Hystrix It. J. Mamm (n.s.) 16 (2) (2005): 127-133

AN ON-LINE TISSUE BANK FOR MARINE MAMMALS OF THE MEDITERRANEAN SEA AND ADJACENT WATERS

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ABSTRACT - This paper reports on the activities of the Mediterranean Tissue Bank for Marine Mammals, established in January 2002. The bank collects fragments of tissues sampled from marine mammals stranded along the Mediterranean coastline and distributes them to scientists working in the field. Tissues are a critical resource for biomedical and innovative research in anatomy, histo-pathology, genetics and toxicology, and the bank exploits the potentials of stranded animals to serve the scientific community of dolphin and whale investigators.

Key words: whales, dolphins, tissue bank, strandings, Mediterranean Sea

RIASSUNTO - ***Una banca* on line *per i tessuti dei Mammiferi marini del Mediterraneo e dei mari limitrofi***. Questo articolo presenta le attività della Banca per i tessuti dei Mammiferi Marini del Mediterraneo, istituita nel gennaio 2002. I tessuti sono una risorsa critica e innovativa per le ricerche biomediche in anatomia, istopatologia, genetica e tossicologia. La banca raccoglie campioni di tessuto dai mammiferi marini spiaggiati lungo le coste del Mediterraneo e li distribuisce ai ricercatori che lavorano nel settore.

Parole chiave: balene, delfini; banca tessuti, spiaggiamenti, Mar Mediterraneo

INTRODUCTION

Behavioral studies on cetaceans living in the wild yield a vast amount of data of unsurpassed importance concerning social interactions, diving habits, and even physiology of whales and dolphins. Cetaceans are protected almost everywhere in the world, and invasive experimentation requires approval by the competent national Authorities (Italian animal experimentation is regulated by the Ministry of Health and follows European guidelines, as expressed by the D.L. 27.1.92, n. 116), and CITES authorization, after careful evaluation by the appointed ethical committees. Some experiments can be performed on (or with) trained bottlenose dolphins or other small cetaceans, but surely not with large whales. Therefore, several questions of interest remain unanswered, and stranded dolphins and whales still represent a useful source of

data on the biology of cetaceans: tissues obtained from stranded animals retain a great scientific potential, including details on age determination, structure and connections of nervous pathways, blood dynamics during diving, and many other issues of cetacean basic biology and physiology otherwise not accessible through other means.

Necropsies also provide data on the incidence of interactions with humans, including collision with ships, entanglements, damage by hooks, and marine debris ingestion.

Furthermore, collecting specimens from stranded animals for future retrospective analysis is widely recognized as an important field of research (Aguilar, 1985; Borrell *et al.*, 2001; Fossi *et al.*, 1999; Marsili *et al.*, 2001). In the last decades, the improvement of laboratory techniques has allowed further elaborations of data acquired from stranded specimens: microwave histology (Login *et al*., 1998; Ichihara *et al*., 2001), the use of nervous tracers working in the fixed brain (Honing and Hume, 1989), amplified immunohistochemistry (Gaétan and Bendayan, 1997), DNA and RNA extraction from fixed materials (Douzery, 1993; Rosenbaum, 1997; Masuda *et al*., 1999; Lewis *et al*., 2001; Specht *et al.*, 2001) are just few examples of these possibilities.

Finally, the existence of some elusive species of marine mammals is known practically only through stranded remains, as in the case of the genus *Caperea* and of some species of the genus *Mesoplodon* (Azzaroli, 1968; Jefferson *et al.*, 1993; Rice, 1998; Podestà *et al*., 2005).

Italy has a quite long coastline facing the Mediterranean Sea. The Italian national stranding network (Centro Studi Cetacei, CSC; www.centrostudicetacei.org) coordinates observation and recovery of marine mammals and reptiles stranded along national and adjacent coasts. Starting from the second half of 2001, tissues recovered from stranded cetaceans and collected by volunteers of the CSC (Centro Studi Cetacei, 2001) and other organizations have been delivered to the facilities of the Mediterranean Marine Mammal tissue bank (Anfuso *et al*., 2001 and 2002), officially established in January 2002 and located in the Department of Experimental Veterinary Science of the University of Padua (NE Italy).

The Mediterranean Marine Mammal tissue bank (see the bank on-line site at http://www.sperivet.unipd.it/tissuebank/) works under CITES recognition and benefits from the joint efforts of CSC and other research organizations, including the Central Governmental Institute for Sea-Applied Research (ICRAM; www.icram.org), to establish an archive of tissues available to all concerned researchers, similar to those (see Appendix) already present in other countries (Becker *et al*., 1990; Hameedi, 1990; Becker *et al.*, 1993; Lillestolen *et al*., 1993; Odsjö, 1993; Giege and Odsjö, 1993; Becker *et al.*, 1997). The bank operates within the Agreement of the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS).

The aim of this paper is to introduce the field and laboratory activities of the bank.

METHODS

In optimal conditions, the necropsy and collection of samples are performed by a veterinarian at the stranding site or at a nearby Institution. However, most of the dissections occurring in the field have been performed by personnel with no specific veterinarian expertise. Detailed instructions on how to sample tissues at the stranding site have been reported on a CD available for free on request (Cozzi, 2003).

The bank also stores samples (including body fluids) derived from the necropsy of animals which have been kept in a controlled environment.

Samples delivered to the bank are either fixed in buffered formalin, carefully washed in phosphate buffer 0.1 M pH 7.4 and processed for paraffin embedding or frozen and stored in deep freeze (from -20 °C to -30 °C). Paraffin blocks and frozen tissues are classified and filed in the on-line database (http://www.sperivet.unipd.it/tissuebank/). A limited number of samples is stored in dimethyl sulfoxide (DMSO). The use of alcohol as a fixative is discouraged for the negative effects of shrinking.

CITES authorization allows the bank to distribute sections of tissue samples in Italy and other countries of the European Community. Export and/or import licenses for non-European countries must be asked to the Competent Governmental Authorities and customs clearing procedures be arranged in advance. The bank provides the material and specimen data to the scientific community for free and offers further optional services including specific staining, immunohistochemistry, blood tests, diagnostics on parasites, necropsy and references.

RESULTS

The overall number of samples collected is approximately 1,200. Tissues derive from over 80 animals of 11 different species of marine mammals (Fig. 1) belonging to stocks living in the Mediterranean Sea or adjacent waters: *Delphinus delphis, Stenella coeruleoalba, Tursiops truncatus, Steno bredanensis, Grampus griseus, Globicephala melas, Ziphius cavirostris, Mesoplodon europaeus, Physeter macrocephalus, Kogia sima, Balaenoptera* sp.

The bank stores samples from all the organs of the body. However, due to the irregular procedures by which necropsies are performed on the beach, some organs are more represented than others: for example muscle (83%) kidney (73%) or spleen (46%) samples are delivered more often than bladder (16%) or trachea (14%) samples. In 2001, when advertising for samples collection started, the bank received tissues from 8 out of a total of 194 (4.1%) stranded cetaceans. In 2002 tissues from 23 out of 146 (15.7%) stranded cetaceans were collected and sent to the Bank.

The bank includes also a small collection of brains from *S. coeruleoalba, D. delphis, T. truncatus* and *G. griseus*. Sections from specific areas of these brains are available upon request.

The bank stores information about gender, age, date and location of the stranding site, body total length and - whenever possible - details about the life-history of the animal (including therapy, cause of death, body condition and condition code according to the protocol of Kuiken and Hartmann, 1991; http://www.sperivet.unipd.it/tissue bank/).

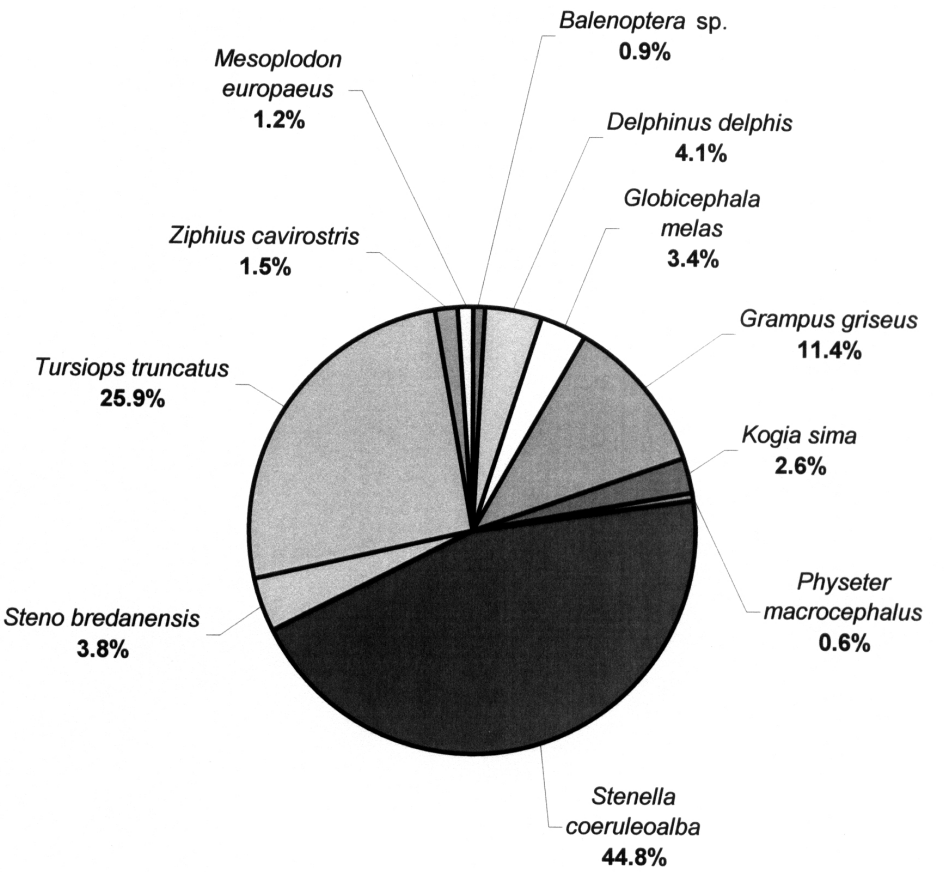

Figure 1 - Per cent distribution of the species of marine mammals stored in the bank.

DISCUSSION

Since its foundation, the tissue bank based at the University of Padua has received growing attention and interest from Italian and foreign research groups. We are aware that maintaining a national standard procedure for measurements and specimen collection is fundamental to optimize data and compare results. With the intent of creating an efficient network of Mediterranean collecting stations, contacts have been established with other similar Institutions in Europe under the ACCOBAMS patronage, and especially with those operating along the Mediterranean coasts (Aguilar and Borrell, 2002a and 2002b).

Furthermore, the bank cooperates with Italian research centers and Universities on different research projects and doctoral thesis on anatomy, physiology and pathology, as well as with foreign Universities and international Institutions.

Further goals of the bank may include collection of blood, semen and urine from live animals or - whenever possible - from recently dead animals under sterile conditions. Preservation of body fluids increases the possibilities of

genetic researches on cetacean populations of the Mediterranean Sea (Schroeder and Keller, 1990; Hartmann, 2000).

Collected images of cetacean organs constitute the basis for an atlas of dolphin anatomy and pathology, which is part of a far-reaching effort of documentation of Mediterranean marine mammals (Cozzi, 2005). Furthermore, the realization of a collection of microscopic images of the cetacean body is an ongoing project still far from completion. The current efforts of the bank research group are aimed at filing an updated photographic database of macro- and microscopic images of stranded cetaceans, in order to have pictorial archives of environmental- and human-induced pathologies at our disposal. Such archives respond to precise goals recently established by ACCOBAMS and by the International Whaling Commission.

ACKNOWLEDGEMENTS

The Bank has been established thanks to the efforts of several Institutions and individuals. We would like to thank the Board of the CSC-onlus for the helpful cooperation, ICRAM for suggestions and concrete support, the Department of Public Health, Comparative Pathology and Veterinary Hygiene of the University of Padua for contributing on pathological anatomy and postmortem diagnosis, and finally Luigi Cagnolaro of the Museum of Natural History of Milan for starting it all in his mind several years ago.

We also acknowledge the continuous support of the Department of Experimental Veterinary Science of the University of Padua, that willingly guaranteed space and scientific instruments used by the bank.

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Appendix - Web-sites of foreigner tissue banks.

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http://esb.naturforvaltning.no/faroe.htm, (Farøese Environmental Specimen bank)
www.absc.usgs.gov/research/ammtap/ammtap.htm, (Alaska Marine Mammal Tissue, Archival Project AMMTAP)
www.nmfs.noaa.gov/prot_res/PR2/Health_and_Stranding_Response_Program/q&as.html, (NMMTB, National Marine Mammal Tissue Bank)
www.accobams.org/activities/tissue_banks.htm, (ACCOBAMScience)
http://www.nrm.se/mg/mpb.html.en (Swedish Environmental Specimen Bank)
http://www.chem.unep.ch/gmn/05_ESB.htm (Germany Environmental Specimen Bank)
http://www.nist.gov/public_affairs/gallery/specimen.htm (NIST Biomonitoring Specimen Bank)

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