Cetacean Ultrastructural Cochlear Imaging Through Scanning Electron Microscopy

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Abstract

The control of noise interaction between artificial and biological sources is essential to assess the development of sustainable marine technologies. Therefore, there is an emergent need to conduct morphological analysis of the acoustic pathways of marine organisms and detect possible structural alterations as a consequence of sound exposure. Cetaceans, because of their use of sounds in their daily activities, represent today the best bioindicators of the acoustic balance of the oceans. To access this information it is necessary to extract the ears of very fresh stranded individuals. One of the challenging steps after extraction and fixation of the samples is to decalcify the bone envelope to access the cochlea without damaging the soft tissues. A fast commercial decalcifier (RDO®) was used in 93 ears from 11 different odontocete species stranded in the Mediterranean Sea, the North Atlantic and the North Sea. Depending on the tympanic-periotic volume of the species, the decalcification time ranged from several hours to a few days, instead of taking few months with other decalcification agents, allowing a subsequently faster observation of the cochlear structures. Here we present images from cetacean cochlear ultrastructure through scanning electron microscopy. Following this protocol it is possible to obtain a fast diagnostic of possible acoustic trauma and relate the results to documented sound exposure. The output of this analysis will help calibrating theoretical results derived from deep-sea observatories.

Keywords

Decalcification, noise pollution, cochlea, ultrastructure, scanning electron microscopy

I. INTRODUCTION

While there is an increasing human pressure on the oceans, very little is known about the effect of underwater noise on marine organisms. Because of their vital dependence on acoustic information and their role in the food chain as top predator, the study of the effects of noise on cetaceans (Mammalia, Cetacea) has recently become ecologically essential [1]. Although some of these effects can be found in organs not directly related to the acoustic pathways [2], other lesions are expected to affect hearing, particularly the organ of Corti and its associated hair cells [3]. The problem relies in accessing fresh samples and in determining the relationship between a pathological change in the cochlea morphology and a possible sound exposure. Moreover, basic morphological and comparative descriptions of the cetacean ears are still lacking, probably because of the difficulty in obtaining suitable material, and a reliable protocol for analysis.

A detailed description of the cochlea morphology was presented for *Tursiops truncatus* [4, 5, 6] and studies of the basilar membrane and *osseus spiral laminae* in different odontocete species have been conducted to compare their hearing capabilities [7, 8, 9]. Despite of these early findings in a limited number of cetacean species, little data are available to comparatively describe inner ear structures. Stranding events may represent a unique opportunity to help building knowledge on cetacean hearing morphology and potential sensitivity when exposed to noise.

II. METHODOLOGY

A. Decalcification

Ninety three (93) ears from 11 different odontocete species that stranded in the Mediterranean Sea, Spanish North Atlantic and North Sea have been extracted. Specifically, the species processed were: *Phocoena phocoena* (n=48), *Stenella coeruleoalba* (n=13), *Stenella frontalis* (n=13), *Tursiops truncatus* (n=8), *Delphinus delphis* (n=2), *Kogia simus* (n=2), *Kogia breviceps* (n=2), *Globicephala macrorhynchus* (n=1), *Globicephala melas* (n=1), *Steno bredanensis* (n=2) and *Lagenodelphis hosei* (n=1).

After extraction, the samples were fixed with 10% buffered formaline or 2,5% glutaraldehyde and used subsequently to precisely determine the decalcification time with different concentrations of RDO®. RDO® is a rapid decalcifier based on hydrochloric acid (Apex Engineering Products Corporation, Aurora, Illinois, USA). Specifically we tried with 100% RDO®, 80% RDO® (diluted with 80% ethanol), 75% RDO® (diluted with distilled water) and 50% RDO® (diluted with distilled water and changing the media after 24h by or 50% RDO® or 25% RDO®, also diluted with distilled water).

B. Scanning Electron Microscopy (SEM)

Twenty three very fresh ears from *Stenella coeruleoalba* (n=14), *Stenella frontalis* (n=4), *Tursiops truncatus* (n=1), *Delphinus delphis* (n=1), *Lagenodelphis hosei* (n=1), *Globicephala melas* (n=1), *Ziphius cavirostris* (n=1) have been processed for the observation through SEM using the facilities of CRIC (Montpellier) and the Universitat Autònoma de Barcelona (UAB).

III. RESULTS

Following a routine protocol with a specific dilution of RDO®, the odontocete ear decalcification time ranged from

several hours to a few days, depending on the tympanic-periotic volume of the species [10].

Some of the samples observed with SEM presented an advanced decomposition stage and it was not possible to identify the ultrastructure of the organ of Corti, but in the fresher samples we could identify the outer hair cells prints on the tectorial membrane (Fig. 1).

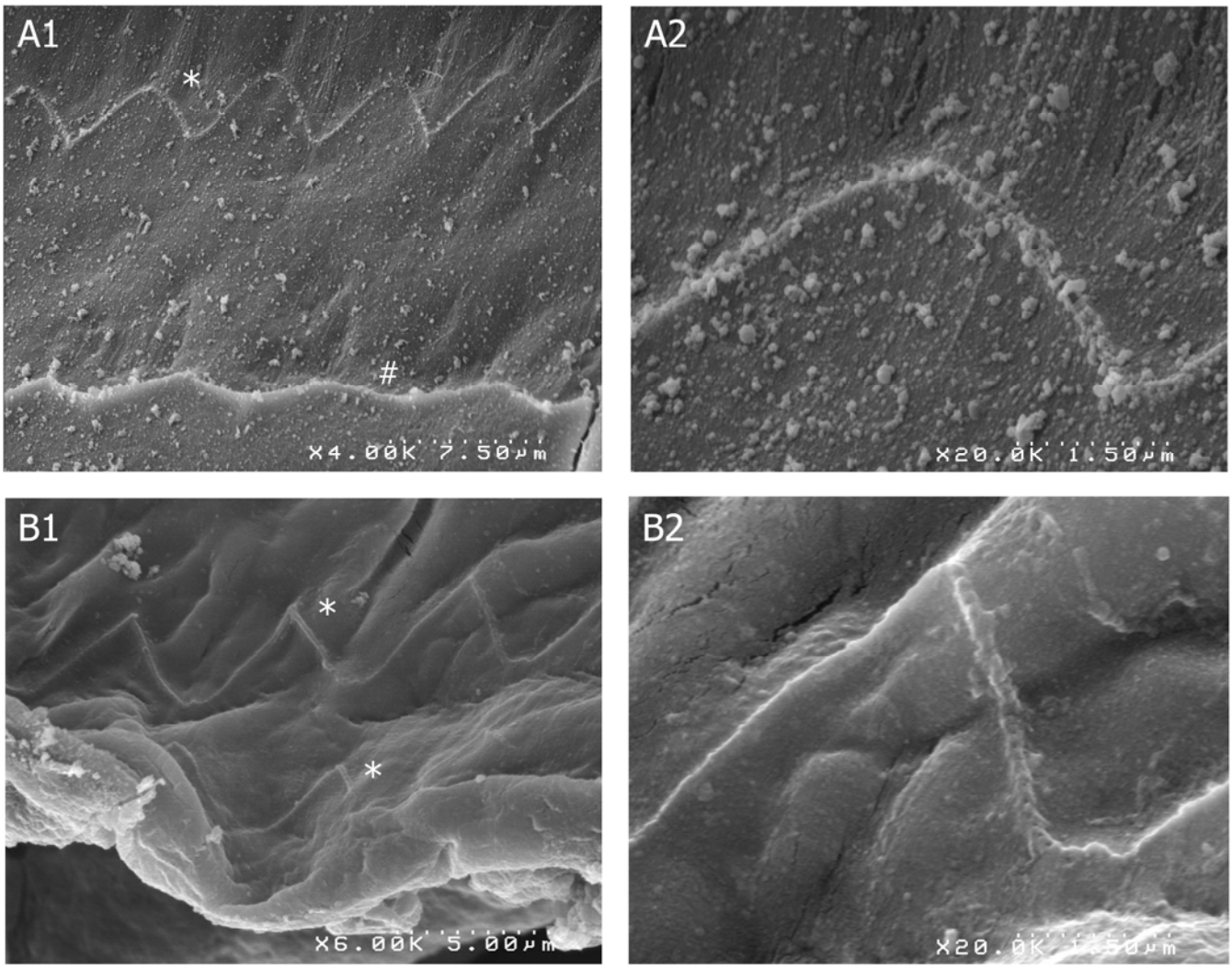

Fig. 1. SEM images of the tectorial membrane of a A) *Delphinus delphis* in 4.00k and 20.00k magnification and B) *Stenella coeruleoalba* in 6.00k and 20.00k magnification. In A1 and B1 are highlighted the position of the outer hair cells rows (*) and Hensen Stripe (#) while in A2 and B2 it is possible to observe the outer hair cells stereocilliae prints

IV. DISCUSSION

Following this protocol it is possible to obtain a fast diagnostic of possible acoustic trauma and relate the results to documented sound exposure, although further studies should be done with fresher and better conserved and fixed ears. The preliminary results presented here represent a step forward in the inner ear ultrastructural morphology study. The output of this analysis will help calibrating theoretical results derived from deep-sea observatories.

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REFERENCES

[1] W. J. Richardson, C. R. Greene Jr., C. I. Malme, and D. H. Thomson, *Marine mammals and noise*. San Diego, CA.: Academic Press, 1995.

[2] P. D. Jepson, M. Arbelo, R. Deaville, I. A. P. Patterson, P. Castro, J. R. Baker, E. Degollada, H. M. Ross, P. Herraez, A. M. Pocknell, F. Rodriguez, F. E. Howie, A. Espinosa, R. J. Reid, J. R. Jaber, V. Martin, A. A. Cunningham, and A. Fernandez, "Gas-bubble lesions in stranded cetaceans - Was sonar responsible for a spate of whale deaths after an Atlantic military exercise?," *Nature*, vol. 425, pp. 575-576, Oct 2003.

[3] M. H. Lurie, H. Davis, and J. E. Hawkins Jr, "Acoustic trauma of the organ of Corti in the guinea pig," *Laryngoscope*, vol. 54, pp. 375-386, 1944.

[4] E. G. Wever, J. G. McCormick, J. Palin, and S. H. Ridgway, "Cochlea of Dolphin, Tursiops-Truncatus - General Morphology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 68, pp. 2381-&, 1971.

[5] E. G. Wever, J. G. McCormick, J. Palin, and S. H. Ridgway, "Cochlea of Dolphin, Tursiops-Truncatus .2. Basilar Membrane," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 68, pp. 2708-&, 1971.

[6] E. G. Wever, J. G. McCormick, J. Palin, and S. H. Ridgway, "Cochlea of Dolphin .3. Tursiops-Truncatus - Hair Cells and Ganglion Cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 68, pp. 2908-&, 1971.

[7] D. R. Ketten and D. Wartzok, "Three-dimensional reconstructions of the dolphin ear," in *Sensory Abilities of Cetaceans*, J. Thomas and R. Kastelein, Eds. New York: Plenum Press, 1990, pp. 81-105.

[8] D. R. Ketten, "The cetacean ear: form frequency and evolution.," in *Marine Mammal Sensory Systems*, J. A. Thomas, R. A. Kastelein, and A. Y. Supin, Eds. New York: Plenum, 1992, pp. 56-69.

[9] D. R. Ketten, "Functional analyses of whale ears: adaptations for underwater hearing," in *Oceans' 94 Proceedings*, 1994, pp. 264-270.

[10] M. Morell, E. Degollada, J. M. Alonso, T. Jauniaux, and M. André, Decalcifying odontocete ears following a routine protocol with RDO®, *Journal of Experimental Marine Biology and Ecology* vol. 376, pp 55-58, 2009.