

CETACEAN EAR EXTRACTION AND FIXATION PROTOCOL

Maria Morell and Michel André, 2009

Introduction

There is an increasing concern about the impacts of anthropogenic underwater noise on cetacean populations. For this reason, the analysis of the ears and especially the presence of possible lesions in the organ of Corti represents a fundamental effort to assess the implication of acoustic trauma in stranding events, otherwise not detectable by routine histopathology techniques.

The difficulty relies in obtaining fresh material rapidly fixed by proper solutions and in accessing the cochlea by decalcifying methods without affecting the inner ear soft structures.

We have developed a fast decalcification protocol for use with most of the common odontocete species (see Figure 1) that allows a fast diagnosis of acoustic trauma if needed.



Figure 1.- Periotic bone decalcification results from a harbour porpoise (*Phocoena phocoena*) after an exposition of 26 hours with the rapid decalcifier RDO®. While other decalcifiers need around one month for a similar complex size, RDO® allows obtaining very fast results.

TYMPANIC-PERIOTIC COMPLEX

The tympanic and periotic bones house the middle and inner ear, respectively. These structures are partially fused forming the tympanic-periotic complex (Figure 2). The tympanic-periotic complex is surrounded by aerial sinuses called peribullar sinuses and suspended in the peribullar cavity through ligaments that hold it fixed and acoustically isolated it from the rest of the bones of the skull, except the sperm whales and some beaked whales who present the tympanic-periotic complex partially fused to the temporal bone.

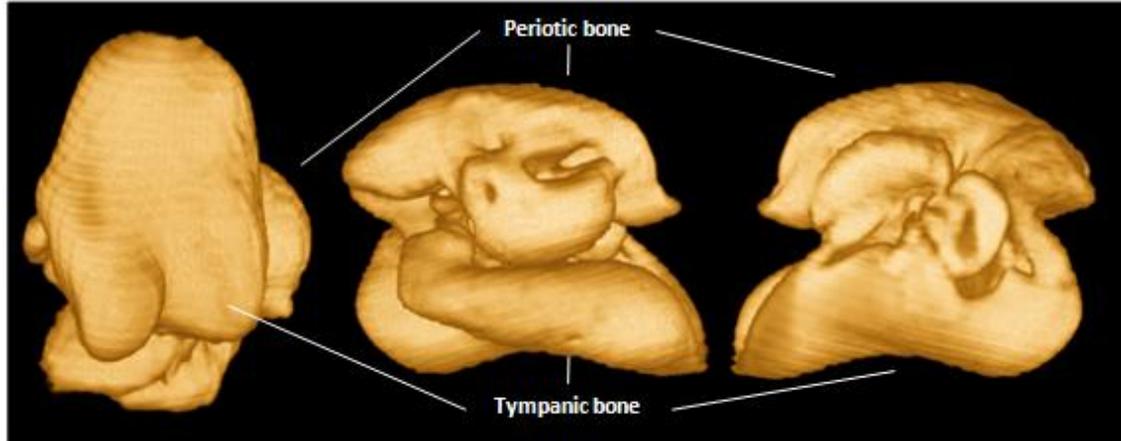


Figure 2.- Computerized tomography images 3D reconstruction from the tympanic-periotic complex of a bottlenose dolphin *Tursiops truncatus* in ventral, medial and lateral vision from left to right, respectively.

Extraction

1.- With small specimens, it is recommended to cut the head of the animal for an easier manipulation (Figure 3).

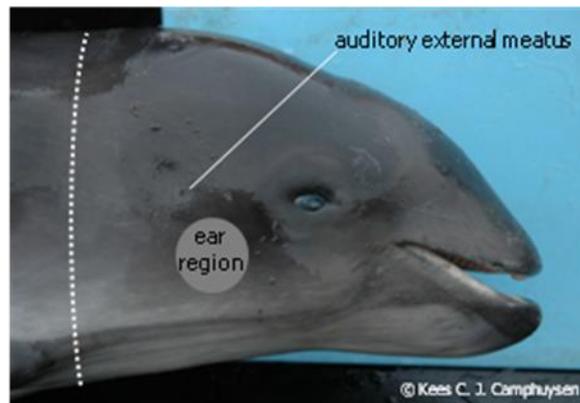


Figure 3.- The position of the tympanic-periotic complex and auditory external meatus is indicated. The dotted line marks the incision path to separate the head from the rest of the body. Alternatively, the digestive system can be extracted from the head to facilitate the access to the ears.

2.- Taking into account the localization of the tympanic-periotic complex (Figures 3 and 4), the easiest way to access the ears is to carefully remove the lower jaw.



Figure 4.- Sagittal cut of a bottlenose dolphin head where the location of the tympanic-periotic complex is indicated.

3.- Situating the head in a ventral position and removing the soft tissues and ligaments (Figure 5) allows to proceed to the tympanic-periotic complex extraction.

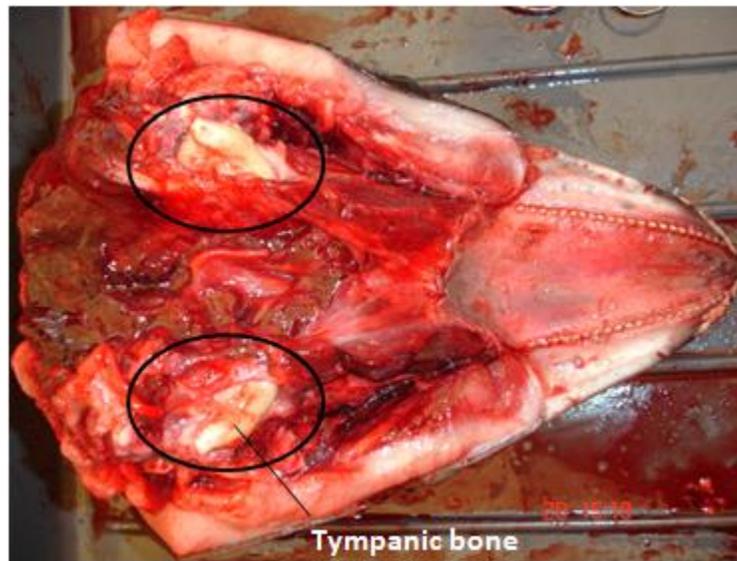


Figure 5.- Image taken during the necropsy of a *Phocoena phocoena*. This image reflects how the tympanic-periotic complex appears after removing the lower jaw (no effort has been made here to clean the area of extraction)

4.- Incise **gently around** the tympanic-periotic complex with a small knife (a scalpel can be used for the final stage of the extraction) to cut the ligaments that maintain the ears in the paraotic sinus (see Figure 6).

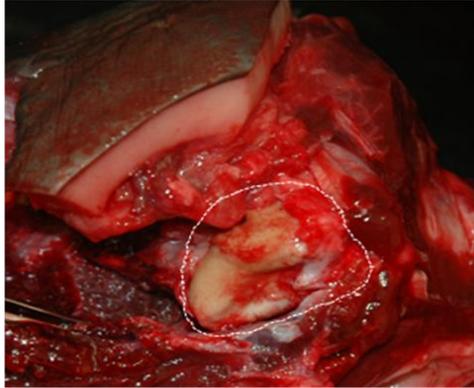


Figure 6.- Image taken during a *Phocoena phocoena* necropsy. The dotted line illustrates the location where the knife should be placed to extract the tympanic-periotic complex.

Fixation

5a.- At that stage, the ear could be fixed simply placing it in a fixative solution:

- **10% neutral buffered formalin**, or
- **4% paraformaldehyde** (pH 7.3-7.4), or
- 2.5% glutaraldehyde with 0.1M phosphate buffer or with 0.1M cacodylate buffer (pH 7.3-7.4), or
- mixture of 0.5% paraformaldehyde with 1% glutaraldehyde with 0.1M phosphate buffer (pH 7.3-7.4).

However, for a better result we recommend to follow the protocol described in point 5b.

5b.- If already experienced with the perfusion protocol, you may want to:

- 1) separate the periotic from the tympanic bone (Figure 7);
- 2) cut the stapedial ligament and remove the stapes. If it does not come off easily, it helps passing a scalpel through the junction;
- 3) make a **little and very superficial** hole to the oval and round window membranes;
- 4) using a soft catheter from the same diameter as the windows size (or the tip of a plastic pipette), **progressively and very slowly (with very little pressure)** introduce the fixative solution (Figure 8) through the oval window and the round window until the solution gets out through the other one during some seconds.

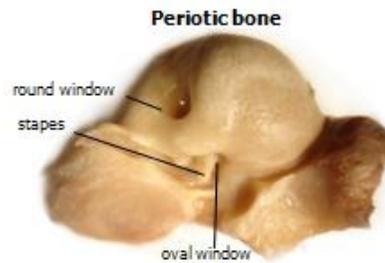


Figure 7.- Localization of the oval and round windows in the periotic bone.

The perfusion is a very delicate process and if you do not feel comfortable with it, please contact us. It is important to mention if the ear has been perfused or not when sending it.

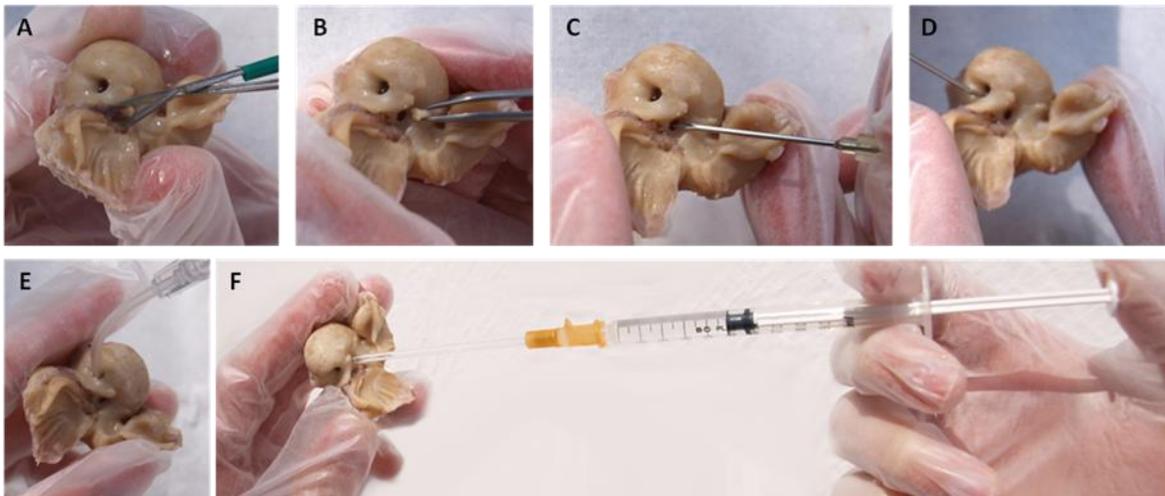


Figure 8.- *Tursiops truncatus* periotic bone used to illustrate all the injection process: A) cut of the stapedial ligament, B) stapes extraction, C and D) realization of a little and very superficial hole to the oval and round window membranes respectively, E and F) very slow and progressive perfusion (with very little pressure) of the fixative through the oval window and the round window until the solution gets out through the other one during some seconds.

6.- Place the ears in jars that contain the fixative liquids (see point 5).

7.- If 2.5% glutaraldehyde has been used as a fixative, change the solution next day or after two days with 0.1M cacodylate buffer for long term storage in the fridge.

Contact

8.- You can send the ears completely immersed in fixative by express mail to the following address:

Maria Morell
Institute for Neurosciences of Montpellier
Inserm UMR 1051
80, rue Augustin Fliche
34091 Montpellier
France

For any further question, do not hesitate to contact us at the following phone number:
+33 499636048 or at the mail address maria.morell@inserm.fr.