

Development Of Biosonar-Related Structures Of Dolphins (Odontoceti: Delphinoidea)

Guilherme Frainer

Universidade Federal do Rio Grande do Sul - UFRGS, Brazil

+555191297190

gui.frainer@gmail.com

SUMMARY

The threat of neonate franciscana dolphins due to increased bycatch may be caused among other things by immature development of biosonar-relevant structures. To test whether this thread could also be significant for other small odontocete species, my Ph.D. project is investigating the comparative anatomy of the complete ontogeny of the biosonar-relevant structures in Delphinidae, Phocoenidae and Pontoporiidae, with additional comparisons of the changes of the skull topography related to biosonar in some key species. This proposal aims to subsidize the morphometrical approach and an opportunity to perform dissections on Sousa specimens, for which forehead anatomy remains unknown.

Project Description

General description

The biosonar of odontocetes (Cetartiodactyla: Odontoceti) is a complex system for navigation and hunting. Lindberg and Pyenson (2007) proposed that the origin of echolocation in toothed whales dates from the early Oligocene (33.9 – 28.4 m.y.) and is related to nocturnal epipelagic feeding on cephalopods and demersal fishes who perform diel migration from deep to less deep waters. The authors pointed out some morphological innovations in fossil records, which could be related to the advent of echolocation in this group, such as the transition from heterodont to homodont forms (Fordyce 2003), the origin of an asymmetric skull (Cranford et al. 1996, Heyning 1989), and the enlargement of the brain (Huggenberger 2008, Marino et al. 2004, Marino et al. 2003, Oelschläger et al. 2009).

Since Norris et al. (1961) verified that a blindfolded dolphin can detect objects placed rostradorsal to the mouth, but not below the rostrum line, it has been accepted that the sound beam formation for echolocation is originated in the epicranial (nasal) complex (Madsen et al. 2013). These vibrations are reflected anteriorly by the skull, the nasal air sacs, and tough connective tissue into the melon (Madsen et al. 2013). The click sounds are reflected from objects in front of the animal, so that their echoes can be used for environment inspection. Accordingly, sound is transmitted via intramandibular fat bodies to the tympano-periotic complex and the middle ear, respectively (Cranford et al. 2010).

Investigations concerning the post-natal development of the biosonar-relevant structures in dolphins supported the understanding of life history aspects and allowed us to investigate the bycatch of some small dolphins from a functional anatomy perspective (Frainer et al. 2015). The comparative anatomy of the pre and post-natal morphology of the biosonar-relevant structures in different dolphin species are investigated for the first time in my Ph.D. project. Herein, this proposal aims to get funding to perform a sub-project of the latter, which mainly includes comparisons on the skull ontogeny of some key taxa of Delphinoidea using three dimensional morphometric geometrics (Galatius & Goodall 2016).

Objectives

The main objective of my Ph.D. project is to investigate the comparative anatomy of the complete ontogeny of the biosonar-relevant structures in Delphinidae, Phocoenidae and Pontoporiidae, with additional comparisons of the changes of the skull topography related to biosonar structures in some key dolphin species. For this I am analyzing the early formation of the biosonar-relevant structures in fetal, perinatal, neonate, and adult specimens of Delphinidae, Phocoenidae and Pontoporiidae. This proposal aims to subsidize a sub-project comparing the skull ontogeny related to the biosonar structures among nine dolphin species (listed below) using modern morphometrical methods. These odontocete taxa are characterized by different types of biosonar systems regarding click frequency and shape as well as hearing capabilities. Additionally, the proposed stay in South Africa will also facilitate an unprecedented opportunity to perform dissections on Sousa specimens (i.e. neonate and adult, including CT scans), for which biosonar-related anatomy remains largely unknown.

MATERIAL AND METHODS

A 3D geometric morphometrics approach will be used to compare the skull ontogenies of *Tursiops truncatus*, *Tursiops aduncus*, *Cephalorhynchus commersonii*, *Sousa plumbea*, *Lagenorhynchus albirostris*, *Phocoena phocoena*, *Delphinapterus leucas*, *Inia geoffrensis*, and *Pontoporia blainvillei*. Three-dimensional coordinates of already established landmarks for the skull (n=26) (Galatius & Goodall 2016) and mandible (n=12) (Barroso et al. 2012) will be registered on at least, 45 skulls and mandibles of each species (i.e. divided into 15 neonates, 15 young/sub-adult and 15 adults) using a Microscribe 3D digitizer. Further proper analyses will be performed (Galatius & Goodall 2016). Additional information regarding the skull and mandible shape development will be described following Frainer et al. (2015) and Miller (1923). Up to now, I performed macroscopical dissections on *Tursiops truncatus* (neonate, n=2) and *Phocoena phocoena* (perinatal, n=4; neonate, n=3; adult, n=1). For the proposed sub-project I will additionally perform dissections on *Sousa* specimens (neonate, n=1; adult, n=1). Existing CT scans from *T. truncatus* (neonate, n=1; adult, n=1) and *P. phocoena* (perinatal, n=1; neonate, n=1; adult, n=2) as well as data from *Pontoporia* (neonate, n=1; adult, n=1; see Frainer et al. (2015)) will be used to compare the CT scans that will be made from the *Sousa plumbea* specimens. The dissections will complete the CT scans so that the tissue type (e.g. connective tissue vs. fat tissue) can be determined. At the end of my complete Ph.D. project, all images obtained will serve to create 3D-models of the general topography of the biosonar-relevant structures on each developmental stage investigated using 3D-Slicer (<http://slicer.org/>).

PROJECT SIGNIFICANCE

Although the gross anatomy of sound generating and receiving structures in the toothed whale head had been described in some detail (Berta et al. 2014, Huggenberger et al. 2009, Cranford et al. 1996), developmental studies are scarce (Galatius et al. 2011, Galatius & Gol'din 2011, Haddad et al. 2012, Moran et al. 2011, Rauschmann et al. 2006, Lancaster et al. 2015) and there is, to our best knowledge, only one detailed descriptions on the postnatal development of head soft tissues (Frainer et al. 2015).

Biosonar-relevant head structures of toothed whales develop in advance of other structures and mature earlier in comparison to homologous structures in terrestrial mammals. This was interpreted as biosonar-relevant structures achieving the adult stage of development in juvenile toothed whales due a fully functional echolocation system being essential in the survival of young animals (Haddad et al. 2012, Rauschmann et al. 2006, Lancaster et al. 2015). However, Frainer et al. (2015) proposed that young individuals of the endangered franciscana dolphin might be more susceptible to entanglement in fishery gill nets than adults, because of an immature anatomy of echolocation-relevant structures. The improvement of echolocation skills is reflected in postnatal morphological changes of the biosonar structures (Frainer et al. 2015) and supported by diet shift (Riccialdelli et al. 2013, Rodríguez et al. 2002) during the first three years of life.

In comparison to the findings in franciscana dolphins described above (Frainer et al. 2015), here we focus on the postnatal development of sonar-relevant structures in three toothed whale species, which are also prone to bycatch in gill nets. Similar to the situation of franciscanas in South America, accidental bycatch during fishing activities is a major threat for harbor porpoises in the North and Baltic Seas (Benke et al. 2014, Osinga & Morick 2008) and young porpoises (calves and subadults) are more vulnerable to bycatch than adults (Siebert et al. 2006).

In the same way, the population of bottlenose dolphins (*Tursiops aduncus*) from the east coast of South Africa has been in decline, because of continued bycatch in shark nets placed along the KwaZulu-Natal coast (KZN) (Cockcroft 1990, Peddemors 1999). 30% of all captured calves presented fresh tooth marks indicative of epimeletic behavior (Cockcroft & Sauer 1990), suggesting that some mothers attempt to free their entangled calves and may subsequently also be captured in the process. Thus, lactating females might detect gill nets, but not fast enough to prevent the entanglement of calves. Humpback dolphins (*Sousa plumbea*) exhibit a similar scenario in the KZN waters, in which mainly males between 2.2 and 2.5 m in length and juveniles are threatened by accidental entanglement in shark nets (Plön et al. 2015, Atkins et al. 2013). The cause of why adolescent males exhibit higher bycatch rates is still a matter of speculation.

In line with the bycatch situation described above our working hypothesis is that the higher bycatch rates of neonates and sub-adults of some toothed whale species (*P. phocoena*, *T. aduncus*) may be caused by the immature state of the biosonar-relevant structures, similar to the situation described for franciscanas

(Frainer et al. 2015), while other species, such as *S. plumbea*, are less threatened, possibly due to smaller changes during postnatal ontogeny of echolocation-relevant structures. Accordingly, the proposed project has is relevant to species conservation as it addresses bycatch of young odontocetes.

Morphological changes throughout biosonar ontogeny in phylogenetically distinct groups within Delphinoidea may not only provide information on functional anatomy and life history (Cranford et al. 1996, Frainer et al. 2015, Huggenberger et al. 2016), but may also form the basis for future investigations on the evolution of these echolocating mammals. Although toothed whale evolution is inextricably related to the ability to echolocate, few studies have addressed the biosonar anatomy and its development, in the light of taxonomy and phylogenetics (Mead 1975, Heyning 1989). Kluge (1985) pointed out that observations on ontogeny could be useful to support homology determinations. Also, morphological comparisons between soft tissues and related osteological data among odontocetes are directly useful in comparison with extinct taxa (Heyning 1989).

Regarding human resources, this financial support (if successful) would provide the opportunity for the applicant to successfully complete this sub-project and solidify existing collaborations with Dr. Anders Galatius – Aarhus University, Denmark (which may provide great assistance on the morphometric approach) and Dr. Stephanie Plön - Nelson Mandela Metropolitan University, South Africa (responsible for the Tursiops and Sousa material in Durban, South Africa). A well-established collaboration with Dr. Stefan Huggenberger – University of Cologne, Germany – has been of great importance and is intensified by an ongoing one-year scholarship (i.e. from the Science Without Borders program – “Ciência sem Fronteiras”) granted to the applicant to study and live in Cologne, Germany, throughout 2016 (the latter program cannot include the travel expenses proposed here). This Ph.D. project is conducted by the applicant in the Post-Graduate Program on Animal Biology at Federal University of Rio Grande do Sul (Programa de Pós-graduação em Biologia Animal, UFRGS) under supervision of Dr. Ignacio B. Moreno.

PROJECT TIMELINE

Below follows the timeline for the entire Ph.D. project. The activities which could be covered by this proposal are marked with asterisks (*).

June – September, 2016

- Analysis of Delphinidae and Phocoenidae soft tissue anatomy, including Digital Imaging approach (7t-MRI, MRI and CT scans), histological series and macroscopical dissections.

* September, 2016

- Visit to the Natural History Museum of Denmark, Copenhagen – Denmark – to analyze skulls of *P. phocoena*, *L. albirostris* and *D. leucas*.

- Visit to the Section for Marine Mammal Research at Aarhus University, Roskilde - Denmark, to study and learn three-dimensional morphometric geometrics with Dr. Anders Galatius.

* October, 2016

- Visit to Port Elizabeth Museum, Port Elizabeth - South Africa – to analyze skulls of *S. plumbea* and *T. aduncus*.

- Visit to the KwaZulu-Natal Sharks Board Maritime Centre of Excellence (KZNSB) to perform dissections on three *Tursiops aduncus* calves and two *Sousa plumbea* females (i.e. neonate and adult), which are already stored frozen.

November, 2016

- Visit to the Natural Museum of Natural History (Naturalis), Leiden – Netherlands, to analyze skulls of *T. truncatus*.

December, 2016

- Finish the remaining dissections in Cologne, Germany, and return to Brazil.

January – November, 2017

- Visit to remaining marine mammal collections to analyze skulls of *I. geoffrensis* (Museu Paraense Emílio Goeldi, Belém – northern Brazil), *P. blainvillei* (Museu Oceanográfico da Universidade de Rio Grande, Rio Grande – southern Brazil) and *C. commersonii* (Museo Acatushún, Tierra del Fuego – Argentine).

December, 2016 – December, 2017

- Data processing and statistical analysis of morphometrical approach;

- Writing and submission of manuscripts and Ph.D. thesis.

PROJECT BUDGET

The budget presented below covers the expenses related to travel to Denmark and South Africa as described above, including accommodation. This sub-project does not have any other financial support.

Item Cost

Air ticket round trip from Cologne (GER) to Copenhagen (DEN) \$100
Subsistence/accommodation in Roskilde, Denmark \$250
Air ticket round trip from Cologne (GER) to Port Elizabeth (RSA) \$900
Subsistence/accommodation in Port Elizabeth and Durban, South Africa \$250

PERMITS

This work is done in compliance with any relevant marine mammal protection regulations. No ethical permitting needed.

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