- Freeland, *Molecular Ecology*- 2005. John Wiley & Sons, Ltd. ISBN 978-0-470-09062-6 400 pp.
- Gambell, R.1974. A review of population assessments of Antarctic sei whales. Report of the International Whaling Commission, Special Issue 1, 44-49.
- Häussermann V, Gutstein CS, Beddington M, Cassis D, Olavarria C, Dale AC, Valenzuela-Toro AM, Perez-Alvarez MJ, Sepúlveda HH, McConnell KM, Horwitz FE, Försterra G. 2017. Largest baleen whale mass mortality during strong El Niño event is likely related to harmful toxic algal bloom. *PeerJ* 5: e3123. DOI: 10.7717/peerj.3123.
- Jackson J, C Baker, M Vant, D Steel, L Medrano Gonzalez L, S Palumbi. 2009 Big and slow: phylogenetic estimates of molecular evolution in baleen whales (Suborder Mysticeti). *Mol. Biol. Evol.* 26, 2427–2440.
- Kanda N., Goto M., Pastene, L.A. 2006. Genetic characteristics of western North Pacific sei whales, *Balaenoptera borealis*, as revealed by microsatellites. *Marine Biotechnology* 8: 86.

- Pérez-Álvarez M.J. 2018. Behind the scene of the world's largest whale mass mortality: historical and contemporary population structure and the impact of whaling on sei whales. Project CONICYT Iniciación 11170182.
- Reilly, S.B., Bannister, J.L., Best, P.B., Brown, M., Brownell Jr., R.L., Butterworth, D.S., Clapham, P.J., Cooke, J., Donovan, G.P., Urbán, J. & Zerbini, A.N. 2008. *Balaenoptera borealis*. The IUCN Red List of Threatened Species 2008: e.T2475A9445100.
- Rice D. 1998 Marine mammals of the world:systematics and distribution. Mar. Mamm. Sci. 4,67-78.
- Ross, H. A., G. M. Lento, M. L. Dalebout, M. Goode, G. Ewing, P. McLaren, A. G. Rodrigo, S. Lavery, and C. S. Baker. (2003) DNA Surveillance: Web-based molecular identification of whales, dolphins and porpoises. Journal of Heredity 94(2): 111-114.
- Steeman M, M Hebsgaard, E Fordyce, S Ho, D Rabosky, R Nielsen, C Rahbek, H Glenner, M Sorensen, E Willerslev. 2009 Radiation of extant cetaceans driven by restructuring of the oceans. Syst Biol. 58:573-585.
- Tebbutt S, R Stewart & D Hill. 2000). Isolation and characterisation of DNA from whale bone. Journal of the Royal Society of New Zealand, 30(4), 365-371.
- Tomilin A. 1957. Mammals of the U.S.S.R and adjacent countries. IX. Cetacea. Nauk SSSR, Moscu. 717 pp.
- Wada S., Numachi K. 1991. Allozyme analyses of genetic differentiation among the populations and species of the *Balaenoptera*. Rep Int Whal Commun (special issue 13): 125-154.