



GUIDELINES FOR THE ESTABLISHMENT OF A SYSTEM OF TISSUE BANKS WITHIN THE ACCOBAMS AREA AND THE ETHICAL CODE

Introduction

The Scientific Committee of ACCOBAMS has recognized the need for Institutions dedicated to the preservation of body parts from marine mammals of the Mediterranean and Black Seas. Such Institutions, from now onward identified as Tissue Banks, should a) promote non-invasive or post-mortal collection of samples from cetaceans living in the Mediterranean and Black Seas and adjacent waters; b) prepare such samples for long term storage; and c) distribute them to the community of marine mammal researchers.

Tissue samples hold an enormous potential for scientific exploitation. Sampling skin fragments from living animals by non-lethal methods or removing tissues and organs from stranded animals may allow extensive studies of population health and dynamics, body structure and pathology, including viral pathology. Tissues may be studied comparing materials derived from geographically separated sites, or a given organ may be investigated in a series of animals that died several years apart. Furthermore, the availability of tissues from cetaceans may greatly improve studies on viral incidence, making it possible to compare lesions and/or viral genetics in outbreaks of epidemics that occurred several years one from the other or simultaneously in distant locations.

The importance of Tissue Banks increases when a single bank is flanked by a series of cooperating Institutions, each dedicated to preservation of body parts of marine mammals in a specific marine area. A network of banks (possibly one for each Member State) could ensure information and exchanges that are vital for scientific studies and could also promote prompt action in case of environmental emergencies (i.e. viral epidemics).

Following thorough examination of primary issues on cetacean mortality, anatomy, pathology and toxicology, and on methods for the collection and preservation of tissue samples, the ACCOBAMS Scientific Committee has approved the purpose of establishing a network of Tissue Banks dedicated to harvesting, manipulation and storage of tissues sampled from cetaceans of the Mediterranean and the Black Sea.

Goals of the effort

The objective of the present Guidelines is to establish a network of Tissue Banks operating along the shores of the Mediterranean and Black Seas to collect tissues from stranded marine mammals and serve the Agreement by making available biological material, mostly deriving from stranded and by caught cetaceans, to the scientific community. Such material would then be used to promote knowledge, *inter alia*, on mortality causes, functional anatomy, physiology (including respiratory and diving physiology), toxicology, pathology (including infectious diseases), population structure, and trophic relationships of the region's cetaceans.

In an ideal context each ACCOBAMS Member State should work toward establishing a National Tissue Bank to serve the adjacent waters.

Samples from each Tissue Bank should be made available to the scientific community of cetacean researchers for free or at the lowest possible cost. Such costs should be – whenever possible – covered by Institutional funding to increase research opportunities.

Countries of the ACCOBAMS Agreement should support the tissue bank system in the general interest of environmental safety and animal and human health protection.

A coordinated network should be established to link all ACCOBAMS Tissue Banks and a Coordinator should be selected among the scientific personnel of the banks (see also below Super national Integration). The link should be extended also to scientist working toward establishing a Tissue Bank in a specific member State even before the bank opens officially, to ensure all potential assistance and support.

Goal of the network is also to prepare and maintain *on-line* databases available to the scientific community. Researchers may check the availability of a given specimen in real time and send motivated scientific requests for it. Each bank should be connected to the others by a continuous exchange of information and possess a specific CITES authorization to directly export/import from/to similar Institutions, avoid undue delays and fully operate within International authorizations.

A list of active tissue bank can be found in Appendix 1.

Procedures for the establishment and maintenance

Individual Tissue Banks who intend to work within the ACCOBAMS agreement should apply to the ACCOBAMS Secretariat providing an individual action plan based on the present Guidelines or eventual future revisions.

The action plan should include a Section dedicated to the existing equipment and personnel and to the funding perspectives.

The Secretariat will approve the action plan after consultation with the Scientific Committee and the Tissue Bank Working Group. Approval from ACCOBAMS Authorities will allow the new Tissue Bank to enter the existing network and ensure support from the Secretariat in every endeavour to obtain recognition and financial aid from Authorities of the relative Member State.

Once part of the ACCOBAMS network, each Tissue Bank should operate harmonically with the other similar Institutions and promote open exchanges of tissues and information with the other Tissue Banks. Eventual area conflicts and disagreements should be discussed within the Tissue Bank Working Group and possibly resolved with mutual cooperation. Failures to comply will be reported to the Scientific Committee and Secretariat for further arbitrate.

The existing Tissue Banks will establish contacts with all governmental and non-governmental Organizations interested in cetacean investigations and welfare. This action should take place within two years following approval of the present Guidelines. A specific ethical code is presented in Annex 1.

Contacts, exchanges, research programs should follow CITES protocols and International and National regulations concerning protected species. According to ACCOBAMS Resolution 2.10, each Country should designate a specific CITES structure responsible for the Permit procedures.

Super national Integration

The Tissue Bank network will communicate by establishing a *Tissue Bank Working Group* under the responsibility of a Coordinator elected every three years among the scientific personnel of the Tissue Banks. The *Tissue Bank Working Group* will report periodically to the Scientific Committee and will present a report of the activities at the Meetings of the Scientific Committee.

The activities of the *Tissue Bank Working Group* will be reported also to the Secretariat who will suggest specific action plans, research goals and topics of discussion according to the transnational situation.

Objectives, priorities of research and tissues to be stored for the existing and potential future Tissue Banks are established by continuous cooperation between the *Tissue Bank Working Group* and the ACCOBAMS Scientific Committee, and by periodical scientific meetings organized on a regular basis by the interested Institutions.

The ACCOBAMS Secretariat and Scientific Committee are responsible for changes and modification of the Guidelines for establishing Tissue Banks, with the Coordinator of the *Tissue Bank Working Group* acting as a Consultant of the Secretariat.

How to establish a Tissue Bank in an ACCOBAMS member State

Here follows a short summary of the ideal characteristics of an ACCOBAMS tissue bank

- The Tissue Bank should be hosted within an official Institution to ensure the proper scientific background, expertise, equipment and continuity. Candidate institutions are Museums of Natural History, Oceanographic Museums, Universities (Faculties of Sciences or Veterinary Medicine), Public Health Institutions (Animal Health Departments), Environmental Agencies (Marine Monitoring Institutions) or even Ministries of Environment.
- The Tissue Bank should have an adequate number of rooms and/or offices, even in coexistence with other functions (i.e.: a few dedicated rooms may be equivalent to a whole floor in coexistence with other parties). Tissues should be stored in a dedicated space or storage room, furnished with refrigerators or cabinets depending on the nature of the tissues (frozen or fixed). Tissues stored in the bank should not be maintained together with specimens meant for other purposes.
- Each bank should have a fixed yearly budget desirably provided by public funding. The budget could vary according to the different States, but should ensure the coverage of the basic expenses including laboratory equipment (freezers, cabinets) and reagents (formalin, DMSO, etc), and also current operative costs including mail, telephone, energy.
- Public funding should also cover the cost of at least one dedicated employee (laboratory technician or investigator). A long-term position is desirable to ensure continuity in the developing activities of the bank.
- Each bank should open a dedicated web-site in which scientists from the outside could look into the list of preserved materials and possibly request them directly on-line.

Guidelines for tissue harvesting and storage

Tissues should be harvested from every marine mammal found dead after stranding or floating at sea, provided that the operating conditions (including safety health procedures for personnel responsible of the sampling) allow it.

Non-invasive collection of skin samples or bodily fluid is also acceptable, provided the operating party possesses the required Authorizations to perform such biopsy or sampling from the National Ministry of Environment (for CITES regulation) and Ministry of Health (according to EEC Directive 86/609 and later integrations concerning animal protection).

Sampling should be performed under guidance from expert personnel (veterinarians, biologists with specific training, laboratory technicians).

Cubes of sampled tissues should not exceed 1 cm³. Larger samples will be harder to preserve. Samples meant for molecular biology should be either immediately frozen and stored at -30-80 C° or

immersed in DMSO. Samples meant for histology should be immersed in buffered formalin. Detailed instructions on how to perform sampling are contained in:

Bruno Cozzi (editor) *Marine mammals of the Mediterranean and Black Sea Natural History and Biomedicine*, Massimo Valdina Editore, Milan, 2006 I.S.B.N. 88-88176-06-3 Special edition prepared for ACCOBAMS

Tissues should be sampled from every organ of the body. If and when available, at least one tooth should be removed from the mandible to provide data on age of the animal. If tooth removal proves impossible, an X-ray or densitometry of the pectoral fin will also allow insights on age.

If and when possible, the brain should be removed as a whole, and subsequently subdivided into transverse (coronal) section not thicker than 1 cm and immersed in buffered formalin. Focal cerebral areas intended for molecular biology should be frozen following the procedures outlined for the other tissues.

Parasites should be photographed and preserved in alcohol or formalin according to specific research purposes.

Detailed pictures should be taken during sampling. If no veterinarian is present on the spot, photographs of all external signs on the body should be taken before opening the body cavities and organ sampling. Pictures of the organs will also help the pathologists in their diagnosis.

Upon arrival at the Tissue Bank, tissues should be classified and prepared for long term storage, either in deep-freezers (frozen tissues for molecular biology) or specific cabinets (for DMSO and formalin-fixed samples).

An updated database should be available on-line containing information on the stranded animal and the tissues available.

Guidelines for Tissue Bank advertisement and tissue distribution

Stored tissues should be made available to the community of marine mammal researchers for free or at the lowest possible cost. To regulate tissue distribution an Ethical Code is presented in Appendix 2.

Tissue Banks should be widely known and recognized as open sources of biological material. To this effect, the establishment of web-sites is encouraged. A quick research through the on-line database should help scientists from the outside to select tissues and species of interest. When available, data on age, length and body condition of the animal at the moment of sampling should also be available.

Tissue samples could be asked *on-line* or by mail writing a request complete with full address, details on the Institution requiring the samples and a short explanation of the research for which the samples will be analyzed.

Scientists asking for the samples should allow their names and Institutional addresses to be entered into the Bank database and recognize the source of the samples in the Materials & Methods and Acknowledgement sections of their published studies.

Appendix 1

A list of active tissue bank

La Rochelle Bank

Centre de Recherche sur les Mammifères Marins, Université de La Rochelle
23, Av. A. Einstein, 17071 La Rochelle cedex
France
Tel. : +33 54 644 99 10
e-mail: vridoux@univ-lr.fr

Padua Bank

Mediterranean Marine Mammal Tissue Bank - Banca per i tessuti dei mammiferi marini del Mediterraneo
Scientific Coordinator professor Bruno Cozzi
Department of Experimental Veterinari Science, University of Padua
Viale dell'Università 16 35020 Legnaro – Agripolis (PD) - ITALY
phone: +39.049.8272626 - +39.049.8272621, fax: +39.049.8272669
e-mail: bruno.cozzi@unipd.it
web site : <http://www.sperivet.unipd.it/tissuebank/>

Barcelona Bank

Barcelona BMA Tissue Bank
Scientific Coordinator professor Alex Aguilar
GRUMM-GBC, Department of Animal Biology (Vertebrates), Faculty of Biology, University of Barcelona
08028 Barcelona - Spain
Telephone: (+34) 93 402 14 53; Fax: (+34) 93 403 44 26
e-mail: alexa@bio.ub.es

Appendix 2

Ethical Code for cetacean tissue banks active within the ACCOBAMS Agreement

This ethical code is provided by the ACCOBAMS Secretariat for Tissue Banks active within the Agreement. All tissue banks must accept the Code to operate within the ACCOBAMS Tissue Bank and Stranding network.

Periodical revision of the ethical code will be undertaken every three years by the Tissue Bank working Group and approved by the Scientific Committee.

GENERAL DISPOSITIONS

Definition

ACCOBAMS Tissue Banks are public Institutions dedicated to harvesting, preparing, conserving and distributing tissues derived from marine mammals living in the Mediterranean Sea, Black Sea and adjacent waters.

General principles

2. Tissue Banks must operate according to relevant rules and regulations of the host country.
3. Their activity must follow procedures approved by the competent State Authorities for treatment of live or dead animals under CITES. Accordingly, Tissue Banks must follow CITES procedures during the acquisition, processing and distribution of tissue fragments or bodily parts.
4. Contacts, exchanges, research programs concerning Tissue Banks alone or in relation to the scientific community must follow CITES protocols and international and national regulations concerning protected species.
5. Tissue Banks must avoid any harm to any marine mammal or vertebrate occurring either directly or indirectly in relation to their activity.
6. Tissue Banks are non-profit institutions. Samples from each Tissue Bank should be made available to the scientific community free of charge. Tissue distribution costs may be met either with public institutional contributions or eventually shared with the requesting parties (i.e. scientists asking for specific tissues for scientific purposes). In this latter case the Tissue Bank should net no profit or gain from the transaction but only aim at covering live expenses.
7. Each National Tissue Bank must operate with the network of ACCOBAMS Tissue Banks.

Goals

ACCOBAMS Tissue Banks should:

1. Encourage non-invasive or post-mortal collection of samples from cetaceans living in the Mediterranean and Black Seas and adjacent waters.
2. Be in line with the guidelines on granting exceptions when a special permit is granted.
3. Prepare such samples for long-term storage.
4. Make samples available to the community of cetacean researchers.
Biological material distributed by Tissue Banks should be used to promote knowledge on mortality causes, functional anatomy, physiology (including respiratory and diving physiology), toxicology, pathology (including infectious diseases), population structure, and trophic relationships of the region's cetaceans.

RELATIONSHIP AMONG TISSUE BANKS

1. Individual Tissue Banks which intend to work within the ACCOBAMS framework should apply to the ACCOBAMS Secretariat for inclusion in the network. The Secretariat will approve the programme of work after consultation with the Scientific Committee and the Tissue Bank Working Group.
2. Approval from ACCOBAMS Authorities will allow the new Tissue Bank to enter the existing network and ensure support from the Secretariat in every endeavour to obtain recognition and financial aid from Authorities of the relative Member State.
3. Once part of the ACCOBAMS network, each Tissue Bank should operate harmoniously with other similar Institutions and promote open exchange of tissues and information with the other Tissue Banks. Eventual area conflicts and disagreements should be discussed within the Tissue Bank Working Group and possibly resolved with mutual cooperation. Failures to comply will be reported to the Scientific Committee and Secretariat for further arbitrate.

SPECIFIC DISPOSITIONS

1. It is desirable that the Tissue Bank be hosted within an official Institution to ensure the proper scientific background, expertise, equipment and continuity in the long-term. Candidate institutions include Museums of Natural History, Oceanographic Museums, Universities (Faculties of Sciences or Veterinary Medicine), Public Health Institutions (Animal Health Departments), Environmental Agencies (Marine Science Institutions) or even Ministries of Environment;
2. The Institution should be registered within the CITES according to the Resolution CITES Conf 11.15 and the ACCOBAMS Resolution 2.10 in order to facilitate tissue exchanges;
3. The Tissue Bank should be given adequate space, even in coexistence with other functions (i.e.: a few dedicated rooms may be equivalent to a whole floor in coexistence with other parties). Tissues should be stored in a dedicated space or storage room, furnished with refrigerators or cabinets depending on the nature of the tissues (frozen or fixed). Tissues stored in the bank should not be maintained together with specimens meant for other purposes;
4. Each bank should have a fixed yearly budget desirably provided by public funding. The budget could vary according to the different cases, but should ensure the coverage of the basic expenses including laboratory equipment (freezers, cabinets) and reagents (formalin, DMSO, etc), and also current operative costs including mail, telephone, internet access and website, energy. Adequate backup must be provided in the eventuality of a power shortage. Public funding should also cover the cost of at least one dedicated employee (laboratory technician or investigator). A long-term position is desirable to ensure continuity in the developing activities of the bank;
5. Each bank should open a dedicated web-site in which scientists from the outside could look into the list of preserved materials and possibly request them directly on-line

TISSUE HARVESTING AND STORAGE

1. Tissues should be harvested from every cetacean found dead after stranding or floating at sea, provided that the operating conditions (including safety health procedures for personnel responsible of the sampling) allow it. Stranding networks should actively contribute to harvesting tissue samples and properly deliver them to the National Tissue Bank or to a local reference Institution for subsequent transport to the closest ACCOBAMS Tissue Bank.
2. Non-invasive collection of skin samples or bodily fluid is also acceptable, provided the operating party possesses the required authorizations to perform such biopsy or sampling from the competent Authorities.
3. Sampling should be performed under guidance from expert personnel (veterinarians,

biologists with specific training, laboratory technicians) and follow the ACCOBAMS for Tissue Banks.

4. Detailed pictures should be taken during sampling. If no veterinarian is present on the spot, photographs of all external signs on the body should be taken before opening the body cavities and organ sampling. Pictures of the organs will also help the pathologists in their diagnosis.

DATABASE, INFORMATION AND PRIVACY ISSUES

1. An updated *on-line* database should be available containing information on the stranded animal and the tissues available.
2. Information on the distribution and use of the samples distributed by the National Tissue Bank should be included in the National Report.

Scientists asking for the samples should allow their names and institutional addresses to be entered into the Bank database and recognize the source of the samples in the Materials & Methods and Acknowledgement sections of their published studies.