# MARINE MAMMALS STRANDING

# GUIDELINES FOR POST-MORTEM INVESTIGATIONS OF CETACEANS & PINNIPEDS

# &

# 13rd CETACEAN NECROPSY WORKSHOP

## SPECIAL ISSUE ON NECROPSY AND SAMPLINGS

## (LIEGE 2019)

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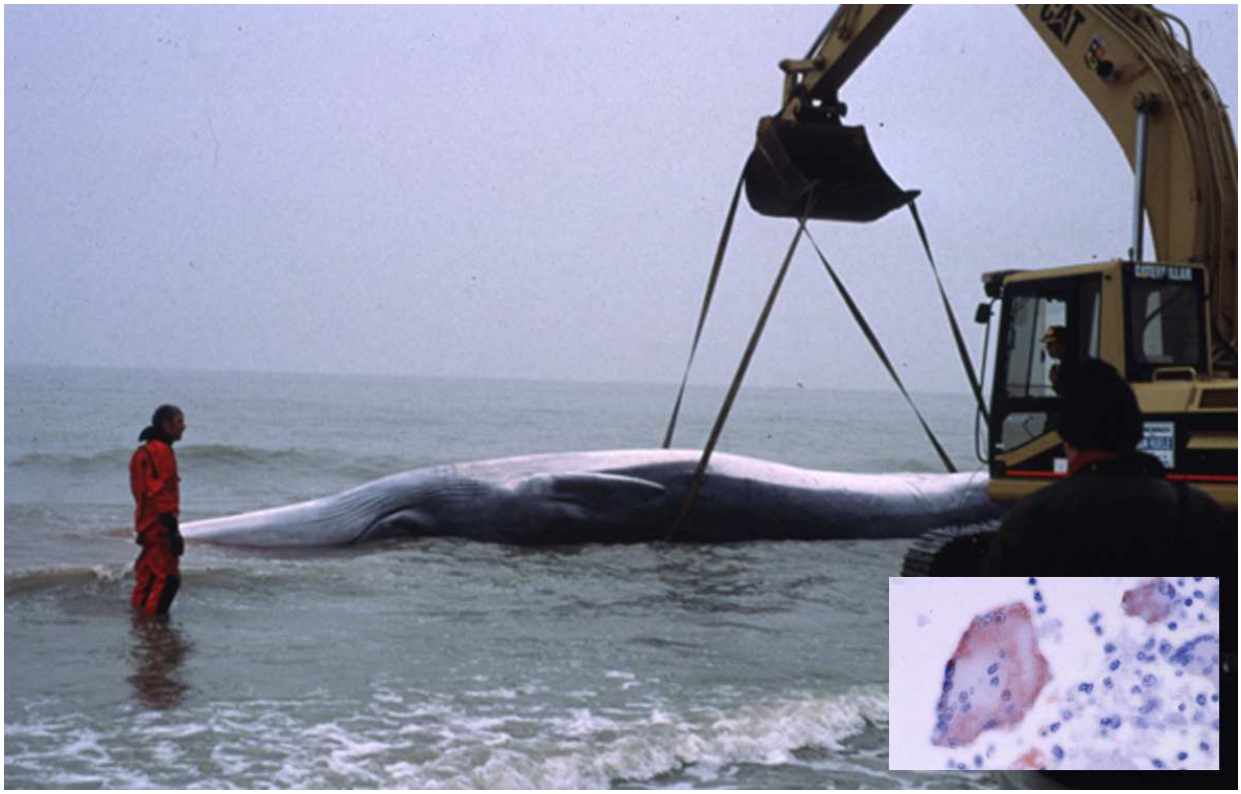

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With the financial support of the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area (ACCOBAMS)

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# 1. INTRODUCTION

Single or mass strandings of whales and dolphins have always intrigued people and must have occurred from the time cetaceans have been present in the oceans. Seals, having also a terrestrial behaviour, are better known and perhaps less intriguing. A mass stranding is defined as an event in which two or more animals run ashore alive at roughly the same place and time (excluding cow-calf pairs). Many theories have attempted to explain that phenomenon. In most cases, it cannot be attributed to a single cause, but is the result of a complex interaction of physical and biological factors such as ocean currents, tides and coastal configuration, the animals' migratory and social behaviour, food availability, echolocation or orientation failure, and diseases with debilitating effects.

All those factors must be taken into account for the evaluation of the cause of the stranding. Emergence of new diseases such as morbillivirus infection, brucellosis … or new theories of strandings such as linked with anthropogenic activities (pollution, sonar) justify to perform post-mortem evaluation of all stranded animals. Multidisciplinary investigations (pathology, microbiology, toxicology, life history …) are necessary to improve our knowledge concerning the causes of stranding and the health status of marine mammal populations.

The aim of the present document is to provide a guideline for post-mortem investigations for marine mammal necropsies (cetaceans and seals) while the necropsy workshops should be considered as a training to dissect marine mammals with emphasis on the cetacean's inner ear extraction and fixation.

All the information contained in the present document is also available on http://www.marinemammals.be/Autopsy.php.

## 2. MARINE MAMMAL STRANDING NETWORKS

Management of marine mammal stranding and necropsy requires to be well organized and needs significant investment of money and time. To be realized adequately and regularly, all the investigations should be performed by a multidisciplinary research program, involving biological, pathological and toxicological laboratories and natural history museum or alike institutes, devoted to the determination of health monitoring and protection of wild marine mammals. Such teams need to work in close collaboration to form a network and require to have scientific coordinator on site, responsible for the collection of all data, and a technical coordinator responsible for the work on the beach. The roles of the network are 1) to provide a rapid and effective response in case of stranding, including the making of any pertinent decision (animal rescue and transportation, euthanasia, necropsy) or providing scientific advice to decision makers and 2) to collect all information and data for the associated research program. Before necropsy, it should be well established if it is necessary to preserve the skeleton or not. Sometimes, local museums show spontaneous interest in anatomical material; however, it should be made clear to all requesting parties that obtaining such material usually is costly (time, money and personnel consuming).

Such networks exist for the European countries bordering the North Sea. For Belgium, the Marine Animal Research & Intervention Network (MARIN) is in charge of determining the causes of death of marine mammals stranded along the Belgian coast and surrounding areas such as the northern part of France (in collaboration with the "*Centre de Recherche sur les Mammifères Marins*, University of La Rochelle"), also providing support to the Netherlands.

# 3. NECROPSY

The aim of necropsies is (1) to provide information about lesions, (2) to ascertain the cause of death and its potential origin and therewith provide information about the health status of and the mains threats to a population and (3) to collect samples. Investigations on samples collected during necropsy can provide data on biology (reproductive status, life history ...), physiology and anthropogenic pollutant burdens of the marine mammals, amongst others.

It is always better to perform the necropsy as soon as possible after death but, in practice, that is not always possible. As such, in case of a mass stranding or epizootics (e.g. morbillivirus), there are too many animals and other solutions have to be found. It is recommended to perform a necropsy on as many animals as possible, and the best solution is to freeze animals (at -20°C) that can be not examined immediately (only feasible for small cetaceans and seals). Ideally, the necropsy should be done by a trained pathologist.

As all information (e.g. about pathology, toxicology, life history ...) that can be obtained by means of a necropsy, is valuable to expand our knowledge of marine mammals (which is very limited for most large whale species), major efforts should be undertaken to collect as many samples and data as possible. Considering the necessary effort, and to gain as much sensible data and samples, a list should be available beforehand that states the required samples (see annex) and processing laboratories (see annex). The provided list can certainly be expanded for further and more detailed research and should be updated on a regular basis. It should not be forgotten that scientific information is exploitable only when carefully documented. While there are probably as many ways to dissect a cetacean, as there are pathologists, the aim of this report is to propose a standard procedure for the management of a marine mammal stranding event and for the necropsy with tissue sampling for an intervention level 3 (see below). This protocol can be used as a guideline for improving post-mortem investigation on those species.

The methodology presented below is organized in two parts: (1) general considerations of marine mammals necropsy (2) particularities for small cetaceans, pinnipeds and large cetaceans.

**GENERAL PROCEDURE**

For biosecurity reasons, all participants should wear adequate protective equipment (clothes, gloves, boots, and mask). A necropsy reference number or code (one and only one) should be given to every animal, and the reference should be present on every sample collected. All organs must be examined carefully and sliced, ideally at regular intervals. Lesions must be described before and after incision (taking into account shape, color, content, consistence, aspect ...), they are sampled (see below) and photographed with the necropsy reference of the animal and a ruler indicating the size. In addition to lesions, some tissues are systematically collected for histopathological, parasitological, bacteriological, virological, toxicological and live history investigations (see annex). This sampling can be done in no specific order, but tissues for bacteriology, virology and electron microscopy should be collected as early in the process as possible. Moreover, to prevent contamination by microorganisms from the gastro-intestinal system, the intestinal tract should be left intact until the end.

*External examination*

The body condition is estimated using the condition code (see table). Photographs should be taken in lateral view, with the head, body openings and external lesions in particular.

The nutritional status is evaluated by measuring blubber thickness and evaluating muscle aspect; a bad nutritional state is characterized by a thin blubber layer and muscle atrophy (hallow aspect lateral to dorsal fin and protrusion of lateral processes of lumbar vertebrae).

The skin is carefully examined for lesions and ectoparasites, particularly around body openings. Special attention should be paid to scars (with photographs and precise description of localization, number, size ...) allowing for animal identification. A skin sample is taken for life history. Lesions are collected for histology and for microbiology.

The body openings, including mouth (tongue, palate, teeth or baleen plates), eyes, blow-hole(s), ear openings, genital slit and anus, are examined and any lesions, parasites or discharges are described and sampled. Photographs are taken when appropriate. In females, the mammary glands are examined and sampled for histopathology. If present, milk is collected for toxicology and biochemistry. In neonates, the umbilicus is examined and collected for histology.

*Subcutis and muscle*

The blubber and its interfaces with skin and muscle are particularly investigated for the presence of bruises, parasites and parasitic cysts. Some of these cysts have nearly the same colour as blubber fat and therefore difficult to identify. Blubber samples, for toxicology, are taken when blubber thickness is measured at the caudal insertion of the dorsal fin, and should include the skin and the entire blubber thickness. Muscle is collected for toxicology (heavy metals and organochlorines) below the place of blubber sampling (same place, same time).

*Abdominal cavity opening*

The peritoneum is investigated for the presence of parasites and parasite cysts.

The abdominal organs are sampled for bacteriology and virology as soon as the abdominal cavity is opened, except for the intestinal tract. Frequently, the gastrointestinal tract, dilated by putrefaction gases, protrudes out of the opening of the abdomen. In such cases, incisions are made along the greater curvature of the stomach compartments. The intestines are ligated cranially, close to the stomach and removed by dissection of the mesentery. Care should be taken as the intestinal tract ruptures easily.

The liver is observed *in situ* on both sides and sampled for toxicology (heavy metals and organochlorines), histopathology, virology and parasitology. The organ is then sliced at regular intervals for internal examination, with special attention for the presence of gas bubbles. The bile ducts are examined for the presence of parasites.

The kidneys are observed *in situ*, sliced at regular intervals and sampled for toxicology (heavy metals and organochlorines), histopathology, virology and parasitology. Samples should include complete reniculi with renal medulla and cortex. Renal tissue and blood vessels are examined for the presence of parasites.

The urinary bladder is observed *in situ* and sampled for histopathology. The content is described (volume, colour ...) and should be collected at the lower part of the bladder for parasitological examination (parasite eggs).

The gastric compartments are opened along the greater curvature and the content is collected in a strainer (life history and parasitology). The stomach can be rinsed with water to collect smaller parts (otolithes). Particular attention should be given to the presence of parasites, lesions, otolithes, fishbones and cephalopod beaks present in between gastric folds.

The testis are examined *in situ,* and sliced and sampled for life history. Special attention should be given to the presence of abscesses inside the testis and epididymis.

The ovaries are examined *in* situ, and sliced and sampled for life history. Note *any corpora lutea* or *albicantia* or follicles on each ovary.

The uterus is opened and examined. In pregnant females, the foetus is examined and sampled according to the dissection protocol.

The adrenal glands, situated between kidney and diaphragm, are collected for histopathology. Particular attention should given to cysts. The cyst's content should be collected (sterile syringe and needle) and kept frozen (-20°C) for biochemical analyses.

The pancreas and pancreatic ducts are examined in particular for the presence of parasites. Tissue samples for histopathology need to be taken as soon as possible, as this organ gets affected quickly and severely by autolysis and putrefaction.

The spleen and mesenteric lymph nodes are examined and sampled for histopathology and virology.

The mesenteric vessels should be examined directly after opening the abdomen for the presence of gas bubbles and chyle. In fresh animals, gas bubbles not associated with putrefaction are collected adequately for analysis. The intestines are examined last, outside of the abdominal cavity. Any lesion or parasite is carefully examined and collected. Intestinal content is described if present. Ligated segments are collected for bacteriology and parasitology.

*Thoracic cavity opening*

If the skeleton is to be preserved, the ribs should be detached carefully from the vertebrae and from the sternum. If not, these can be sawn through. Samples for bacteriology, virology and electron microscopy should be collected as soon as the cavity is opened.

All thoracic organs should be examined *in situ*, after which the respiratory system with the heart, major blood vessels and oesophagus are removed from the thoracic cavity.

The lungs are examined, carefully described (size, colour, consistency, aspect ...) and sampled (histopathology, bacteriology and virology). Before and after slicing, the lungs should be examined with special attention for the presence of gas bubbles. Tissue is sliced at regular intervals and the parenchyma is examined for the presence of liquids (blood, serous ...) or oozing foam. If present, parasites are collected.

The bronchioles, bronchi and trachea are examined and the lumen content, if present, is described. All parasites are collected.

The heart is separated from the lungs by cutting through the major vessels. Blood can be collected from the heart lumen, from major vessels or from elsewhere for virology, parasitology (blood parasites), toxicology and biochemistry. Left and right ventricles, and atria are opened and heart tissue is collected for histopathology. All parasites are collected.

All major blood vessels are examined and parasites are collected.

*Neck and head*

The lower jaw is dissected and cut at its temporo-mandibular joint. The hyoid bones are cut close to the skull. This way, the entire oral cavity can be examined as well as basis of the tongue with the tonsil area and larynx.

Dissection of this area allows for examination of larynx, trachea and oesophagus. The latter should be entirely examined for the presence of preys. If fishes are present, their orientation (head of fish towards stomach or oral cavity) must be specified. Dissection of tissue overlaying the trachea allows for identification of thyroid gland and thymus (not easily observed). They are examined and sampled (histopathology).

**SMALL CETACEANS**

For cetaceans that can be transported easily, it is recommended to perform the necropsy in a dissection or necropsy room.

Particularities of small cetacean necropsy are presented hereafter.

*External examination*

Photographs should be taken in lateral view, with special attention to the presence of net marks on the head, around rostrum and flippers. Pictures of the dorsal fin can be helpful for animal identification. If teeth marks (indication of inter- and/or intraspecies interaction) are present, they are described, measured and photographed. Any evidence of predation by a seal (claw and/or teeth marks) should reported.

The body length (see annex) is measured by placing a measuring tape next to the carcass (laying ventrally) in a straight line parallel to the longitudinal body axis. The total body length is taken from most cranial point of the rostrum to the notch in the tail fluke. After skin and blubber are incised vertically from the cranial insertion of the dorsal fluke down to the ventral midline, the blubber thickness is measured, at three locations along the cut: dorsally, laterally and ventrally.

In males, the penis is externally not visible, as it is wrapped in a subcutaneous sheath (preputial pouch) and in the pouch by the contraction of an S-curvature. To examine the penis, it is necessary to pull it out or to open the preputial pouch. Sample is collected for histology.

*Abdominal and thoracic cavity opening*

For practical reasons, it is recommended to position the animal on its right side, and to cut the left flank, opening the abdominal and thoracic cavity at the same time. To do this, a horizontal incision is made through skin and blubber, along the lateral processes of the vertebrae from the neck to the anus level. Two more incisions are made perpendicular to the first incision down to the ventral midline, one between neck and throat, the other to the anus. A strip of skin and blubber is removed together with the scapula and the left flipper. The prescapulary lymph node can be collected for histology and virology. To open the abdominal cavity, the topographical reference to find is the caudal extremity of the last rib. The extremity is pulled upwards and the abdominal wall is opened by cutting through the musculature along the last rib, without damaging the intra-abdominal organs and continued caudally along the ventral midline to the anus. The left side of the rib cage is removed in one piece, cutting through the articulations between ribs and vertebrae and ribs and sternum. A rib can be collected for toxicology. The windows allow for a general overview of the topography and an inspection of the organs *in situ.*

The examination of abdominal and thoracic organs and sampling procedures are similar to what it is described in the general procedure.

Neck and head

The skin and subcutaneous tissues are removed ventrally from the intermandibular space to the entry of the thorax. After examination and sampling of tissues such as tongue, oesophagus, trachea, thyroid, and thymus (in young animals), the head is separated from the rest of the body by dissecting the atlanto-occipital joint. The synovial liquid is examined macroscopically and collected for microbiology if necessary. The mandibula-temporal joint is cut, the lower jaw separated from the head and teeth (at least 4) are collected from the halfway to be stored frozen for age determination. The acoustic fat within and around the posterior part of the mandible is examined in situ and the mandible is sawed through halfway to detect any evidence of haemorrhage or acoustic fat bleeding. Acoustic fat is collected for histology.

*Ear extraction and fixation protocol*

TYMPANIC-PERIOTIC COMPLEX (T-P complex)

The tympanic and periotic bones house the middle and inner ear, respectively. These structures are partially fused forming the tympanic-periotic complex (Figure 1). 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, with the exception of sperm whales and some beaked whales who present the tympanic-periotic complex partially fused to the temporal bone.

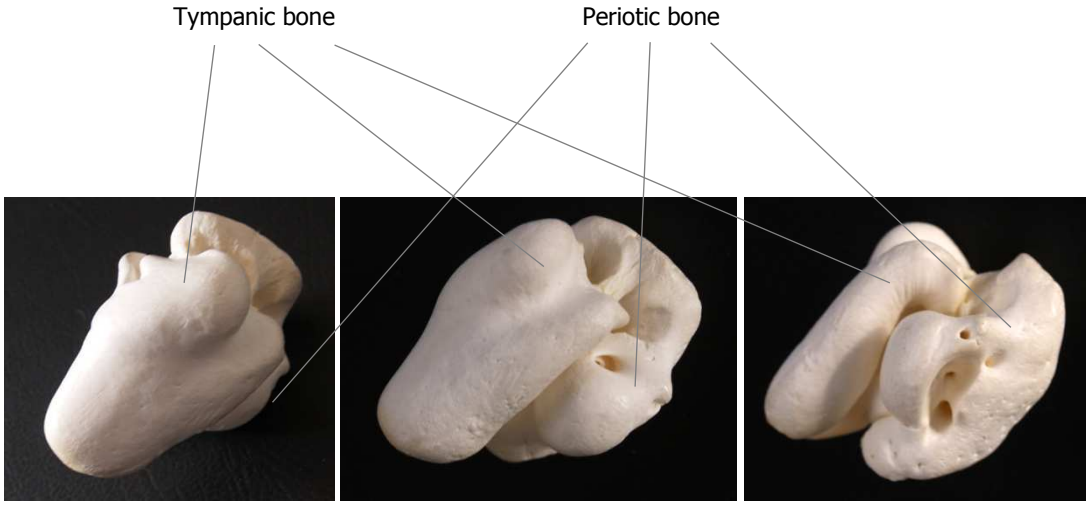


Figure 1. - Tympanic-periotic complex of a *Stenella coeruleoalba* stranded in Almeria, Spain. Sample provided by Promar

## Extraction

1. - In small specimens, it is recommended to cut the head of the animal for easier manipulation (Figure 2).

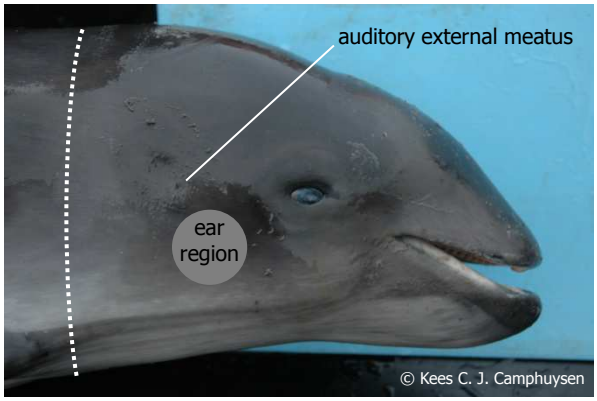


Figure 2. - The position of the tympanic-periotic complex and auditory external meatus is indicated. The dotted line marks the incision path to separate the head from the rest of the body. Alternatively, the digestive system can be extracted from the head to facilitate the access to the ears.

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


Figure 3. - Sagittal cut of a bottlenose dolphin head where the location of the tympanic-periotic complex is indicated.

3. - Exposing the ventral part of the head and removing the soft tissues and ligaments (Figure 4) allows to proceed to the tympanic-periotic complex extraction. The ventral air sinuses are dissected and examined for the presence of parasites and fluid.

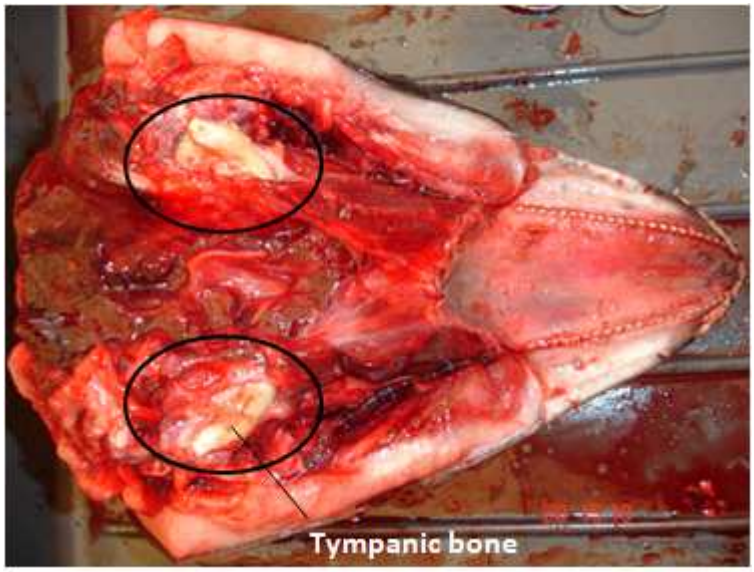


Figure 4. - Image taken during the necropsy of a *Phocoena phocoena*. This image reflects how the tympanic-periotic complex appears after removing the lower jaw (no effort has been made here to clean the area of extraction)

4. - Incise **gently around** the tympanic-periotic complex with a small knife (a scalpel can be used for the final stage of the extraction) and cut the ligaments that hold the ears in the peribullar sinus (see Figure 5).

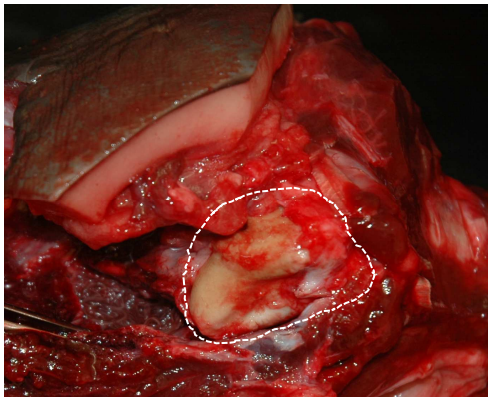

Figure 5. - Image taken during a *Phocoena phocoena* necropsy. The dotted line illustrates the location where the knife should be placed to extract the tympanic-periotic complex.

**Fixation**

5a. - At that stage, the ear could be fixed simply placing it in a fixative solution: glutaraldehyde 2,5 % with phosphate buffer 0,1M2 or a mixture of paraformaldehyde 0,5% with glutaraldehyde 1% with phosphate buffer 0,1M3. If the mentioned fixative solutions are lacking, the tissues can be also fixed with formaldehyde 10%.

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

5b. - The fixative solution (glutaraldehyde 2,5% with phosphate buffer 0,1M or a mixture of paraformaldehyde 0,5% with glutaraldehyde 1% with phosphate buffer 0,1M) should be introduced through the oval window (after removing the stapes and making a small and superficial hole in both windows) and the round window (Figure 6) using a soft catheter from the same diameter as the windows size (Figure 7).

If the above fixative solutions are lacking, the ears can alternatively be injected with formaldehyde 10%.

The injection is a very delicate operation and should be done by trained people. It is important to mention if the ear has been injected or not when sending it. In any case, before performing the injection, you are welcome to contact the Laboratori d'Aplicacions Bioacústiques1.

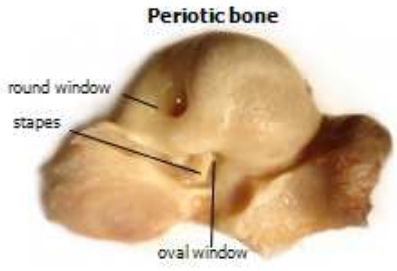


Figure 6. - Localization of the oval and round windows in the periotic bone.

1 Maria Morell or Michel André. Laboratori d'Aplicacions Bioacústiques. Universitat Politècnica de Catalunya. Avda. Rambla Exposició s/n 08800- Vilanova i la Geltrú (Barcelona) – SPAIN. Phone: +34 618521819 or +34 938967227 http://www.lab.upc.edu maria.morell@lab.upc.edu

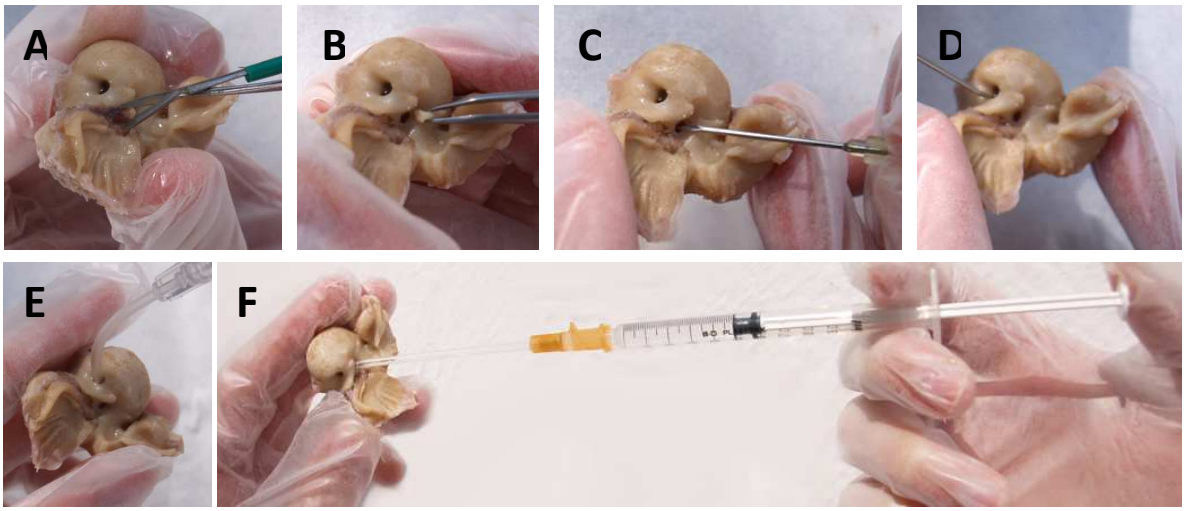


Figure 7.- *Tursiops truncatus* periotic bone used to illustrate all the steps in the injection process: A) cut of the stapedial ligament, B) removal of the stapes, C and D) the performance of a little a superficial hole in the oval and round windows respectivelly, E and F) introduction of the fixative solution progressively and very slowly (very little pressure) through the oval window and the round window using a soft catheter from the same diameter as the windows size, until the solution gets out through the other one for some seconds.

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

*Brain or central nervous system tissue*

If spinal cord sample is necessary (histology or microbiology), it can be collected after the head separation within the atlas vertebrae. To gain access to the brain, the skull can be sawed longitudinally. The blowhole and nasal passages can be examined for the presence of parasites, fluids or foam. The pituitary gland (mid-ventral part of the brain, in the *Sella turcica*) is sampled separately for histology. A complete cerebral hemisphere is collected for histology while the other hemisphere is sampled for virology, toxicology and bacteriology. The ventral air sinuses are dissected and examined for the presence of parasites and fluid.

**PINNIPEDS**

Most pinnipeds in European waters are easily transportable so the necropsy should be performed in well-equipped facilities such as dissection and necropsy rooms.

Particularities of pinniped necropsy are presented hereafter.

*External examination*

Body length (see annex) is measured by placing a measuring tape next to the carcass (laying ventrally) in a straight line parallel to the longitudinal body axis. The total body length is taken from most rostral point of the head to the caudal extremities of the hind flippers. The blubber thickness is measured dorsally after skin and blubber incision.

As in cetaceans, the penis is not protrude externally and should be examined and sampled as described hereinbefore.

*Abdominal and thoracic cavity opening*

Pinnipeds are positioned on their back before opening the interior cavities. The first incision is made along the ventral midline from the intermandibular region to the anus with two additional incisions from the xiphoid process of the sternum to the foreflippers (V-shaped incision). The skin and subcutaneous tissues are removed. The abdominal cavity is opened along the hypochondrium and after by two backward incision. The ribs are cut or sawed halfway through and the ribcage is removed. A rib can be collected for toxicology. This window allows for a general overview of the topography and the organs *in situ.*

The examination of abdominal and thoracic organs and sampling procedures are similar to what it is described in the general procedure. Unlike in cetaceans, the stomach is singular.

*Neck and head*

After examination and sampling of tissues in the neck area (tongue, esophagus, trachea, thyroid, thymus), the head is separated from the rest of the body by dissection of the atlanto-occipital articulation. The lower jaw is separated from the head and a canine tooth is collected and stored frozen for age determination.

*Brain or central nervous system tissue*

If a spinal cord sample is necessary (histology or microbiology), this can be collected after the head separation within the atlas vertebra. To gain access to the brain, the skull is sawed longitudinally. Nostrils and air passages can be examined for the presence of parasites, liquids or foam. The pituitary gland (mid-ventral part of the brain, in the *sella turcica*) is sampled for histology. A complete cerebral hemisphere is collected for histology while the other hemisphere is sampled for virology, toxicology and bacteriology.

## LARGE CETACEANS

*On spot organization*

The necropsy of large cetaceans, or many large cetaceans in case of mass stranding such as frequently observed for sperm whales and pilot whales or unusual mass stranding as for beaked whales, presents a major logistical and scientific challenge if a complete examination is to be performed. It requires an effective collaboration between public authorities, scientists, media and public. We consider as large cetaceans animals that are too large to be necropsied away from the stranding site because their transportation is too money-, time- and manpower-consuming. Large cetaceans include sperm whales, baleen whales, large beaked whales and pilot whales.

To perform a gross pathological examination of the carcass of a large whale on the beach and to collect the aforementioned information and samples requires heavy equipment and considerable manpower. In touristic areas, such as most coastlines along the southern part of the North Sea, the beaches must be left in the same state as before the stranding and dissection took place, for both biosecurity and economic reasons. It is -more often than not- not possible to let a whale carcass lie to rot where it is and to let tide, weather and scavengers take care of it as is possible in some parts of the world. Nor can the carcass be towed out to sea, as in most cases it may drift back to shore, driven by currents, winds and tides, and moreover, large floating carcasses may represent a major danger for marine traffic. Burying the carcass on site can be hazardous as there is possible environmental contamination and the potential of whale carcasses "refloating" on the sand, e.g. unburying themselves, during the process of decomposition. To date, there is no valid solution for on site carcass disposal and stranded animals must be removed from the site and disposed of elsewhere. Again, this requires heavy equipment and manpower. The best solution is to collaborate with a team in charge of the carcass disposal, benefiting from their equipment. It should be considered potentially dangerous (dissemination of pathogenic organisms,...) to open a whale carcass without the equipment required to eliminate the waste (muscle, blubber flats, visceral organs) from the beach. When material is not available or if collaboration is not directly possible, heavy-duty equipment should be obtained from the state, municipal civil services or from private sources.

The technical coordinator has an essential role the organization for all operations on the beach. This person should coordinate all technical support, ensure the security of workers, handle the press and by-standers alike, and communicate effectively with local and public authorities. The coordinator should ensure that the team in charge of the carcass disposal and the heavy-duty equipment is on site as soon as possible. The coordinators, both scientific and technical, should consider all political and legal aspects. As there are many unexpected characteristics implied in these events, such coordination should be well prepared beforehand and not improvised on the spot. In areas like the North Sea where strandings of large cetaceans and mass strandings frequently occur, it is essential to hold periodic meetings (national and international) with all intervening parties (coordinators, scientists, authorities,...), without any excitation as occurs during an event, in order to facilitate field collaboration and coordination. This meeting should define:

1) stranding interests -scientific, public exhibition,...-; 2) the regional status of marine mammals; 3) the political and legal framework such as national and international agreements; 4) the role of research programs on marine mammals; 5) possible financial support for carcass disposal; 6) technical availability. Previous stranding experiences and results should also be presented during such meetings, and to be discussed on a round table to improve the stranding management and the protocols for necropsy and sampling.

With well-trained and well-coordinated teams, a necropsy can be done well and in a reasonable timeframe with a certain comfort. In addition, a mass stranding of sperm whales requires several necropsy teams, and the post-mortem examination itself should only be performed by trained scientists. Such organization requires collaboration between pathologists in charge of marine mammals necropsy, and volunteers like students in veterinary medicine or biology. As previously described, such logistics should be prepared in advance and not improvised on the beach.

A stranding of one or many large cetaceans is very attractive for the public and media, and major efforts should be made to provide journalists with the right information. As soon as possible, the coordinators and scientists must organize a press conference on the spot with the first results of the on-site investigations (first observations of animal(s), first necropsy results,...). A second press conference could be organized later (after event excitement) with results from laboratories involved in the network.

Large cetacean necropsy is very different to that of small marine mammals made in a well-equipped necropsy room. It is usually performed on the beach near the place of stranding. Basic necropsy equipment for large cetaceans should be ready and available beforehand. So, it is important to have an updated checklist of material needed. It is useful to have phone lists to contact and mobilize: 1) personnel and volunteers; 2) public and local authorities; 3) Civil Protection, Police and Fire brigades and 4) and to obtain additional material when required (heavy-duty equipment).

Field material should be assembled as kits, each in is own transport box.

Plastic badges are stakeholder to identify people on the beach with name, institutional affiliation and function (coordinator, pathologist ...). Badges should also be provided to volunteers. In addition, for night work each person should have reflective safety clothing.

Manipulation of large cetacean carcasses requires heavy and haulage equipment working on the beach. This is either provided through the collaboration with the team in charge of the carcass disposal or it should be provided by the state, civil services or by a private source.

As soon as possible (before necropsy), an operation centre with phone and internet line close to the stranding site should be established (hotel, communal building ...) where coordination meetings, teams briefings, press conferences... will be organized.

Every intervention should be carried out with care for human safety. Large cetaceans stranding events usually cause major agitation and excitement of authorities, scientists, volunteers and public, with the latter often extremely curious and sometimes willing to help. For safety reasons, the number of people with direct access to the carcass(es) should be restricted to the working teams and local authorities (police, fire brigade, civil protection). The latter should limit the access with barriers at sufficient distance to the animal (25 m minimum) to allow the scientists and heavy machinery to work. In some animals in advanced decay, there is a substantial risk of explosion-like evisceration due to the elevated internal pressure because of the production of decomposition gases. This is an additional reason to keep the public at a significant distance to the animal. Identification badges help police services to only allow access to authorized persons.

The second step of the intervention is to avoid the carcass from drifting away with the next high tide. The body can be dragged ashore at the maximum high tide and sand barriers around the whale, erected by bulldozers, will delay the body immersion at the next tide.

Putrefaction and associated post-mortem tissue damage and alteration are processes that step in very fast in large cetaceans, and especially in sperm whales. Therefore, the necropsy and tissues sampling should be carried out as soon as possible, and require a concurrent quick, effective and safe waste disposal.

*Necropsy team & procedure*

The necropsy of a large cetacean, especially in case of a mass stranding, should be performed by a team of at least 3 persons, including a trained pathologist, an assistant in charge of photographs and taking notes of the necropsy findings, and one assistant in charge of sampling (storage and labelling). Ideally, these assistants should have basic scientific knowledge. When more persons or qualified volunteers are present, it is useful to have one person storing samples (dirty work) and one person in charge of labelling (clean work). Additional manpower is needed for the manipulation of organs. The animal should get a reference number as soon as possible, useful for the sampling.

Before the necropsy, it is necessary to hold a briefing to define the general rules of action, safety and biosecurity on the beach. All parties involved (coordinator, scientists, volunteers, authorities, carcasses disposal team ...) should participate in this briefing. Similarly, it is recommended to hold a debriefing after completion of the necropsy and removal of the carcass. This will allow to gather all information (e.g. for press conference) and to thank all participants. A liaison person, usually one of the coordinators, should maintain contact with all non-scientific parties involved after the event and inform them of the scientific findings.

*Mass strandings*

Each animal must have one (and only one) reference number and this reference number must be used for labelling the samples.

Depending on several conditions, we define three levels of intervention, from very limited (level 1) to a full execution of the herein described protocol (level 3). These parameters include the number of stranded animals, the accessibility to the stranding site, the availability of heavy-duty equipment and manpower, the availability of necropsy teams

and the body condition of the animals. Considering all of these, it is more or less possible or useful to perform the post-mortem investigations. In case of a mass stranding of more than 5 animals on a not easily accessible site (e.g. sandbank), or when heavy equipment or necropsy teams are not present, the intervention should be limited to level 1. In case of a mass stranding of 5 or less animals, occurring on easily accessible area with heavy equipment quickly available and a necropsy team per animal, intervention should not exclude any point of the present protocol (level 3).

In case of mass strandings of more than 5 cetaceans, necropsy should begin and be limited to animals with the best condition score.

If animals strand on sandbank in relative small distance of an accessible beach, they should be dragged ashore as soon as possible. Although this implies a delay to the intervention, but should not keep necropsy and tissues sampling from happening. Carcasses disposal and necropsies are two different activities performed by two different teams but they can operate simultaneously and in collaboration. It is recommended to give preference to animal in good condition to be dragged ashore (if possible) so the two teams can start work immediately.

*Necropsy*

All organs must be examined carefully and sliced, ideally at intervals of 10-20 cm. Lesions must be described before and after incision (taking into account shape, colour, content, consistency, aspect,...), sampled (see below) and photographed with a ruler indicating the size. In addition to lesions, certain tissues are systematically collected for histopathological, parasitological, bacteriological, virological, toxicological, and life history investigations (see annex 3). This sampling can be done in no specific order but tissues for bacteriology, virology and electron microscopy should be collected first. Moreover, the intestinal tract should be left intact until last to prevent contamination by micro-organisms from the intestines from occurring. Precautions should be taken to avoid sample contamination by sand (not easy on sandy beach), in particular for histopathology and toxicology.

*External examination*

The body condition is estimated using the condition code (see table). Photographs should be taken of lateral view, of the dorsal and pectoral fins (ideally ventral view), and of body openings and lesions.

The body length is measured by placing tape next to the carcass in a straight line parallel to the longitudinal body axis. The total body length is taken from most rostral point of the head to the notch in the tail fluke. The blubber thickness is measured at the caudal insertion of the pectoral fin.

The nutritional status is evaluated taking into account blubber thickness and muscle aspect; a bad nutritional state is characterized by a thin blubber layer and muscle atrophy (hallow aspect lateral to dorsal fin and protrusion of lateral processes of lumbar vertebrae).

The skin is carefully examined for lesions and ectoparasites, particularly around body openings. Special attention should be paid to any scars (precise description with photographs, localization, number, size ...) allowing for the whale's identification, and for presence of parasites between the ventral throat grooves. A skin sample is taken for life history.

The body openings, including mouth (tongue, palate, teeth or baleen plates), eyes, blow-hole(s), ear openings, genital slit and anus are examined, and any lesions, parasites or discharges are described and sampled. Pictures are taken when needed. In females, the mammary glands are examined and sampled for histopathology. If present, milk is collected for toxicology and biochemistry. In large male cetaceans and particularly the sperm whale, the penis protrudes soon after death due to internal pressure building up in the body cavities; this makes it easy to examine. Particular attention should be given to the external ear of both baleen whales and sperm whales: the outer ear opening and the ear canal should be sampled for histopathology and microbiology, the ear plug in mysticetes is collected for life history.

Abdominal cavity opening

In case the animal is in a state of advanced decomposition (condition code 4; in sperm whales also condition code 3), it would be dangerous to open the abdominal cavity directly because of the chance of "explosion-like evisceration". First, the internal pressure should be reduced either by making a pressure-relieve cut on the whale’s back (standing out of the way of possible extruding visceral organs) or -in more advanced cases of bloating- by introduction

of rigid pipes through abdominal wall. After the internal abdominal pressure dropped, the decaying animal should be investigated last in case of a mass stranding; the internal examination -other than for life history samples- has limited value.

To gain access to the abdominal cavity, it is necessary to cut a window in the belly. The skin and muscles are cut with relative ease, but the rather fibrous blubber layer of sperm whales, which can reach 15 to 20 cm thickness, is very hard to incise. Although there are several techniques to open the abdomen, which is one of the most time and strength consuming procedures of the entire necropsy, preference is given to the following with the help of bulldozers to pull long horizontal strips of skin and blubber: an horizontal incision is made through skin and blubber, from the pectoral flipper to the level above the anus. Next, two vertical cuts, the first from the cranial end of the horizontal cut to the ventral midline and the second from the caudal end of the horizontal cut to the anus. Next, ropes or chains are attached to the skin and blubber flaps at the horizontal incision and pulled by bulldozers (or men) for dissection of the abdominal blubber and exposure of the abdominal muscles. After the strip of tissue has been removed, the subcutaneous tissue and blubber are examined for lesions, parasites and parasite cysts. Some of these cysts have nearly the same colour as the blubber and are not identified easily. Sampling for toxicology should be done at the caudal insertion of dorsal fin and should be performed when blubber thickness is measured.

The same stripping technique is used to remove the abdominal muscle layers and peritoneum.

If necessary to climb onto the whale, it is useful to cover it with sand to avoid slipping.

While blood can be collected from the heart, blood samples from vessels of the skin or abdominal wall can be taken more easily and are in most cases free of contamination.

Muscle is collected for toxicology (heavy metals and organochlorines) below the place of blubber sample (same place, same time).

The peritoneum is investigated for the presence of parasites and parasite cysts.

Sampling for bacteriology and virology should take place as soon as the abdominal cavity is opened, with the exception of the intestinal tract. Frequently, the tract is dilated by putrefaction gases and protrudes out through the opening of the abdomen. In such case, incisions are made along the greater curvature of the stomach compartments. The intestines are ligatured cranially, close to the stomach, and removed by dissecting the mesentery while

helpers pull the intestines. Care should be taken as the intestinal tract is easily torn and it is relatively hard to pull it out of the abdomen.

The liver is observed *in situ* on both sides as far as possible and sampled (toxicology - heavy metals and organochlorines, histopathology, virology, parasitology). The organ is then sliced for internal examination, ideally at intervals of 10 cm, and the bile ducts in particular are examined for the presence of parasites.

The kidneys are observed *in situ*, sliced at intervals of 10 cm and sampled (toxicology - heavy metals and organochlorines, histopathology, virology, parasitology) including complete *reniculi* with renal medulla and cortex. Renal tissue and its larger blood vessels are examined for the presence of parasites.

The urinary bladder is observed *in situ* and sampled for histopathology. Its content is described (volume, colour ...) and collected at the lowest part of the bladder for parasitological examination (parasite eggs).

The gastric compartments are opened along greater curvature and their content is collected. Particular attention should be given to the presence of parasites, lesions and cephalopod beaks in between the gastric folds. If the stomach seems empty, an incision should be made at the sloping area to collect the any liquids it contains (life history and parasitology).

The testis are examined *in situ*, sliced and sampled for life history

The ovaries are examined *in situ*, sliced and sampled for life history. Note any *corpora lutea, albicantia* or follicles on each ovary.

The uterus is opened and examined. In pregnant females, the foetus is examined and sampled according to its own dissection protocol.

The adrenal glands are difficult to spot, but should be collected for histopathology. Particular attention should be given to the presence of cysts. The cysts content should be collected (sterile syringe and needle) and kept frozen (-20°C) for biochemical analyses.

The pancreas is examined and pancreatic ducts are opened for the presence of parasites. As this organ is affected rapidly by decomposition, tissue samples for histopathology should be taken as soon as possible.

The spleen and the mesenteric lymph nodes are examined and sampled for histopathology and virology.

The intestines should be examined last, outside of the abdominal cavity. They should not be opened on the sand (potential micro-organism and waste dissemination), but rather

in the mechanical shovel of a bulldozer before being discarded in the waste container. Any lesion or parasite should be carefully examined and collected. The intestinal content is described if present. Ligated segments are collected for bacteriology and parasitology.

All organs and especially lymph nodes, mesenteric blood vessels and the liver should be carefully examined for the presence of gas bubbles.

Thoracic cavity opening

If the skeleton is to be preserved, the opening of the thoracic cavity requires a careful detachment of the ribs from the vertebrae and the sternum. First, it is necessary to strip the ribs from its muscles and connective tissue; then, the ribs are removed one by one (this procedure is very time-consuming). During this operation, samples for bacteriology, virology and electron microscopy should be collected as soon as possible. If the skeleton is not to be preserved, the ribs can be sawn through. If it is not possible to completely open the thoracic cavity, for instance due to lack of time or of manpower, there are two alternative ways to open the thoracic cavity: after removing abdominal organs, the thoracic cavity is approached from the abdomen by cutting through the diaphragm, and cranially, after severing the head from the body, through the cranial thoracic opening. Alternatively, for a fast access to one lung, the thoracic cavity is opened by cutting a window between two ribs. This allows for visual examination and palpation of a very small part of one lung.

In the thoracic cavity, the organs are not easily examined *in situ*. Depending on the circumstances, it may be useful to remove the respiratory systems with the heart and major blood vessels from the thoracic cavity. Otherwise, examination and sampling are performed inside the cavity.

The lungs are examined, carefully described (size, colour, consistence, aspect ...) and sampled (histopathology, bacteriology and virology). The tissue is sliced at regular intervals and the cut surface is examined for the presence of liquids (haemorrhagic, serous...) or foam oozing out of parenchyma. All parasites are collected.

The bronchioles, bronchi and trachea are examined and the lumen content, if present, is described. All parasites are collected.

The heart is separated from the lungs by cutting the major vessels. Blood is collect from the heart lumen, from major vessels or from elsewhere for virology, parasitology (blood parasites), toxicology and biochemistry. Left and right ventricles, and atria are opened and heart tissue is collected for histopathology. All parasites are collected.

All major blood vessels are examined and all parasites are collected.

*Neck and head*

If possible, the lower jaw is dissected and the mandibula-temporal joint is cut. The hyoid bones are cut close to the skull. This way, it is possible to examine the entire oral cavity as well as basis of the tongue with the tonsil area and larynx.

Dissection of this area allows for examination of larynx, trachea and oesophagus.

The oesophagus is inspected for the presence of prey over its entire course. If fishes are present, their orientation (head of fish to stomach or to oral cavity) must be specified.

Dissection of tissue overlying the trachea allows for identification of the thyroid gland and thymus (not easily observed). They are examined and sampled (histopathology).

*Brain and central nervous system tissue*

To transport the animal to the rendering plant (carcass disposal), the body is usually cut in 2 or more pieces. Once the head is detached (classical technique in sperm whale), it is possible to collect brain tissue (histopathology, virology) through the occipital foramen. Spinal cord tissue can be obtained when the body is cut elsewhere.

*Animal weight and blubber thickness*

The total body weight should be calculated after weighing the carcass and necropsy wastes at the rendering plant at the time of the carcass disposal. For sperm whales, there is a correction factor (1.14) to compensate for the loss of bodily fluids during dissection and transportation. A predictive formula of normal weight (W) can be used from the measured length (L), where $W = 0.00218*L^{2.74}$. This calculation allows comparison of estimated weight of stranded sperm whales and weight reported of sperm whales caught during whaling operation. Similarly, blubber thickness can be compared with reference values. All this information is complementary for evaluating the nutritional status (see above).

**COLLECTION AND STORAGE OF SAMPLES**

*Condition code*

The condition code (C.C.) describes the quality of conservation or the state of decomposition of a body.

Warning: In state of moderate decomposition (C.C. 3), there is already pressure building up in the abdominal and thoracic cavities. This has to be taken into account when performing the cut to open these cavities, as pressure-relieve cut should be made ; in advanced stages of decomposition (C.C. 4), it becomes hazardous to open the abdomen and special security measures need to be taken. In our experience, the careful opening of sperm whale carcasses of C.C. 3 has often led to an explosive evisceration of the stomach and intestinal content, dispersing squid beaks and decomposition liquids over more than 5 meters away from the whale. All by-standers should be warned of the imminent opening of the abdomen and security measures should be taken to protect the public.

The condition code determines which samples are useful to take (Table 4). Due to rapid decomposition of internal organs in large cetaceans, the external tissues may sometimes be useful while the internal organs are in an advanced state of decomposition.

*Labelling of samples*

Particular attention should be given to labelling the samples. The best is to have only one person (per animal in case of mass stranding) in charge of sample labelling. It is useful to pre-print sticker labels in doubles, one on the outside and one inside the sample container.

As such,

- each individual animal must have its own reference number, especially in case of a mass stranding and the number must be the same for all samples from that animal.

- each sample must have a specific label that includes a description of the tissue, the animal reference and the sample destination (histopathology, virology,…)

- label should be printed and written with indelible ink, in English, with legible writing and appropriate terminology

- all samples should be kept together until they reach their destination (laboratory).

**SAMPLE SHIPPING**

Perishable samples (virology, bacteriology, biochemistry) should be shipped to the adequate laboratory with an express overnight delivery service with all pertinent documents for transportation. As these samples should be sent as soon as possible after collection, it is useful to have express delivery packages prepared in advance to be shipped directly from the stranding site. Samples should be wrapped in protective paper or plastic with coolants (dry ice) and packed with Styrofoam chips. Make sure that the samples do not leak during transportation, as in many countries, the sender is responsible for all damage arising from such leakage. Inform the overnight delivery service of the nature of the shipment; they will avoid the loss of such a shipment at all costs once they know what consequence (smell) to expect from any delivery delay.

**LIFE HISTORY SAMPLE**

*Teeth (age determination)*

*Collection*: in sperm whales, teeth are present in the lower jaw; protruding upper jaw teeth are rare, but teeth buds are usually embedded in the gingiva. At least one tooth is necessary but typically hard to extract. If the skull is not to be preserved, the lower jaw can be sawed off and teeth extracted by maceration. The extraction of teeth on site should be left for the end of the necropsy. Due to the general misbelief that sperm whale teeth are extremely valuable ivory and as the carcasses often remain on the beach for several days after necropsy, it is advised to cover the head of the whale with sand (by bulldozer) after embedding the lower jaw in a plastic sheet.

*Storage*: Teeth can be frozen (temperature not critical) or fixed in 10% neutral-buffered formalin or 70% ethanol. They should not be stored dry as this may lead to cracking.

*Earplugs (age determination in baleen whales)*

Storage: earplugs should be preserved in 10% neutral-buffered formalin.

*Stomach content*

Storage: stomach content are collected at the lowest part of the organ and can be frozen (temperature not critical) or fixed in 70% ethanol. Do not preserve stomach content

of fish-eating species in formalin, since it may dissolve small bones. Special attention should be given to collecting cephalopod beaks from between the gastric folds.

*Skin (DNA studies)*

Storage: skin fragments can be frozen (-20°C) or fixed in DMSO or 70% ethanol.

*Gonad (reproduction studies)*

Storage: gonads are weighed and samples are fixed in neutral-buffered formalin after slicing to a maximum of 1 cm thickness.

*Skeleton or part of skeleton*

Before necropsy, it should be well clarified if it is necessary to preserve the skeleton. Natural history museums or similar institutes should coordinate this work with the carcass disposal team, to make sure that no bones are lost (e.g., hyoids, T-P complex, pelvic bones and femur in sperm whales). If the skeleton does not need to be preserved, some operations are facilitated (teeth extraction, opening of thoracic cavity). But in any case, pelvic bones, femur and T-P complexes should be collected for morphological study.

**HISTOPATHOLOGICAL SAMPLES**

Collection: A detailed list of systematic samples is given in annex 3. Actually, as many pathological conditions cannot be observed in gross examination, it is advised to collect samples from all organs, even those appearing to be normal. Tissue samples should not exceed 1 cm thickness. All lesions should be collected with an adjacent piece of normal tissue. Use a sharp scalpel and try not to damage the tissue by excessive manipulation. It is better to have many samples of the same organ than one large sample.

Fixation: the most common fixative used is 10% neutral-buffered formalin[2]. Other fixatives such as Bouin's solution should be avoided. Large pieces should be sliced at intervals of 1 cm as tissue penetration is a slow process. Some organs (eye, uterus, intestine…) or lesions (cyst…) should be injected with the fixative. For good fixation, the formalin:tissue volume ratio should be 10:1. Brain samples should be placed in at least 20 times as much formalin as tissue and remain in the fixative for one week.

2 10% neutral-buffered formalin (pH 7.2) should be prepared as follows (for 10l) : dissolve 85.45g Na2HPO4 and 25.45g KH2PO4 in 9l of distilled water. Add 1l of concentrated formalin (37%-40% formaldehyde solution).

If a large amount of fixative is necessary on site, you should preserve the 10% neutral-buffered formalin should for immunohistochemistry, (see below), while tissues for histopathology can be fixed in 10% (unbuffered) formalin solution[3] prepared on site. Samples should be processed within 3 months (risk of saponification of fat-rich tissue).

#### IMMUNOHISTOCHEMISTRY

Samples for immunohistochemistry should be fixed in 10% neutral-buffered formalin and processed (paraffin embedding) after 24 h of fixation.

#### ELECTRON MICROSCOPY

Samples for electron microscopy should be collected as soon as possible, finely diced and kept at 4°C in glutaraldehyde (glass jar).

#### POLYMERASE CHAIN REACTION [PCR]

Samples for PCR should be collected as soon as possible and kept frozen at -70°C as soon as possible.

#### VIROLOGICAL SAMPLES

Collection: A detailed list of samples is given in annex 3. All lesions of suspected viral origin should be collected; similar to histopathological samples, the sampling of all major parenchymatous organs is recommended. Commercial kits are available for the collection and transport of virological samples. The organ surface should be disinfected with 70% alcohol; then, samples (2x2x2 cm) should be collected aseptically and placed in appropriate sterile containers. If they are sent to a laboratory of virology within 24 hours (express delivery courier service in adequate packaging), then the tissue should be kept at 4°C, otherwise it should be frozen at -70°C as soon as possible.

#### BACTERIOLOGICAL SAMPLES

Collection: A detailed list of samples is given in annex 3. All lesions of suspected bacterial origin should be collected. Tissue samples (6x6x6 cm) should be collected

3 10% formalin solution should be prepared as follows (for 10l) : add 1l of concentrated formalin (37%-40% formaldehyde solution) in 9l of water.

aseptically, placed in appropriate sterile containers (aerobes and/or anaerobes) and kept at 4°C. Avoid freezing of samples for bacteriology. Samples should be shipped to a laboratory (express delivery courier service in adequate packaging) and processed as soon as possible. To collect blood from the heart or liquids from tissue (urine, fluid from abscesses, from pericardium,…), first disinfect the organ surface, and collect the sample from lumen using sterile needle and syringe or Pasteur pipette before opening the organ. To collect intestinal content, the sample should be ligated at both extremities before cutting. Alternatively, for tissue blocks, swabs may be used: disinfect the organ's surface with 70% alcohol, incise with a sterile scalpel blade, place the swab into the incision, and then into the sterile tube with transport medium.

Lesions of suspected fungal origin should be collected as tissue blocks.

#### PARASITOLOGICAL SAMPLES

Collection: A detailed list of samples is given in annex 3. Any parasite should be collected and preserved in 70% ethanol with 5% glycerine. The total number of parasites should be counted, estimated or weighed. Samples of lung, kidney, gastric content, ligatured intestine and blood should be collected systematically (parasite and egg identification) and kept at 4°C if investigations are to be performed within 24 hours, otherwise they should be frozen at -20°C as soon as possible. Urine, collected at the lowest part of the urinary bladder, should be examined for the presence of parasite eggs.

#### TOXICOLOGICAL SAMPLES

Collection: A detailed list of samples is given in annex 3. For toxicology, field samples should be large (10 g minimal) to avoid any influence by the sample container. They should be stored at -20°C if investigations are not performed directly. Samples should be weighed before freezing (compensation of storage dehydration) and not thawed during storage.

Samples of blubber, muscle, liver, kidney, blood, and central nervous system tissue should be collected for organochlorine analysis. Blubber (full thickness of the layer with the skin) and muscle should be sampled in front of the dorsal fin. Samples should be large and ideally they are kept in especially cleaned glass, aluminium or Teflon.

Samples of muscles, kidney, liver and blood should be collected for heavy metal analysis. They should not come in contact with any metal other than stainless steel and can be stored in a plastic bag.

In lactating females, milk should be collected for organochlorine analysis.

**BIOCHEMICAL SAMPLES**

For biochemical analyses, blood and serum should be collected. In lactating females, milk and any special fluid should be sampled, like for instance from cysts (adrenal, thymus). These should be sampled with a sterile needle and syringe. If samples are sent to a laboratory of biochemistry within 24 hours, they should be kept at 4°C, otherwise they should be frozen at -20°C as soon as possible.

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# 6. ANNEX

**TABLE 1: KITS AND THEIR POTENTIAL CONTENT**

- Cutting equipment
- Sampling and labelling equipment, vials and containers, formaldehyde, glutaraldehyde, and other preservation chemical required, plastics bags…
- Cooler or transportable fridge
- Dirty clothes container (preferably airtight)
- Dirty equipment container (preferably airtight)
- Cleaning equipment and disinfectants for instruments and beach
- Sample container (preferably airtight)
- First-aid kit
- Several complete clothes set per person
- Towels, soap, … for showering
- Personal equipment

**TABLE 2: INTERVENTION LEVEL AND FOLLOWING POST-MORTEM EXAMINATION**

| Level | Post-mortem examination |
|---|---|
| 1 | Total length, blubber thickness at caudal insertion of pectoral flipper, skin sample, blubber and muscle samples, lower jaw or tooth, blood sample, fluke photo, gross external examination |
| 2 | Level 1 + partial necropsy and samplings following heavy duty equipment availability, necropsy team availability, animal condition code |
| 3 | Complete necropsy and sampling |

**TABLE 3: CONDITION CODE (C.C.) - LARGE CETACEANS – BASED ON OUTER APPEARANCE**

| | |
|---|---|
| C.C. 1 | Live animal, becomes code 2a at death |
| C.C. 2 a | Extremely fresh (just died, no bloating)<br>in sperm whales :<br>-pectoral flippers are in recumbent position next to thoracic wall<br>-penis is not protruded<br>-tongue is in the back of the oral cavity<br>-mouth is closed or somewhat open, rigor mortis possible (very short duration) |
| C.C. 2 b | Fresh (slight bloating, blood imbition visible)<br>In sperm whales :<br>-pectoral flippers begin to rise<br>-penis protruding moderately<br>-tongue begins to occlude back of oral cavity<br>-mouth is open, no rigor mortis |
| C.C. 3 | Moderate decomposition bloating, skin peeling, penis extended in males, organs still intact, excluding post-mortem damage<br>in sperm whales :<br>-bloating of the body becomes visible and continues rapidly until an almost explosive evisceration, mostly through the back, takes place (C.C. 4)<br>-penis is fully extended<br>-tongue occludes mouth more and more, decomposition gases and fluids run from the mouth. Through this continuing process, the mouth is forced to a fully open position, while at the same time gases and fluids force their way out (driven by the bloating pressure) alongside the very swollen, occluding tongue. This process indicates that the core |

| | |
|---|---|
| | temperature of the animal has risen and decomposition is progressing rapidly. At some point, melted spermaceti starts running out of the mouth and coagulates on the ground. |
| C.C. 4 | Advanced decomposition major bloating distended or exploded abdominal cavity (please note: in sperm whales, there is danger of a spontaneous explosive evisceration of the abdomen, usually through the back). Skin peeling, some organs beyond recognition. In some cases, protrusion of abdominal organs through buccal cavity. |
| C.C. 5 | Undetermined mummified carcass or skeletal remains, no organs present.<br>In mysticetes: very advanced decomposition leads to a highly amorphous white mass of several meters length, which represents the remains of the blubber and skin. |

**TABLE 4: SAMPLING RELATED TO C.C.**

| SAMPLES FOR | C.C. |
|---|---|
| Life history | 2-5 |
| Histopathology (including immunocytochemistry) | 2-3 |
| Virology | 2-3 |
| Bacteriology | 2 (-3) |
| Parasitology | 2-4 |
| Toxicology | 2-3 |
| Electron microscopy | 2 |
| Biochemistry | 2 |
| PCR | 2-5 |

**FIELD EQUIPMENT**

*Check lists*

This field equipment list

Telephone lists

Tissue samples list

*Necropsy*

Various sharp high quality knives, including flensing knife if possible

Sharpening steel and electric knife sharpener

Butcher hooks

Butcher saws and spare blades

Scalpel handles and blades

Latex and Kevlar® gloves

Ropes and chains

10 m measuring tape

20 cm tape for photographs

Special container for biological material and used scalpel blades, syringes and needles

Various buckets to collect organ's content, to rinse tissue…

Cleaning equipment and disinfectant for instruments

*Various*

Camera

Mobile phone

*Sampling*

Plastic containers (250 ml, 1 l and 2 l)

Glass jars for electron microscopy

Plastic bags (1 l, 5 l and 100 l)

Syringes and syringe needles

Pasteur pipette

70% alcohol

Blood tubes

Indelible ink pens

Pre-printed sticker labels

Transportable refrigerator or cooling box

Express courier package

For microbiology

- Alcohol
- Sterile swabs
- Sterile containers
- Transport medium (for bacteria, for virus)

For histopathology

- 20l jerrican with formaldehyde 37-40%
- 20l jerrican with 10% neutral buffered formalin
- 20l jerrican with distilled water
- Glutaraldehyde

For parasitology

- 70% ethanol with 5% glycerine

Beach equipment

- Power generator
- Lighting

Heavy-duty equipment to work on the beach:

- Bulldozers
- Mechanical shovel
- Large containers (for disposal of necropsy remains)

Facilities

- Toilet
- Foods & refreshments

For personnel

- Warm and waterproof clothes (raincoat), hats and boots
- First -aid kit
- Hand soap and towel
- Identification plastic badges
- Reflective safety tape

*List of samples*

| | HP. | Viro. | Bact. | Parasi. | Toxico. | Bioch. | LH. |
|---|---|---|---|---|---|---|---|
| skin | | | | | | | |
| body orifices | | | | | | | |
| mammary gland | | | | | | | |
| ear canal | | | | | | | |
| earplug | | | | | | | |
| eye | | | | | | | |
| teeth | | | | | | | |
| blubber | | | | | | | |
| muscle | | | | | | | |
| liver | | | | | | | |
| adrenal | | | | | | | |
| mesenteric lymph node | | | | | | | |
| spleen | | | | | | | |
| gonad | | | | | | | |
| reproductive tract | | | | | | | |
| stomach | | | | | | | |
| food remains | | | | | | | |
| intestine | | | | | | | |
| kidney | | | | | | | |
| urinary bladder | | | | urine | | | |
| pancreas | | | | | | | |
| lung | | | | | | | |
| lymph node | | | | | | | |
| heart | | | blood | | | | |
| thymus | | | | | | | |
| thyroid | | | | | | | |
| tonsil | | | | | | | |
| central nervous system | | | | | | | |
| blood | | | | | | | |
| pelvis bone + femur | | | | | | | |
| hyoid | | | | | | | |
| *bullae tympanica* | | | | | | | |

HP: Histopathology : buffered formaline (* in separate container)

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D/2019/0480/7

ISBN 978-2-87543-138-7