Imaging Techniques to study the effects of low frequency sounds on Cephalopods spp.

Marta Solé[1], Antonio Lombarte[2], Manel López Bejar[3], Alex Mas[1] and Michel André[1]

(1) Laboratori d'Aplicacions Bioacústiques, Universitat Politècnica de Catalunya, 08800 Vilanova i la Geltru, Espanya. www.lab.upc.es
(2) Institut de Ciències del Mar. Consejo superior de Investigaciones Científicas. E-08003 Barcelona, Espanya. http://www.icm.csic.es
(3) Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Espanya. http://www.uab.es

Abstract *: The introduction of artificial sound sources in the marine environment has shown to have negative effects on marine organisms. While marine mammals have attracted most of the attention of the research conducted in that area, invertebrates are also suspected to be negatively affected after an exposure to loud low frequency noise. An ongoing study from the Laboratory of Applied Bioacoustics of the Technical University of Catalonia is studying through imaging techniques (routine histology and SEM) the possible lesions in the statocysts of three cephalopod species (Sepia officinalis, Loligo vulgaris and Octopus vulgaris) as the likely most sensitive organ to high intensity noise.*

Keywords *: cephalopods, noise, statocysts*

INTRODUCTION

Between September and October 2001 and in October 2003 the natural rhythm of annual records of giant squids (*Architeuthis dux*) in the area of the West coast of Asturias, experienced a significant increase [1]. In both cases the stranding and collection of the bodies were related to the proximity of vessels using compressed air guns for geophysical prospecting, producing sound waves of low frequency (below 100 Hz) and high intensity (200 dB re 1 µPa at 1m per airgun).

Some of the specimens had lesions in different tissues and organs, but all presented pathologies in the gills and the receptor of equilibrium or statocists.

Because none of these lesions could be related to known causes of death, the presence of geophysical prospecting vessels suggested that the death of these animals could be related to effects produced by sound waves. However, no further study addressed this problem and the doubt remained on how and if high intensity low frequency pulses could negatively affect cephalopods.

A comprehensive study was therefore needed to assess the direct effects of the acoustic impact on these species. The first step was to choose which organ, found in all cephalopods spp. could be an indicator of noise-induced damage. Amongst other less sensitive-to-noise tissues, the statocysts are presumably the best candidates to injury if exposed to loud sources. All cephalopods have a couple of statocysts generally located within the cephalic cartilage. The statocysts are sophisticated balloon-shape bodies that present two layers of epithelial tissue (inner and outer) separated by a layer of connective

tissue, and include two receptors: the macula-statolith system and the crista-cupula system. The macula indicates the changes in the position according to the gravity and the linear acceleration, while the crista indicates changes in the angular acceleration. These systems are analogous to the vestibular system of the inner ear of vertebrates. However, unlike ciliated cells of the latter, the cephalopods' are quinocilis. The adjacent accessory structures (macula/statolith, crista/cupula) are responsible for the sensory perception. When there is a stimulus, these structures cause tiny deflations in the cilia groups, which in turn stimulate the ciliated cells that transmit the information to the sensory nervous system. Within the central nervous system, the sensory input of the statocysts is used to regulate a wide range of behaviours, including locomotion, posture, control of eye movement and of the pattern of the body coloration [2].

The aim of this ongoing project of the Laboratory of Applied Bioacoustics (Technical University of Catalonia) is to try to experimentally reproduce the sound exposure scenario that took place in Asturias and make a thorough analysis of possible lesions associated to low frequency sources in individuals from three different species of cephalopods (*Sepia officinalis, Loligo vulgaris* and *Octopus vulgaris*) by imaging techniques (histology and electron microscopy, SEM). Here we present the first SEM images that were obtained from control animals.

MATERIAL AND METHODS

Species

Adult and juvenile specimens from *Sepia officinalis, Loligo vulgaris* and *Octopus vulgaris* were taken from the wild and kept in a structure consisting of 2 mechanically filtered tanks PRFV of 2000L capacity, connected to each other. This included a physicochemical self-filtration with activated carbon and sand, driven by a circulation pump and filtration.

Dissection, fixation and removal of tissues

10% of the individuals were sacrificed prior to the application of acoustic pulses (the noise exposure protocol that was applied to the samples is not shown here. It included a ramp-up procedure using increasing frequencies and levels) and a routine necropsy [2,3,4,5] was conducted, collecting samples of different tissues, which were further fixed in formalin 10%. These sample animals were to be used as control tissue for histological assessment and the identification of possible affected organs: mantle, and radial muscle fibres in the two inner collagen tunics surrounding mantle muscle, various organs of the digestive tract-blind digestive gland, branchial hearts, gills and ovary.

The statocysts were also extracted but fixed with Glutaraldehyde 2,5% for posterior observation and analysis in SEM.

RESULTS

The systematic comparison of the histological preparations obtained from exposed individuals with control animals affected by acoustic pulses did not yet allow us to determine whether there were injuries associated with exposure to sound in any of the tissues analyzed.

The analysis of the statocysts showed the regular arrangements of kinocilliary groups of different hair cells that are thought to be the

structures likely to be affected by high intensity sound exposure.

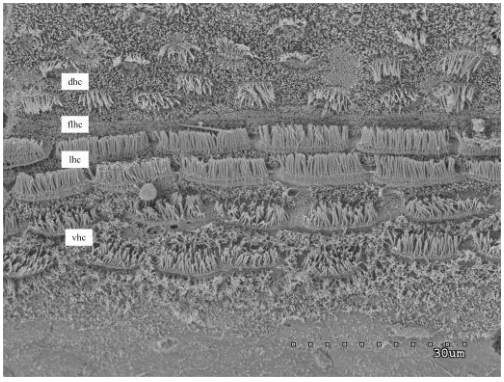

Fig.1. Crista of *Octopus vulgaris*, showing the arrangements of the kinocilliary groups of different hair cells. Small dorsal primary hair cells (dhc), regular row of fairly large (flhc), large secondary hair cells (lhc), small ventral secondary hair cells (vhc).

REFERENCES

[1] Guerra A., González A. F., Rocha F., Gracia J., Vecchione M. (2004) Calamares gigantes varados. Víctimas de explotaciones acústicas. Investigación y ciencia, 334, 35-37.

[2] Budelmann, B.U., Schipp, R., Boletzky, S. von. (1996) Cephalopoda. Microscopic Anatomy of Invertebrates. Vol 6. F.W. Harrison and A.J.Kohn (eds.) Wiley and Liss, New Cork.

[3] Bargmann,W. (1977) Histologie und mikroskopische Anatomie des Menschen. Thieme, Stuttgart.

[4] Nachtigall, W. Mikroskopieren, Gerate, Objekte, Praxis. BLV Verlagsgesellschaft mbH, Manchen, Germany, (1994) (Edición española: Microscopia, materiales, instrumental, métodos. Ediciones Omega S.A. Barcelona, 1997)

[5] Mitsuhashi J. Invertebrate Tissue Culture Methods (Springer Lab. Manual). (2003)Tokyo University of agriculture.